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TARGETED THERAPY POSSIBILITIES FOR METASTATIC MELANOMA

by

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Abstract

The incidence of malignant melanoma of the skin has been steadily rising worldwide during the past decades. Most early detected primary tumors can be removed surgically and the prognosis is good. However, at the same time there still is no permanent cure for metastatic melanoma and its prognosis is poor, although lately new effective drugs have emerged.

In this thesis, four different approaches of experimental therapy for metastatic melanoma were studied. Endogenous *cis*-Urocanic acid (UCA) is found in every individual's skin, where exposure to UV light from the sun generates it from its inactive *trans* conformation. *Cis*- UCA was found to destroy malignant melanoma cells in culture under an acidified pH and sufficient concentration through caspase-3 mediated apoptosis. Furthermore, *cis*-UCA is able to considerably diminish the growth rate in human melanoma tumors on living SCID mice.

Using replication-competent Semliki Forest viruses, human melanoma tumors grown in SCID mice were dramatically shrunken as the fulminant production of viruses in melanoma cells leads them to apoptosis within 72 hours. Small oligopeptides attaching to melanoma cells were identified using *in vivo* phage display. The melanoma-specific peptides found were further tested *in vitro* on adenoviruses. Ultimately, the adenoviral retargeting using the peptides was tested *in vivo*. One peptide homed to human transferrin receptor upregulated on melanoma cells.

In order to kill the malignant melanoma cells with the retargeted adenoviruses, the viruses should carry genetic material producing apoptotic proteins in the cancer tissue. TIMP-3 has been identified as a good candidate for such a protein, as it inhibits malignant cell adhesion as well as promotes apoptosis through a caspase-8 pathway. It is further shown here that adenovirally delivered TIMP-3 is even more potent, as it could kill non-adherent cancer cells, lacking the fully functional death receptor signaling pathway. Adenovirally delivered TIMP-2 also showed marked antitumor effects in human malignant melanoma xenografts on SCID mice both in *ex vivo* and systemic delivery.

Key words: Melanoma, *Cis*-Urocanic Acid, SFV, Adenovirus, Phage display, TIMP

Janne Kallio
Tutkimus pahanlaatuisen melanooman etäpesäkkeiden hoitomahdollisuuksista

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Lyhennelmä

Viime vuosikymmeninä uusien melanoomatapausten määrä on kasvanut tasaisesti ympäri maailmaa. Onneksi monet varhain todetut kasvaimet voidaan kuitenkin poistaa kirurgisesti, jolloin ennuste on hyvä. Pidemmälle ehtineeseen, etäpesäkkeitä muodostaneeseen melanoomaan ei kuitenkaan edelleenkään ole olemassa tehokasta parantavaa hoitoa ja sen ennuste on huono, vaikkakin aivan viime aikoina uusia lääkkeitä on tutkittu menestyksellisesti.

Tässä väitöskirjassa tutkittiin neljää eri keinoa levinneen melanooman pysäyttämiseksi. Sisäsyntyistä *cis*-urokaanihappoa syntyy jokaisen yksilön ihossa, jossa auringon UV-valo muodostaa sitä hapon inaktiivisesta *trans*-muodosta. *Cis*-UCA tuhoaa melanooma- ja muita syöpäsoluja oikeanlaisen pH:n ja konsentraation vallitessa soluviljelmissä kaspaasi-3 -välitteisellä apoptoosilla. Lisäksi SCID-hiirissä kasvatetuissa ihmisen melanoomakasvaimissa *cis*-UCA pystyy huomattavasti hidastamaan kasvaimen kehitystä.

Käyttämällä jakautumiskykyistä Semliki Forest -virusta osoitettiin, että jakautuva virus voi dramaattisesti pienentää kasvaimen kokoa ajamalla syöpäsolut apoptoosiin 72 tunnin kuluessa. Toisessa tutkimuksessa etsittiin lyhyitä, melanoomasoluihin tarttuvia, proteiinipätkiä phage display -menetelmällä. Menetelmää käytettiin hiirissä, joilla oli niskassaan ihmisen melanoomakasvain. Löydettyjä peptidejä testattiin edelleen ilmennettynä adenovirusten pinnalla soluviljelmissä. Lopulta adenovirusten uudelleenkohdentamista peptideillä testattiin *in vivo* melanoomahiirissä. Yksi peptideistä tarttui ihmisen transferriinireseptoriin. Kyseinen reseptori yliesiintyy ihmisen melanoomasoluissa. Muut peptidit tarttuivat melanoomasoluihin vaihtelevasti.

Jotta melanoomaan ohjautuvista viruksista olisi hyötyä, niiden tulisi tappaa infektoimansa solut. TIMP-3 on tunnettu jo pitkään lupaavana ehdokkaana apoptoottiseksi proteiiniksi. Se estää syöpäsolujen tarttumista ja aiheuttaa apoptoosia kaspaasi-8 –rettiä käyttäen. Tässä tutkimuksessa todettiin adenoviruksella ilmennetty TIMP-3 vieläkin lupaavammaksi, sillä se pystyy tuhoamaan myös irrallaan olevia syöpäsoluja, joilta puuttui täysin toimiva apoptoosin kaspaasi-8 -signaalireitti. Myös adenoviruksella ilmennettyä TIMP-2 -proteiinia testattiin kasvainhiirissä melanoomakasvaimissa, joissa se selvästi ehkäisi kasvaimen kasvua niin suoraan syöpäsoluihin kuin systeemisestikin annosteltuna.

Avainsanat: Melanooma, *Cis*-Urokaanihappo, SFV, Adenovirus, Phage display, TIMP

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Abbreviations

ADAM	A disintegrin and metalloproteinase
AKT	Protein found in AK mouse strain with transforming capabilities
BAD	Bcl-2 antagonistic of cell death
Bcl-2	B-cell CLL/Lymphoma 2
BRAF	Proto oncogene serine/treonine protein kinase
cAMP	Cyclic adenosine mono phosphate
CAR	Coxackie-adenovirus receptor
CD	Cluster of differentiation
CDKN2A	Cyclin-dependent kinase inhibitor 2A
CNS	Central nervous system
CT	Computer tomography
CTLA4	Cytotoxic T-lymphocyte antigen 4
ECM	Extracellular matrix
ERK	Extracellular –regulated kinase 1,2
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
GABA	Gamma amino butyric acid
GCP	Good clinical practice
HDAC	Histone deacetylase
HUVEC	Human umbilical vein endothelial cell
Jun	(Japanese ju –nana =) 17 – protein
LDH	Lactate dehydrogenase
IGF	Insulin like growth factor
IL	Interleukin
MAPK	Mitogen activated protein kinase
MEK	Mitogen activated protein kinase kinase
MITF	Micropthalmia associated transcription factor
MMP	Matrix metalloproteinase
MOI	Multiplicity of infection
NF	Nuclear factor
NK	Natural killer
NRAS	Neuroblastoma RAS viral oncogene homologue
PD	Phage display
PET	Positron emission tomography
PI3K	Phoshatidylinositol 3’ kinase
Raf	Proto oncogene serine/threonine protein kinase

RAS	Rat sarcoma oncogene
SCID	Severe combined immunodeficiency
SFV	Semliki forest virus
TGF-β	Transforming growth factor β
TIMP	Tissue inhibitor of metalloproteinases
TNF	Tumor necrosis factor
TRAIL	TNF related apoptosis inducing ligand
TUNEL	Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling
VEGF	Vascular endothelial growth factor
XIAP	X linked inhibitor of apoptosis protein

List of Original Publications:

I: Laihia JK, Kallio JP, Taimen P, Kujari H, Kähäri VM and Leino L. (2010)
Protodynamic intracellular acidification by cis-urocanic acid promotes apoptosis of melanoma cells *in vitro* and *in vivo*
J. Invest. Dermatol. 130(10): 2431-9

II: Vähä-Koskela MJ, Kallio JP, Jansson LC, Heikkilä JE, Zakhartchenko VA, Kallajoki MA, Kähäri VM and Hinkkanen AE. (2006)
Oncolytic capacity of attenuated replicative semliki forest virus in human melanoma xenografts in severe combined immunodeficient mice.
Cancer Res. 66: 7185-94

III: Kallio JP, Work L, Vihinen P, Talve L, Baker AH and Kähäri VM. (2010)
Identification and characterization of a peptide targeted to metastatic melanoma
(Manuscript)

IV: Kallio JP, Hopkins-Donaldson S, Baker AH. and Kähäri VM. (2010)
TIMP-3 promotes apoptosis in non-adherent small cell lung carcinoma cells lacking functional death receptor pathway.
Int. J. Cancer (In press)

Unpublished data on adenovirally delivered TIMP-2 effects on human metastatic melanoma cells and tumors in SCID mice is included in the Results.

1. Introduction:

Increased recreational exposure to UV radiation from the sun, along with with increased life span expectancy, are considered the main reasons for the constantly increasing number of primary cutaneous malignant melanoma in the Western world. The loss of the normal functions of a cell and the gradual development of a cancer is always a multistep process that involves genetic as well as functional changes of right order and magnitude, combined with failure of cells to correct these harmful changes. Melanoma is no different in this respect.

For early detected primary melanoma, the prognosis is fairly good when the tumor is removed by surgical excision. The prognosis for metastatic disease is generally poor, depending on the stage of metastasis, and no efficient cure is yet available.

Possible ways to cure metastatic malignancies include ways of increasing the number of programmed cell deaths or decreasing the number of cells dividing constantly without control. Even slowing down the progression of the disease or more precise information on the location of the metastasis would be beneficial. An additional challenge for the cure is the delivery of the treatment to the site of the metastasis together with the protection of nonmalignant tissue from harmful effects.

2. Review of the Literature

2.1 Malignant melanoma

The types of cancers originating from the skin can be divided into subgroups depending on the cell type origin from which the cancer begins to develop. Malignant melanoma of the skin originates from the melanocytes which are located in the lower epidermis and produce the skin pigment melanin (Gray-Schopfer *et al.* 2007). General steps for melanoma formation include self-sufficiency of growth signals, resistance to growth-inhibitory signals, resistance to apoptosis, unlimited replicative potential, sustained angiogenesis and the acquisition of metastatic potential (Hanahan *et al.* 2000). All typical steps need sufficient time to take place. The primary cause of melanoma is the ultraviolet (UV) light from the sun correlating with the absorbed amount of radiation (Armstrong *et al.* 2001). The most effective way of prevention is considered to be protection against sunlight with clothing, shade or avoiding the peak hours of UV irradiation during the day. When using a sunscreen formulation, one blocking both UV-A and UV-B radiation should be preferred.

2.1.1 Incidence in population and the sensitizing factors

Since the 1960s the incidence of melanoma has increased between 3-8% per year in the European population. However, the survival rate after first detection has improved from 40% in the 1960s to almost 90% today (Beddingfield 2003). In the USA, the trend has been similar and a further rise in melanoma incidents is predicted (http://seer.cancer.gov/statfacts/). Primary melanoma is often found in the skin of the limbs or the head area, which are usually the areas mostly exposed to sun during life. The naevi of the upper torso are also a common source. The rise in general awareness, improvements in diagnostics and the risen general life expectancy naturally play a role in the incidence rate detected. Melanoma seems to be quite resistant to the therapies known today, surgery being the most commonly used method of cure (Soengas *et al.* 2003).
On an individual level, six characteristics have been noted as risk factors contributing to potential future development of malignant melanoma:

1. Multiple naevi (Proportional melanoma risk rises tenfold when the amount of naevi goes from 50 to 120) (Gallagher *et al.* 1990; Kanzler *et al.* 2001)
2. More than 4-5 clinically atypical naevi (Dark, unevenly shaped, large) (Bataille *et al.* 1996)
3. Previous removal of primary melanoma (Roberts *et al.* 2002)
4. Several relatives affected by melanoma (Bajetta *et al.* 2002)

5. Combination of the previously mentioned sensitizing factors (Naeyaert *et al.* 2003) and
6. Large congenital naevi or xeroderma pigmentosum disease (Zaal *et al.* 2004) and in some rare cases inherited mutation in the CDKN2A gene (Tucker *et al.* 1985; Marrett *et al.* 1992; Bliss *et al.* 1995; Goldstein *et al.* 1995; Grulich *et al.* 1999; Bishop *et al.* 2002).

In the Finnish population, the trend has followed global development. According to the Finnish Cancer Registry (http://www.cancerregistry.fi), the number of new skin melanoma cases reported in 2008 was 511 in males and 473 in females. The respective values 20 years ago during the period of 1987-1991 were 241 cases in males and 256 cases in females in total. The Finnish cancer registry annual statistics start from the year 1962, and the incidence rate has increased in both sexes ever since the beginning of the data base. Fortunately, the trend in melanoma deaths has decreased in Finland during the above mentioned 20 years from 27% to 21% in males and from 21% to 15% in females. This is mostly due to improved early detection and excision of primary melanoma.

2.1.2 Clinical signs and markers of melanoma

For melanoma starting from naevi, common clinical signs are asymmetry, border irregularity, color change, diameter greater than 6 mm and evolution – the so called ABCDE system (Friedman *et al.* 1985; Healsmith *et al.* 1994; Robinson *et al.* 2009). However, roughly 5% of all melanomas are non-pigmented (Shaw *et al.* 1981). Melanoma is also rarely diagnosed on the mucous membranes of inner cavities (Cassarino *et al.* 2008; Kumar *et al.* 2008). There have even been reports of melanoma in the nervous system but these are considered rare exceptions (about 1% of all cases) (Levidou *et al.* 2007). Ocular melanoma is considered the most common intra-ocular malignant primary tumor and has therefore been the subject of many recent studies (Nemati *et al.* 2010). The clinical pathological classification of skin melanomas forms four distinctive classes: *lentigo maligna melanomas, superficially spreading melanomas, nodular melanomas and acral melanomas* (Weedon 2002). Other methods for diagnosis are biopsies followed by immunohistochemical (IHC) stainings and dermoscopy, i.e. microscopy of the living skin (Gibson *et al.* 1988; Menzies 1997). Common factors that are upregulated in melanoma and so stained from the paraffin sections by IHC are gp100 (more than 90% of cases) and melanA/MART-1 proteins, together with the proliferation marker Ki-67 (Gould Rothberg *et al.* 2009). Some additional and more novel melanoma markers are Glypican-3 (Nakatsura *et al.* 2004) and MITF (King *et al.* 1999). In advanced metastatic melanoma, increased gene expression of receptor collagen level has been quite recently noted to correlate with the survival rate (Vuoristo *et al.* 2007).

The prognosis of melanoma is determined mainly by two factors: tumor thickness and possible metastasis to regional lymph nodes (Rubin *et al.* 2009). Other, slightly less evident, aspects are possible tumor ulceration, mitotic rate, regression, patient sex and age as well as the site of the tumor. There has been more than a 50-year-long evolution of

staging systems commonly used to assess the progression of melanoma. Each new system has incorporated some new variables and nuances to the process of staging. One of the latest is the AJCC staging system introduced in 2002 (AJCC, Staging manual 6th edition, New York, Springer-Verlag, 2002) (Thompson *et al.* 2004). Internationally, guidelines for melanoma diagnosis and treatment have been provided by the British Association of Dermatologists (B.A.D., http://www.bad.org.uk/) as well as the European Dermatology Forum (EDF, http://www.euroderm.org/). The Finnish Medical Society Duodecim also gives guidelines for standard good clinical practice for diagnosis and treatment of melanoma (Suominen 2005). Melanoma can be staged by observing three different parameters of spreading: T (tumor), N (lymph node) and M (metastasis). In Finland, as in many other countries, two further staging systems have been in use. These are the Breslow class and the Clark classification (Clark *et al.* 1969; Breslow 1970). Melanoma classification has been renewed during year 2010. The use of the Clark classification has been permanently abolished and instead mitosis frequence per square millimeter is in use. This change is based on the new AJCC recommendation which includes Breslow class, mitosis frequence and ulceration, but not the Clark classification. The recomedation is based on statistical observations from 30 496 melanoma patients (Balch *et al.* 2009) (http://www.cancerstaging.org/).

2.1.3 Genetic alterations in melanoma cells and drugs targeted to them

The actual mutations leading to melanoma do not have to be exactly the same each time a tumor develops (Figure 1.). There is a variety of mutations detected only in a particular subset of melanomas. A constitutively activating mutation of NRAS is found in 21% of melanomas (Tsao *et al.* 1998; Dumaz *et al.* 2006). However, the most common mutation of melanomas is the BRAF activating mutation, whose high frequency is a probable indication of its central role in melanocyte physiology. It occurs in 10-30% of all primary melanomas (Dong *et al.* 2003). Moreover, BRAF mutations are also found in 60-80% of benign melanocyte naevi (Dong *et al.* 2003). Both mutations lead to unrestrained proliferation. As in many other cancer types, the disruption of CDKN2A gene products, proteins p16 and p14ARF is central to the development of melanoma. CDKN2A mutations are considered especially crucial in families with inherited melanoma susceptibility (Kefford *et al.* 2003). p16 mutation leads to an inability to control the cell growth cycle (Serrano 1997). p14ARF mutation, in its turn, leads to the degradation of p53 tumor suppressor protein by release of HDM2 (Sharpless *et al.* 2003). The growth of the tumor is not only the result of increased cell proliferation, but usually apoptosis is simultaneously blocked, which leads to the number of viable tumor cells being maximized. Often this is achieved by overexpression of antiapoptotic proteins Bcl-2 and Bcl-X (Soengas *et al.* 2003). Apoptosis effector Apaf-1 is also often inactivated as the disease proceeds (Soengas *et al.* 2001). It seems that some melanoma cells carry a mutated ErbB4 protein that might serve as a new target for drugs in a similar way as the Erb family members have in the case of breast cancer (Culouscou *et al.* 1993; Kurppa *et al.* 2009).

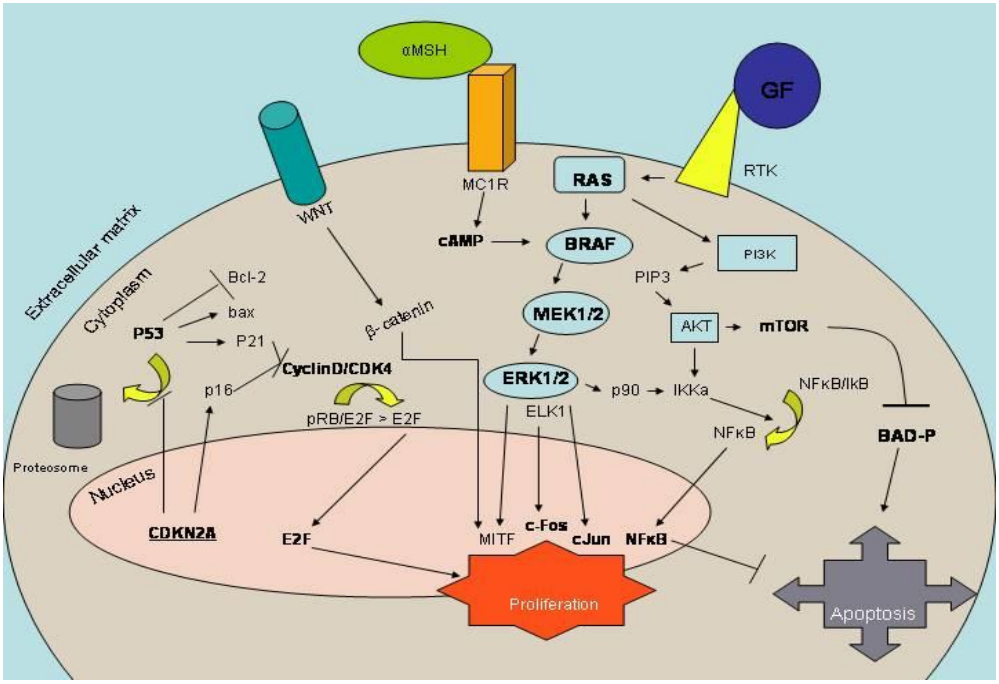


Figure 1. Cellular mechanisms and factors involved in development of cutaneous melanoma as well as normal growth of a cell. GF = growth factor. Adapted from: (Palmieri *et al.* 2009).

Frequently, the problem in modern melanoma diagnostics is that the amount of false positives tends to rise far above the number of real metastasis detected. For example, in one study of chest radiography real detection of tumors was 0.1% whereas the amount of false positives was 15% of the populations with suspicion of the disease. Other methods such as computer tomography (CT) scan, ultrasound and positron emission tomography (PET) are producing similar kind of results (Buzaid *et al.* 1993; Terhune *et al.* 1998; Hafner *et al.* 2004; Starritt *et al.* 2005). However, in the follow-up of high risk melanomas, whole body PET has been applicable (Koskivuo *et al.* 2007). There has been much debate on the correct width of margins in excision of primary melanoma, due to risk of malignant cells left behind. Unless the margins are sufficient, the remaining cells are very prone to spread. It is usually rather the tumor thickness than the margin which determines the outcome (Milton *et al.* 1985). A maximum of 2 cm or less in margins seems to be the optimum depending on the thickness of the tumor area (Haigh *et al.* 2003). Surgery can also help patients with quite highly metastatic case of the disease. For example, subungual melanoma, usually diagnosed at late stage, can be surgically removed, leading to a 40% 5-year survival (Cohen *et al.* 2008). Regional node dissection, when appropriate, has resulted in 13-59% of metastatic disease free patients (Balch *et al.* 2001). One has to note that the number of subjects treated in different studies is rather small and so the statistical significance of the results remains low.

Needle biopsy of the sentinel lymph nodes has been in use in some but not all melanoma treatment centers, although it is highly accurate in assessing the stage of the disease.

Recently, the high value of the biopsies for vital prognostic information has been further studied (Koskivuo *et al.* 2007). For a long time, the same sort of debate has concerned the removal of sentinel lymph nodes closest to the tumor site. Their status is usually checked with radioactive probes and correlates with the prognosis. The actual benefit from complete removal of the so called regional nodes around the cancer is somewhat controversial, since in many cases only sentinel nodes are affected (Jansen *et al.* 2000; Uren *et al.* 2006). Lately, new computer models have been created to more accurately interpret the affected nodes and metastasis (Reynolds *et al.* 2007).

At the moment, the prognosis for patients with advanced metastatic melanoma is poor. If skin, subcutis and lymph nodes are affected, survival will be at least 12 months, but if there is visceral involvement and elevated serum LDH, survival is predicted at an average of 4-9 months (Balch *et al.* 2001; Bedikian *et al.* 2006). Usually cutaneous melanoma first metastasizes to the skin or the closest sentinel lymph nodes. In approximately 30% of metastatic cases, distal metastases are observed (Balch *et al.* 2001). Common sites for metastasis are the lungs, liver and brain (Cohn-Cedermark *et al.* 1999; Manola *et al.* 2000; Wood *et al.* 2001; Barnholtz-Sloan *et al.* 2004). Sometimes melanoma that has spread extensively has started from a region not visible in everyday life, for example, under the nails or on the retina. Recently, a median survival of 8.5 months for such a cases was proposed (Neri *et al.* 2006).

Year 2010 has been succesfull in treatment of metatasized melanoma. Such new drugs as PLX4032 (a.k.a RG 7204) (Whang *et al.* 2009) and Ipilimumab (Morse 2005) have reached the stage of late clinical trials. PLX4032 is a BRAF kinase inhibitor, which targets to a specific mutation form of BRAF kinase called V600E. There glutamic acid is replaced by valine (Flaherty *et al.* 2010). The mutation is causing 90% of the constant activation of BRAF. In the recent phase I study of Flaherty et al., the orally taken drug not only stopped, but even diminished, the growth of metastasis in 81% of the trial subjects. One of the previous golden standars in MAPK inhibitory cancer treatment, sorafenib, although known to inhibit all BRAF mutations, has not shown such melanoma specific effect as the new PLX4032. One speculation has been that sorafenib might never reach large enough concentration at the tumor site due to early rising, severe, side effects.

Ipilimumab has lately performed well in phase III clinical trial (Hodi *et al.* 2010). Ipilimumab considerably improved the survival of previously treated patients with metastatic melanoma compared to vaccination to gp100 antigen on melanoma cells. CTLA4 molecule downregulates T-cell activation and is many times used by melanoma cells to inhibit immune system from recognizing the cancer. Ipilimumab is a CTLA4 blocker, which unmasks the hiding melanoma cells to facilitate immune defence against them. Combination of vaccination against gp100 and ipilimumab was also tried, but in this study there was no improvement of the results. Additional to its effect against melanoma, ipilimumab showed severe immunity related side effects, which could however be controlled with immediate administration of corticosteroids. Vaccination with sole CTLA4 blocker has been tried in clinical trials as well (Ribas *et al.* 2005).

For metastasized disease dacarbazine and interferon-α (IFN-α) seems to be somewhat effective and dacarbazine is recognized by the FDA for melanoma treatment (Gardere *et al.* 1972; Kefford 2003; Garbe *et al.* 2007; Vihinen *et al.* 2010)). Another approved therapy is IL-2, which is unfortunately rather toxic in higher doses (Rosenberg *et al.* 1985; Rosenberg *et al.* 1998). One candidate for an effective cure of metastatic melanoma is IL-24. Both IL-2 and -24 can also be delivered adenovirally and especially the apoptotic profile of IL-24 highly resembles that of TIMP-3, which is introduced later in this thesis (Lebedeva *et al.* 2007; Gopalan *et al.* 2008). Abolishment of some death receptor methylation might sensitize melanoma cells to therapy (Bae *et al.* 2008). One alternative way of approaching metastatic disease is isolated limb perfusions. This can be done either using isotopes or via chemotherapy (Cassumbhoy *et al.* 2007; Yoshimoto *et al.* 2007).

Chemotherapy alone has included dacarbazine (response up to 13% (Middleton *et al.* 2000; Bedikian *et al.* 2006)), temozolomide and fotemustine, which are used widely for other malignancies as well (Del Vecchio *et al.* 2007; Tawbi *et al.* 2007). The effect against melanoma seems to be rather low, especially with temozolomid (Eggermont *et al.* 2004; Mhaidat *et al.* 2007). The use of immunotherapy has raised some hopes recently as noted above. Different co-therapies containing an immuno-element as well as some other drug or therapy have produced promising results (Skak *et al.* 2008; Sundstedt *et al.* 2008). Various other molecular therapy options to be used after post surgical or other first line therapy are constantly being researched. Despite the traditionally low impact of chemotherapy against metastatic melanoma, proteasome inhibitor molecule called bortezomib has recently shown promise in sensitizing melanoma cells to T-cell attack (Seeger *et al.* 2010). Bortezomid has recently been tried in phase II trial for increasing the effects of chemotherapy, but without conciderable success (Croghan *et al.* 2010).

The panel of promising drugs can be sorted by their target area. Oblimersen works as an antisense agent against mitochondrial bcl-2 which promotes normally proliferation in melanoma (Bush *et al.* 2003). Inhibition of the melanoma survival promoting RAS-Raf-ERK signaling route is an area of extensive research. Different combined treatments for example with sorafenib and the classical temozolomide are currently under investigation (Gollob *et al.* 2006; McDermott *et al.* 2008). One option is to target the growth factor receptors over-expressed in melanoma, for example by inhibiting c-kit. Inhibition of kit by imatinib has proven to be especially effective in acral melanoma, where the mutation has been detected the most (Kim *et al.* 2008; Handolias *et al.* 2010). Proteasomes are multi-enzyme aggregates responsible for the intracellular degradation of proteins. They also regulate the expression of many critical proteins in cell cycle and survival. Bortezomib aims to downregulate the proteasomes but has shown lack of effect in rather recent studies (Markovic *et al.* 2005). MS-275 and other histone deacetylase (HDAC) inhibitors try to block the effect of deasetylases in cell growth and survival, this way promoting apoptosis (Hauschild *et al.* 2008). HDACs function as gene silencers of cell cycle arrest and differentiation (Laird 2005). Angiogenesis is fundamental for tumor growth and metastasis and, self evidently, a tempting target for therapy. Among the best candidates of the area is sunitinib, making its impact through inhibition of VEGFR, PDGFR, stem-cell factor receptor and fms-like 3 tyrosine kinases (Cabebe *et al.* 2006;

Ashida *et al.* 2009). However, in some mouse melanomas sunitinib has shown tumor promotion by increased neovascularization. This effect was lately and most importantly suggested to be characteristic to all VEGFR kinase related inhibitors (Cabebe *et al.* 2006; Ebos *et al.* 2009). Additionally, Vitaxin (McNeel *et al.* 2005), CNTO95 (Mullamitha *et al.* 2007), and semaxanib (Peterson *et al.* 2004) all target the crucial neovascularization by blocking receptors and proteins.

Vaccination against melanoma has been tried but without considerable success (Soiffer *et al.* 2003). In a recent study that used dendritic cells as antigen-presenting cells, the projected 5-year survival rate was 54%, which is considered reasonably good (Dillman *et al.* 2009). Interleukins 12 (Hamid *et al.* 2007) and -21 (Peluso *et al.* 2007) have been seen to reinforce the antigen-presenting CD cells as well as NK cells. The polypeptide Tymosin α-1 has had the same supportive effect on interleukins (Garaci *et al.* 2007). Recent phase I study with vaccination against the classical gp100 antigen found in melanoma, using vaccine HLA-A*0201, proved to be rather poor in efficasy (Baba *et al.* 2010).

2.2 Extracellular Matrix of Skin

An extracellular matrix (ECM) consists of all the material surrounding individual cells and cell types and binding them to form larger, more complex structures. The matrix is not a passive framework for cells, but an active and complex part of the tissues and organs. Remodeling of the matrix is required in development, morphogenesis and tissue repair, but also occurs in the course of many diseases. Major proteins of the ECM are collagens, accompanied by various glycoproteins (Sechler *et al.* 1998; Murphy *et al.* 2008).

2.2.1 Matrix metalloproteinases (MMPs)

Matrix metalloproteinases are a group of zinc-dependent endopeptidases collectively capable of degrading essentially all matrix components (Kessenbrock *et al.* 2010). A total of 23 different MMPs are known in humans. The structure of MMPs consists of basically four domains: pre-domain (for secretion), pro-domain (for latency), catalytic domain (degradation) and hemopexin-like domain (helper of interaction with substrate or docking site for TIMPs) (Puente *et al.* 2003). The hemopexin-like domain is absent from MMPs 7 and -26. MMPs modify the ECM in many physiological states and also in fully grown organs, one such event being wound healing (Toriseva *et al.* 2009).

In cancer, MMPs function mainly in the degradation of the ECM and basement membranes, but also promote cell proliferation and angiogenesis (Folgueras *et al.* 2004).

Recently an anti-cancer role has been proposed for MMP-8 (Lopez-Otin *et al.* 2009). Primarily, MMP induction is regulated on the level of gene expression. Induction is enhanced by cytokines and growth factors but also by other *cis*-regulatory elements like PEA-3, AP-2 and NF-κB (Sternlicht *et al.* 2001; Stamenkovic 2003). MMPs are mainly secreted as inactive zymogens. The activation of the MMPs requires removal of the pro-domain, which can be done under experimental conditions at least by alkylating agents, detergents, organomercurial and oxidants (Denhardt *et al.* 1993). In living organisms, MMP activation is performed by other proteins such as plasmin, cathepsin G, neutrophil elastase and oxidative changes of their environment (Saari *et al.* 1990; McCawley *et al.* 2000). MMPs are also capable of activating other MMPs (Wang *et al.* 2000). The family of MMPs is classically divided into subfamilies based on their structure and preferred substrates that they degrade. These are collagens (MMP-1, -8 and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10 and -11) and others (Toriseva *et al.* 2009). While many MMPs are secreted, MMPs 14-17, -24 and -25 contain a transmembrane sequence and are anchored on the cell membranes (Visse *et al.* 2003), they are called membrane-type MMPs.

The same group of metalloproteinases also includes the ADAM (a disintegrin and metalloproteinase) and ADAMTS (a disintegrins and metalloproteinase with thrompospondin-like motif) protein families. ADAMS work as cell surface sheddases while ADAMTS1 is secreted for collagen propeptidase processing and ECM breakdown (White 2003; Apte 2004).

2.2.2 MMPs and ADAMs in cancer

In many studies, a clear relationship between elevated levels of MMPs and tumor progression has been shown (Egeblad *et al.* 2002; Ala-aho *et al.* 2005; Hu *et al.* 2007; Villanueva *et al.* 2008). The degree of breakdown of the ECM is one of the defining aspects when melanoma tumor progression is studied (Stamenkovic 2000). Frequently, MMP induction takes place as a result of changes in transcription promoted by activation of oncogenes. For example, MMP-7 is induced by the src oncogene (Rivat *et al.* 2003). MMP expression is also activated through other proteins, such as EMMPRIN, already present in the cell, as in the case of tissue remodeling (Gabison *et al.* 2005). MMP effects in cancer have been noted not to be limited to breaking the ECM, but to also extend to suppression of apoptosis and promoting of angiogenesis (Yoon *et al.* 2003). On the other hand, on some occasions, MMPs have shown inhibitory activity to cancer by cleaving chemokines such as CXCL12 (McQuibban *et al.* 2001). Recently, MMP-8 has expressed anti-cancer properties in a mouse model (Balbin *et al.* 2003). Only limited evidence exits about the role of ADAMS or ADAMTS in cancer. At least ADAMs 10, -15 and -17 have promoted tumorigenesis and invasion by inducing G-protein coupled EGFR-receptor activation (Huovila *et al.* 2005). ADAMTSs are upregulated at least in breast (Porter *et al.* 2006) and pancreatic (Masui *et al.* 2001) cancers, and ADAMTS1 again has exhibited anti-angiogenic properties by reducing neovascularization (Kuno *et al.* 2004).

Melanoma cells express at least MMPs -1, -2, -9, -13 and MT-1-MMP (Hofmann *et al.* 2000). The expression pattern may also vary between different stages of tumor development. MMP-21 has been shown to be expressed during early melanoma tumor development, but it vanishes as the tumor becomes more aggressive (Kuivanen *et al.* 2005). In addition to the breaking basement membrane, MMP-2 has been shown to promote vascularization in melanoma. The melanoma progression correlates with MMP-2 expression (Väisänen *et al.* 1996; Väisänen *et al.* 1998). Overexpression of MT1-MMP related to melanoma tumor invasion has been discovered (Nakahara *et al.* 1997) and high serum levels of MMP-1 and -9 seem to correlate with rapid progression of the disease (Nikkola *et al.* 2005). Tumor-surrounding cells play a role in the melanoma progression by contributing to the production of MMPs (Hsu *et al.* 2002). MMP-1 expression predicts melanoma invasion and together with MMP-3 seems to correlate with shorter disease-free survival in metastatic disease (Nikkola *et al.* 2002). MMP-9 is special in that it is expressed only during the early phases of melanoma metastasis development, fading away later as the disease progresses (Hofmann *et al.* 2003). Studies linking ADAMs to melanoma are few. In one of them, ADAM-9 has been shown to be expressed specifically on the protruding front of the melanoma tumor (Zigrino *et al.* 2005).

2.2.3 Tissue inhibitors of metalloproteinases (TIMPs)

Tissue inhibitors of metalloproteinases (TIMPs) function primarily in the inhibition of MMPs in both physiological and pathological states, thus controlling the homeostasis of the ECM (Cruz-Munoz *et al.* 2008). TIMPs also inhibit the activity of the disintegrin and metalloproteinase family called ADAMs (Edwards *et al.* 2008). Four human TIMPs are known, named simply TIMP-1, -2, -3 and -4 (Docherty *et al.* 1985; Stetler-Stevenson *et al.* 1989; Greene *et al.* 1996; Hammani *et al.* 1996). Corresponding proteins can be found throughout the animal kingdom, right down to mollusks manifesting high conservation and importance of TIMPs during evolution (Brew *et al.* 2000). It is worth noting that not only TIMPs are responsible for MMP regulation. Molecules like α2-macroglobulin (Sternlicht *et al.* 2001), thrombospondin-1 and -2 (Rhee *et al.* 2002) are also reported to act towards the same goal. TIMP-1 is the most conserved throughout evolution where as TIMPs 2 and -4 are the newcomers of the family (Brew *et al.* 2000). The chromosomal location of TIMPs in humans is as follows: TIMP-1 is located in X-chromosome, TIMP-2 in chromosome 17, TIMP-3 in chromosome 22 and TIMP-4 in chromosome 3 (Pohar *et al.* 1999). TIMPs interact on various processes of tissue remodeling from embryo to wound healing and cancer. In tumor growth, the point of metastasizing induces many MMPs (Liotta *et al.* 2001; Egeblad *et al.* 2002). This is when the TIMP activity usually simultaneously fails, possessing possibilities for intervening in the process by supporting the TIMP activity. The breakdown of the peritumoral connective tissue is often backed up by the stromal cells, leading to the accumulation of degrading molecules on the tumor cell surfaces. This forms an area called an invadopodia.

Different TIMPs all have specific characteristics and they are expressed in quite individual tissues. TIMP-2 is the only TIMP which participates to the activation of pro-

MMP2 (Strongin *et al.* 1995). TIMP-3, on the other hand, inhibits ADAMs 9, -12 and -17 and also regulates specifically ADAMTS-4 and -5 (Amour *et al.* 2000; Kashiwagi *et al.* 2001). TIMP-3 is special in that it is stored into ECM and is the only TIMP so far for which a single gene mutation leading to a disease has been discovered. This disease is Sorsby's Fundus Dystrophy, which causes deterioration of the retina (Weber *et al.* 1994; Yu *et al.* 2000). TIMP-1 is found in bone. TIMP-2 is most widely expressed and found throughout the body. TIMP-3 is expressed in high levels in the kidneys, heart and lungs and TIMP-4 is expressed in the heart, brain, muscles, ovaries and testes (Apte *et al.* 1995; Nuttall *et al.* 2004). The methylation of genes of TIMPs 2-4 seems to be a key feature for the inactivation of TIMPs in many cancers (Bachman *et al.* 1999; Ivanova *et al.* 2004; Dammann *et al.* 2005).

2.2.4 TIMPs in cancer

In cancer, the role of TIMPs seems to be rather dualistic depending on the situation (Figure 2.). Overexpression of TIMP-1 in mouse melanoma as well as TIMPs 2-4 in other murine and human cancers inhibits the growth of the primary tumor (Khokha 1994; Wang *et al.* 1997; Vergani *et al.* 2001; Spurbeck *et al.* 2002). At the same time, there is evidence of poor outcome with high levels of TIMPs (Ruokolainen *et al.* 2005). For TIMP-1, one of the mechanisms of blocking the proliferation is the inhibition of proteolytic release of IGF (Martin *et al.* 1999). Additionally, TIMP-1 downregulates apoptosis, which it facilitates, usually through AKT phosphorylation and PI3Kinase activation. This leads to the prevention of BCL-2 and –XL interactions (Lambert *et al.* 2003). Furthermore, TIMP-1 blocks TRAIL –induced apoptosis at least in breast cancer cells (Liu *et al.* 2005). Reports of MMP-dependent anti-apoptotic effects of TIMP-1 have also been published (Murphy *et al.* 2004). TIMP-1 has an anti-angiogenic effect in tumors as well (Guedez *et al.* 2001; de Lorenzo *et al.* 2003). After primary tumor formation and its extensive vascularization, tumors usually set out to form metastasis. TIMP-1 has been shown to have a rather simple effect in this respect. In short, underexpression promotes loosened cell-cell adhesions whereas overexpression keeps cells tightly adhered (Roeb *et al.* 1999; Ho *et al.* 2001).

TIMP-2 overexpression inhibits proliferation of cells in many ways. The effect of TIMP-2 is mediated through inhibiting collagenolysis in melanoma (Montgomery *et al.* 1994), but also diminishes EGFR phosphorylation together with FGF-2 and PDGF independently of collagenolysis (Hoegy *et al.* 2001). TIMP-2 has been reported to cause apoptosis MMP-dependently and by preventing the disruption of Fas ligand in T-lymphocytes (Lim *et al.* 1999). In one of the most recent studies of malignant melanoma patients, both TIMP-1 and TIMP-2 serum levels were connected to the stage of the disease (Yoshino *et al.* 2008). TIMP-2's effect on vascularization seems to take place MMP-independently. The effect was specifically mapped to the C-terminal loop 6 of TIMP-2 (Fernandez *et al.* 2003). In addition to this, TIMP-2 inhibits angiogenesis MMP-dependently through MT1-MMP inhibition together with blocking the FGFR1 and KDR (Lafleur *et al.* 2002; Seo *et al.* 2003). Furthermore, TIMP-2 has been seen to upregulate

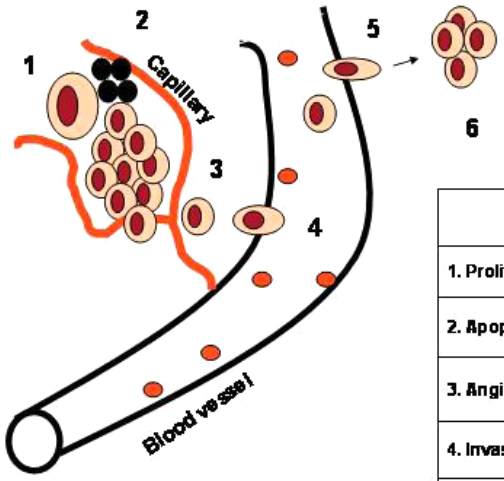
mpk1 and Ptpn16 phosphatases and downregulate VEGF expression, resulting in diminished tumor vascularization (Hajitou *et al.* 2001; Feldman *et al.* 2004). Whit regard to forming metastasis, TIMP-2 functions so that lesser amounts of TIMP-2 results in more migrating cells and overexpression of TIMP-2 diminishes migration as MMP-7 is degraded (Ray *et al.* 1995; Kioi *et al.* 2003).

For melanoma, TIMP-3 has a clear inhibitory effect both *in vitro* and *in vivo* regardless of the method of delivery (Bian *et al.* 1996; Ahonen *et al.* 2002). The mechanisms for TIMP-3 induced apoptosis upregulation have been stabilization of death receptors TNF-RI, TRAIL-RI and FAS in melanoma cells as well as in embryonic kidney cells (Bond *et al.* 2002; Ahonen *et al.* 2003). Overexpression of TIMP-2 and -3 has excerted apoptosis in hepatocellular carcinoma and colon cancer cells (Brand *et al.* 2000; Tran *et al.* 2003). Interestingly, TIMP-3 deficient (-/-) mice show apoptosis of the epithelial cells of the developing mammary gland, but this has been interpreted to be due to problems of cell adhesion in the gland, rather than the lack of TIMP-3 (Fata *et al.* 2001). In one of the most recent studies, the time and expression patterns of the TIMPs 2 and -3 were confirmed immunohistochemically in uveal melanoma (Lai *et al.* 2008). The effect of TIMP-3 on angiogenesis has been widely studied using retroviral gene delivery, and the effect has been anti-angiogenic in both, cell cultures and animals (Anand-Apte *et al.* 1997). The effect is mediated through a decrease in endothelial cell migration and tubule formation (Spurbeck *et al.* 2003; Saunders *et al.* 2006). TIMP-3 works by inhibiting MT1-MMP and thus preventing capillary formation (Collen *et al.* 2003). Even though in TIMP-3 deficient mice vascularization was upregulated in the tumor, it is noteworthy that murine melanoma B16F10 was used in the study instead of human melanoma cells (Cruz-Munoz *et al.* 2006).

It has been suggested that TIMP-4 tumor inhibitory actions are mediated through interactions with growth-regulatory proteins of the nucleus at least in kidney cells, but more studies are required (Celiker *et al.* 2001). Contrary to prevention TIMP-4 has been seen to also prevent apoptosis in some occasions, as with MDA-MB-435 cells (Jiang *et al.* 2001). The reason was suggested to be the promotion of anti-apoptotic BCL-family members. TIMP-4 is still relatively new and requires further study. The suggested role of TIMP-4 in tumor angiogenesis has already been investigated. As TIMP-4 -/- mice do not show reduced MMP-2 activation, it has been speculated that TIMP-4 does not function as an anti-angiogenic protein (English *et al.* 2006). On the other hand, TIMP-4 has diminished capillary formation *in vitro* on fibrin gel as well as *in vivo* (Lafleur *et al.* 2002; Fernandez *et al.* 2006).

Differing from apoptosis, TIMPs have been reported to have some potential in promoting tissue growth. TIMPs -1 and -2 have historically shown strong cell proliferation promoting effects through erythroid potentiating activity and have also enhanced the growth of tumor cells (Hayakawa *et al.* 1992; Stetler-Stevenson *et al.* 1992; Nemeth *et al.* 1993). TIMPs -1 and -2 have been able to induce proliferation independently of MMPs, such as activation of Ras through PKA by TIMP-2 and tyrosine-kinases by TIMP-1 (Brummer *et al.* 1999; Joo *et al.* 1999). TIMP-3 has promoted proliferation in chicken embryo fibroblasts and TIMP-4 in breast cancer cells (Yang *et al.* 1992; Celiker *et al.*

2001). TIMPs have been suggested immunosuppressive role, thus promoting tumor metastasis, although the proof is still minimal. TIMPs -1 and -2 inhibit T-cell mediated cytotoxicity and TIMP-3 has downregulated inflammation (Oelmann *et al.* 2002; Mohammed *et al.* 2004; Gill *et al.* 2010). All in all, it is good to keep in mind that overexpression of TIMPs clearly decreases invasion and metastasis of cancer cells (Walther *et al.* 1996; Wang *et al.* 1997; Ahonen *et al.* 1998; Li *et al.* 2001; Elezkurtaj *et al.* 2004),



	TIMP-1	TIMP-2	TIMP-3	TIMP-4
1. Proliferation	+ / -	+ / -	-	+ / -
2. Apoptosis	-	+ / -	+	+ / -
3. Angiogenesis	-	-	-	-
4. Invasion	-	-	-	-
5. Local migration	-	-	-	-
6. Local growth	-			

Figure 2. The role of TIMP's in metastatic cancer. Adapted from (Cruz-Munoz *et al.* 2008).
(+) promotes, (-) inhibits

2.3 *Cis*-urocanic acid

As a compound, the *cis*-Urocanic acid (*cis*-UCA) has been known for long. Historically it has been considered as immunosuppressive and thus promoting tumor development (Anglin *et al.* 1968; Räsänen *et al.* 1987). The role of an acid-base regulator has also been proposed (Krien *et al.* 2000). Endogenous UCA has a maximum concentration of 8.9 mM in the skin depending on the thickness of the epidermis (Laihia *et al.* 1998). The expression of UCA throughout the skin is constant, with the exception of the feet where it is the highest (Kavanagh *et al.* 1995). There are no large differences in its expression between children and adults (de Fine Olivarius *et al.* 1998). As UV radiation reaches the skin, it affects mainly two things: DNA and *trans*-urocanic acid [3-(1H-imidazol-4-ly)-2-propenoic acid]. UV radiation converts the *trans*-UCA, normally physiologically synthesized from histidine, to *cis*-isomer dose dependently until dynamic stability of equal amounts of the isomers is formed (De Fabo *et al.* 1983). The optimal wavelength for the photoisomerization is 300-310 nm (Gibbs *et al.* 1993). The excess *cis*-UCA is mostly secreted in sweat (Illana *et al.* 1997), whereas the *trans* conformation is degraded in the liver by an enzyme called urocanase (Rao *et al.* 1960). Some *cis*-UCA has been detected in the serum and urine as well (Moodycliffe *et al.* 1993; Kammeyer *et al.* 1995). Usually, the persistence of *cis*-UCA in the skin after photoisomerization is approximately two days (Moodycliffe *et al.* 1993). It has recently been suggested that the receptor through which the immunosuppressive effect would be conducted would be the 5-hydroxytryptamine receptor (5-HT) of the skin (Walterscheid *et al.* 2006). There is also some evidence of the receptor being the GABA(A)-receptor, which can be found in the brain as well (Laihia *et al.* 1998).

2.3.1 Cis-UCA and cancer

Patients with melanoma and basal cell carcinoma have been reported to have somewhat higher *cis*-UCA production in their skin than general population (De Fine Olivarius *et al.* 1998). There has also been a comparative study of *cis*-UCA expression in non-melanoma cancer patients and controls, that takes into account exposed skin areas versus unexposed skin. No significant differences were found in that study, although only 49 subjects were included in the material (De Simone *et al.* 2001). As noted, *cis*-UCA has originally been suggested to be cancer-promoting. The role of immunomodulatory cytokine IL-12 in abrogating the immunosuppressive characteristics of *cis*-UCA has been studied, inspired by the similar behaviour of IL-12 in other UV radiation-induced pathways. It has been found that IL-12 is capable of completely blocking the immunosuppressive effects of *cis*-UCA and so supressing the formation of skin cancer in mice (Beissert *et al.* 2001). The above study of IL-12 was rather artificial: it used *ex vivo* treatment of antigen-presenting cells (Langerhans cells) combined with much higher than physiological concentrations of apically administered *cis*-UCA. As the authors note, the study does not give an answer to the role of endogenous *cis*-UCA in photocarcinogenesis.

2.4 Viral vectors

The goal of using different viruses in gene delivery to eukaryotic cells is to bring a limited amount of genetic material inside the target cells, which is to be expressed in those cells only. The introduced genetic material aims either to replace a defective function or, as in the case of cancer, to treat the existing condition with additional material. The aim can also be preventative as in the case of vaccination with genetic material. Target cells can be infected *ex vivo*, outside the organism, and transferred inside later on (Aiuti *et al.* 2002). Infection can also take place *in vivo*, inside or on the organism itself. Considering the treatment of cancer, the latter *in vivo* approach would be much more desirable. Non-essential viral gene material is usually replaced by a foreign gene of interest when using viruses as gene transfer vectors. By choosing different viral vector types, one can have modifying effect on the vector homing, infection efficiency to target cells, amount of gene material produced and possible severity of the immune reaction. The first real clinical trial of using viral gene delivery was in 1973, when an attempt was made to express arginase activity in patients cells by the Shope papilloma virus (Rogers *et al.* 1973). Experimental success in viral gene transfer was gained only in 1990 in advanced melanoma, when tumor-infiltrating lymphocytes were genetically modified *ex vivo* by retroviruses to resist the cell toxin neomycin (Rosenberg *et al.* 1990).

There are five major classes of well characterized viral vectors known today. These can be further divided into two categories depending on whether the viral genome integrates into the host genome (**oncoretroviruses and lentiviruses**) or persists as extrachromosomal episomes (**adeno associated viruses, adenoviruses and herpes viruses**) (Thomas *et al.* 2003). The four main and most potent viral vectors at the moment are adeno-associated viruses (AAV), retroviral vectors, adenoviral vector and the herpes simplex virus -1 (HSV-1), with many others being currently under investigation. The four vectors above range in size from 20 nm to 300 nm and can carry a load of genetic material from 5 kb to 100 kb respectively. The adenoviral vector is currently the most popular gene therapy vector when measured by the use in clinical trials (Bouard *et al.* 2009). If one thinks of the modification and production of viral vectors for clinical use, there are a number of issues to be considered. First, there are the properties gained from the vectors' wild type ancestor concerning the host spesificity, infectivity of the cells and the response of the individual's immunity as already mentioned. Second, large scale production and purification may form a bottle neck. Third, vectors must somehow escape the immune defense that has evolved during thousands of years specifically to destroy such invaders. This brings us to targeting. In addition to escaping immunity, the vector must target the right kind of cells and not cause massive infection of innocent healthy bystander cells. Usually detargeting from the original homing molecule is required preliminary to the actual targeting to the new host in order to achieve an optimal result. After homing and successful entering to the cell, maintenance of the transgene expression for sufficient time and amount is also required. Finally, the biology and characteristics of the used viral vector must be studied to a sufficient extent for it to be considered safe and acceptable for clinical use by the authorities.

2.5 Adenoviruses

2.5.1 History and structure

Adenoviruses were first characterized in 1953 (Rowe *et al.* 1953). About 50 serotypes of adenoviruses are known today, including various non-human strains (Davison *et al.* 2003; Campos *et al.* 2007). From the beginning two serotypes, adenoviral serotype (Ad) 5 and Ad2 became the most frequently used ones in different vector applications. The Ad5 genome does not integrate to the host genome and thus offers a suitable platform for short-term expression of transgenes.

The wild type adenovirus structure consists of 12 distinct polypeptides and 36 kDa of double stranded DNA. The structure has been mainly revealed by electron microscopy (EM), especially cryoEM with the help of x-ray crystallography. Classically, the adenoviral capsid has been known to be made up of four major components: Hexons forming the icosahedral core with twenty facets and penton base proteins linking out sticking fibers ending with a distinct knob on each corner to the core (Valentine *et al.* 1965; Stewart *et al.* 1991), Figure 3. The length of the fiber shaft varies with different serotypes (Rux *et al.* 2004). In addition to the main building blocks of adenoviruses, so called minor capsid components have been found. Those are proteins IIIA, VI (actually a 23 kDa proteasome), VIII and IX (Sundquist *et al.* 1973; Colby *et al.* 1981; van Oostrum *et al.* 1985; Stewart *et al.* 1993). Aside from the capside proteins there are two helper proteins inside the capsid called VII and IX (Hassell *et al.* 1978; Rancourt *et al.* 1995). The role of the minor components seems to lie in the disassembly of the virion during infection, their abolishment leading to a too rigid a structure and an inability to enter the host nucleus (Hannan *et al.* 1983). Even further, the adenoviral genome seems to be associated with the proteins V and X in addition to VII and IX. The core proteins organize the genome as well as facilitate the viral cycle (van Oostrum *et al.* 1985; Matthews *et al.* 1998; Wiethoff *et al.* 2005).

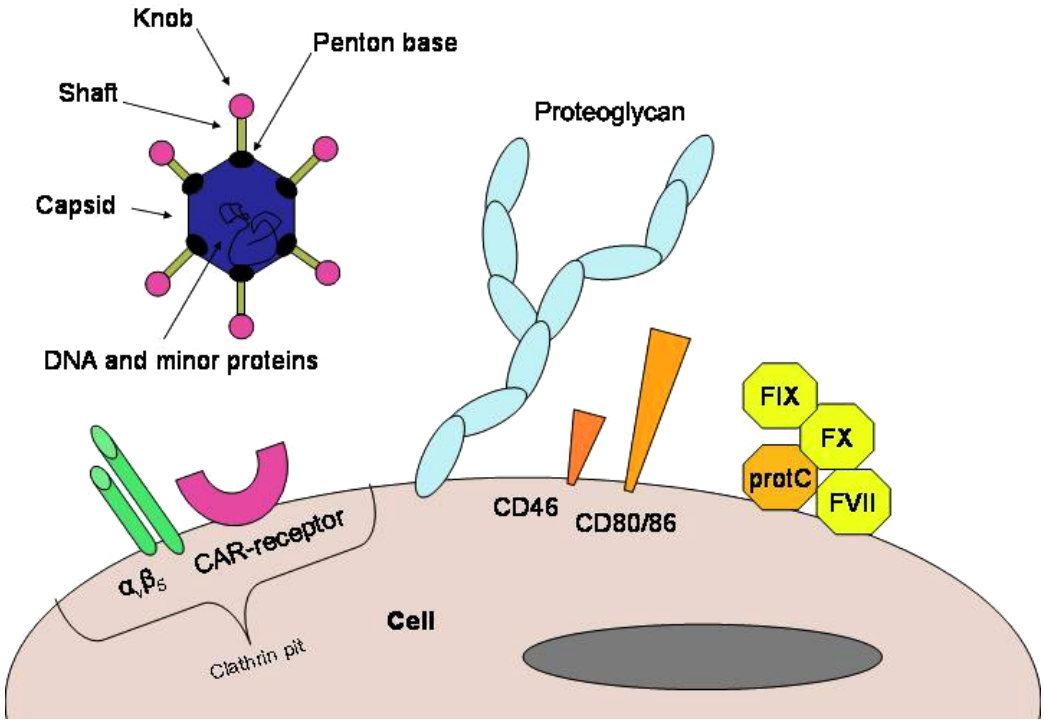


Figure 3. Simplified general structure of adenovirus and the currently known receptors it uses in entry on different cell types.

In order to inhibit uncontrolled infection, some areas of the Ad5 genome had to be deleted. At first, E1 genes were deleted, preventing the virus from using its late genes and E2 area for own DNA synthesis, capsid protein formation and consequently replication. Also, E3 deletion was found useful as it mostly abolished the anti-host immunity. In order to multiply the crippled virus for experiments, a special cell line contributing these deleted gene areas had to be established. The 293 packaging cell line of embryonic kidney origin complementing the essential genes was created, allowing production of titers up to 10^{13} viral particles (vp)/ml with viruses carrying up to 8 kb of foreign DNA (Graham *et al.* 1977; Volpers *et al.* 2004). Today the development of Ad-vectors has proceeded to the level of viruses not having a viral genome, but only some *cis*-elements for replication and capsidation (Amalfitano 1999). These latest models offer a packing capacity of around 35 kb of foreign DNA (Palmer *et al.* 2005). Maximal room for foreign gene material can be obtained by using helper dependent adenoviruses. In them, most adenoviral gene material is removed and only packing sequence and terminal repeats are left behind (Parks *et al.* 1996). As the name implies these vectors have to be assembled using special helper cells that provide the building blocks for these adenoviruses. This approach reduces the residual viral gene expression in host, present in other vectors, to a minimum. The problems of adenoviral vector gene delivery *in vivo* were also discovered

right in early research. These include innate immunity, cytokine inflammation, liver toxicity and thrombocytopenia.

2.5.2 Process of entry to the cell nucleus

To infect cells, the virus has to first dock onto them. Classically, Coxsackievirus B and adenovirus receptor CAR, a type I transmembrane receptor, has been identified as the primary docking site on the cell surface (Bergelson *et al.* 1997). For the viral capsid, the primary site for docking to the cell is the distal knob of the fiber shaft (Bewley *et al.* 1999), Figure 3. Lately, differences in viral homing among different adenoviral serotypes have been discovered, especially in subgroup B which seems to utilize also CD46, CD80/86 and, apparently, even sialic acid (Gaggar *et al.* 2003; Wu *et al.* 2003; Short *et al.* 2004; Tuve *et al.* 2006). Heparan sulfate proteoglycans have been shown to dock adenoviral vectors as well (Dechecchi *et al.* 2001; Smith *et al.* 2002). It has been suggested that some serotypes might be able to distinguish cancer cells through cell-specific proteoglycans without the need of additional targeting. For example, Ad3 natively binds to HeLa cells (Stevenson *et al.* 1995). The viruses tend to bind the cell receptors on special areas of the cell surface: either clathrin-coated pits or caveloae facilitating lipid raft endocytosis (Smith *et al.* 2004). Also, micropinocytosis has been suggested (Meier *et al.* 2002). As the fiber shaft seems to control the proper association with CAR (Shayakhmetov *et al.* 2000), the penton base homes to $\alpha_v\beta_5$ integrin, further activating PI3K (phosphatidylinositide-3OH kinase), which is thought to be a crucial trigger in adenoviral internalizing through clathrin pits (Li *et al.* 1998; Chiu *et al.* 1999). The time frame of the binding and following uptake is rather short, with 80-85% of virions being endocytosed in 5-10 minutes (Greber *et al.* 1993). The course of action inside the cell is thought to be rapid as well, as escape from the endosome to cytoplasm takes place on average 15-20 minutes post infection (Greber *et al.* 1993). In cytoplasm, the adenoviral vector capsid interacts with dynein (Kelkar *et al.* 2004), which transports the capsid to the nuclear pore, where CAN/nup214 nuclear pore protein along with histone H1 are associated with the entering of the viral DNA to the nucleus (Trotman *et al.* 2001; Pelkmans *et al.* 2005). All in all, the entire event from the cell surface to the nuclear pore is thought to take 30-60 minutes depending on conditions (Meier *et al.* 2004). The interest for viral infection cycle from the gene therapy point of view usually stops to the nucleus. The course of events in wild type viruses is usually such, that in the nucleus virus converts the cell to produce its genetic material and proteins for viral capsids, viruses are assembled inside the cell and finally new viruses bud out of the cell. The faith of the host cells is usually apoptosis.

2.5.3 Cancer therapy using adenoviral vector gene delivery

Even though it is not yet in global clinical therapeutic use, there are numerous studies concerning the area of viral gene therapy. Previously, many studies have approached the requirements of apoptosis and targeting in melanoma treatment from an immunologic point of view. Generally in that approach an antibody against a known melanoma surface marker is expressed on an adenoviral surface while the normal adenovirus docking protein, Coxsackievirus B and adenovirus receptor (CAR), is switched off. The result is a melanoma-homing adenoviral vector which usually creates its effect by simply massively replicating in the target cells and thus leading to apoptosis of the tumor tissue (Sebestyen *et al.* 2007). Oncolysis of this kind has been shown not to affect human dendritic cells essential to forming proper immune defense (Schierer *et al.* 2008).

A combination of adenoviral vector therapy has shown promise in experimental setup *in vitro* (Quirin *et al.* 2007). Replication-incompetent adenoviral vectors are still considered the most likely tool to first reach common therapeutic use in cancer. Constructs already in Phase II/III studies include replication incompetent adenos expressing TNF-α under control of egr-1 radiation and chemotherapy enhanced promoter (MacGill *et al.* 2007), as well as adenoviral vectors with TK gene for glioma treatment (Immonen *et al.* 2004). Considered very potent and now in Phase I studies is a replication-incompetent adenoviral vector comprising of mda-7 and IL-24 (Gopalan *et al.* 2007; Sarkar *et al.* 2007). Brain tumors form an area where conventional therapies and surgery frequently face difficulties due to the sensitive nature of the tissue. For viral therapies of the brain, one of the biggest problems is access through the blood brain barrier impermeable to viruses. Attempts to overcome this have also been made, one being the use of brain accumulating melanotransferrin (P97) expression on viruses, with some success in experiments (Tang *et al.* 2007). Some oncolytic adenoviral vectors against solid brain tumors and their post-surgical treatment show promising results (Pesonen *et al.* 2010), with surgery, in this case, providing an easy access route to the site of the tumor cavity. At present, China is the only country where adenoviral vector treatment of cancer has been accepted for clinical use. In China, patients with various cancers are allowed to be treated with adenoviral vectors carrying the p53 tumor suppressor gene (Gendicine) and oncolytic adenovirus called H101 (Oncorine) (Yuan *et al.* 2003; Yuan *et al.* 2003; Wolf *et al.* 2004; Peng 2005). However, a shadow of doubt is cast on the efficacy and proper conduct of trials of these therapeutic vectors. The studies in question are published exclusively in Chinese papers, trial structure is rather weak and some phase III trials are totally missing. Furthermore, when some results were translated to English, it appears that the translations were not totally in line with the original text (Guo *et al.* 2006). More extensive study of these therapeutic vectors is required before they could be accepted and released on the global market.

2.5.4 Adenoviral vectors, de- targeting and re-targeting

Ideally, adenoviral vector gene therapy would be conducted by the systemic injection of viruses to the circulation where the largest obstacle, apart from the immune system, is the liver. The liver filters and retains most of the viruses intended for therapy as well as suffers the consequences of a large intake in the form of toxicity and cell death. This has been one of the primary reasons for criticism against adenoviral vector therapy. A clinical trial patient treated in 1999 in Philadelphia, USA died of liver-originating multi-organ failure (Marshall 1999). He was treated for inherited deficiency of ornithinetranscarbamylase of ammonia metabolism by liver injection of $3{,}8x10^{13}$ viral particles carrying the corrective gene material. These events were certainly one reason to turn the focus of virally administered gene therapy from straight targeting to the direction of preventing unwanted homing.

One of the most recent findings in preventing the unwanted liver targeting has been the blocking of the liver with anti-coagulant warfarin. It has been found that instead of the classic CAR route, vitamin K-dependent coagulation factors FIX, FX, protein C and FVII mediate the transduction of adenoviral vectors in the liver, and this partly explains the high infectivity of the liver also with CAR-mutated adenoviral vector constructs. Furthermore, it has been shown that from the adenoviral vector serotype 5 side it is actually the capsid hexons rather than fiber which, in the above case, facilitate the transduction (Waddington *et al.* 2008). Another novel way has been to try to prevent the already liver-infecting adenoviruses from replicating by the use of micro RNAs (miRs) (Ylösmäki *et al.* 2008). This approach belongs to a field of conditionally replicating vectors where the focus is more on the genetic material of the virus rather than its outer surface. By replacing part of the viral E1A gene normally needed for replication with mRNA complementary to liver specific gene area, a retargeted adenovirus was produced. This virus was highly attenuated in liver cells, but replicative in others.

There are many known viral gene therapy candidates today, ranging from adenoviral vectors to the Semliki Forest virus. Adenoviral surface structure has been in the scope of this particular study and so its targeting to certain tissues as well as cells will be discussed further in the following.

In order to gain retargeting, several approaches have been utilized. Fiber pseudotyping where the step of engineering the viral genome is totally skipped (Gall *et al.* 1996; Von Seggern *et al.* 2000) and cloning of a new peptide sequence to be expressed in the tip of the fiber protein (Michael *et al.* 1995) have been tried (Wickham *et al.* 1997). Bispesific antibody conjugates between adenoviral vector and target (Douglas *et al.* 1996) and coating of adenoviruses with polymers with targeting peptides have also been used as targeting approaches (Romanczuk *et al.* 1999). Replacing the fiber with a fiber from another adenovirus serotype with binding preferences differing from CAR can be done as well (Krasnykh *et al.* 1996). These systems have been used for replication-competent adenoviruses as well, for example by combining the adenoviral vector 5 with adenoviral vector 35 serotype targeting to CD46 protein widely expressed in head and neck cancers

(Suominen *et al.* 2006; Toivonen *et al.* 2009) or in the case of renal carcinoma (Guse *et al.* 2007). If no targeting is done at all, the adenoviral vectors are rapidly cleared from the circulation by Kupffer cells of the liver, assisted by macrophage as well as monocytes (Alemany *et al.* 2000). To overcome this and to retain efficacy of the infection, the easiest and simplest way would be just to increase the viral load. This would, however, ultimately lead to increased toxicity. Exact targeting or blocking of the unwanted docking alternatives is a necessity in order to achieve efficient therapy.

The most straightforward method is modification of the viral tropism, which is to express new desired surface structures on the adenoviral vector capsid. Many times this is combined with turning off the usual CAR docking (Dmitriev *et al.* 1998; Von Seggern *et al.* 1999).

In the work of Nicklin *et al*. (2001), the CAR binding of adenoviral vector serotype 5 was switched off and a peptide SIGYPLP, first identified by phage display (Nicklin *et al.* 2000), was expressed on the capside of the virus targeting to vascular endothelial cells (Nicklin *et al.* 2001). Before this the adenoviral vector had already been modified similarly in other studies, but then the CAR-binding had been preserved (Dmitriev *et al.* 1998). The sequence of the desired peptide was cloned to the adenovirus HI-loop gene after the amino acid T542, as suggested by previous studies and literature (Krasnykh *et al.* 1998). A system was used for assembling the virus, where 293T cells were transfected with the fiber containing plasmid and also infected with replication-incompetent adenos (genes E1, E3 and L5 deleted) (Von Seggern *et al.* 2000; Jakubczak *et al.* 2001). Furthermore, the genome of the infecting adenoviral vectors was equipped with the green fluorescent protein (GFP) gene for easy detection (Wu *et al.* 2001). The silencing of CAR binding was achieved by a mutation called KO1 (areas S408E and P409E of the fiber mutated) (Jakubczak *et al.* 2001).

On many occasions, only a fraction of a foreign peptide has been required to be expressed on a capsid in order to achieve the change in tropism. Well-utilized areas have been HI-loop (Dmitriev *et al.* 1998), C-terminus of the fiber knob (Wickham *et al.* 1996), RGD-loop of the penton base (Wickham *et al.* 1996) as well as HVR of the hexon (Crompton *et al.* 1994). It seems the re-targeting can be obtained using several points of the viral structure, all involved in the viral docking and internalization. One classical and early example is the insertion of the RGD-peptide to the fiber HI-loop facilitating docking to integrins of cells and also to cells even totally lacking CAR (Pasqualini *et al.* 1996). From small inserted peptides, the development has been towards the insertion of more complex protein domains facilitating higher affinity targeting and other applications (Le *et al.* 2006). One central issue is that, whatever the size, it is often not possible to obtain the desired capsid-ligand interaction, as there is a need for folding or bridging modifications synthetically unavailable. Attempts have been made to overcome this by using molecular adaptors in between the virus and the receptor (Glasgow *et al.* 2006). The problem of this approach is that the interactions are usually non-covalent and considered weak and nonfunctional in real situations. A third general strategy has been chemical modification of the capsid (Lanciotti *et al.* 2003; Kreppel *et al.* 2008). The advantage of the chemical

approach is that with it, the destruction or preservation of the original tropism can be rather easily and consistently modified for different experiments.

From the above mentioned strategies, different combinatorial resolutions have emerged (Parrott *et al.* 2000). In fiber swapping, the feature that adenoviral vector serotype 5 fibers can be swapped with other serotypes is utilized (Rivera *et al.* 2004). It is generally the adenoviral vector serotype 5 fiber tail that is combined with the shaft and knob of other adenoviral serotypes. In fiber replacement, the whole or most of the fiber protein is replaced to a different one (van Beusechem *et al.* 2000; Schagen *et al.* 2008). Perhaps the biggest problem with this technique is to achieve adequate encapsidation of the chimeric protein. Another issue is the propagation of adenoviral vectors usually conducted in 293 cells, as the chimera has now lost its usual way of infection and no new host cells can be infected. For this purpose, special wild type fiber expressing cell lines have been made (Von Seggern *et al.* 2000). Another method of facilitating the production is so called adenovirus stripping, where an engineered knob first enables production in the normal 293 cells and can later be cleaved chemically after the purification of the retargeted viruses (Hong *et al.* 2003).

2.6 Alphaviruses

Alphaviruses are enveloped, positive-strand RNA viruses belonging to a family of Togaviridae. There are 27 members in the family including such viruses as Sindbis, Enchephalitis and Semliki Forest virus (SFV). SFV is named after a forest in Uganda habituated by mosquitoes from which the virus was extracted (McIntosh *et al.* 1961; Riezebos-Brilman *et al.* 2006). Alphaviruses are spherical and have a diameter of 65-70nm., the capsid has 20 faces and is formed of 240 copies of a similar kind of capsid protein (Schlesinger 2001). SFV is transmitted from mosquitoes to vertebrates and the infection leads to shutdown of the cells' own RNA production. Alphaviruses use the route of endocytotic clathrin-coated pits on cell surfaces for internalization to the host cells, although the specific receptor for SFV is still unknown (Helenius *et al.* 1980). Entry is followed by the release of viral RNA to the cytoplasm. First, minus strand RNA is created from the positive strand and it serves as a model for the positive RNA of developing viruses. Simultaneously, the endogenous gene functions of the cell are shut down and the production of viral proteins commences. Finally, new virions are assembled and release of the viruses by budding from the cell surface takes place. The usually apoptotic effect of oncolytic replication competent SFV in different cell lines is considered to happen because of the sheer speed and extent of the viral propagation, rather than because of differences in cell tropism or signaling pathways (Glasgow *et al.* 1997). Apart from apoptosis, SFV has been reported to cause necrosis at least in cerebellar neurons. Despite their strong oncolytic potentials, alphaviruses are not major human pathogens; SFV usually causes only a mild fever or flu in humans (Riezebos-Brilman *et al.* 2006).

2.6.1 Alphavirus vector systems

Basically three kinds of vector systems have been developed from alphaviruses: replication-deficient vectors (a.k.a replicons) (Xiong *et al.* 1989), replication-competent vectors, used in this thesis, (Raju *et al.* 1991) and DNA-based vectors (DiCiommo *et al.* 1998). The genome of replication-deficient vectors lacks the structural genes of the viral vector, therefore there is no possibility of formation of the next generation of viruses in the host cells, which leads to a single round of infection. Ideally, this vector could be used as a transportation vehicle of gene material to the target tissue. Replication-competent viruses, on the other hand, have those structural genes included and replicate and infect new cells until the infection is suppressed by the immune system or lack of host cells. Their potential is based on the straight capability of destroying harmful cells by pure replication causing apoptosis, but they can be used as transportation vehicles as well. DNA-based vectors are an application of the replication-deficient viral vectors. They are made in order to ease the cloning of foreign DNA to the normally RNA-based vector of alphaviruses. The use of DNA has been facilitated by changing the original SP6 RNA polymerase promoter of the viral genome to CMV promoter. The Semliki forest virus has become particularly popular in alphavirus vector studies because of its broad host range and high expression levels, as well as the fact the virus is inherently oncolytic (Wahlfors *et al.* 2000; Lundström *et al.* 2001). Being highly neurovirulent, SFV has gained popularity especially in neuroscience and it does not infect the liver to the same extent as adenoviruses (Owe-Larsson *et al.* 1999; Loot *et al.* 2004). In the case of the replication-competent viruses, the fulminant shut down of the endogenous gene activity does not play such a big role, but this can be a problem for the replication-deficient vectors (Lundström 2005).

2.6.2 VA7-EGFP

The development of gene therapy applications using replication-incompetent SFV has started mainly during the 21st century, although several SFV strains have been known for decades. The first trials utilizing pure oncolysis of some SFV strains date back to 1950s. Infection of human prostate tumor cells *in vitro* (Hardy *et al.* 2000) and B16 mouse melanoma tumors *in vivo* (Asselin-Paturel *et al.* 1999) are among the first studies. As regards the gene therapy use of replication-competent SFV, the results are even more recent. Traditionally, oncolytic or conditionally replicative viral vectors have been made of totally different virus types, mostly adenoviruses (Khuri *et al.* 2000) or herpes simplex virus 1 (Rampling *et al.* 2000).

The replication-competent oncolytic VA7-EGFP has its roots in the avirulent vector A7(74) found originally in the central nervous system of mice. It was further purified in several rounds through neonatal mouse brain and twice in chicken embryo cells to extract the most avirulent clone (Bradish *et al.* 1971). The A7(74) strain was originally reported to be harmless in mice older than three weeks, contrary to the wild type form of SFV which is lethal to mice of all ages. However, in SCID mice, A7(74) causes fur ruffling, lethargy and ultimately, paralysis of the limbs regardless of age (Amor *et al.* 1996). From

day 14 onwards, the infective stress of A7(74) is diminished to moderate, thus prolonging the time before the first symptoms of paralysis appear (Oliver *et al.* 1997). Even in mice with a fully working immunodefence, avirulent SFV has been reported to cause demyelination around week 3 post injection of the virus, although further neurodegeneration is not evident (Suckling *et al.* 1978; Amor *et al.* 1996). In immunocompetent animals, it seems IgM antibodies are clearing the viremia whereas IgG antibodies are reducing infectious viruses. The change for better tolerance of SFV infection in mice during development is due to the maturation of the nervous system. Increase in tolerance develops the same way even in mice without working T-cell driven immunity, like the SCID mice (Griffin *et al.* 1994). By cloning to the original A7(74) genome additional 26S viral promoter, multiple cloning sites for possible transportation of gene material and green fluorescent protein for detection, replication-competent expression vector VA7-EGFP was created (Tuittila *et al.* 2000; Vähä-Koskela *et al.* 2003). Since the publication in this thesis, oncolytic SFV strain VA7-EGFP has been used in studies of rodent tumor models (Määttä *et al.* 2007), adenocarcinoma xenografts in nude mice (Määttä *et al.* 2008) and osteosarcoma model (Ketola *et al.* 2008). In all experiments, the virus showed clear tumor-suppressive effects. Previous attempts to treat malignant melanoma using SFV vector have been few. Among the best is gene transfer of inter leukine 12 by SFV to target murine B16 melanoma with clear tumor-suppressive effects (Asselin-Paturel *et al.* 1999).

The major concern of gene therapy applications has been the spread of SFV to the central nervous system when given systematically leading to a fulminant immune reaction. In addition to CNS infection, peripheral infections in SCID and nude mice have been detected especially in the skeletal muscles, heart, and pancreas (Pusztai *et al.* 1971). Despite its long history in research, SFV has not been used in clinical trials due to its neuropathic effects. In the experimental lung cancer model of mice, SFV has recently been able to diminish the tumor size when administered locally, but the limited efficacy of systemic administration seems to pose a problem for clinical oncolytic SFV applications (Määttä *et al.* 2008).

2.7 Peptide targeting

The need to identify short protein stretches, oligopeptides, capable of attaching firmly to different target molecules and surfaces has been an important goal in academic research as well as in pharma industry for quite some time. Variable approaches have been developed. The common idea of all the targeting peptides is that after the wanted peptides have been picked out of the collection or library of several candidates, they can be removed from the experimental context, propagated and used as drugs, experimental tools or targeting tags in various applications. Two main approaches to oligopeptide libraries have been utilized: biological or synthetic peptide libraries (Falciani *et al.* 2005). Synthetic libraries have classically been built on some solid monolayer, flushed over by the possible binder candidates. In attaching the peptide library on the surface, several different techniques have been used, such as pins and glass chips (Geysen *et al.* 1984; Fodor *et al.* 1991). The synthetic library can also be cleaved from its solid support to form a free-flowing library of pure oligopeptides (Houghten *et al.* 1991). Several protein ligands and kinase inhibitors have been recognized using the synthetic library tools. In biological applications, a phage library for presenting the collection of peptides have become very popular. There, the peptide sequence is fused to the phage genome and so is expressed on the surface of the phage capsid. This has better facilitated homing to cancer cells as well as several *in vivo* applications, in addition to the achievements of the synthetic methods (George *et al.* 2003). Many different phage serotypes have been tried as a peptide presenting vector, but the M13 bacteriophage seems to dominate the field.

2.7.1 Phage display (PD)

In order to find peptides for homing to certain desired tissue, a method called phage display, using M13 bacteriophages, was developed during the 1990s (Matthews *et al.* 1993), following the discovery that peptides can be expressed on phage and panned either as a solution or bound on a surface against the desired target by Smith and others (Smith 1985; Parmley *et al.* 1988). The general idea in PD is to first make a library of bacteriophages expressing various oligopeptides on their surfaces (Figure 4.). This library is then panned (that is, laid over in liquid) on cell monolayers or injected into circulation. By repeating the cycle of panning, amplifying the library to the same size between every round using pilus positive bacteria, the aim is to achieve enrichment of a phage population that is homing to the desired tissue. The bacteria are unique in that they do not lyse when infected, but instead survive and produce numerous phages into the surrounding media. The system can be enhanced by introducing phases of pre-clearings, which means that the library is exposed to a number of unwanted cell types or surfaces prior to proceeding to actual panning, in order to clean as much unwanted background as possible.

Other phage types, such as labda-phage, also emerged but M13 became the most widely used one (Mizusawa *et al.* 1982). In one of the first applications, M13 phage were bound to antibodies in a solution, had tetracycline selection gene and were purified through a streptavidin dish (Parmley *et al.* 1988). This method utilizes viruses whose host is bacterium in such a way that peptide is expressed as a fusion with a bacteriophage coat protein. At the early stage, this was the phage surface protein pIII and pVIII (Parmley *et al.* 1988; Smith *et al.* 1993). As a result, a large number of peptides homing to tumor cell antigens or tumor cell supporting molecules have been identified (Brown 2000). Bacteriophage pIII capsid protein binds to the F-pilus of the bacteria, thus facilitating the internalization of the virus (Pratt *et al.* 1969; Gray *et al.* 1981). There are altogether 5 copies of the pIII protein on the phage surface (Lin *et al.* 1980). The library is created by ligating a huge mixture of short inserts made with PCR (Mullis *et al.* 1987) to a suitable amount of open cut vectors like fUSE5. After ligation, the vectors are then electroporated to bacteria, further producing the bacteriophage (Parmley *et al.* 1988).

Devlin *et al.* constructed a new plasmid called M13LP67 which, differing from the Smith study in the previous paragraph, introduced β-lactamase as a reporter gene (Devlin *et al.* 1990). They also introduced 15mer inserts more than doubling the insert size of 6mers used by Scott *et al.* (Scott *et al.* 1990). PD provided a long-searched cost-effective method for high throughput screening of epitopes. In the beginning, titers reached values of $4x10^6$ phage / ml on λgt11 (Cold Spring Harbor laboratory manual -89) compared to the commercial $2x10^{13}$ phage / ml on M13 easily available today (NEB biosciences, USA). The libraries of different phages can be obtained (Parmley *et al.* 1988; Ferrer *et al.* 1999) or made locally using specified vectors like fUSE5 (Scott *et al.* 1990; Wrighton *et al.* 1996). Both *in vitro* and *in vivo* use of libraries is possible. Contrary to simple linear oligos, constrained peptides can be expressed as well. In these peptides, a rigid three-dimensional structure is usually achieved by using Cys-Cys-disulfide bridges within the

oligopeptide. This is thought to lead into more specific and strong binding as the wobbling of the peptide is reduced (McLafferty *et al.* 1993). Not only the existence but also the amount of Cys-bridges seems to play a role in the binding (Koivunen *et al.* 1995). Peptides that mimic the structure of a folded protein are called mimotopes and are usually of constrained sort. Mimotopes are actually able to compete with carbohydrates in binding, in spite of being peptides (Oldenburg *et al.* 1992). The approach with the 3D peptides seems to work especially well when panning against a prepared and known monolayer *in vitro*. Phages maintain their infectivity even in cell lysates containing proteases and hostile pH values (Pasqualini *et al.* 1996).

The length of the peptides that can be expressed on phage varies greatly between few to 30 or even more amino acids. Many times it is not the whole peptide, but a smaller sequence in the middle of it that is responsible for the binding. One of the milestones of the practical applications of PD is the finding of the RGD peptide capable of docking to integrins (Ruoslahti *et al.* 1987). Integrins are transmembrane heterodimeric cell-surface receptors expressed in a variety of cancer cells as well as endothelial cells. They mediate adhesion further to vitronectin, fibrinogen and laminin. The finding of the RGD was associated with the search for markers of vasculature, like in many experiments since (Koivunen *et al.* 1999). Furthermore, it has been discovered that the distribution of target peptides in human vasculature is specific to different locations of the body and that it is thus possible to map different areas of vasculature with the help of PD (Arap *et al.* 2002). The same kind of approach has been used for a library of human cancer cell lines, and the results suggest that many types of surface peptides are overexpressed irrespective of tumor origin (Kolonin *et al.* 2006). Short sequences within oligo as the RGD have been utilized later in other contexts as targeting sites in cells that are known to use such features, such as the major histocompatibility complex of the immunity (Davenport *et al.* 1996). The mapping of human vasculature in a living patient with terminal phase cancer was performed already in 2002 (Arap *et al.* 2002). Since then, even multiple rounds of panning in a single patient has been tried (Krag *et al.* 2006). Recently, lung tumor targeted peptides were used as markers on nanoparticles (Giordano *et al.* 2009).

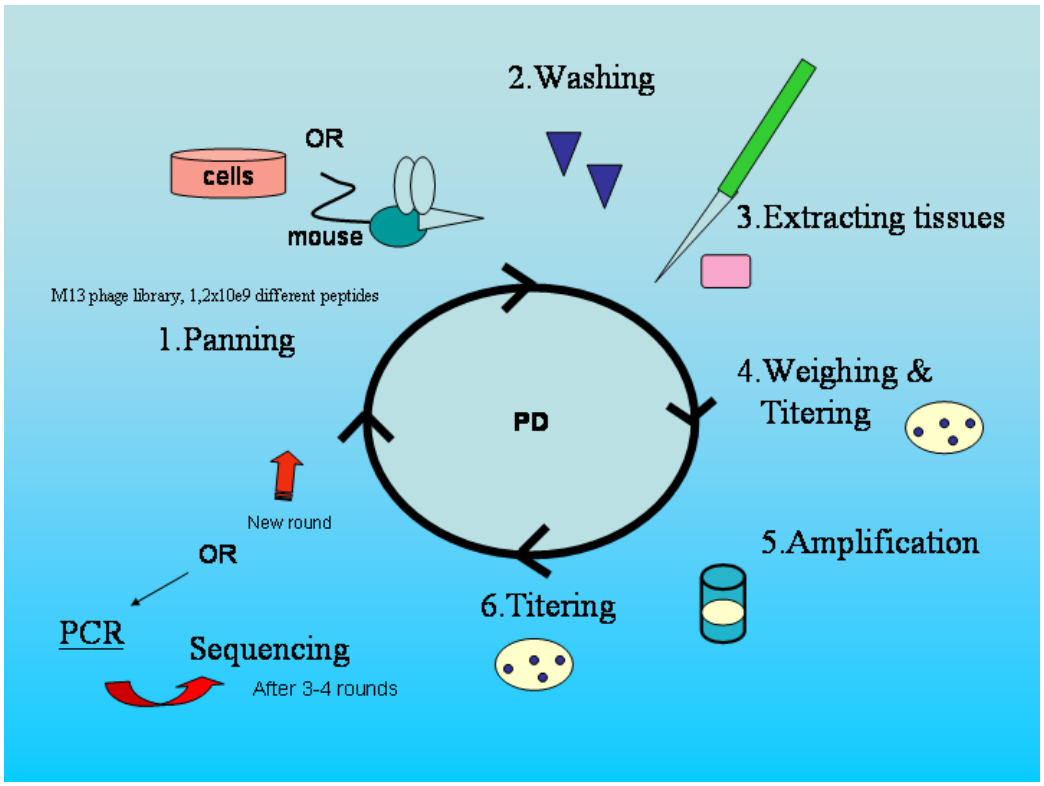


Figure 4. A simplified view of phage display method. Bacteriophage/peptide library is exposed to cells or an animal, unbound phage is washed away, cells or organs collected, phage extracted and tittered. Phage library is amplified near to its original size using ER2738 bacteria as hosts. Amplified library is titrated in order to provide equal amount of phages to each panning round. As an end result bacterial colonies, each hosting a different phage, are sequenced and hopefully a specifically bound subpopulation of peptide sequence expressed on phage revealed.

The applications of the PD system are many. It has been used in epitope mapping (Scott *et al.* 1990; Koivunen *et al.* 1999), mapping of protein-protein contacts (Hong *et al.* 1995), as well as the identification of peptide mimics of non-peptide ligands (Oldenburg *et al.* 1992). In the tumor, targeting monoclonal antibodies derived from PD as well as recombinant antibody fragments have been mostly used in experiments of cancer imagining and therapeutics (Goldenberg 2002; Popkov *et al.* 2004). Not only peptides for surface structures can be found, but the system also works vice versa, as bacteriophages are strong antigens and hence induce antibodies against the expressed oligopetide (Cortese *et al.* 1996).

Following the phage display experiments, numerous peptide candidates for various applications have emerged. The targeting has not only been limited to receptors but enzymes, carbohydrates (Peletskaya *et al.* 1997) and other ligands have also been targeted. In many cases, the peptide has facilitated blocking an original docking site. From a cancer prevention point of view, many peptides have been identified, one being ErbB-2 receptor (Houimel *et al.* 2001; Urbanelli *et al.* 2001). With vasculature formation

playing an essential role in tumor formation and metastasis, it has also been a key area of peptide search and has produced ephrin -A-2 (Koolpe *et al.* 2005), FGF (Maruta *et al.* 2002), IGF (Skelton *et al.* 2001), VEGF (Zhu *et al.* 1998; Binetruy-Tournaire *et al.* 2000)and uPA (Goodson *et al.* 1994) binding peptides. MMPs have been panned, as well, and there are specific peptides found to bind at least MMP-2, MMP-9, MMP-11 and MMP-14 (Pan *et al.* 2003) (Koivunen *et al.* 1999; Suojanen *et al.* 2009).

Considering all the viral vectors, gene therapy tools and techniques mentioned in this review, it seems that to a great extent, general interest has moved from the pure, simple targeting of viral vectors towards prevention of unwanted targeting to liver and other nonmalignant tissues. There are very few targeting peptides known that have real tumor specificity, and this has its effect on the commercial applications for the clinical treatment of patients. As many times in the past, today as well, science and the potential in the research laboratory is vastly more developed of approved treatments that still represent the very first generation and mechanisms of applications found in targeted gene therapy. Despite the large amount of knowledge available today, there are still some gaps to overcome concerning the safety and efficacy of the targeted gene therapy vectors for clinical use. The most likely step forward will be combining the approved gene therapy applications with the traditional cancer drug cocktails, rather than having gene therapy be a standalone means of cure (Parker *et al.* 2008; Rodon *et al.* 2010).

3. Specific Aims

The need for therapy against advanced metastatic melanoma initially led to the general aim of exploring possibilities for controlling the disease in an expreimental model. The initial goal was to create and systemically administer a melanoma-targeted adenovirus.

Aim 1: To study the possibilities offered by *Cis*-urocanic acid in killing melanoma cells *in vitro* and *in vivo*.

Aim 2: To explore the ability of SFV to kill human metastatic melanoma cells *in vitro* and *in vivo*.

Aim 3: To identify metastatic melanoma homing peptides *in vivo* using phage display and to use these to construct melanoma-targeted adenoviruses.

Aim 4: To further characterize TIMP-3 as proapoptotic molecule against cancer cells.

4. Materials and Methods

Below are the main methods used in original publications in table format for quick reference. The roman numeral indicates the corresponding publication:

Table 1.

I	
Urocanic acid	Photoisomerization of *trans*-UCA (Sigma Aldrich, Acros Organics)+ exchange chromatography
Cell culture	A2058, Epithelial fibtocarcinoma; HT-1080, Epithelial adenocarcinoma of cervix; HeLa (All ATCC)
Cell viability	Cell Titer 96 Aqueous One Solution Proliferation assay (Promega)
Binding assay	Annexin V-FITC conjugate (Caltag, Burlingame), Flow cytometry (BD FACScan, CyFlo)
Western Blotting technique	10% SDS PAGE, Cleaved Caspase-3 ab (Cell Signaling), ECL (Amersham)
SCID/SCID mice	(Charles River Laboratories), camptothecin (Sigma-Aldrich) (Animal experiment permit from State Provincial Office of South-Western Finland, institutional lisence: STO/1053(8.9.1999), 1132/712–92, 1286/712–86)
Immunohistochemistry	Paraffin embedded, Ki-67 (Dako), Cleaved Caspase-3 (Cell Signaling), ABC kit (Vector, Vectastain)
II	
SFV	VA7-EGFP
Cell Culture	Baby Hamster Kidney; BHK-21, MBA-13, Human colon adenocarcinoma; LS174T, Human colon adenocarcinoma SW620, Human lung carcinoma A549, A2058, SK-Mel-5, Mouse neuroblastoma; C-1300 (All ATCC)
Detection of Cell Death	TUNEL kit (Roche Switzerland), PARP-1 antibody (BD PharMingen, USA), Horse anti-mouse secondary Ab (Vector

	laboratories, USA), PARP-1 (BD, USA), Alexa fluor 546-conjugated sterptavidin (Molecular probes, USA), MTT-assay (Roche, Switzerland), FACS (FACSCalibur, BD, USA)
SCID/SCID mice	Harlan laboratories, Netherlands, (Animal experiment permit from State Provincial Office of South-Western Finland, institutional lisence: STO/1053(8.9.1999), 1132/712–92, 1286/712–86)
Histology	H&E, anti-SFV ab, anti-PARP ab, TUNEL, van Gieson, Hoechst 33342, Melan-A, S100
In Situ Hybridization	^{33}P-probe, EGFP-probe, X-ray film
III	
Cell culture	A2058, SK-mel-5, WM115, WM2664, HepG2, HUVEC (All ATCC), BML extracted from blood samples.
Phage Display	Ph.D.-7 and Ph.D.-C7C (New England BioLabs)
SCID/SCID mice, their terminal anesthesia	Charles River, Germany / University of Turku animal facility – Isoflurane /O_2 (Animal experiment permit from State Provincial Office of South-Western Finland, institutional lisence: STO/1053(8.9.1999), 1132/712–92, 1286/712–86)
Assembly of candidate adenoviral vectors	See the following chapters of mat.met.
β-gal detection; staining of fixed cells, spectrophotometric A420	Promega, Perkin Elmer
Blocking of adenoviral liver infection in mice	Warfarin i.v.
Immunohistochemistry of paraffin sections	hTfR (Zymed, USA/Dako, Denmark), B-gal(Cappel, France/Vectastain, USA)
IV	
Cell culture	A2058 (ATCC), SW2 and N417 (Hopkins-Donaldson *et al.* 2003)
Antibodies & recombinants	TIMP-3 (R&D Systems, USA), FAS activating CH-11 (MBL International, USA), Flag tagged TRAIL (Alexis, Switzerland), M2 cross linker (Sigma,

	USA), Batimastat BB94 (British biotech, UK), *Western Blotting antibodies*; C-20, H-130, H-5 (Santa Cruz Biotechnology, USA), TIMP-3 (AB802, Chemicon, USA)
Pulling down death receptors	EZ-link-sulfo-NHS-LC-biotin (Pierce, USA), Immobilized streptavidin beads (Immunopure, Pierce, USA)
Adenoviral vectors	RAdTIMP-3, RAdLacZ, RAd66 (Dr. Andrew H. Baker, Glasgow, UK and Dr Gavin W.G. Wilkinson, Univ. of Cardiff, Wales, UK)(Baker *et al.* 1996; Baker *et al.* 1998)
Cell viability	Cell titer 96 Aqueous non-radioactive cell proliferation assay (Promega, USA)
Caspase Inhibitors	Z-VAD-FMK (Promega, USA), Caspase-3 inhibitor (Merck, Germany)
Histochemistry and hybridization	Hoechst 33342, Tunel kit (Roche, Switzerland)

Unpublished TIMP-2 data	
Cell culture	A2058
Adenoviral vectors	RAdTIMP-1, RAdTIMP-2 RadLacZ (Dr. Andrew H. Baker, Glasgow, UK and Dr Gavin W.G. Wilkinson, Univ. of Cardiff, Wales, UK)
SCID/SCID mice	Charles River, Germany / University, $1x10^6$ A2058 cells in PBS s.c. to the neck. (Animal experiment permit from State Provincial Office of South-Western Finland, institutional lisence: STO/1053(8.9.1999), 1132/712–92, 1286/712–86)

Adenoviral vectors

Regular CAR homing replication-deficient adnenoviral vectors, whether carrying transgene like TIMP-3 or not, were constructed as described in (Baker *et al.* 1996; Baker *et al.* 1998) and were of the adenoviral vector serotype (Ad)5. The recombinant adenovirus (RAd)LacZ has been propagated locally from a strain (RAd35, based on Ad5) originally donated by Dr. Gavin W.G. Wilkinson (University of Cardiff, Wales, UK)(Wilkinson *et al.* 1992).

Recombinant non-CAR adenoviral vectors were constructed so that as the same time as the new oligopeptides of same structure were expressed on their surfaces, the usual CAR binding ability was abolished from the viruses. The plasmids expressing peptides at the Ad5 HI loop were constructed by inserting the required peptide sequence into the restriction site on the HI loop gene. Stocks of recombinant pseudotyped adenoviruses were generated by transfection of 293T cells (ATCC) with the modified plasmids described above followed by superinfection with a fiber-deleted adenoviral vector serotype 5. Briefly, 293T cells were transfected with the appropriate fiber-expressing plasmid. Sixteen hours later cells were superinfected with an E1, E3, fiber-deleted rAd5 (Ad5ΔF) (β-galactosidase) at 2000 virus particles (VP)/cell. Virus particles were purified by CsCl ultracentrifugation and dialyzed into 10 mM TRIS (pH 8.1), 150 mM EDTA and 10% glycerol. Virus particles were quantified by BCA protein assay with bovine serum albumin (BSA) standards according to the conversion: 1 μg protein = 4×10^9 VP.

5. Results

(I) *Cis*-urocanic acid promotes apoptosis of malignant melanoma cells in slightly acidic pH *in vitro* and *in vivo*.

Cis-UCA inhibits cancer cell proliferation in an acidic environment in vitro

Cis-UCA was shown to inhibit the growth of A2058 malignant melanoma cells, as well as control cervical carcinoma and fibrosarcoma cells in an extracellular pH of 6.5, mimicking the conditions inside a tumor (I, Fig.1). As hypothesized, *cis*-UCA did not affect the cells cultured in a pH of 7.4, which is close to the physiological pH value of ECM. For A2058 cells, the inhibition was 31% in slightly acidic surroundings compared to 3% in the physiological state. To confirm that the acidic pH alone did not cause the cell death, cells were cultured for 44 hours in an acidic environment without any signs of reduction in viability (I Fig.1b). Thirdly, the recovery of A2058 cells from *cis*-UCA treatment was tested. Whereas cells treated for 20 hours in a pH of 6.5 and 10mM *cis*-UCA could recover during 44 hours in a recovery culture in a medium absent of *cis*-UCA, cells treated with 30mM *cis*-UCA the similar manner showed 69% inhibition of tumor cell viability at the end of the experiment (I, Fig. 1c)..

Cis-UCA promotes intracellular acidification of cancer cells

For the first time, *cis*-UCA was shown to decrease the cytoplasmic pH of A2058 metastatic melanoma cells in a dose-dependent manner, reaching a maximum decrease of 0.5 pH units with the dose of 30mM *cis*-UCA (I, Fig.1d). When the same experiment was done with *trans*-UCA or with *cis*–UCA in a pH of 7.4, a maximum decrease of only 0.1-0.18 pH units was observed.

Cis-UCA treatment of adequate concentration results in apoptosis of melanoma cells

When Annexin V binding to the cell membrane of A2058 cells was used as an apoptotic marker (I, Fig. 2b), positive control camptothecin 10 µM could induce 81% of cell death in a pH 6.5 for 16 hours, whereas *cis*-UCA induced 33%, *trans*-UCA 18%, and negative control 7% respectively. Cleaved caspase-3 was visible around the 24 hour time point in 30mM treated A2058 cells in Western blotting (I, Fig. 2d). Caspase-3 -like apoptotic activity was detected only in cells treated with 30mM *cis*-UCA for more than 6 hours in a pH of 6.5 and exceeded the endogenous levels of caspase-3 detected around 36 hour time point(I, Fig. 2e). The difference in the viability of cells treated with 30mM *cis*-UCA in pH 6.5 compared to pH 7.4 was 30 % after 36 hours. Gradual acidification affected the 7.4 pH medium during that time, so that the final difference of medium pH was only 0.5 units (I, Fig. 2f and g).

Cis-UCA inhibits human melanoma tumor growth in vivo in SCID mice

The effects of *cis*-UCA were studied *in vivo* in xenograft tumors of human A2058 metastatic melanoma cells grown on the backs of SCID/SCID mice. Intratumoral injection of 30mM *cis*-UCA reduced tumor area as well as tumor mass for more than 50% compared to the control and equal to1µM champtotecin when *cis*-UCA was used (I, Fig. 3). The process of apoptosis was verified by detection of activated caspase-3 and Ki-67 by immunohistochemistry of the tumors. It was also noted that the necrotic area of the tumors doubled in size (I, Fig. 4a and c). At the same time, no adverse reactions in the mice were detected. Activated caspase-3 immunopositive cells undergoing apoptosis were detected slightly more in the *cis*-UCA -treated mice, and the number of proliferating cells was around 20% smaller in the *cis*-UCA group when compared to the control cells (I, Fig. 4b and d).

(II) Semliki Forest virus VA7-EGFP diminishes melanoma tumors *in vivo*.

Infectivity of SFV on different tumor cell lines

Initially, infectivity of different cell lines with SFV was tested and BHK-21 (baby hamster kidney), A2058 (human metastatic melanoma), A549 (human lung carcinoma), LS174T (human colon adenocarcinoma), SK-MEL-5 (metastatic melanoma) and SW620 (human colon adenocarcinoma) showed marked signs of infection and gradual degradations, whereas C-1300 human neuroblastoma cells remained resistant to SFV infection (II, Table 1.). The replication efficiency in the above mentioned cell lines was measured by observing the green fluorescent protein production (II, Table 2.). The properties of the observed gradual cell death were studied using PARP-1 and TUNEL stains, and the mode was confirmed to be apoptosis (II, Fig. 1). However, propidium iodide uptake revealed that a fraction of the cells were dying in some other manner, characterized by increased permeability of the cell membrane (Fig. 1c).

Effect of VA7-EGFP on human melanoma xenografts on SCID mice

When VA7-EGFP virus was injected to SCID/SCID mice bearing A2058 tumors via i.v. (intravenous), i.p. (intraperitoneal) and i.t. (intratumoral) routes, it was observed that here, as well as in *cis*-UCA studies, tumor size corresponded well to tumor mass. This was notable since in publication (I) only the tumor area was measured and the mass predicted with suitable equation. Vector injection lead to notable reduction of tumor size in all of the administration methods used (II, Fig. 2). The side effects of instant fur ruffling followed by ataxia and paralysis appearing approximately two weeks after vector administration could be expected based on previous studies on the effects of SFV infection in young mice (Atkins *et al.* 1982). In addition, weight loss of about 5-20% was noted. This was also noted in the untreated control group. The end result after the SFV had had its full effect on the original tumor was a group of yellowish encapsulated

nodules separated by connective tissue. This same pattern of small nodules was observed in all SFV treated mice. Control tumors were at the same time juicy, large and vascularized (II, Fig. 3). Notable necrotic areas in the tumor were observed in both treated as well as control mice.

Apoptosis in tumors and the viral distribution in tumors and the body

Apoptotic as well as viable tumor cells could be seen in all treatment groups, mostly in the inner part of the tumors (II, Fig. 4). The infection of the tumor area was confirmed using *in situ* hybridization with an SFV specific probe. Indeed, the vector had proliferated in all of the observed tumors, forming a patchy pattern (II, Fig. 5a-f). Furthermore, the infection seemed to nest in the cell layer between living and dying tumor cells, although a significant amount of viral RNA was detected in the necrotic core of the tumors, as well (II, Fig. 5g-h). When looking at other organs in the mice bodies, SFV could be detected in the brain, spinal cord and muscles, whereas other organs were negative for the virus (II, Fig. 5 i and j).

To further characterize the viral infection, virus from the tumors was extracted and it showed infectivity not differing from fresh stock when introduced to cell cultures. Furthermore, the still living melanoma cells were extracted from the tumors and, surprisingly, they showed resistance to SFV infection (II, supplementary Fig. S3). This small subpopulation could be grown further and was confirmed to be melanoma cells with S100 and Melan-A immunohistochemical stainings.

(III) Identification and characterization of a peptide targeted to metastatic melanoma.

In this study, *in vivo* phage display was used to find peptide candidates targeting to metastatic melanoma. The peptides found were expressed on adenoviruses and the targeting abilities were tested *in vivo.* At the same time the unwanted liver homing was blocked. This study provided additional information on the ability to use phage display in a model where phages have to penetrate *in vivo* beyond vessel walls to the actual tumor cells.

One major difference, especially *in vivo,* compared to PD studies focusing on endothelium (White *et al.* 2004) was that here, library candidates had to extravasate in order to reach actual melanoma cells. This was achieved simply by prolonging the library circulation time up to one hour. Melanoma-specific oligopeptides were not just characterized but their efficacy was also tested on adenoviral vectors. Out of each candidate found *in vivo,* four were also found previously *in vitro* in a large panning experiment of different melanoma cell lines using both linear and constrained peptide libraries (Data not shown). Only one candidate was found exlusivly *in vitro* and this was also the only constrained oligopeptide that had shown promise in previous high titer phage studies of the lab. This same candidate LPMSKNS was utilized as a low binding

control in all *in vivo* studies. Although adenoviruses are used in these experiments, targeted oligopeptides could be easily used in other vector models like adeno-associated virus (AAV) as well (Work *et al.* 2005).

Phage display

For *in vivo* phage display, both types of libraries, linear and constrained, were originally used. Linear seemed to out-perform constrained and will be further discussed here. The circulation time was chosen to be rather long right from the beginning. Two sets of experiments were made on SCID/SCID mice carrying human SK-mel-5 (Human metastatic melanoma) tumors on their backs. After the sequencing, linear sequence data obtained was combined with all the linear *in vitro* data and sorted according to the amino acid charge of the different oligos. From this sorting by charge altogether 101 matches were found. However, after the sorting was taken to the second level and actual amino acid sequences were compared, only five good candidate oligos were identified. The characteristics of finding the peptide HAIYPRH and GETRAPL are summarized in the table 2. of (III). Peptide GETRAPL, found in several similar setups as artifact, was also found here both *in vitro* and *in vivo* (Work *et al.* 2004).

Testing the oligopeptides by expressing them on adenoviruses

As a second stage, stocks of adenoviral vector serotype 5 based adenoviruses were constructed. These adenoviral vectors do not have the natural CAR-receptor homing ability, but express candidate oligo on their knob protein in such a way that the candidate is critical in viral docking on target cell (Jakubczak *et al.* 2001). Furthermore, these viruses have the *E.Coli* β-galactoside (β-gal) gene for easy detection.

Oligo candidates on adenoviral vectors were tested on different melanoma cell lines first *in vitro* (III, Fig.1a). In these experiments the differences of the surface structure and the nature of various malignant melanoma cell lines became evident, as noted previously by others, as well (Hoashi *et al.* 2001). The results varied markedly from the rather high and stable β-gal production of WM115 (human primary melanoma) to large differences between different lines of for e.g. A2058. MTT-assay and Western blot method have been widely used in other hTfR expression studies and MTT-assay was used here as well (III, Fig.1b). WM115 made an exception, showing low hTfR expression. The high infectivity of CAR deleted adenoviral vectors, which were considered the best and the worst of the candidates, might be due to the high proteoglycan expression of the cell line in question.

To further verify the transferrin receptor content of human melanomas, although already demonstrated in many occasions (Holzmann *et al.* 1985), melanoma cell lines were tested for hTfR expression using Western blot method (III, Fig. 2a). Some tissue samples of patient melanoma metastases were also stained for hTfR production and as anticipated, results of wide hTfR expression in tumor cells were detected (III, Fig.2b). The patient material used for all the IHC slides of the publications is summarized in the table 1. of (III), including the amount of hTfR in the sample.

When candidate oligos expressed on adenoviral vectors were injected straight to the tumor, the tumors' ability to retain viruses after 48 hours was determined. HAIYPRH was seen to be among the best in binding to the tumor tissue when compared to the low binder constrained LPMSKNS (III, Fig.3**).** pDV111 binding CAR and used as a control manifested the similar infectivity of the SK-mel- 5 line already described in the Figure1. When liver was analyzed, the results were opposite to the tumor results, confirming the high potenrtial of the HAIYPRH (III, Fig.3**)**.

Finally, the ability of HAIYPRH peptide to target to melanoma was examined in systemic administration. The liver was first blocked with warfarin and the adenoviral vectors were injected to SCID mice trough the tail vein. Again, HAIYPRH was targeted more to tumor than liver when compared to pDV111 or LMNSKNS. To verify the connection between hTfR and CAR deleted adenovirus expressing HAIYPRH, a set of staining for both hTfR and β-gal produced by adenoviruses was prepared of the SK-mel-5 tumors from SCID mice (III, Fig.4b). Interestingly, in this setup, the CAR-binding adeno did not show extensive binding, probably due to the numerous CAR receptors displayed along the way to the liver and tumor shown here.

(IV) TIMP-3 promotes apoptosis also in suspension cells lacking fully functional death receptor pathway

Small cell lung carcinoma (sclc) cell lines SW2 and N417 are suspension-growing cells that form aggregates floating in the medium when cultured. They also lack a functional caspase-8 route (Hopkins-Donaldson *et al.* 2003). They were used for testing if loss of adhesion plays a significant role in adenovirally induced apotosis and if TIMP-3 is capable of using other routes of apoptosis than a death receptor-induced caspase-8 pathway. This was done in order to try to broaden the apoptotic potential of TIMP-3 for the possible use of it in gene therapy applications. Previously, Ahonen *et al.* (1998, 2002, 2003) had shown that TIMP-3 kills monolayer malignant melanoma cells *in vitro* and solid melanoma tumors *in vivo* through death receptor originated apotosis. However, the question remained whether the simple detachment of cells from the monolayer surface was a reason for promoted apoptosis. The substantial variability in morphology and aggressiveness of these locally extracted melanoma cells are possibly due to differences in their signaling pathways. One should also consider the systemic travel of migrating metastatic melanoma cells on their way from the primary tumor to the site of metastasis. For example $\alpha_v\beta_3$ integrin overexpression has been connected to the transformation of melanoma cells from vertical to a horizontal growth state and has been associated with elevated resistance to apoptosis (Brassard *et al.* 1999; Tzukert *et al.* 2010). As there are not any malignant melanoma cell lines, which grow in suspension and would also have nonfunctional caspase-8 pathway, sclc cells were used instead.

First the lack of functional caspase-8 pathway in sclc cells was confirmed by activating the pathways with death receptor ligands (TNF-α, TRAIL and FAS activating ab) and then detecting the amounts of pro caspase-8 and cleaved caspase-3 in SW2 by Western

blot technique using A2058 melanoma cell line as positive control (IV Fig.1a). Recombinant TIMP-3 -induced cell surface FAS levels could also be detected. Sclc lines showed no detectable aggregation of FAS receptors, which is considered the starting point of caspase-8 -mediated apoptosis. FAS aggregation was noted on A2058 cell membrane as anticipated (IV Fig.1a).

The susceptibility of sclc cells to infect by adenoviral vectors was tested using recombinant adenovirus RAdLacZ capable of producing β-galactosidase enzyme that is detectable in cells and so showing the potential of infectivity in this case. As moi 100 was sufficient to produce almost 100% infection of control A2058 cell line it accounted only 50% infectivity in sclc cell lines (IV Fig.2a). Inspite of that, the infection of cells with RAdTIMP-3 induced apoptosis and a rate of cell death almost equal to A2058 control cells. The increase of TIMP-3 expression was confirmed by Western blotting technique of the lysed cells (IV Fig.2b). The apoptotic effect was confirmed by Tunel staining and MTT-cell viability assay. Infection with control viruses had no effect on cell viability (IV Fig.2c and 3a). The apoptotic effect was even confirmed to be persistent by following the viability of cells for up to 6 days after infection (IV Fig.2d).

To further confirm the caspase-8 -independent apoptosis, pan-caspase inhibitor Z-VAD-FMK and caspase-3 inhibitor (also inhibiting caspases 6, 7, 8 and 10) were used. 20μM concentrations of either inhibitor were not able to block the effect of RAdTIMP-3. On the other hand, 20μM concentration of caspase inhibitors had no effect on cell viability of RAdLacZ -infected cells (IV Fig.3b).

Adenovirally delivered TIMP-2 kills melanoma cells *in vivo*

Characterization of TIMP-2 was performed. Although high-concentration TIMP-2's capabilities as an inducer of cell death in melanoma tissue have been known for quite some time, adenoviral delivery of TIMP-2 in melanoma, as well as differences in methods of adenoviral delivery to the cancer cells, have received less attention (Hofmann *et al.* 2000). When A2058 malignant melanoma cells were first infected with adenoviruses in cell culture conditions, after which the cells were injected to animals, the ones infected with TIMP-2 adenovirus did not show tumor growth at all (Fig.5). Injections of TIMP-2 adenovirus on three days per week for several weeks resulted in clear hindrance to tumor progression (Fig.6).

A2058 human melanoma cells were chosen to represent the cell line population seemingly unaffected by TIMP-2 *in vitro*. Figure 5. shows the effect of adenovirally delivered TIMP-2 on *ex vivo* infected A2058 cells (moi 100), injected s.c. to the neck of SCID/SCID mice. Results are clear as both control cells infected with recombinant adenovirus RAdLacZ control vector, as well as RAdTIMP-1 -infected cells produced large tumors, whereas in mice with TIMP-2 -infected cells tumor was not detected at any point of the observation period. Contrary to the earlier *in vitro* studies with different cell

lines, in this case TIMP-1 did not seem to have at least a marked growth stimulatory effect. Rather large variability in growth of the tumor could be seen between different mice bearing melanoma cells that were infected with similar adenovirus (Table 2.).

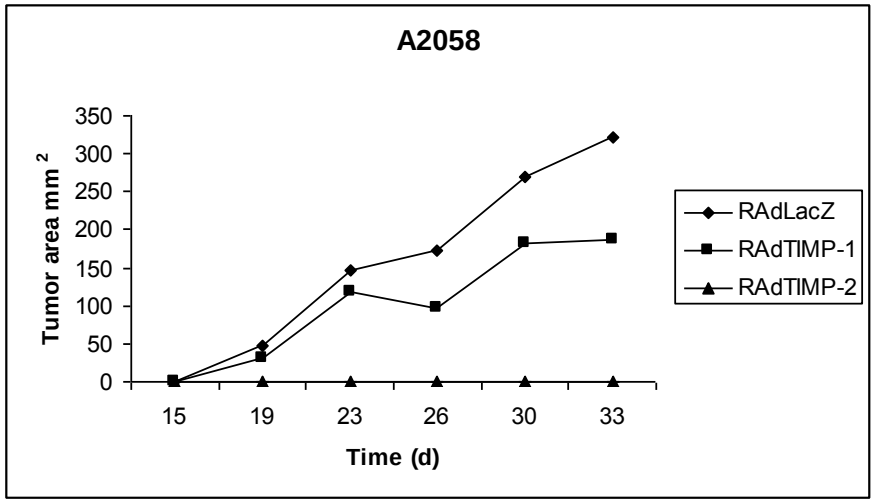


Figure 5. Development of the tumor areas in SCID/SCID mice carrying A2058 malignant melanoma tumors. A2058 cells were infected *ex vivo* with corresponding recombinant adenoviral vectors by moi 100. Result of t-test between RAdLacZ and RAdTIMP-2 during the whole observation period: 0,0102 (Student t-test, unpaired, two-tailed, equal variance).

Table 2. Minimum and maximum tumor areas of the treatment groups in Figure 5. Each group had 5 parallel mice.

		Days:	15	19	23	26	30	33
RAdLacZ	mm^2	Max	0	127,5	227,5	207,68	327,81	334,99
		Min	0	24	90,95	113,9	213,92	260
RAdTIMP-1		Max	0	73,45	151,9	239,54	344,19	408
		Min	0	2	90	6	2	32
RAdTIMP-2		Max	0	0	0	0	0	0
		Min	0	0	0	0	0	0

To investigate further the possibilities of adenovirally delivered TIMP-2 as an anti-melanoma molecule, an experiment utilizing i.v. injections of RAdTIMP-2 was conducted. Here, the A2058 tumors were first grown in SCID/SCID mice and after they had reached the size of a palpable pearl (dimensions approximately 3x3x2 mm), the mice were injected with RAdTIMP-2 as well as control viruses through the tail vein (Figure 6.). The growth-inhibitory effect on cancer cells by TIMP-2 *in vivo* was evident, although not as dramatic as in the case of *ex vivo* adenoviral infections.

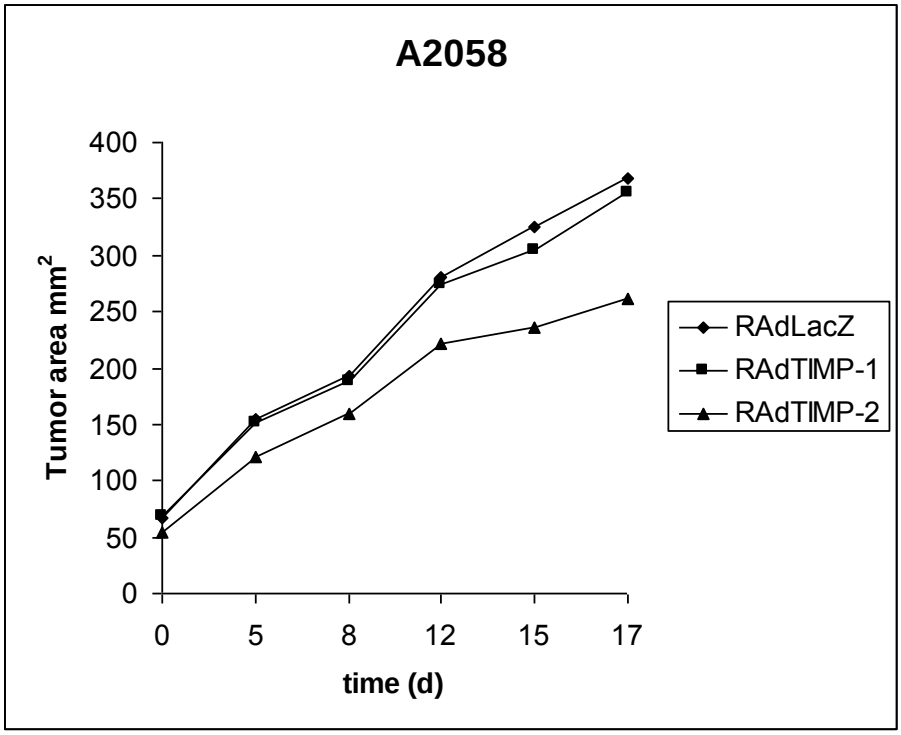


Figure 6. Effect of single injection of adenoviral vectors to tail vein of mice, bearing A2058 malignant melanoma tumors, at day 0. Injections contained $2{,}4x10^7$ plaque forming units / ml (PFU/ml) of adenoviral vector in 100µl PBS per mouse. Six parallel mice per treatment group were used. Result of t-test on day 17 between control RAdLacZ and RAdTIMP-2 is 0,069 (Student t-test, unpaired, two-tailed, equal variance).

6. Discussion

6.1 Effect of *cis*-UCA administration to malignant melanoma cells

Despite its suggested role in promoting cancer, *cis*-urocanic acid has been found to have some potential in preventing re-growth of cancer, as well (Hart *et al.* 2001; Rinaldi *et al.* 2006). It seems that *cis*-UCA inhibitis cancer cell proliferation based on the second acid dissociation constant and enhances the effects of ebirubicin *in vitro* when bladder cancer cells are used (Laihia *et al.* 2009). However, there are very few publications on the topic and further research is required. Recently, Laihia *et al.* presented their concept of protodynamic therapy for bladder cancer cells in cell culture (Laihia *et al.* 2009). There, bladder cancer cell lines 5637 and T24 showed significant decrease in cell viability in a slightly acidic pH of 6.65 whereas cells in the physiological pH did not respond correspondingly.

The reason for the formation of an acidic environment inside a tumor is largely due to enhanced metabolism and reduced blood flow, which results in the accumulation of lactate and other acidic metabolites. In study (I), it was postulated for the first time that not only can the tumor-surrounding matrix be acidified, but with the help of an adequate concentration of *cis*-UCA, the cytoplasm of the tumor cells can be acidified as well. This leads to apoptosis of the tumor cells also *in vivo*. According to these studies; time, *cis*-UCA concentration and pH appear to play a critical role in the course of events. Probably due to rather small changes in concentration, cells can resist the *cis*-UCA effect for quite some time. This is not necessarily a disadvantage, as from another perspective it gives time and opportunities for protecting the non-malignant tissues from destruction, as the study at hand also firmly proves that the effects of *cis*-UCA are due to apoptosis rather than any reversible actions. The large necrotic areas observed inside the tumors *in vivo* are very unlikely to be connected to *cis*-UCA. Previous studies using the malignant melanoma model in SCID mice have initially produced the same phenotype of tumors whether treated or not (Ahonen *et al.* 1998; Ahonen *et al.* 2002; Ahonen *et al.* 2003). All of them have included lumps of dividing cancer cells separated by walls and fibers of fat and connective tissue, and as the tumor has reached a certain size, all of them have developed an ascites-filled necrotic hole inside the primary tumor. In our studies, vascularization has not been extensive with any of the melanoma tumor cell lines used to date. The differences in the tumor growth rate and necrotic tumors in xenografts of SCID mice have been noted in other studies as well (Samkoe *et al.* 2010).

The high usefullness of *cis*-UCA in the treatment of metastasis is that it is a compound already found in the body at the physiological state which considerably reduces the chances of adverse reactions. Regarding malignant melanoma, *cis*-UCA might pose even more intriguing possibilities if administered to the site of the primary tumor, which most likely would be rather easy to reach with the correct concentration. In such a way, securing the site of the tumor after its removal would probably be beneficial in the long

term. A systemically delivered application of *cis*-UCA for metastasis would be rather challenging due to the requirement of an adequate concentration.

6.2 Semliki Forest virus VA7-EGFP replication-competent virus and malignant melanoma tumors

The malignant cell-killing effects combined with the adverse events of neuropathic nature have also been seen in one form or another previously when replication-competent avirulent SFV strains have been used (Tuittila *et al.* 2004; Graham *et al.* 2006). In addition to the drastic effect of tumor diminishment, a rarely seen factor of interest here is the mode of cell death other than apoptosis that was present also *in vivo*. The neuropathic effects seen might be partly due to the lack of adaptive immune system in SCID mice. Further studies with immuno-competent mice are needed in order to see to what extent the fully functional immune system can deal with the fulminant SFV infection and what kind of effects that has on the kinetics of the anti-cancer effects of SFV.

The administration of SFV to primary tumor may play crucial role in the tumor ability to develop resistance to SFV. In the present study, resistant cells were collected from the tumors infected originally by viruses from i.p. injections. Considering the compartmentalized structure of the malignant melanoma tumors, one could hypothesize that intratumoral injections in multiple sites might reduce the number of SFV-resistant cells. Based on this study it seems that at some point, a certain kinetic balance between replicating virus and growing cancer cells was established.

6.3 SCID/SCID mice as melanoma tumor model

The mouse model for severe combined immunodeficiency was developed in 1983 and has been the source of many applications since (Bosma *et al.* 1983). Despite the lack of T-cell mediated immunodefence in those animals, they still possess immunity in the form of natural killer (NK) cells and a functional complement system (Dorshkind *et al.* 1985). A phenomenon called T-cell leakiness has also been observed in SCID mice, meaning that some T-cells may develop during the maturation of the animal. In practice all the SCID mice finally develop some sort of T-cell immunity by the end of their lifespan (Petrini *et al.* 1990). Prior to SCID, the best model was the athymic and hairless nude mouse of the 1960s (Flanagan 1966). Self-evidently, the mice had plenty of relevance in the immunoresearch field as well. One of the first models in SCID mice was a lung cancer tumor model, where tumor cells were injected subcutaneously into the mice (Reddy *et al.* 1987). Complete tumors have also been planted surgically to mice (Kamel-Reid *et al.* 1989). The suitability for the tumor model has been compared between SCID and nude mice but the only difference has been that SCID might possess a slightly better metastasizing potential (Xie *et al.* 1992). The use of human melanomas has been quite popular in SCID mouse models, especially due to easy plantation of cells subcutaneously and the possibility to observe metastasis formation (Hill *et al.* 1991; Mueller *et al.* 1992). However, concerning the missing cell-mediated immunity, the model is somewhat

handicapped in predicting the immunoreactions that are likely to take place in immunocompetent animals treated with viruses. The immunoreaction works two ways, on the other hand, causing the fulminant and potentially harmful reaction to the host but also strongly diminishing the effect and infectivity of the viral vector used. From another perspective, there have been reports of SCID mice not being sensitive enough if one wants to study metastasis or use locally extracted cell lines straight from patients, which tend to be much less viable than the commercial cell lines available (Quintana *et al.* 2008).

The tumor weight calculated from the tumor area in *cis*-UCA publication (I) correlated well with the observations from the SFV studies (II), where the tumor mass was actually measured from the excised tumors. This observation confimed the strength of the equation used to calculate the approximate tumor mass from the surface area of the tumor. Despite the treatment model chosen, the tumors in mice have very commonly shown a large necrotic area in the middle of the tumor, as well as the compartmentalization of the tumors by extracellular matrix and connective tissue of the animal. The same kind of results have been obtained from similar experimental setups with reports of slightly more solid tumors in the untreated controls (Fodstad *et al.* 1994; Rofstad *et al.* 1996; White *et al.* 2009). Both features mentioned above have persisted to the end point of the chosen treatment. A notable fact is also the poor predictability of the tumor growth rate and size at the end point following the injection of the initial tumor cell under the skin of the mice, which leads to considerable variety between tumor sizes among treatment groups. Others have tried to block the back flow of injected cells from the needle hole by using matrigel dissolved in PBS, when injecting the subcutaneous tumor (White *et al.* 2009). The individual abilities of different mice to grow the tumor might play a role in this respect, as might the difficulty of preserving the cancer cell suspension in PBS homogenous prior to the mouse injections. There have been recent comparisons of different mouse strains concerning their ability to grow human melanoma (A375) xenografts (Carreno *et al.* 2009). According to this study, an SCID strain with an absolute deficiency of natural killer cells was considered the most sensitive for growing the xenografts. Rearranging the mice according to the tumor at later stages was unfortunately ethically impossible, as changes in the hierarchy of the animal group usually lead to injuries to some mice and so it was forbidden by the animal unit rules.

6.4 Re-targeting of adenovirus to melanoma metastasis

The great advantage of PD, the easy and fast propagation of the library in plain Luria Broth medium, is also the weakness of the system. As bacteriophages can be found everywhere, even the clean facilities, the risk of getting a wild type (wt) phage contamination increases heavily during every round of panning. This limits the maximum rounds to 3-5 depending on the application. After every step of amplification there is a step of titering, in order to find out the equal titer for the next round. Normally the library phages have the ability to produce blue colonies on the dilution series of x-gal plates, whereas wt colonies are usually white. To find out the final composition of the library in the end, certain amount of the colonies is separately grown up and the DNA of a colony-

hosting phage that is expressing single type of oligopeptide is extracted and sequenced. By doing this to hundreds of colonies the possible propagation of the subpopulation of certain bound phage is revealed.

For targeting to tumor cells *in vivo* using PD, the entry to the target cells is more complex than e.g. when targeting vasculature. Phages have to extravasate through leaking capillary walls and find their way to cancer cells, usually mixed with adipose and connective tissue. The frequent poor vasculature of the emerging tumor also possesses a challenge for the phage. On the other hand, such an approach is more than likely to promote cell-specific binding as the library given systemically is exposed to thorough pre-clearing on its way to the target tissue. In an experiment, targeting to PC3 prostate tumor cells was achieved using PD and mice bearing the human tumor (Newton *et al.* 2006). In the case of melanoma mainly studies with murine melanomas have been conducted, showing proof of principle in verification of tumor binding like PC3 as well as targeting to neovasculature of tumors (Burg *et al.* 1999; Newton *et al.* 2007). One highly interesting study was done by Howell et al. (2007), in which they identified 24 promising peptides for human melanin using a linear commercial heptamer library and tested them in A2058 human melanoma line. They used the classical approach of coating a monolayer with melanin before panning. Peptides were coupled to radio isotope 188-rhenium and were noted to bind only melanin expressed outside the cells (Howell *et al.* 2007). The best homing peptide was NPNWGPR. Interestingly, none of the best peptides were found in our study. However, the authors postulate that their panel was created against eumelanin, whereas A2058 cells express pheomelanin that differs somewhat in color as well as molecular structure from the latter.

Many other panning experiments have produced promising oligopeptide candidates in PD using human tumor cells; including SCC of the head and neck (Nothelfer *et al.* 2009), colon cancer (Kubo *et al.* 2008) and breast cancer (Schier *et al.* 1995), to name just a few. By further characterizing peptides found in our studies, but not yet published, it might be possible to recognize new melanoma surface antigens and possible melanoma specific targets for homing. For example detection of β-galactosidase in PET scan, that was rather unexplored and uncertain field a couple of years ago, seems to be feasible in a short while (Celen *et al.* 2009). Keeping that in mind the distribution of the adenoviruses expressing the candidate oligopeptides could be detected in mouse using the PET facility in university of Turku. More information concerning the distribution would be needed also noting that in the mouse model the comparison between tumor and liver includes human tumor cells and mouse liver.

As interesting as adenoviral targeting and therapy potential is, the application for treatment of metastatic melanoma in the near future is more likely to be found elsewhere. Despite the uncertainty of the adenoviral approach, the peptides themselves may possess higher value. Lately artemisin-HAIYPRH conjugate was used succesfully in an experimental setup for destruction of leukemia cells. As already discussed earlier in this thesis, there are new small molecules like PLX4032. For several other cancer types, for example the many times unoperable glioma, adenovirally delivered genes and gene products might offer more promising solutions than for melanoma. Very recently,

recombinant vaccinia virus JX-594 significantly improved survival by inhibiting tumor growth in rodents carrying intracranial and brain tumors (Lun *et al.* 2010).

6.5 The potential of TIMP-3 in killing cancer cells in addition to monolayer metastatic melanoma

Based on the previous findings, it seems that the pure detachment of the cells due to adenoviral infection does not markedly increase the rate of apoptotic death. Previously it was hypothesized that sudden abolishment of cell contact of monolayer cells to the surface or basal membrane might trigger the apoptotic pathways. Here, cells forming aggregates and growing freely in suspension could be killed with RAdTIMP-3. Even the cells within large aggregates seemed to be dying, manifesting the bystander effect of TIMP-3 previously detected. Of further note are the apoptotic effects without the fully functional caspase-8 pathway. Caspases are known to be able to compensate for each other, but use of critical caspase inhibitors should have blocked the apoptosis. For example, caspase-10, previously shown to compensate for caspase-8, was blocked with both caspase inhibitors used. The speculation about an unknown cell death inducement linked to SFV induced tumor shrinkage might also be true with TIMP-3 induced cell death. Here the ratio between apoptosis and possible other modes of cell death was not determined, although in TUNEL staining the proportion of apoptotic nuclei seemed rather dominant.

Following the findings presented here, it is quite clear that the full potential of TIMP-3 as a cancer cell killer is yet to be mapped. The possibilities are many. In some of our data, slight aggregation of cell surface death receptors could be seen in sclc cells yet uncapable of using caspase-8. This might point to the direction of the somewhat controversial caspase-independent cell death (CICD) (Franke *et al.* 2010). On the other hand, in TUNEL staining, clear DNA fragmentation could be seen in all cell lines, which is not considered a sign of CICD (Chipuk *et al.* 2005).

Apotosis independently of caspase-8 could be possible considering the capability of different caspases to interact and substitute each other in certain circumstances. There have been speculation that death receptor activation might lead to an excess number of reactive oxygen species, which would rapidly destroy mitochondrial functions and cause cell death (Chen *et al.* 2009). However, the simplest explanation might hold true in this case too. Caspase-10 has been shown to be able to independently cleave caspase-3 (Fernandes-Alnemri *et al.* 1996). There has been an accumulating amount of data suggesting caspase-10 as an initiator caspase, especially in the case of FASL-induced apoptosis (Milhas *et al.* 2005).

The role of TIMP-3 as a major inhibitor of MMP-2 and metastasis formation has been demonstrated in several studies (Cruz-Munoz *et al.* 2006; Cruz-Munoz *et al.* 2006). Moreover, adenovirally delivered TIMP-3 has also been shown to be an effective cancer killer in some, but not all, non-small cell carcinoma cell lines *in vitro* (Finan *et al.* 2006). Interestingly, in a recent microarray study of TIMP-3 -induced apoptosis in glial cells,

especially caspase-1 and caspase-4 seemed to be upregulated (Lam *et al.* 2005). Caspase-1 is known to be involved in CD95 -induced apoptosis, although this route is not so well characterized (Watanabe-Fukunaga *et al.* 1992; Enari *et al.* 1995). Caspase -4, on the other hand, is rather poorly known and may have a role in the caspase-1 maturation, as it has a very similar tissue distribution (Faucheu *et al.* 1995). All in all, TIMP-3 is likely to be exploited for therapy in a form or another, as it is being widely studied in vascularization, caspase pathways and other cancer types than melanoma (Chetty *et al.* 2008; Angoulvant *et al.* 2009; Kim *et al.* 2010).

6.6 Adenovirally delivered TIMP-2 and metastatic melanoma tumors

These experiments verify for the first time that even adenovirally delivered TIMP-2 is capable of reducing the tumor growth of human malignant melanoma. Furthermore, RAdTIMP-2 is capable of doing so even when given systemically, without any marked adverse effects to the animals. The differences in morphology as well as physiology between different melanoma cell lines and even from primary tumor to metastasis was also evident here.

The previously speculated mechanism of TIMP-2 in tumor suppression is further strengthened by these preliminary studies (Valente *et al.* 1998). It indeed seems that the viral load reaching the target cells plays a crucial role in the process of diminishing tumor growth. For its part, this further underlines the need to target the adenoviral vectors in order to minimize the possible side effects and maximize the homing of systematically administered adenoviruses.

7. Summary and Conclusions

Adequate concentration of *Cis*-urocanic acid inhibits melanoma growth and promotes apoptosis *in vitro* and *in vivo* by promoting intracellular acidification of cancer cells.
At present the use of *cis*-UCA in patients might most likely culminate in apical administration of the compound on the primary tumor or its excision site. By using, for example, liposomes targeted to melanoma cells by specific peptides, sufficient concentration of *cis*-UCA might be possible to be administered also to the site of multiple metastasis (Chemin *et al.* 2010).

Semliki Forest virus VA7-EGFP kills most but not all human malignant melanoma cells in a xenograft tumor on SCID mice. This results in considerable reduction of the tumor size. However, the treated mice develop gradually progressing neuropathic symptoms leading to paralysis. Due to its neuropathic characteristics SFV needs to be studied further in order to better control the adverse effects.

Search for oligopeptides homing to human malignant melanoma cells using phage display technique produced several promising oligopeptide candidates. Linear peptide library and *in vivo* approach were most effective in the search for peptides. As a proof of principle, peptide HAIYPRH targeting to human transferrin receptor was found. This receptor is known to be over expressed in melanomas. Adenovirus expressing HAIYPRH was also among the ones attaching the least to the liver cells. It is possible to find and target specific proteins over expressed on malignant melanoma cells using recombinant adenoviruses. The efficacy and safety of these modified vectors needs to be investigated thoroughly, before any clinical applications can be developed.

Adenovirally delivered TIMP-3 promotes apoptosis even in cells without fully functional caspase-8 pathway. The apoptotic effects seen previously are also independent of the consequences of adenoviral infection and cell detachment due to it. Adenovirally delivered TIMP-2 has also shown potential in blocking growth of malignant melanoma cells *in vivo*. Both tissue inhibitors of metalloproteases TIMP-2 and TIMP-3 can be considered as interesting anti-cancer molecules in gene therapy applications against malignant melanoma.

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9. References:

Ahonen, M., R. Ala-Aho, A. H. Baker, S. J. George, R. Grenman, U. Saarialho-Kere and V. M. Kahari (2002). "Antitumor activity and bystander effect of adenovirally delivered tissue inhibitor of metalloproteinases-3." Mol Ther **5**(6): 705-15.

Ahonen, M., A. H. Baker and V. M. Kahari (1998). "Adenovirus-mediated gene delivery of tissue inhibitor of metalloproteinases-3 inhibits invasion and induces apoptosis in melanoma cells." Cancer Res **58**(11): 2310-5.

Ahonen, M., M. Poukkula, A. H. Baker, M. Kashiwagi, H. Nagase, J. E. Eriksson and V. M. Kahari (2003). "Tissue inhibitor of metalloproteinases-3 induces apoptosis in melanoma cells by stabilization of death receptors." Oncogene **22**(14): 2121-34.

Aiuti, A., S. Slavin, M. Aker, F. Ficara, S. Deola, A. Mortellaro, S. Morecki, G. Andolfi, A. Tabucchi, F. Carlucci, E. Marinello, F. Cattaneo, S. Vai, P. Servida, R. Miniero, M. G. Roncarolo and C. Bordignon (2002). "Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning." Science **296**(5577): 2410-3.

Ala-aho, R. and V. M. Kahari (2005). "Collagenases in cancer." Biochimie **87**(3-4): 273-86.

Alemany, R., K. Suzuki and D. T. Curiel (2000). "Blood clearance rates of adenovirus type 5 in mice." J Gen Virol **81**(Pt 11): 2605-9.

Amalfitano, A. (1999). "Next-generation adenoviral vectors: new and improved." Gene Ther **6**(10): 1643-5.

Amor, S., M. F. Scallan, M. M. Morris, H. Dyson and J. K. Fazakerley (1996). "Role of immune responses in protection and pathogenesis during Semliki Forest virus encephalitis." J Gen Virol **77 (Pt 2)**: 281-91.

Amour, A., C. G. Knight, A. Webster, P. M. Slocombe, P. E. Stephens, V. Knauper, A. J. Docherty and G. Murphy (2000). "The in vitro activity of ADAM-10 is inhibited by TIMP-1 and TIMP-3." FEBS Lett **473**(3): 275-9.

Anand-Apte, B., M. S. Pepper, E. Voest, R. Montesano, B. Olsen, G. Murphy, S. S. Apte and B. Zetter (1997). "Inhibition of angiogenesis by tissue inhibitor of metalloproteinase-3." Invest Ophthalmol Vis Sci **38**(5): 817-23.

Anglin, J. H., Jr. and W. H. Batten (1968). "Studies on cis urocanic acid." J Invest Dermatol **50**(6): 463-6.

Angoulvant, D., S. Fazel, R. D. Weisel, T. Y. Lai, P. W. Fedak, L. Chen, S. Rafati, C. K. Seneviratne, N. Degousee and R. K. Li (2009). "Cell-based gene therapy modifies matrix remodeling after a myocardial infarction in tissue inhibitor of matrix metalloproteinase-3-deficient mice." J Thorac Cardiovasc Surg **137**(2): 471-80.

Apte, S. S. (2004). "A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motifs: the ADAMTS family." Int J Biochem Cell Biol **36**(6): 981-5.

Apte, S. S., B. R. Olsen and G. Murphy (1995). "The gene structure of tissue inhibitor of metalloproteinases (TIMP)-3 and its inhibitory activities define the distinct TIMP gene family." J Biol Chem **270**(24): 14313-8.

Arap, W., M. G. Kolonin, M. Trepel, J. Lahdenranta, M. Cardo-Vila, R. J. Giordano, P. J. Mintz, P. U. Ardelt, V. J. Yao, C. I. Vidal, L. Chen, A. Flamm, H. Valtanen, L. M. Weavind, M. E. Hicks, R. E. Pollock, G. H. Botz, C. D. Bucana, E. Koivunen, D. Cahill, P. Troncoso, K. A. Baggerly, R. D. Pentz, K. A. Do, C. J. Logothetis and R. Pasqualini (2002). "Steps toward mapping the human vasculature by phage display." Nat Med **8**(2): 121-7.

Armstrong, B. K. and A. Kricker (2001). "The epidemiology of UV induced skin cancer." J Photochem Photobiol B **63**(1-3): 8-18.

Ashida, A., M. Takata, H. Murata, K. Kido and T. Saida (2009). "Pathological activation of KIT in metastatic tumors of acral and mucosal melanomas." Int J Cancer **124**(4): 862-8.

Asselin-Paturel, C., N. Lassau, J. M. Guinebretiere, J. Zhang, F. Gay, F. Bex, S. Hallez, J. Leclere, P. Peronneau, F. Mami-Chouaib and S. Chouaib (1999). "Transfer of the murine interleukin-12 gene in vivo by a Semliki Forest virus vector induces B16 tumor regression through inhibition of tumor blood vessel formation monitored by Doppler ultrasonography." Gene Ther **6**(4): 606-15.

Atkins, G. J. and B. J. Sheahan (1982). "Semliki forest virus neurovirulence mutants have altered cytopathogenicity for central nervous system cells." Infect Immun **36**(1): 333-41.

Baba, T., M. Sato-Matsushita, A. Kanamoto, A. Itoh, N. Oyaizu, Y. Inoue, Y. Kawakami and H. Tahara (2010). "Phase I clinical trial of the vaccination for the patients with metastatic melanoma using gp100-derived epitope peptide restricted to HLA-A*2402." J Transl Med **8**(1): 84.

Bachman, K. E., J. G. Herman, P. G. Corn, A. Merlo, J. F. Costello, W. K. Cavenee, S. B. Baylin and J. R. Graff (1999). "Methylation-associated silencing of the tissue inhibitor of metalloproteinase-3 gene suggest a suppressor role in kidney, brain, and other human cancers." Cancer Res **59**(4): 798-802.

Bae, S. I., V. Cheriyath, B. S. Jacobs, F. J. Reu and E. C. Borden (2008). "Reversal of methylation silencing of Apo2L/TRAIL receptor 1 (DR4) expression overcomes resistance of SK-MEL-3 and SK-MEL-28 melanoma cells to interferons (IFNs) or Apo2L/TRAIL." Oncogene **27**(4): 490-8.

Bajetta, E., M. Del Vecchio, C. Bernard-Marty, M. Vitali, R. Buzzoni, O. Rixe, P. Nova, S. Aglione, S. Taillibert and D. Khayat (2002). "Metastatic melanoma: chemotherapy." Semin Oncol **29**(5): 427-45.

Baker, A. H., G. W. Wilkinson, R. M. Hembry, G. Murphy and A. C. Newby (1996). "Development of recombinant adenoviruses that drive high level expression of the human metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 and -2 genes: characterization of their infection into rabbit smooth muscle cells and human MCF-7 adenocarcinoma cells." Matrix Biol **15**(6): 383-95.

Baker, A. H., A. B. Zaltsman, S. J. George and A. C. Newby (1998). "Divergent effects of tissue inhibitor of metalloproteinase-1, -2, or -3 overexpression on rat vascular smooth muscle cell invasion, proliferation, and death in vitro. TIMP-3 promotes apoptosis." J Clin Invest **101**(6): 1478-87.

Balbin, M., A. Fueyo, A. M. Tester, A. M. Pendas, A. S. Pitiot, A. Astudillo, C. M. Overall, S. D. Shapiro and C. Lopez-Otin (2003). "Loss of collagenase-2 confers increased skin tumor susceptibility to male mice." Nat Genet **35**(3): 252-7.

Balch, C. M., J. E. Gershenwald, S. J. Soong, J. F. Thompson, M. B. Atkins, D. R. Byrd, A. C. Buzaid, A. J. Cochran, D. G. Coit, S. Ding, A. M. Eggermont, K. T. Flaherty, P. A. Gimotty, J. M. Kirkwood, K. M. McMasters, M. C. Mihm, Jr., D. L. Morton, M. I. Ross, A. J. Sober and V. K. Sondak (2009). "Final version of 2009 AJCC melanoma staging and classification." J Clin Oncol **27**(36): 6199-206.

Balch, C. M., S. J. Soong, J. E. Gershenwald, J. F. Thompson, D. S. Reintgen, N. Cascinelli, M. Urist, K. M. McMasters, M. I. Ross, J. M. Kirkwood, M. B. Atkins, J. A. Thompson, D. G. Coit, D. Byrd, R. Desmond, Y. Zhang, P. Y. Liu, G. H. Lyman and A. Morabito (2001). "Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system." J Clin Oncol **19**(16): 3622-34.

Barnholtz-Sloan, J. S., A. E. Sloan, F. G. Davis, F. D. Vigneau, P. Lai and R. E. Sawaya (2004). "Incidence proportions of brain metastases in patients diagnosed (1973 to 2001) in the Metropolitan Detroit Cancer Surveillance System." J Clin Oncol **22**(14): 2865-72.

Bataille, V., J. A. Bishop, P. Sasieni, A. J. Swerdlow, E. Pinney, K. Griffiths and J. Cuzick (1996). "Risk of cutaneous melanoma in relation to the numbers, types and sites of naevi: a case-control study." Br J Cancer **73**(12): 1605-11.

Beddingfield, F. C., 3rd (2003). "The melanoma epidemic: res ipsa loquitur." Oncologist **8**(5): 459-65.

Bedikian, A. Y., M. Millward, H. Pehamberger, R. Conry, M. Gore, U. Trefzer, A. C. Pavlick, R. DeConti, E. M. Hersh, P. Hersey, J. M. Kirkwood and F. G. Haluska (2006). "Bcl-2 antisense (oblimersen

sodium) plus dacarbazine in patients with advanced melanoma: the Oblimersen Melanoma Study Group." J Clin Oncol **24**(29): 4738-45.

Beissert, S., D. Ruhlemann, T. Mohammad, S. Grabbe, A. El-Ghorr, M. Norval, H. Morrison, R. D. Granstein and T. Schwarz (2001). "IL-12 prevents the inhibitory effects of cis-urocanic acid on tumor antigen presentation by Langerhans cells: implications for photocarcinogenesis." J Immunol **167**(11): 6232-8.

Bergelson, J. M., J. A. Cunningham, G. Droguett, E. A. Kurt-Jones, A. Krithivas, J. S. Hong, M. S. Horwitz, R. L. Crowell and R. W. Finberg (1997). "Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5." Science **275**(5304): 1320-3.

Bewley, M. C., K. Springer, Y. B. Zhang, P. Freimuth and J. M. Flanagan (1999). "Structural analysis of the mechanism of adenovirus binding to its human cellular receptor, CAR." Science **286**(5444): 1579-83.

Bian, J., Y. Wang, M. R. Smith, H. Kim, C. Jacobs, J. Jackman, H. F. Kung, N. H. Colburn and Y. Sun (1996). "Suppression of in vivo tumor growth and induction of suspension cell death by tissue inhibitor of metalloproteinases (TIMP)-3." Carcinogenesis **17**(9): 1805-11.

Binetruy-Tournaire, R., C. Demangel, B. Malavaud, R. Vassy, S. Rouyre, M. Kraemer, J. Plouet, C. Derbin, G. Perret and J. C. Mazie (2000). "Identification of a peptide blocking vascular endothelial growth factor (VEGF)-mediated angiogenesis." Embo J **19**(7): 1525-33.

Bishop, D. T., F. Demenais, A. M. Goldstein, W. Bergman, J. N. Bishop, B. Bressac-de Paillerets, A. Chompret, P. Ghiorzo, N. Gruis, J. Hansson, M. Harland, N. Hayward, E. A. Holland, G. J. Mann, M. Mantelli, D. Nancarrow, A. Platz and M. A. Tucker (2002). "Geographical variation in the penetrance of CDKN2A mutations for melanoma." J Natl Cancer Inst **94**(12): 894-903.

Bliss, J. M., D. Ford, A. J. Swerdlow, B. K. Armstrong, M. Cristofolini, J. M. Elwood, A. Green, E. A. Holly, T. Mack, R. M. MacKie and et al. (1995). "Risk of cutaneous melanoma associated with pigmentation characteristics and freckling: systematic overview of 10 case-control studies. The International Melanoma Analysis Group (IMAGE)." Int J Cancer **62**(4): 367-76.

Bond, M., G. Murphy, M. R. Bennett, A. C. Newby and A. H. Baker (2002). "Tissue inhibitor of metalloproteinase-3 induces a Fas-associated death domain-dependent type II apoptotic pathway." J Biol Chem **277**(16): 13787-95.

Bosma, G. C., R. P. Custer and M. J. Bosma (1983). "A severe combined immunodeficiency mutation in the mouse." Nature **301**(5900): 527-30.

Bouard, D., D. Alazard-Dany and F. L. Cosset (2009). "Viral vectors: from virology to transgene expression." Br J Pharmacol **157**(2): 153-65.

Bradish, C. J., K. Allner and H. B. Maber (1971). "The virulence of original and derived strains of Semliki forest virus for mice, guinea-pigs and rabbits." J Gen Virol **12**(2): 141-60.

Brand, K., A. H. Baker, A. Perez-Canto, A. Possling, M. Sacharjat, M. Geheeb and W. Arnold (2000). "Treatment of colorectal liver metastases by adenoviral transfer of tissue inhibitor of metalloproteinases-2 into the liver tissue." Cancer Res **60**(20): 5723-30.

Brassard, D. L., E. Maxwell, M. Malkowski, T. L. Nagabhushan, C. C. Kumar and L. Armstrong (1999). "Integrin alpha(v)beta(3)-mediated activation of apoptosis." Exp Cell Res **251**(1): 33-45.

Breslow, A. (1970). "Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma." Ann Surg **172**(5): 902-8.

Brew, K., D. Dinakarpandian and H. Nagase (2000). "Tissue inhibitors of metalloproteinases: evolution, structure and function." Biochim Biophys Acta **1477**(1-2): 267-83.

Brown, K. C. (2000). "New approaches for cell-specific targeting: identification of cell-selective peptides from combinatorial libraries." Curr Opin Chem Biol **4**(1): 16-21.

Brummer, O., S. Athar, L. Riethdorf, T. Loning and H. Herbst (1999). "Matrix-metalloproteinases 1, 2, and 3 and their tissue inhibitors 1 and 2 in benign and malignant breast lesions: an in situ hybridization study." Virchows Arch **435**(6): 566-73.

Burg, M. A., R. Pasqualini, W. Arap, E. Ruoslahti and W. B. Stallcup (1999). "NG2 proteoglycan-binding peptides target tumor neovasculature." Cancer Res **59**(12): 2869-74.

Bush, J. A. and G. Li (2003). "The role of Bcl-2 family members in the progression of cutaneous melanoma." Clin Exp Metastasis **20**(6): 531-9.

Buzaid, A. C., A. B. Sandler, S. Mani, A. M. Curtis, W. J. Poo, J. L. Bolognia and S. Ariyan (1993). "Role of computed tomography in the staging of primary melanoma." J Clin Oncol **11**(4): 638-43.

Cabebe, E. and H. Wakelee (2006). "Sunitinib: a newly approved small-molecule inhibitor of angiogenesis." Drugs Today (Barc) **42**(6): 387-98.

Campos, S. K. and M. A. Barry (2007). "Current advances and future challenges in Adenoviral vector biology and targeting." Curr Gene Ther **7**(3): 189-204.

Carreno, B. M., J. R. Garbow, G. R. Kolar, E. N. Jackson, J. A. Engelbach, M. Becker-Hapak, L. N. Carayannopoulos, D. Piwnica-Worms and G. P. Linette (2009). "Immunodeficient mouse strains display marked variability in growth of human melanoma lung metastases." Clin Cancer Res **15**(10): 3277-86.

Cassarino, D. S., E. S. Cabral, R. V. Kartha and S. M. Swetter (2008). "Primary dermal melanoma: distinct immunohistochemical findings and clinical outcome compared with nodular and metastatic melanoma." Arch Dermatol **144**(1): 49-56.

Cassumbhoy, R. and A. G. Pitman (2007). "Isolated limb infusion for local control of lower limb melanoma: radiologic aspects." Australas Radiol **51**(6): 543-9.

Celen, S., J. Cleynhens, C. Deroose, T. de Groot, A. Ibrahimi, R. Gijsbers, Z. Debyser, L. Mortelmans, A. Verbruggen and G. Bormans (2009). "Synthesis and biological evaluation of (11)C-labeled beta-galactosyl triazoles as potential PET tracers for in vivo LacZ reporter gene imaging." Bioorg Med Chem **17**(14): 5117-25.

Celiker, M. Y., M. Wang, E. Atsidaftos, X. Liu, Y. E. Liu, Y. Jiang, E. Valderrama, I. D. Goldberg and Y. E. Shi (2001). "Inhibition of Wilms' tumor growth by intramuscular administration of tissue inhibitor of metalloproteinases-4 plasmid DNA." Oncogene **20**(32): 4337-43.

Chemin, C., J. M. Pean, A. Le Pape, J. M. Delbos, M. German-Fattal, P. Wuthrich and P. Couvreur (2010). "Biodistribution and anticancer activity of a new vinca alkaloid encapsulated into long-circulating liposomes." J Liposome Res **20**(1): 62-72.

Chen, T. Y., K. H. Chi, J. S. Wang, C. L. Chien and W. W. Lin (2009). "Reactive oxygen species are involved in FasL-induced caspase-independent cell death and inflammatory responses." Free Radic Biol Med **46**(5): 643-55.

Chetty, C., S. S. Lakka, P. Bhoopathi, S. Kunigal, R. Geiss and J. S. Rao (2008). "Tissue inhibitor of metalloproteinase 3 suppresses tumor angiogenesis in matrix metalloproteinase 2-down-regulated lung cancer." Cancer Res **68**(12): 4736-45.

Chipuk, J. E. and D. R. Green (2005). "Do inducers of apoptosis trigger caspase-independent cell death?" Nat Rev Mol Cell Biol **6**(3): 268-75.

Chiu, C. Y., P. Mathias, G. R. Nemerow and P. L. Stewart (1999). "Structure of adenovirus complexed with its internalization receptor, alphavbeta5 integrin." J Virol **73**(8): 6759-68.

Clark, W. H., Jr., L. From, E. A. Bernardino and M. C. Mihm (1969). "The histogenesis and biologic behavior of primary human malignant melanomas of the skin." Cancer Res **29**(3): 705-27.

Cohen, T., K. J. Busam, A. Patel and M. S. Brady (2008). "Subungual melanoma: management considerations." Am J Surg **195**(2): 244-8.

Cohn-Cedermark, G., E. Mansson-Brahme, L. E. Rutqvist, O. Larsson, T. Singnomklao and U. Ringborg (1999). "Metastatic patterns, clinical outcome, and malignant phenotype in malignant cutaneous melanoma." Acta Oncol **38**(5): 549-57.

Colby, W. W. and T. Shenk (1981). "Adenovirus type 5 virions can be assembled in vivo in the absence of detectable polypeptide IX." J Virol **39**(3): 977-80.

Collen, A., R. Hanemaaijer, F. Lupu, P. H. Quax, N. van Lent, J. Grimbergen, E. Peters, P. Koolwijk and V. W. van Hinsbergh (2003). "Membrane-type matrix metalloproteinase-mediated angiogenesis in a fibrin-collagen matrix." Blood **101**(5): 1810-7.

Cortese, I., R. Tafi, L. M. Grimaldi, G. Martino, A. Nicosia and R. Cortese (1996). "Identification of peptides specific for cerebrospinal fluid antibodies in multiple sclerosis by using phage libraries." Proc Natl Acad Sci U S A **93**(20): 11063-7.

Croghan, G. A., V. J. Suman, W. J. Maples, M. Albertini, G. Linette, L. Flaherty, J. Eckardt, C. Ma, S. N. Markovic and C. Erlichman (2010). "A study of paclitaxel, carboplatin, and bortezomib in the treatment of metastatic malignant melanoma: a phase 2 consortium study." Cancer **116**(14): 3463-8.

Crompton, J., C. I. Toogood, N. Wallis and R. T. Hay (1994). "Expression of a foreign epitope on the surface of the adenovirus hexon." J Gen Virol **75 (Pt 1)**: 133-9.

Cruz-Munoz, W. and R. Khokha (2008). "The role of tissue inhibitors of metalloproteinases in tumorigenesis and metastasis." Crit Rev Clin Lab Sci **45**(3): 291-338.

Cruz-Munoz, W., I. Kim and R. Khokha (2006). "TIMP-3 deficiency in the host, but not in the tumor, enhances tumor growth and angiogenesis." Oncogene **25**(4): 650-5.

Cruz-Munoz, W., O. H. Sanchez, M. Di Grappa, J. L. English, R. P. Hill and R. Khokha (2006). "Enhanced metastatic dissemination to multiple organs by melanoma and lymphoma cells in timp-3(-/-) mice." Oncogene.

Culouscou, J. M., G. D. Plowman, G. W. Carlton, J. M. Green and M. Shoyab (1993). "Characterization of a breast cancer cell differentiation factor that specifically activates the HER4/p180erbB4 receptor." J Biol Chem **268**(25): 18407-10.

Dammann, R., M. Strunnikova, U. Schagdarsurengin, M. Rastetter, M. Papritz, U. E. Hattenhorst, H. S. Hofmann, R. E. Silber, S. Burdach and G. Hansen (2005). "CpG island methylation and expression of tumour-associated genes in lung carcinoma." Eur J Cancer **41**(8): 1223-36.

Davenport, M. P., C. L. Quinn, P. Valsasnini, F. Sinigaglia, A. V. Hill and J. I. Bell (1996). "Analysis of peptide-binding motifs for two disease associated HLA-DR13 alleles using an M13 phage display library." Immunology **88**(4): 482-6.

Davison, A. J., M. Benko and B. Harrach (2003). "Genetic content and evolution of adenoviruses." J Gen Virol **84**(Pt 11): 2895-908.

De Fabo, E. C. and F. P. Noonan (1983). "Mechanism of immune suppression by ultraviolet irradiation in vivo. I. Evidence for the existence of a unique photoreceptor in skin and its role in photoimmunology." J Exp Med **158**(1): 84-98.

De Fine Olivarius, F., J. Lock-Andersen, F. G. Larsen, H. C. Wulf, J. Crosby and M. Norval (1998). "Urocanic acid isomers in patients with basal cell carcinoma and cutaneous malignant melanoma." Br J Dermatol **138**(6): 986-92.

de Fine Olivarius, F., H. C. Wulf, J. Crosby and M. Norval (1998). "Urocanic acid isomers and photosensitivity in healthy children." Br J Dermatol **139**(4): 605-9.

de Lorenzo, M. S., G. V. Ripoll, H. Yoshiji, M. Yamazaki, U. P. Thorgeirsson, D. F. Alonso and D. E. Gomez (2003). "Altered tumor angiogenesis and metastasis of B16 melanoma in transgenic mice overexpressing tissue inhibitor of metalloproteinases-1." In Vivo **17**(1): 45-50.

De Simone, C., C. Masini, M. S. Cattaruzza, C. Guerriero, D. Cerimele and M. Norval (2001). "Urocanic acid isomers in patients with non-melanoma skin cancer." Br J Dermatol **144**(4): 858-61.

Dechecchi, M. C., P. Melotti, A. Bonizzato, M. Santacatterina, M. Chilosi and G. Cabrini (2001). "Heparan sulfate glycosaminoglycans are receptors sufficient to mediate the initial binding of adenovirus types 2 and 5." J Virol **75**(18): 8772-80.

Del Vecchio, M., S. Canova, A. Messina and E. Bajetta (2007). "Impressive objective response in a patient with extensive metastatic melanoma including the brain." Melanoma Res **17**(5): 332-4.

Denhardt, D. T., B. Feng, D. R. Edwards, E. T. Cocuzzi and U. M. Malyankar (1993). "Tissue inhibitor of metalloproteinases (TIMP, aka EPA): structure, control of expression and biological functions." Pharmacol Ther **59**(3): 329-41.

Devlin, J. J., L. C. Panganiban and P. E. Devlin (1990). "Random peptide libraries: a source of specific protein binding molecules." Science **249**(4967): 404-6.

DiCiommo, D. P. and R. Bremner (1998). "Rapid, high level protein production using DNA-based Semliki Forest virus vectors." J Biol Chem **273**(29): 18060-6.

Dillman, R. O., S. R. Selvan, P. M. Schiltz, E. F. McClay, N. M. Barth, C. DePriest, C. de Leon, C. Mayorga, A. N. Cornforth and K. Allen (2009). "Phase II trial of dendritic cells loaded with antigens from self-renewing, proliferating autologous tumor cells as patient-specific antitumor vaccines in patients with metastatic melanoma: final report." Cancer Biother Radiopharm **24**(3): 311-9.

Dmitriev, I., V. Krasnykh, C. R. Miller, M. Wang, E. Kashentseva, G. Mikheeva, N. Belousova and D. T. Curiel (1998). "An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor-independent cell entry mechanism." J Virol **72**(12): 9706-13.

Docherty, A. J., A. Lyons, B. J. Smith, E. M. Wright, P. E. Stephens, T. J. Harris, G. Murphy and J. J. Reynolds (1985). "Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity." Nature **318**(6041): 66-9.

Dong, J., R. G. Phelps, R. Qiao, S. Yao, O. Benard, Z. Ronai and S. A. Aaronson (2003). "BRAF oncogenic mutations correlate with progression rather than initiation of human melanoma." Cancer Res **63**(14): 3883-5.

Dorshkind, K., S. B. Pollack, M. J. Bosma and R. A. Phillips (1985). "Natural killer (NK) cells are present in mice with severe combined immunodeficiency (scid)." J Immunol **134**(6): 3798-801.

Douglas, J. T., B. E. Rogers, M. E. Rosenfeld, S. I. Michael, M. Feng and D. T. Curiel (1996). "Targeted gene delivery by tropism-modified adenoviral vectors." Nat Biotechnol **14**(11): 1574-8.

Dumaz, N., R. Hayward, J. Martin, L. Ogilvie, D. Hedley, J. A. Curtin, B. C. Bastian, C. Springer and R. Marais (2006). "In melanoma, RAS mutations are accompanied by switching signaling from BRAF to CRAF and disrupted cyclic AMP signaling." Cancer Res **66**(19): 9483-91.

Ebos, J. M., C. R. Lee, W. Cruz-Munoz, G. A. Bjarnason, J. G. Christensen and R. S. Kerbel (2009). "Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis." Cancer Cell **15**(3): 232-9.

Edwards, D. R., M. M. Handsley and C. J. Pennington (2008). "The ADAM metalloproteinases." Mol Aspects Med **29**(5): 258-89.

Egeblad, M. and Z. Werb (2002). "New functions for the matrix metalloproteinases in cancer progression." Nat Rev Cancer **2**(3): 161-74.

Eggermont, A. M. and J. M. Kirkwood (2004). "Re-evaluating the role of dacarbazine in metastatic melanoma: what have we learned in 30 years?" Eur J Cancer **40**(12): 1825-36.

Elezkurtaj, S., C. Kopitz, A. H. Baker, A. Perez-Canto, M. J. Arlt, R. Khokha, B. Gansbacher, M. Anton, K. Brand and A. Kruger (2004). "Adenovirus-mediated overexpression of tissue inhibitor of

metalloproteinases-1 in the liver: efficient protection against T-cell lymphoma and colon carcinoma metastasis." J Gene Med **6**(11): 1228-37.

Enari, M., H. Hug and S. Nagata (1995). "Involvement of an ICE-like protease in Fas-mediated apoptosis." Nature **375**(6526): 78-81.

English, J. L., Z. Kassiri, I. Koskivirta, S. J. Atkinson, M. Di Grappa, P. D. Soloway, H. Nagase, E. Vuorio, G. Murphy and R. Khokha (2006). "Individual Timp deficiencies differentially impact pro-MMP-2 activation." J Biol Chem **281**(15): 10337-46.

Falciani, C., L. Lozzi, A. Pini and L. Bracci (2005). "Bioactive peptides from libraries." Chem Biol **12**(4): 417-26.

Fata, J. E., K. J. Leco, E. B. Voura, H. Y. Yu, P. Waterhouse, G. Murphy, R. A. Moorehead and R. Khokha (2001). "Accelerated apoptosis in the Timp-3-deficient mammary gland." J Clin Invest **108**(6): 831-41.

Faucheu, C., A. Diu, A. W. Chan, A. M. Blanchet, C. Miossec, F. Herve, V. Collard-Dutilleul, Y. Gu, R. A. Aldape, J. A. Lippke and et al. (1995). "A novel human protease similar to the interleukin-1 beta converting enzyme induces apoptosis in transfected cells." Embo J **14**(9): 1914-22.

Feldman, A. L., W. G. Stetler-Stevenson, N. G. Costouros, V. Knezevic, G. Baibakov, H. R. Alexander, Jr., D. Lorang, S. M. Hewitt, D. W. Seo, M. S. Miller, S. O'Connor and S. K. Libutti (2004). "Modulation of tumor-host interactions, angiogenesis, and tumor growth by tissue inhibitor of metalloproteinase 2 via a novel mechanism." Cancer Res **64**(13): 4481-6.

Fernandes-Alnemri, T., R. C. Armstrong, J. Krebs, S. M. Srinivasula, L. Wang, F. Bullrich, L. C. Fritz, J. A. Trapani, K. J. Tomaselli, G. Litwack and E. S. Alnemri (1996). "In vitro activation of CPP32 and Mch3 by Mch4, a novel human apoptotic cysteine protease containing two FADD-like domains." Proc Natl Acad Sci U S A **93**(15): 7464-9.

Fernandez, C. A., C. Butterfield, G. Jackson and M. A. Moses (2003). "Structural and functional uncoupling of the enzymatic and angiogenic inhibitory activities of tissue inhibitor of metalloproteinase-2 (TIMP-2): loop 6 is a novel angiogenesis inhibitor." J Biol Chem **278**(42): 40989-95.

Fernandez, C. A. and M. A. Moses (2006). "Modulation of angiogenesis by tissue inhibitor of metalloproteinase-4." Biochem Biophys Res Commun **345**(1): 523-9.

Ferrer, M. and S. C. Harrison (1999). "Peptide ligands to human immunodeficiency virus type 1 gp120 identified from phage display libraries." J Virol **73**(7): 5795-802.

Finan, K. M., G. Hodge, A. M. Reynolds, S. Hodge, M. D. Holmes, A. H. Baker and P. N. Reynolds (2006). "In vitro susceptibility to the pro-apoptotic effects of TIMP-3 gene delivery translates to greater in vivo efficacy versus gene delivery for TIMPs-1 or -2." Lung Cancer **53**(3): 273-84.

Flaherty, K. T., I. Puzanov, K. B. Kim, A. Ribas, G. A. McArthur, J. A. Sosman, P. J. O'Dwyer, R. J. Lee, J. F. Grippo, K. Nolop and P. B. Chapman (2010). "Inhibition of mutated, activated BRAF in metastatic melanoma." N Engl J Med **363**(9): 809-19.

Flanagan, S. P. (1966). "'Nude', a new hairless gene with pleiotropic effects in the mouse." Genet Res **8**(3): 295-309.

Fodor, S. P., J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu and D. Solas (1991). "Light-directed, spatially addressable parallel chemical synthesis." Science **251**(4995): 767-73.

Fodstad, O. and I. Kjonniksen (1994). "Microenvironment revisited: time for reappraisal of some prevailing concepts of cancer metastasis." J Cell Biochem **56**(1): 23-8.

Folgueras, A. R., A. M. Pendas, L. M. Sanchez and C. Lopez-Otin (2004). "Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies." Int J Dev Biol **48**(5-6): 411-24.

Franke, J. C., M. Plotz, A. Prokop, C. C. Geilen, H. G. Schmalz and J. Eberle (2010). "New caspase-independent but ROS-dependent apoptosis pathways are targeted in melanoma cells by an iron-containing cytosine analogue." Biochem Pharmacol **79**(4): 575-86.

Friedman, R. J., D. S. Rigel and A. W. Kopf (1985). "Early detection of malignant melanoma: the role of physician examination and self-examination of the skin." CA Cancer J Clin **35**(3): 130-51.

Gabison, E. E., T. Hoang-Xuan, A. Mauviel and S. Menashi (2005). "EMMPRIN/CD147, an MMP modulator in cancer, development and tissue repair." Biochimie **87**(3-4): 361-8.

Gaggar, A., D. M. Shayakhmetov and A. Lieber (2003). "CD46 is a cellular receptor for group B adenoviruses." Nat Med **9**(11): 1408-12.

Gall, J., A. Kass-Eisler, L. Leinwand and E. Falck-Pedersen (1996). "Adenovirus type 5 and 7 capsid chimera: fiber replacement alters receptor tropism without affecting primary immune neutralization epitopes." J Virol **70**(4): 2116-23.

Gallagher, R. P., D. I. McLean, C. P. Yang, A. J. Coldman, H. K. Silver, J. J. Spinelli and M. Beagrie (1990). "Suntan, sunburn, and pigmentation factors and the frequency of acquired melanocytic nevi in children. Similarities to melanoma: the Vancouver Mole Study." Arch Dermatol **126**(6): 770-6.

Garaci, E., C. Favalli, F. Pica, P. Sinibaldi Vallebona, A. T. Palamara, C. Matteucci, P. Pierimarchi, A. Serafino, A. Mastino, F. Bistoni, L. Romani and G. Rasi (2007). "Thymosin alpha 1: from bench to bedside." Ann N Y Acad Sci **1112**: 225-34.

Garbe, C. and T. K. Eigentler (2007). "Diagnosis and treatment of cutaneous melanoma: state of the art 2006." Melanoma Res **17**(2): 117-27.

Gardere, S., S. Hussain and D. H. Cowan (1972). "Treatment of metastatic malignant melanoma with a combination of 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide (NSC-45388), cyclophosphamide (NSC-26271), and vincristine (NSC-67574)." Cancer Chemother Rep **56**(3): 357-61.

George, A. J., L. Lee and C. Pitzalis (2003). "Isolating ligands specific for human vasculature using in vivo phage selection." Trends Biotechnol **21**(5): 199-203.

Geysen, H. M., R. H. Meloen and S. J. Barteling (1984). "Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid." Proc Natl Acad Sci U S A **81**(13): 3998-4002.

Gibbs, N. K., M. Norval, N. J. Traynor, M. Wolf, B. E. Johnson and J. Crosby (1993). "Action spectra for the trans to cis photoisomerisation of urocanic acid in vitro and in mouse skin." Photochem Photobiol **57**(3): 584-90.

Gibson, L. E. and J. R. Goellner (1988). "Amelanotic melanoma: cases studied by Fontana stain, S-100 immunostain, and ultrastructural examination." Mayo Clin Proc **63**(8): 777-82.

Gill, S. E., I. Huizar, E. M. Bench, S. W. Sussman, Y. Wang, R. Khokha and W. C. Parks (2010). "Tissue inhibitor of metalloproteinases 3 regulates resolution of inflammation following acute lung injury." Am J Pathol **176**(1): 64-73.

Giordano, R. J., J. K. Edwards, R. M. Tuder, W. Arap and R. Pasqualini (2009). "Combinatorial ligand-directed lung targeting." Proc Am Thorac Soc **6**(5): 411-5.

Glasgow, G. M., M. M. McGee, B. J. Sheahan and G. J. Atkins (1997). "Death mechanisms in cultured cells infected by Semliki Forest virus." J Gen Virol **78 (Pt 7)**: 1559-63.

Glasgow, J. N., M. Everts and D. T. Curiel (2006). "Transductional targeting of adenovirus vectors for gene therapy." Cancer Gene Ther **13**(9): 830-44.

Goldenberg, D. M. (2002). "Targeted therapy of cancer with radiolabeled antibodies." J Nucl Med **43**(5): 693-713.

Goldstein, A. M. and M. A. Tucker (1995). "Genetic epidemiology of familial melanoma." Dermatol Clin **13**(3): 605-12.

Gollob, J. A., S. Wilhelm, C. Carter and S. L. Kelley (2006). "Role of Raf kinase in cancer: therapeutic potential of targeting the Raf/MEK/ERK signal transduction pathway." Semin Oncol **33**(4): 392-406.

Goodson, R. J., M. V. Doyle, S. E. Kaufman and S. Rosenberg (1994). "High-affinity urokinase receptor antagonists identified with bacteriophage peptide display." Proc Natl Acad Sci U S A **91**(15): 7129-33.

Gopalan, B., M. Shanker, S. Chada and R. Ramesh (2007). "MDA-7/IL-24 suppresses human ovarian carcinoma growth in vitro and in vivo." Mol Cancer **6**: 11.

Gopalan, B., M. Shanker, A. Scott, C. D. Branch, S. Chada and R. Ramesh (2008). "MDA-7/IL-24, a novel tumor suppressor/cytokine is ubiquitinated and regulated by the ubiquitin-proteasome system, and inhibition of MDA-7/IL-24 degradation enhances the antitumor activity." Cancer Gene Ther **15**(1): 1-8.

Gould Rothberg, B. E., M. B. Bracken and D. L. Rimm (2009). "Tissue biomarkers for prognosis in cutaneous melanoma: a systematic review and meta-analysis." J Natl Cancer Inst **101**(7): 452-74.

Graham, A., R. Walker, P. Baird, C. N. Hahn and J. K. Fazakerley (2006). "CNS gene therapy applications of the Semliki Forest virus 1 vector are limited by neurotoxicity." Mol Ther **13**(3): 631-5.

Graham, F. L., J. Smiley, W. C. Russell and R. Nairn (1977). "Characteristics of a human cell line transformed by DNA from human adenovirus type 5." J Gen Virol **36**(1): 59-74.

Gray, C. W., R. S. Brown and D. A. Marvin (1981). "Adsorption complex of filamentous fd virus." J Mol Biol **146**(4): 621-7.

Gray-Schopfer, V., C. Wellbrock and R. Marais (2007). "Melanoma biology and new targeted therapy." Nature **445**(7130): 851-7.

Greber, U. F., M. Willetts, P. Webster and A. Helenius (1993). "Stepwise dismantling of adenovirus 2 during entry into cells." Cell **75**(3): 477-86.

Greene, J., M. Wang, Y. E. Liu, L. A. Raymond, C. Rosen and Y. E. Shi (1996). "Molecular cloning and characterization of human tissue inhibitor of metalloproteinase 4." J Biol Chem **271**(48): 30375-80.

Griffin, D. E., B. Levine, W. R. Tyor, P. C. Tucker and J. M. Hardwick (1994). "Age-dependent susceptibility to fatal encephalitis: alphavirus infection of neurons." Arch Virol Suppl **9**: 31-9.

Grulich, A. E., X. Wan, M. G. Law, M. Coates and J. M. Kaldor (1999). "Risk of cancer in people with AIDS." Aids **13**(7): 839-43.

Guedez, L., A. J. McMarlin, D. W. Kingma, T. A. Bennett, M. Stetler-Stevenson and W. G. Stetler-Stevenson (2001). "Tissue inhibitor of metalloproteinase-1 alters the tumorigenicity of Burkitt's lymphoma via divergent effects on tumor growth and angiogenesis." Am J Pathol **158**(4): 1207-15.

Guo, J. and H. Xin (2006). "Chinese gene therapy. Splicing out the West?" Science **314**(5803): 1232-5.

Guse, K., T. Ranki, M. Ala-Opas, P. Bono, M. Sarkioja, M. Rajecki, A. Kanerva, T. Hakkarainen and A. Hemminki (2007). "Treatment of metastatic renal cancer with capsid-modified oncolytic adenoviruses." Mol Cancer Ther **6**(10): 2728-36.

Hafner, J., M. H. Schmid, W. Kempf, G. Burg, W. Kunzi, C. Meuli-Simmen, P. Neff, V. Meyer, D. Mihic, E. Garzoli, K. P. Jungius, B. Seifert, R. Dummer and H. Steinert (2004). "Baseline staging in cutaneous malignant melanoma." Br J Dermatol **150**(4): 677-86.

Haigh, P. I., L. A. DiFronzo and D. R. McCready (2003). "Optimal excision margins for primary cutaneous melanoma: a systematic review and meta-analysis." Can J Surg **46**(6): 419-26.

Hajitou, A., N. E. Sounni, L. Devy, C. Grignet-Debrus, J. M. Lewalle, H. Li, C. F. Deroanne, H. Lu, A. Colige, B. V. Nusgens, F. Frankenne, A. Maron, P. Yeh, M. Perricaudet, Y. Chang, C. Soria, C. M. Calberg-Bacq, J. M. Foidart and A. Noel (2001). "Down-regulation of vascular endothelial growth factor by tissue inhibitor of metalloproteinase-2: effect on in vivo mammary tumor growth and angiogenesis." Cancer Res **61**(8): 3450-7.

Hamid, O., J. C. Solomon, R. Scotland, M. Garcia, S. Sian, W. Ye, S. L. Groshen and J. S. Weber (2007). "Alum with interleukin-12 augments immunity to a melanoma peptide vaccine: correlation with time to relapse in patients with resected high-risk disease." Clin Cancer Res **13**(1): 215-22.

Hammani, K., P. Henriet, S. M. Silbiger and Y. A. DeClerck (1996). "Cloning and partial structure of the gene encoding human tissue inhibitor of metalloproteinases-3." Gene **170**(2): 287-8.

Hanahan, D. and R. A. Weinberg (2000). "The hallmarks of cancer." Cell **100**(1): 57-70.

Handolias, D., R. Salemi, W. Murray, A. Tan, W. Liu, A. Viros, A. Dobrovic, J. Kelly and G. A. McArthur (2010). "Mutations in KIT occur at low frequency in melanomas arising from anatomical sites associated with chronic and intermittent sun exposure." Pigment Cell Melanoma Res **23**(2): 210-5.

Hannan, C., L. H. Raptis, C. V. Dery and J. Weber (1983). "Biological and structural studies with an adenovirus type 2 temperature-sensitive mutant defective for uncoating." Intervirology **19**(4): 213-23.

Hardy, P. A., M. J. Mazzini, C. Schweitzer, K. Lundstrom and L. M. Glode (2000). "Recombinant Semliki forest virus infects and kills human prostate cancer cell lines and prostatic duct epithelial cells ex vivo." Int J Mol Med **5**(3): 241-5.

Hart, P. H., M. A. Grimbaldeston and J. J. Finlay-Jones (2001). "Sunlight, immunosuppression and skin cancer: role of histamine and mast cells." Clin Exp Pharmacol Physiol **28**(1-2): 1-8.

Hassell, J. A. and J. Weber (1978). "Genetic analysis of adenovirus type 2. VIII. Physical locations of temperature-sensitive mutations." J Virol **28**(3): 671-8.

Hauschild, A., U. Trefzer, C. Garbe, K. C. Kaehler, S. Ugurel, F. Kiecker, T. Eigentler, H. Krissel, A. Schott and D. Schadendorf (2008). "Multicenter phase II trial of the histone deacetylase inhibitor pyridylmethyl-N-{4-[(2-aminophenyl)-carbamoyl]-benzyl}-carbamate in pretreated metastatic melanoma." Melanoma Res **18**(4): 274-8.

Hayakawa, T., K. Yamashita, K. Tanzawa, E. Uchijima and K. Iwata (1992). "Growth-promoting activity of tissue inhibitor of metalloproteinases-1 (TIMP-1) for a wide range of cells. A possible new growth factor in serum." FEBS Lett **298**(1): 29-32.

Healsmith, M. F., J. F. Bourke, J. E. Osborne and R. A. Graham-Brown (1994). "An evaluation of the revised seven-point checklist for the early diagnosis of cutaneous malignant melanoma." Br J Dermatol **130**(1): 48-50.

Helenius, A., J. Kartenbeck, K. Simons and E. Fries (1980). "On the entry of Semliki forest virus into BHK-21 cells." J Cell Biol **84**(2): 404-20.

Hill, L. L., R. Korngold, C. Jaworsky, G. Murphy, P. McCue and D. Berd (1991). "Growth and metastasis of fresh human melanoma tissue in mice with severe combined immunodeficiency." Cancer Res **51**(18): 4937-41.

Ho, A. T., E. B. Voura, P. D. Soloway, K. L. Watson and R. Khokha (2001). "MMP inhibitors augment fibroblast adhesion through stabilization of focal adhesion contacts and up-regulation of cadherin function." J Biol Chem **276**(43): 40215-24.

Hoashi, T., T. Kadono, K. Kikuchi, T. Etoh and K. Tamaki (2001). "Differential growth regulation in human melanoma cell lines by TIMP-1 and TIMP-2." Biochem Biophys Res Commun **288**(2): 371-9.

Hodi, F. S., S. J. O'Day, D. F. McDermott, R. W. Weber, J. A. Sosman, J. B. Haanen, R. Gonzalez, C. Robert, D. Schadendorf, J. C. Hassel, W. Akerley, A. J. van den Eertwegh, J. Lutzky, P. Lorigan, J. M. Vaubel, G. P. Linette, D. Hogg, C. H. Ottensmeier, C. Lebbe, C. Peschel, I. Quirt, J. I. Clark, J. D. Wolchok, J. S. Weber, J. Tian, M. J. Yellin, G. M. Nichol, A. Hoos and W. J. Urba (2010). "Improved survival with ipilimumab in patients with metastatic melanoma." N Engl J Med **363**(8): 711-23.

Hoegy, S. E., H. R. Oh, M. L. Corcoran and W. G. Stetler-Stevenson (2001). "Tissue inhibitor of metalloproteinases-2 (TIMP-2) suppresses TKR-growth factor signaling independent of metalloproteinase inhibition." J Biol Chem **276**(5): 3203-14.

Hofmann, U. B., A. A. Eggert, K. Blass, E. B. Brocker and J. C. Becker (2003). "Expression of matrix metalloproteinases in the microenvironment of spontaneous and experimental melanoma metastases reflects the requirements for tumor formation." Cancer Res **63**(23): 8221-5.

Hofmann, U. B., J. R. Westphal, G. N. Van Muijen and D. J. Ruiter (2000). "Matrix metalloproteinases in human melanoma." J Invest Dermatol **115**(3): 337-44.

Holzmann, B., J. P. Johnson, P. Kaudewitz and G. Riethmuller (1985). "In situ analysis of antigens on malignant and benign cells of the melanocyte lineage. Differential expression of two surface molecules, gp75 and p89." J Exp Med **161**(2): 366-77.

Hong, S. S. and P. Boulanger (1995). "Protein ligands of the human adenovirus type 2 outer capsid identified by biopanning of a phage-displayed peptide library on separate domains of wild-type and mutant penton capsomers." Embo J **14**(19): 4714-27.

Hong, S. S., M. K. Magnusson, P. Henning, L. Lindholm and P. A. Boulanger (2003). "Adenovirus stripping: a versatile method to generate adenovirus vectors with new cell target specificity." Mol Ther **7**(5 Pt 1): 692-9.

Hopkins-Donaldson, S., A. Ziegler, S. Kurtz, C. Bigosch, D. Kandioler, C. Ludwig, U. Zangemeister-Wittke and R. Stahel (2003). "Silencing of death receptor and caspase-8 expression in small cell lung carcinoma cell lines and tumors by DNA methylation." Cell Death Differ **10**(3): 356-64.

Houghten, R. A., C. Pinilla, S. E. Blondelle, J. R. Appel, C. T. Dooley and J. H. Cuervo (1991). "Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery." Nature **354**(6348): 84-6.

Houimel, M., P. Schneider, A. Terskikh and J. P. Mach (2001). "Selection of peptides and synthesis of pentameric peptabody molecules reacting specifically with ErbB-2 receptor." Int J Cancer **92**(5): 748-55.

Howell, R. C., E. Revskaya, V. Pazo, J. D. Nosanchuk, A. Casadevall and E. Dadachova (2007). "Phage display library derived peptides that bind to human tumor melanin as potential vehicles for targeted radionuclide therapy of metastatic melanoma." Bioconjug Chem **18**(6): 1739-48.

Hsu, M. Y., F. Meier and M. Herlyn (2002). "Melanoma development and progression: a conspiracy between tumor and host." Differentiation **70**(9-10): 522-36.

http://seer.cancer.gov/statfacts/.

http://www.cancerregistry.fi.

Hu, J., P. E. Van den Steen, Q. X. Sang and G. Opdenakker (2007). "Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases." Nat Rev Drug Discov **6**(6): 480-98.

Huovila, A. P., A. J. Turner, M. Pelto-Huikko, I. Karkkainen and R. M. Ortiz (2005). "Shedding light on ADAM metalloproteinases." Trends Biochem Sci **30**(7): 413-22.

Illana, M., A. Prada, C. Verastegui, M. C. Gonzalez Macias and F. J. Fernandez-Trujillo (1997). "Study of the distribution of glycosidic residues in eccrine sweat glands, with special reference to the content of sialic acid." Eur J Histochem **41**(1): 41-6.

Immonen, A., M. Vapalahti, K. Tyynela, H. Hurskainen, A. Sandmair, R. Vanninen, G. Langford, N. Murray and S. Yla-Herttuala (2004). "AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant glioma: a randomised, controlled study." Mol Ther **10**(5): 967-72.

Ivanova, T., S. Vinokurova, A. Petrenko, E. Eshilev, N. Solovyova, F. Kisseljov and N. Kisseljova (2004). "Frequent hypermethylation of 5' flanking region of TIMP-2 gene in cervical cancer." Int J Cancer **108**(6): 882-6.

Jakubczak, J. L., M. L. Rollence, D. A. Stewart, J. D. Jafari, D. J. Von Seggern, G. R. Nemerow, S. C. Stevenson and P. L. Hallenbeck (2001). "Adenovirus type 5 viral particles pseudotyped with

mutagenized fiber proteins show diminished infectivity of coxsackie B-adenovirus receptor-bearing cells." J Virol **75**(6): 2972-81.

Jansen, L., O. E. Nieweg, J. L. Peterse, C. A. Hoefnagel, R. A. Olmos and B. B. Kroon (2000). "Reliability of sentinel lymph node biopsy for staging melanoma." Br J Surg **87**(4): 484-9.

Jiang, Y., M. Wang, M. Y. Celiker, Y. E. Liu, Q. X. Sang, I. D. Goldberg and Y. E. Shi (2001). "Stimulation of mammary tumorigenesis by systemic tissue inhibitor of matrix metalloproteinase 4 gene delivery." Cancer Res **61**(6): 2365-70.

Joo, Y. E., K. S. Seo, J. Kim, H. S. Kim, J. S. Rew, C. S. Park and S. J. Kim (1999). "Role of tissue inhibitors of metalloproteinases (TIMPs) in colorectal carcinoma." J Korean Med Sci **14**(4): 417-23.

Kamel-Reid, S., M. Letarte, C. Sirard, M. Doedens, T. Grunberger, G. Fulop, M. H. Freedman, R. A. Phillips and J. E. Dick (1989). "A model of human acute lymphoblastic leukemia in immune-deficient SCID mice." Science **246**(4937): 1597-600.

Kammeyer, A., M. B. Teunissen, S. Pavel, M. A. de Rie and J. D. Bos (1995). "Photoisomerization spectrum of urocanic acid in human skin and in vitro: effects of simulated solar and artificial ultraviolet radiation." Br J Dermatol **132**(6): 884-91.

Kanzler, M. H. and S. Mraz-Gernhard (2001). "Primary cutaneous malignant melanoma and its precursor lesions: diagnostic and therapeutic overview." J Am Acad Dermatol **45**(2): 260-76.

Kashiwagi, M., M. Tortorella, H. Nagase and K. Brew (2001). "TIMP-3 is a potent inhibitor of aggrecanase 1 (ADAM-TS4) and aggrecanase 2 (ADAM-TS5)." J Biol Chem **276**(16): 12501-4.

Kavanagh, G., J. Crosby and M. Norval (1995). "Urocanic acid isomers in human skin: analysis of site variation." Br J Dermatol **133**(5): 728-31.

Kefford, R. F. (2003). "Adjuvant therapy of cutaneous melanoma: the interferon debate." Ann Oncol **14**(3): 358-65.

Kefford, R. F. and G. J. Mann (2003). "Is there a role for genetic testing in patients with melanoma?" Curr Opin Oncol **15**(2): 157-61.

Kelkar, S. A., K. K. Pfister, R. G. Crystal and P. L. Leopold (2004). "Cytoplasmic dynein mediates adenovirus binding to microtubules." J Virol **78**(18): 10122-32.

Kessenbrock, K., V. Plaks and Z. Werb (2010). "Matrix metalloproteinases: regulators of the tumor microenvironment." Cell **141**(1): 52-67.

Ketola, A., A. Hinkkanen, F. Yongabi, P. Furu, A. M. Maatta, T. Liimatainen, R. Pirinen, M. Bjorn, T. Hakkarainen, K. Makinen, J. Wahlfors and R. Pellinen (2008). "Oncolytic Semliki forest virus vector as a novel candidate against unresectable osteosarcoma." Cancer Res **68**(20): 8342-50.

Khokha, R. (1994). "Suppression of the tumorigenic and metastatic abilities of murine B16-F10 melanoma cells in vivo by the overexpression of the tissue inhibitor of the metalloproteinases-1." J Natl Cancer Inst **86**(4): 299-304.

Khuri, F. R., J. Nemunaitis, I. Ganly, J. Arseneau, I. F. Tannock, L. Romel, M. Gore, J. Ironside, R. H. MacDougall, C. Heise, B. Randlev, A. M. Gillenwater, P. Bruso, S. B. Kaye, W. K. Hong and D. H. Kirn (2000). "a controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer." Nat Med **6**(8): 879-85.

Kim, E. M., E. J. Shin, J. H. Choi, H. J. Son, I. S. Park, T. H. Joh and O. Hwang (2010). "Matrix metalloproteinase-3 is increased and participates in neuronal apoptotic signaling downstream of caspase-12 during endoplasmic reticulum stress." J Biol Chem **285**(22): 16444-52.

Kim, K. B., O. Eton, D. W. Davis, M. L. Frazier, D. J. McConkey, A. H. Diwan, N. E. Papadopoulos, A. Y. Bedikian, L. H. Camacho, M. I. Ross, J. N. Cormier, J. E. Gershenwald, J. E. Lee, P. F. Mansfield, L. A. Billings, C. S. Ng, C. Charnsangavej, M. Bar-Eli, M. M. Johnson, A. J. Murgo and V. G. Prieto

(2008). "Phase II trial of imatinib mesylate in patients with metastatic melanoma." Br J Cancer **99**(5): 734-40.

King, R., K. N. Weilbaecher, G. McGill, E. Cooley, M. Mihm and D. E. Fisher (1999). "Microphthalmia transcription factor. A sensitive and specific melanocyte marker for MelanomaDiagnosis." Am J Pathol **155**(3): 731-8.

Kioi, M., K. Yamamoto, S. Higashi, N. Koshikawa, K. Fujita and K. Miyazaki (2003). "Matrilysin (MMP-7) induces homotypic adhesion of human colon cancer cells and enhances their metastatic potential in nude mouse model." Oncogene **22**(54): 8662-70.

Koivunen, E., W. Arap, D. Rajotte, J. Lahdenranta and R. Pasqualini (1999). "Identification of receptor ligands with phage display peptide libraries." J Nucl Med **40**(5): 883-8.

Koivunen, E., W. Arap, H. Valtanen, A. Rainisalo, O. P. Medina, P. Heikkila, C. Kantor, C. G. Gahmberg, T. Salo, Y. T. Konttinen, T. Sorsa, E. Ruoslahti and R. Pasqualini (1999). "Tumor targeting with a selective gelatinase inhibitor." Nat Biotechnol **17**(8): 768-74.

Koivunen, E., B. Wang and E. Ruoslahti (1995). "Phage libraries displaying cyclic peptides with different ring sizes: ligand specificities of the RGD-directed integrins." Biotechnology (N Y) **13**(3): 265-70.

Kolonin, M. G., L. Bover, J. Sun, A. J. Zurita, K. A. Do, J. Lahdenranta, M. Cardo-Vila, R. J. Giordano, D. E. Jaalouk, M. G. Ozawa, C. A. Moya, G. R. Souza, F. I. Staquicini, A. Kunyiasu, D. A. Scudiero, S. L. Holbeck, E. A. Sausville, W. Arap and R. Pasqualini (2006). "Ligand-directed surface profiling of human cancer cells with combinatorial peptide libraries." Cancer Res **66**(1): 34-40.

Koolpe, M., R. Burgess, M. Dail and E. B. Pasquale (2005). "EphB receptor-binding peptides identified by phage display enable design of an antagonist with ephrin-like affinity." J Biol Chem **280**(17): 17301-11.

Koskivuo, I., L. Talve, P. Vihinen, M. Maki, T. Vahlberg and E. Suominen (2007). "Sentinel lymph node biopsy in cutaneous melanoma: a case-control study." Ann Surg Oncol **14**(12): 3566-74.

Koskivuo, I. O., M. P. Seppanen, E. A. Suominen and H. R. Minn (2007). "Whole body positron emission tomography in follow-up of high risk melanoma." Acta Oncol **46**(5): 685-90.

Krag, D. N., G. S. Shukla, G. P. Shen, S. Pero, T. Ashikaga, S. Fuller, D. L. Weaver, S. Burdette-Radoux and C. Thomas (2006). "Selection of tumor-binding ligands in cancer patients with phage display libraries." Cancer Res **66**(15): 7724-33.

Krasnykh, V., I. Dmitriev, G. Mikheeva, C. R. Miller, N. Belousova and D. T. Curiel (1998). "Characterization of an adenovirus vector containing a heterologous peptide epitope in the HI loop of the fiber knob." J Virol **72**(3): 1844-52.

Krasnykh, V. N., G. V. Mikheeva, J. T. Douglas and D. T. Curiel (1996). "Generation of recombinant adenovirus vectors with modified fibers for altering viral tropism." J Virol **70**(10): 6839-46.

Kreppel, F. and S. Kochanek (2008). "Modification of adenovirus gene transfer vectors with synthetic polymers: a scientific review and technical guide." Mol Ther **16**(1): 16-29.

Krien, P. M. and M. Kermici (2000). "Evidence for the existence of a self-regulated enzymatic process within the human stratum corneum -an unexpected role for urocanic acid." J Invest Dermatol **115**(3): 414-20.

Kubo, N., N. Akita, A. Shimizu, H. Kitahara, A. L. Parker and S. Miyagawa (2008). "Identification of oligopeptide binding to colon cancer cells separated from patients using laser capture microdissection." J Drug Target **16**(5): 396-404.

Kuivanen, T., K. Ahokas, S. Virolainen, T. Jahkola, E. Holtta, O. Saksela and U. Saarialho-Kere (2005). "MMP-21 is upregulated at early stages of melanoma progression but disappears with more aggressive phenotype." Virchows Arch **447**(6): 954-60.

Kumar, S. K., C. F. Shuler, P. P. Sedghizadeh and J. R. Kalmar (2008). "Oral mucosal melanoma with unusual clinicopathologic features." J Cutan Pathol **35**(4): 392-7.

Kuno, K., K. Bannai, M. Hakozaki, K. Matsushima and K. Hirose (2004). "The carboxyl-terminal half region of ADAMTS-1 suppresses both tumorigenicity and experimental tumor metastatic potential." Biochem Biophys Res Commun **319**(4): 1327-33.

Kurppa, K. and K. Elenius (2009). "Mutated ERBB4: a novel drug target in metastatic melanoma?" Pigment Cell Melanoma Res **22**(6): 708-10.

Lafleur, M. A., M. M. Handsley, V. Knauper, G. Murphy and D. R. Edwards (2002). "Endothelial tubulogenesis within fibrin gels specifically requires the activity of membrane-type-matrix metalloproteinases (MT-MMPs)." J Cell Sci **115**(Pt 17): 3427-38.

Lai, K., R. M. Conway, R. Crouch, M. J. Jager and M. C. Madigan (2008). "Expression and distribution of MMPs and TIMPs in human uveal melanoma." Exp Eye Res **86**(6): 936-41.

Laihia, J. K., M. Attila, K. Neuvonen, P. Pasanen, L. Tuomisto and C. T. Jansen (1998). "Urocanic acid binds to GABA but not to histamine (H1, H2, or H3) receptors." J Invest Dermatol **111**(4): 705-6.

Laihia, J. K., L. Pylkkanen, M. Laato, P. J. Bostrom and L. Leino (2009). "Protodynamic therapy for bladder cancer: in vitro results of a novel treatment concept." BJU Int.

Laird, P. W. (2005). "Cancer epigenetics." Hum Mol Genet **14 Spec No 1**: R65-76.

Lam, P., K. Sian Lim, S. Mei Wang and K. M. Hui (2005). "A microarray study to characterize the molecular mechanism of TIMP-3-mediated tumor rejection." Mol Ther **12**(1): 144-52.

Lambert, E., C. Boudot, Z. Kadri, M. Soula-Rothhut, M. L. Sowa, P. Mayeux, W. Hornebeck, B. Haye and E. Petitfrere (2003). "Tissue inhibitor of metalloproteinases-1 signalling pathway leading to erythroid cell survival." Biochem J **372**(Pt 3): 767-74.

Lanciotti, J., A. Song, J. Doukas, B. Sosnowski, G. Pierce, R. Gregory, S. Wadsworth and C. O'Riordan (2003). "Targeting adenoviral vectors using heterofunctional polyethylene glycol FGF2 conjugates." Mol Ther **8**(1): 99-107.

Le, L. P., H. N. Le, I. P. Dmitriev, J. G. Davydova, T. Gavrikova, S. Yamamoto, D. T. Curiel and M. Yamamoto (2006). "Dynamic monitoring of oncolytic adenovirus in vivo by genetic capsid labeling." J Natl Cancer Inst **98**(3): 203-14.

Lebedeva, I. V., L. Emdad, Z. Z. Su, P. Gupta, M. Sauane, D. Sarkar, M. R. Staudt, S. J. Liu, M. M. Taher, R. Xiao, P. Barral, S. G. Lee, D. Wang, N. Vozhilla, E. S. Park, L. Chatman, H. Boukerche, R. Ramesh, S. Inoue, S. Chada, R. Li, A. L. De Pass, P. J. Mahasreshti, I. P. Dmitriev, D. T. Curiel, A. Yacoub, S. Grant, P. Dent, N. Senzer, J. J. Nemunaitis and P. B. Fisher (2007). "mda-7/IL-24, novel anticancer cytokine: focus on bystander antitumor, radiosensitization and antiangiogenic properties and overview of the phase I clinical experience (Review)." Int J Oncol **31**(5): 985-1007.

Levidou, G., P. Korkolopoulou, A. Papetta, E. Patsouris and E. Agapitos (2007). "Leptomeningeal melanoma of unknown primary site: two cases with an atypical presentation of acute meningitis." Clin Neuropathol **26**(6): 299-305.

Li, E., D. Stupack, R. Klemke, D. A. Cheresh and G. R. Nemerow (1998). "Adenovirus endocytosis via alpha(v) integrins requires phosphoinositide-3-OH kinase." J Virol **72**(3): 2055-61.

Li, H., F. Lindenmeyer, C. Grenet, P. Opolon, S. Menashi, C. Soria, P. Yeh, M. Perricaudet and H. Lu (2001). "AdTIMP-2 inhibits tumor growth, angiogenesis, and metastasis, and prolongs survival in mice." Hum Gene Ther **12**(5): 515-26.

Lim, M. S., L. Guedez, W. G. Stetler-Stevenson and M. Stetler-Stevenson (1999). "Tissue inhibitor of metalloproteinase-2 induces apoptosis in human T lymphocytes." Ann N Y Acad Sci **878**: 522-3.

Lin, T. C., R. E. Webster and W. Konigsberg (1980). "Isolation and characterization of the C and D proteins coded by gene IX and gene VI in the filamentous bacteriophage fl and fd." J Biol Chem **255**(21): 10331-7.

Liotta, L. A. and E. C. Kohn (2001). "The microenvironment of the tumour-host interface." Nature **411**(6835): 375-9.

Liu, X. W., M. E. Taube, K. K. Jung, Z. Dong, Y. J. Lee, S. Roshy, B. F. Sloane, R. Fridman and H. R. Kim (2005). "Tissue inhibitor of metalloproteinase-1 protects human breast epithelial cells from extrinsic cell death: a potential oncogenic activity of tissue inhibitor of metalloproteinase-1." Cancer Res **65**(3): 898-906.

Loot, A. E., R. H. Henning, L. E. Deelman, R. A. Tio, P. Schoen, J. C. Wilschut, W. H. van Gilst and A. J. Roks (2004). "Semliki Forest virus is an efficient and selective vector for gene delivery in infarcted rat heart." J Mol Cell Cardiol **37**(1): 137-42.

Lopez-Otin, C., L. H. Palavalli and Y. Samuels (2009). "Protective roles of matrix metalloproteinases: from mouse models to human cancer." Cell Cycle **8**(22): 3657-62.

Lun, X., J. Chan, H. Zhou, B. Sun, J. J. Kelly, O. O. Stechishin, J. C. Bell, K. Parato, K. Hu, D. Vaillant, J. Wang, T. C. Liu, C. Breitbach, D. Kirn, D. L. Senger and P. A. Forsyth (2010). "Efficacy and Safety/Toxicity Study of Recombinant Vaccinia Virus JX-594 in Two Immunocompetent Animal Models of Glioma." Mol Ther.

Lundström, K. (2005). "Biology and application of alphaviruses in gene therapy." Gene Ther **12 Suppl 1**: S92-7.

Lundström, K., C. Schweitzer, D. Rotmann, D. Hermann, E. M. Schneider and M. U. Ehrengruber (2001). "Semliki Forest virus vectors: efficient vehicles for in vitro and in vivo gene delivery." FEBS Lett **504**(3): 99-103.

MacGill, R. S., T. A. Davis, J. Macko, H. J. Mauceri, R. R. Weichselbaum and C. R. King (2007). "Local gene delivery of tumor necrosis factor alpha can impact primary tumor growth and metastases through a host-mediated response." Clin Exp Metastasis **24**(7): 521-31.

Manola, J., M. Atkins, J. Ibrahim and J. Kirkwood (2000). "Prognostic factors in metastatic melanoma: a pooled analysis of Eastern Cooperative Oncology Group trials." J Clin Oncol **18**(22): 3782-93.

Markovic, S. N., S. M. Geyer, F. Dawkins, W. Sharfman, M. Albertini, W. Maples, P. M. Fracasso, T. Fitch, P. Lorusso, A. A. Adjei and C. Erlichman (2005). "A phase II study of bortezomib in the treatment of metastatic malignant melanoma." Cancer **103**(12): 2584-9.

Marrett, L. D., W. D. King, S. D. Walter and L. From (1992). "Use of host factors to identify people at high risk for cutaneous malignant melanoma." Cmaj **147**(4): 445-53.

Marshall, E. (1999). "Gene therapy death prompts review of adenovirus vector." Science **286**(5448): 2244-5.

Martin, D. C., J. L. Fowlkes, B. Babic and R. Khokha (1999). "Insulin-like growth factor II signaling in neoplastic proliferation is blocked by transgenic expression of the metalloproteinase inhibitor TIMP-1." J Cell Biol **146**(4): 881-92.

Maruta, F., A. L. Parker, K. D. Fisher, M. T. Hallissey, T. Ismail, D. C. Rowlands, L. A. Chandler, D. J. Kerr and L. W. Seymour (2002). "Identification of FGF receptor-binding peptides for cancer gene therapy." Cancer Gene Ther **9**(6): 543-52.

Masui, T., R. Hosotani, S. Tsuji, Y. Miyamoto, S. Yasuda, J. Ida, S. Nakajima, M. Kawaguchi, H. Kobayashi, M. Koizumi, E. Toyoda, S. Tulachan, S. Arii, R. Doi and M. Imamura (2001). "Expression of METH-1 and METH-2 in pancreatic cancer." Clin Cancer Res **7**(11): 3437-43.

Matthews, D. A. and W. C. Russell (1998). "Adenovirus core protein V is delivered by the invading virus to the nucleus of the infected cell and later in infection is associated with nucleoli." J Gen Virol **79 (Pt 7)**: 1671-5.

Matthews, D. J. and J. A. Wells (1993). "Substrate phage: selection of protease substrates by monovalent phage display." Science **260**(5111): 1113-7.

McCawley, L. J. and L. M. Matrisian (2000). "Matrix metalloproteinases: multifunctional contributors to tumor progression." Mol Med Today **6**(4): 149-56.

McDermott, D. F., J. A. Sosman, R. Gonzalez, F. S. Hodi, G. P. Linette, J. Richards, J. W. Jakub, M. Beeram, S. Tarantolo, S. Agarwala, G. Frenette, I. Puzanov, L. Cranmer, K. Lewis, J. Kirkwood, J. M. White, C. Xia, K. Patel and E. Hersh (2008). "Double-blind randomized phase II study of the combination of sorafenib and dacarbazine in patients with advanced melanoma: a report from the 11715 Study Group." J Clin Oncol **26**(13): 2178-85.

McIntosh, B. M., C. B. Worth and R. H. Kokernot (1961). "Isolation of Semliki Forest virus from Aedes (Aedimorphus) argenteopunctatus (Theobald) collected in Portuguese East Africa." Trans R Soc Trop Med Hyg **55**: 192-8.

McLafferty, M. A., R. B. Kent, R. C. Ladner and W. Markland (1993). "M13 bacteriophage displaying disulfide-constrained microproteins." Gene **128**(1): 29-36.

McNeel, D. G., J. Eickhoff, F. T. Lee, D. M. King, D. Alberti, J. P. Thomas, A. Friedl, J. Kolesar, R. Marnocha, J. Volkman, J. Zhang, L. Hammershaimb, J. A. Zwiebel and G. Wilding (2005). "Phase I trial of a monoclonal antibody specific for alphavbeta3 integrin (MEDI-522) in patients with advanced malignancies, including an assessment of effect on tumor perfusion." Clin Cancer Res **11**(21): 7851-60.

McQuibban, G. A., G. S. Butler, J. H. Gong, L. Bendall, C. Power, I. Clark-Lewis and C. M. Overall (2001). "Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1." J Biol Chem **276**(47): 43503-8.

Meier, O., K. Boucke, S. V. Hammer, S. Keller, R. P. Stidwill, S. Hemmi and U. F. Greber (2002). "Adenovirus triggers macropinocytosis and endosomal leakage together with its clathrin-mediated uptake." J Cell Biol **158**(6): 1119-31.

Meier, O. and U. F. Greber (2004). "Adenovirus endocytosis." J Gene Med **6 Suppl 1**: S152-63.

Menzies, S. W. (1997). "Surface microscopy of pigmented skin tumours." Australas J Dermatol **38 Suppl 1**: S40-3.

Mhaidat, N. M., X. D. Zhang, J. Allen, K. A. Avery-Kiejda, R. J. Scott and P. Hersey (2007). "Temozolomide induces senescence but not apoptosis in human melanoma cells." Br J Cancer **97**(9): 1225-33.

Michael, S. I., J. S. Hong, D. T. Curiel and J. A. Engler (1995). "Addition of a short peptide ligand to the adenovirus fiber protein." Gene Ther **2**(9): 660-8.

Middleton, M. R., J. J. Grob, N. Aaronson, G. Fierlbeck, W. Tilgen, S. Seiter, M. Gore, S. Aamdal, J. Cebon, A. Coates, B. Dreno, M. Henz, D. Schadendorf, A. Kapp, J. Weiss, U. Fraass, P. Statkevich, M. Muller and N. Thatcher (2000). "Randomized phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma." J Clin Oncol **18**(1): 158-66.

Milhas, D., O. Cuvillier, N. Therville, P. Clave, M. Thomsen, T. Levade, H. Benoist and B. Segui (2005). "Caspase-10 triggers Bid cleavage and caspase cascade activation in FasL-induced apoptosis." J Biol Chem **280**(20): 19836-42.

Milton, G. W., H. M. Shaw and W. H. McCarthy (1985). "Resection margins for melanoma." Aust N Z J Surg **55**(3): 225-6.

Mizusawa, S. and D. F. Ward (1982). "A bacteriophage lambda vector for cloning with BamHI and Sau3A." Gene **20**(3): 317-22.

Mohammed, F. F., D. S. Smookler, S. E. Taylor, B. Fingleton, Z. Kassiri, O. H. Sanchez, J. L. English, L. M. Matrisian, B. Au, W. C. Yeh and R. Khokha (2004). "Abnormal TNF activity in Timp3-/- mice leads to chronic hepatic inflammation and failure of liver regeneration." Nat Genet **36**(9): 969-77.

Montgomery, A. M., B. M. Mueller, R. A. Reisfeld, S. M. Taylor and Y. A. DeClerck (1994). "Effect of tissue inhibitor of the matrix metalloproteinases-2 expression on the growth and spontaneous metastasis of a human melanoma cell line." Cancer Res **54**(20): 5467-73.

Moodycliffe, A. M., M. Norval, I. Kimber and T. J. Simpson (1993). "Characterization of a monoclonal antibody to cis-urocanic acid: detection of cis-urocanic acid in the serum of irradiated mice by immunoassay." Immunology **79**(4): 667-72.

Morse, M. A. (2005). "Technology evaluation: ipilimumab, Medarex/Bristol-Myers Squibb." Curr Opin Mol Ther **7**(6): 588-97.

Mueller, B. M., R. A. Reisfeld, T. S. Edgington and W. Ruf (1992). "Expression of tissue factor by melanoma cells promotes efficient hematogenous metastasis." Proc Natl Acad Sci U S A **89**(24): 11832-6.

Mullamitha, S. A., N. C. Ton, G. J. Parker, A. Jackson, P. J. Julyan, C. Roberts, G. A. Buonaccorsi, Y. Watson, K. Davies, S. Cheung, L. Hope, J. W. Valle, J. A. Radford, J. Lawrance, M. P. Saunders, M. C. Munteanu, M. T. Nakada, J. A. Nemeth, H. M. Davis, Q. Jiao, U. Prabhakar, Z. Lang, R. E. Corringham, R. A. Beckman and G. C. Jayson (2007). "Phase I evaluation of a fully human anti-alphav integrin monoclonal antibody (CNTO 95) in patients with advanced solid tumors." Clin Cancer Res **13**(7): 2128-35.

Mullis, K. B. and F. A. Faloona (1987). "Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction." Methods Enzymol **155**: 335-50.

Murphy, F., J. Waung, J. Collins, M. J. Arthur, H. Nagase, D. Mann, R. C. Benyon and J. P. Iredale (2004). "N-Cadherin cleavage during activated hepatic stellate cell apoptosis is inhibited by tissue inhibitor of metalloproteinase-1." Comp Hepatol **3 Suppl 1**: S8.

Murphy, G. and H. Nagase (2008). "Progress in matrix metalloproteinase research." Mol Aspects Med **29**(5): 290-308.

Määttä, A. M., T. Liimatainen, T. Wahlfors, T. Wirth, M. Vaha-Koskela, L. Jansson, P. Valonen, K. Hakkinen, O. Rautsi, R. Pellinen, K. Makinen, J. Hakumaki, A. Hinkkanen and J. Wahlfors (2007). "Evaluation of cancer virotherapy with attenuated replicative Semliki forest virus in different rodent tumor models." Int J Cancer **121**(4): 863-70.

Määttä, A. M., K. Makinen, A. Ketola, T. Liimatainen, F. N. Yongabi, M. Vaha-Koskela, R. Pirinen, O. Rautsi, R. Pellinen, A. Hinkkanen and J. Wahlfors (2008). "Replication competent Semliki Forest virus prolongs survival in experimental lung cancer." Int J Cancer **123**(7): 1704-11.

Naeyaert, J. M. and L. Brochez (2003). "Clinical practice. Dysplastic nevi." N Engl J Med **349**(23): 2233-40.

Nakahara, H., L. Howard, E. W. Thompson, H. Sato, M. Seiki, Y. Yeh and W. T. Chen (1997). "Transmembrane/cytoplasmic domain-mediated membrane type 1-matrix metalloprotease docking to invadopodia is required for cell invasion." Proc Natl Acad Sci U S A **94**(15): 7959-64.

Nakatsura, T., T. Kageshita, S. Ito, K. Wakamatsu, M. Monji, Y. Ikuta, S. Senju, T. Ono and Y. Nishimura (2004). "Identification of glypican-3 as a novel tumor marker for melanoma." Clin Cancer Res **10**(19): 6612-21.

Nemati, F., X. Sastre-Garau, C. Laurent, J. Couturier, P. Mariani, L. Desjardins, S. Piperno-Neumann, O. Lantz, B. Asselain, C. Plancher, D. Robert, I. Peguillet, M. H. Donnadieu, A. Dahmani, M. A. Bessard, D. Gentien, C. Reyes, S. Saule, E. Barillot, S. Roman-Roman and D. Decaudin (2010). "Establishment and characterization of a panel of human uveal melanoma xenografts derived from primary and/or metastatic tumors." Clin Cancer Res **16**(8): 2352-62.

Nemeth, J. A. and C. L. Goolsby (1993). "TIMP-2, a growth-stimulatory protein from SV40-transformed human fibroblasts." Exp Cell Res **207**(2): 376-82.

Neri, B., L. Vannozzi, C. Fulignati, P. Pantaleo, D. Pantalone, C. Paoletti, F. Perfetto, M. Turrini and R. Mazzanti (2006). "Long-term survival in metastatic melanoma patients treated with sequential biochemotherapy: report of a Phase II study." Cancer Invest **24**(5): 474-8.

Newton, J. R., K. A. Kelly, U. Mahmood, R. Weissleder and S. L. Deutscher (2006). "In vivo selection of phage for the optical imaging of PC-3 human prostate carcinoma in mice." Neoplasia **8**(9): 772-80.

Newton, J. R., Y. Miao, S. L. Deutscher and T. P. Quinn (2007). "Melanoma imaging with pretargeted bivalent bacteriophage." J Nucl Med **48**(3): 429-36.

Nicklin, S. A., S. J. White, S. J. Watkins, R. E. Hawkins and A. H. Baker (2000). "Selective targeting of gene transfer to vascular endothelial cells by use of peptides isolated by phage display." Circulation **102**(2): 231-7.

Nicklin, S. A., D. J. Von Seggern, L. M. Work, D. C. Pek, A. F. Dominiczak, G. R. Nemerow and A. H. Baker (2001). "Ablating adenovirus type 5 fiber-CAR binding and HI loop insertion of the SIGYPLP peptide generate an endothelial cell-selective adenovirus." Mol Ther **4**(6): 534-42.

Nikkola, J., P. Vihinen, T. Vlaykova, M. Hahka-Kemppinen, V. M. Kahari and S. Pyrhonen (2002). "High expression levels of collagenase-1 and stromelysin-1 correlate with shorter disease-free survival in human metastatic melanoma." Int J Cancer **97**(4): 432-8.

Nikkola, J., P. Vihinen, M. S. Vuoristo, P. Kellokumpu-Lehtinen, V. M. Kahari and S. Pyrhonen (2005). "High serum levels of matrix metalloproteinase-9 and matrix metalloproteinase-1 are associated with rapid progression in patients with metastatic melanoma." Clin Cancer Res **11**(14): 5158-66.

Nothelfer, E. M., S. Zitzmann-Kolbe, R. Garcia-Boy, S. Kramer, C. Herold-Mende, A. Altmann, M. Eisenhut, W. Mier and U. Haberkorn (2009). "Identification and characterization of a peptide with affinity to head and neck cancer." J Nucl Med **50**(3): 426-34.

Nuttall, R. K., C. L. Sampieri, C. J. Pennington, S. E. Gill, G. A. Schultz and D. R. Edwards (2004). "Expression analysis of the entire MMP and TIMP gene families during mouse tissue development." FEBS Lett **563**(1-3): 129-34.

Oelmann, E., H. Herbst, M. Zuhlsdorf, O. Albrecht, A. Nolte, C. Schmitmann, O. Manzke, V. Diehl, H. Stein and W. E. Berdel (2002). "Tissue inhibitor of metalloproteinases 1 is an autocrine and paracrine survival factor, with additional immune-regulatory functions, expressed by Hodgkin/Reed-Sternberg cells." Blood **99**(1): 258-67.

Oldenburg, K. R., D. Loganathan, I. J. Goldstein, P. G. Schultz and M. A. Gallop (1992). "Peptide ligands for a sugar-binding protein isolated from a random peptide library." Proc Natl Acad Sci U S A **89**(12): 5393-7.

Oliver, K. R., M. F. Scallan, H. Dyson and J. K. Fazakerley (1997). "Susceptibility to a neurotropic virus and its changing distribution in the developing brain is a function of CNS maturity." J Neurovirol **3**(1): 38-48.

Owe-Larsson, B., M. Berglund, K. Kristensson, H. Garoff, D. Larhammar, L. Brodin and P. Low (1999). "Perturbation of the synaptic release machinery in hippocampal neurons by overexpression of SNAP-25 with the Semliki Forest virus vector." Eur J Neurosci **11**(6): 1981-7.

Palmer, D. J. and P. Ng (2005). "Helper-dependent adenoviral vectors for gene therapy." Hum Gene Ther **16**(1): 1-16.

Palmieri, G., M. Capone, M. L. Ascierto, G. Gentilcore, D. F. Stroncek, M. Casula, M. C. Sini, M. Palla, N. Mozzillo and P. A. Ascierto (2009). "Main roads to melanoma." J Transl Med **7**: 86.

Pan, W., M. Arnone, M. Kendall, R. H. Grafstrom, S. P. Seitz, Z. R. Wasserman and C. F. Albright (2003). "Identification of peptide substrates for human MMP-11 (stromelysin-3) using phage display." J Biol Chem **278**(30): 27820-7.

Parker, A. L., S. A. Nicklin and A. H. Baker (2008). "Interactions of adenovirus vectors with blood: implications for intravascular gene therapy applications." Curr Opin Mol Ther **10**(5): 439-48.

Parks, R. J., L. Chen, M. Anton, U. Sankar, M. A. Rudnicki and F. L. Graham (1996). "A helper-dependent adenovirus vector system: removal of helper virus by Cre-mediated excision of the viral packaging signal." Proc Natl Acad Sci U S A **93**(24): 13565-70.

Parmley, S. F. and G. P. Smith (1988). "Antibody-selectable filamentous fd phage vectors: affinity purification of target genes." Gene **73**(2): 305-18.

Parrott, M. B. and M. A. Barry (2000). "Metabolic biotinylation of recombinant proteins in mammalian cells and in mice." Mol Ther **1**(1): 96-104.

Pasqualini, R. and E. Ruoslahti (1996). "Organ targeting in vivo using phage display peptide libraries." Nature **380**(6572): 364-6.

Peletskaya, E. N., V. V. Glinsky, G. V. Glinsky, S. L. Deutscher and T. P. Quinn (1997). "Characterization of peptides that bind the tumor-associated Thomsen-Friedenreich antigen selected from bacteriophage display libraries." J Mol Biol **270**(3): 374-84.

Pelkmans, L., E. Fava, H. Grabner, M. Hannus, B. Habermann, E. Krausz and M. Zerial (2005). "Genome-wide analysis of human kinases in clathrin- and caveolae/raft-mediated endocytosis." Nature **436**(7047): 78-86.

Peluso, I., M. C. Fantini, D. Fina, R. Caruso, M. Boirivant, T. T. MacDonald, F. Pallone and G. Monteleone (2007). "IL-21 counteracts the regulatory T cell-mediated suppression of human CD4+ T lymphocytes." J Immunol **178**(2): 732-9.

Peng, Z. (2005). "Current status of gendicine in China: recombinant human Ad-p53 agent for treatment of cancers." Hum Gene Ther **16**(9): 1016-27.

Pesonen, S., P. Nokisalmi, S. Escutenaire, M. Sarkioja, M. Raki, V. Cerullo, L. Kangasniemi, L. Laasonen, C. Ribacka, K. Guse, E. Haavisto, M. Oksanen, M. Rajecki, A. Helminen, A. Ristimaki, A. Karioja-Kallio, E. Karli, T. Kantola, G. Bauerschmitz, A. Kanerva, T. Joensuu and A. Hemminki (2010). "Prolonged systemic circulation of chimeric oncolytic adenovirus Ad5/3-Cox2L-D24 in patients with metastatic and refractory solid tumors." Gene Ther.

Peterson, A. C., S. Swiger, W. M. Stadler, M. Medved, G. Karczmar and T. F. Gajewski (2004). "Phase II study of the Flk-1 tyrosine kinase inhibitor SU5416 in advanced melanoma." Clin Cancer Res **10**(12 Pt 1): 4048-54.

Petrini, J. H., A. M. Carroll and M. J. Bosma (1990). "T-cell receptor gene rearrangements in functional T-cell clones from severe combined immune deficient (scid) mice: reversion of the scid phenotype in individual lymphocyte progenitors." Proc Natl Acad Sci U S A **87**(9): 3450-3.

Pohar, N., T. A. Godenschwege and E. Buchner (1999). "Invertebrate tissue inhibitor of metalloproteinase: structure and nested gene organization within the synapsin locus is conserved from Drosophila to human." Genomics **57**(2): 293-6.

Popkov, M., C. Rader and C. F. Barbas, 3rd (2004). "Isolation of human prostate cancer cell reactive antibodies using phage display technology." J Immunol Methods **291**(1-2): 137-51.

Porter, S., P. N. Span, F. C. Sweep, V. C. Tjan-Heijnen, C. J. Pennington, T. X. Pedersen, M. Johnsen, L. R. Lund, J. Romer and D. R. Edwards (2006). "ADAMTS8 and ADAMTS15 expression predicts survival in human breast carcinoma." Int J Cancer **118**(5): 1241-7.

Pratt, D., H. Tzagoloff and J. Beaudoin (1969). "Conditional lethal mutants of the small filamentous coliphage M13. II. Two genes for coat proteins." Virology **39**(1): 42-53.

Puente, X. S., L. M. Sanchez, C. M. Overall and C. Lopez-Otin (2003). "Human and mouse proteases: a comparative genomic approach." Nat Rev Genet **4**(7): 544-58.

Pusztai, R., E. A. Gould and H. Smith (1971). "Infection patterns in mice of an avirulent and virulent strain of Semliki Forest virus." Br J Exp Pathol **52**(6): 669-77.

Quintana, E., M. Shackleton, M. S. Sabel, D. R. Fullen, T. M. Johnson and S. J. Morrison (2008). "Efficient tumour formation by single human melanoma cells." Nature **456**(7222): 593-8.

Quirin, C., A. Mainka, A. Hesse and D. M. Nettelbeck (2007). "Combining adenoviral oncolysis with temozolomide improves cell killing of melanoma cells." Int J Cancer **121**(12): 2801-7.

Raju, R. and H. V. Huang (1991). "Analysis of Sindbis virus promoter recognition in vivo, using novel vectors with two subgenomic mRNA promoters." J Virol **65**(5): 2501-10.

Rampling, R., G. Cruickshank, V. Papanastassiou, J. Nicoll, D. Hadley, D. Brennan, R. Petty, A. MacLean, J. Harland, E. McKie, R. Mabbs and M. Brown (2000). "Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma." Gene Ther **7**(10): 859-66.

Rancourt, C., H. Keyvani-Amineh, S. Sircar, P. Labrecque and J. M. Weber (1995). "Proline 137 is critical for adenovirus protease encapsidation and activation but not enzyme activity." Virology **209**(1): 167-73.

Rao, D. R. and D. M. Greenberg (1960). "Studies on the enzymic decomposition of urocanic acid. II. Properties of products of urocanase reaction." Biochim Biophys Acta **43**: 404-18.

Ray, J. M. and W. G. Stetler-Stevenson (1995). "Gelatinase A activity directly modulates melanoma cell adhesion and spreading." Embo J **14**(5): 908-17.

Reddy, S., D. Piccione, H. Takita and R. B. Bankert (1987). "Human lung tumor growth established in the lung and subcutaneous tissue of mice with severe combined immunodeficiency." Cancer Res **47**(9): 2456-60.

Reynolds, H. M., P. R. Dunbar, R. F. Uren, S. A. Blackett, J. F. Thompson and N. P. Smith (2007). "Three-dimensional visualisation of lymphatic drainage patterns in patients with cutaneous melanoma." Lancet Oncol **8**(9): 806-12.

Rhee, J. S. and L. M. Coussens (2002). "RECKing MMP function: implications for cancer development." Trends Cell Biol **12**(5): 209-11.

Ribas, A., L. H. Camacho, G. Lopez-Berestein, D. Pavlov, C. A. Bulanhagui, R. Millham, B. Comin-Anduix, J. M. Reuben, E. Seja, C. A. Parker, A. Sharma, J. A. Glaspy and J. Gomez-Navarro (2005). "Antitumor activity in melanoma and anti-self responses in a phase I trial with the anti-cytotoxic T lymphocyte-associated antigen 4 monoclonal antibody CP-675,206." J Clin Oncol **23**(35): 8968-77.

Riezebos-Brilman, A., A. de Mare, L. Bungener, A. Huckriede, J. Wilschut and T. Daemen (2006). "Recombinant alphaviruses as vectors for anti-tumour and anti-microbial immunotherapy." J Clin Virol **35**(3): 233-43.

Rinaldi, M., P. Moroni, L. Leino, J. Laihia, M. J. Paape and D. D. Bannerman (2006). "Effect of cis-urocanic acid on bovine neutrophil generation of reactive oxygen species." J Dairy Sci **89**(11): 4188-201.

Rivat, C., N. Le Floch, M. Sabbah, I. Teyrol, G. Redeuilh, E. Bruyneel, M. Mareel, L. M. Matrisian, H. C. Crawford, C. Gespach and S. Attoub (2003). "Synergistic cooperation between the AP-1 and LEF-1 transcription factors in activation of the matrilysin promoter by the src oncogene: implications in cellular invasion." Faseb J **17**(12): 1721-3.

Rivera, A. A., J. Davydova, S. Schierer, M. Wang, V. Krasnykh, M. Yamamoto, D. T. Curiel and D. M. Nettelbeck (2004). "Combining high selectivity of replication with fiber chimerism for effective adenoviral oncolysis of CAR-negative melanoma cells." Gene Ther **11**(23): 1694-702.

Roberts, D. L., A. V. Anstey, R. J. Barlow, N. H. Cox, J. A. Newton Bishop, P. G. Corrie, J. Evans, M. E. Gore, P. N. Hall and N. Kirkham (2002). "U.K. guidelines for the management of cutaneous melanoma." Br J Dermatol **146**(1): 7-17.

Robinson, J. K. and S. Ortiz (2009). "Use of photographs illustrating ABCDE criteria in skin self-examination." Arch Dermatol **145**(3): 332-3.

Rodon, J., J. Perez and R. Kurzrock (2010). "Combining targeted therapies: practical issues to consider at the bench and bedside." Oncologist **15**(1): 37-50.

Roeb, E., R. Winograd, B. Breuer, H. Nguyen and S. Matern (1999). "Increased TIMP-1 activity results in increased expression of gelatinases and altered cell motility." J Cell Biochem **75**(2): 346-55.

Rofstad, E. K. and H. Lyng (1996). "Xenograft model systems for human melanoma." Mol Med Today **2**(9): 394-403.

Rogers, S., A. Lowenthal, H. G. Terheggen and J. P. Columbo (1973). "Induction of arginase activity with the Shope papilloma virus in tissue culture cells from an argininemic patient." J Exp Med **137**(4): 1091-6.

Romanczuk, H., C. E. Galer, J. Zabner, G. Barsomian, S. C. Wadsworth and C. R. O'Riordan (1999). "Modification of an adenoviral vector with biologically selected peptides: a novel strategy for gene delivery to cells of choice." Hum Gene Ther **10**(16): 2615-26.

Rosenberg, S. A., P. Aebersold, K. Cornetta, A. Kasid, R. A. Morgan, R. Moen, E. M. Karson, M. T. Lotze, J. C. Yang, S. L. Topalian and et al. (1990). "Gene transfer into humans--immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction." N Engl J Med **323**(9): 570-8.

Rosenberg, S. A., M. T. Lotze, L. M. Muul, S. Leitman, A. E. Chang, S. E. Ettinghausen, Y. L. Matory, J. M. Skibber, E. Shiloni, J. T. Vetto and et al. (1985). "Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer." N Engl J Med **313**(23): 1485-92.

Rosenberg, S. A., J. C. Yang, D. J. Schwartzentruber, P. Hwu, F. M. Marincola, S. L. Topalian, N. P. Restifo, M. E. Dudley, S. L. Schwarz, P. J. Spiess, J. R. Wunderlich, M. R. Parkhurst, Y. Kawakami, C. A. Seipp, J. H. Einhorn and D. E. White (1998). "Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma." Nat Med **4**(3): 321-7.

Rowe, W. P., R. J. Huebner, L. K. Gilmore, R. H. Parrott and T. G. Ward (1953). "Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture." Proc Soc Exp Biol Med **84**(3): 570-3.

Rubin, K. M. and D. P. Lawrence (2009). "Your patient with melanoma: staging, prognosis, and treatment." Oncology (Williston Park) **23**(8 Suppl): 13-21.

Ruokolainen, H., P. Paakko and T. Turpeenniemi-Hujanen (2005). "Tissue inhibitor of matrix metalloproteinase-1 is prognostic in head and neck squamous cell carcinoma: comparison of the circulating and tissue immunoreactive protein." Clin Cancer Res **11**(9): 3257-64.

Ruoslahti, E. and M. D. Pierschbacher (1987). "New perspectives in cell adhesion: RGD and integrins." Science **238**(4826): 491-7.

Rux, J. J. and R. M. Burnett (2004). "Adenovirus structure." Hum Gene Ther **15**(12): 1167-76.

Räsänen, L., C. T. Jansen, T. Reunala and H. Morrison (1987). "Stereospecific inhibition of human epidermal cell interleukin-1 secretion and HLA-DR expression by cis-urocanic acid." Photodermatol **4**(4): 182-6.

Saari, H., K. Suomalainen, O. Lindy, Y. T. Konttinen and T. Sorsa (1990). "Activation of latent human neutrophil collagenase by reactive oxygen species and serine proteases." Biochem Biophys Res Commun **171**(3): 979-87.

Samkoe, K. S., A. Chen, I. Rizvi, J. A. O'Hara, P. J. Hoopes, S. P. Pereira, T. Hasan and B. W. Pogue (2010). "Imaging tumor variation in response to photodynamic therapy in pancreatic cancer xenograft models." Int J Radiat Oncol Biol Phys **76**(1): 251-9.

Sarkar, D., I. V. Lebedeva, P. Gupta, L. Emdad, M. Sauane, P. Dent, D. T. Curiel and P. B. Fisher (2007). "Melanoma differentiation associated gene-7 (mda-7)/IL-24: a 'magic bullet' for cancer therapy?" Expert Opin Biol Ther **7**(5): 577-86.

Saunders, W. B., B. L. Bohnsack, J. B. Faske, N. J. Anthis, K. J. Bayless, K. K. Hirschi and G. E. Davis (2006). "Coregulation of vascular tube stabilization by endothelial cell TIMP-2 and pericyte TIMP-3." J Cell Biol **175**(1): 179-91.

Schagen, F. H., H. C. Graat, J. E. Carette, J. Vellinga, M. A. van Geer, R. C. Hoeben, T. S. Dermody and V. W. van Beusechem (2008). "Replacement of native adenovirus receptor-binding sites with a new attachment moiety diminishes hepatic tropism and enhances bioavailability in mice." Hum Gene Ther **19**(8): 783-94.

Schier, R., J. D. Marks, E. J. Wolf, G. Apell, C. Wong, J. E. McCartney, M. A. Bookman, J. S. Huston, L. L. Houston, L. M. Weiner and et al. (1995). "In vitro and in vivo characterization of a human anti-c-erbB-2 single-chain Fv isolated from a filamentous phage antibody library." Immunotechnology **1**(1): 73-81.

Schierer, S., A. Hesse, I. Muller, E. Kampgen, D. T. Curiel, G. Schuler, A. Steinkasserer and D. M. Nettelbeck (2008). "Modulation of viability and maturation of human monocyte-derived dendritic cells by oncolytic adenoviruses." Int J Cancer **122**(1): 219-29.

Schlesinger, S. (2001). "Alphavirus vectors: development and potential therapeutic applications." Expert Opin Biol Ther **1**(2): 177-91.

Scott, J. K. and G. P. Smith (1990). "Searching for peptide ligands with an epitope library." Science **249**(4967): 386-90.

Sebestyen, Z., J. de Vrij, M. Magnusson, R. Debets and R. Willemsen (2007). "An oncolytic adenovirus redirected with a tumor-specific T-cell receptor." Cancer Res **67**(23): 11309-16.

Sechler, J. L., S. A. Corbett, M. B. Wenk and J. E. Schwarzbauer (1998). "Modulation of cell-extracellular matrix interactions." Ann N Y Acad Sci **857**: 143-54.

Seeger, J. M., P. Schmidt, K. Brinkmann, A. A. Hombach, O. Coutelle, P. Zigrino, D. Wagner-Stippich, C. Mauch, H. Abken, M. Kronke and H. Kashkar (2010). "The proteasome inhibitor bortezomib sensitizes melanoma cells toward adoptive CTL attack." Cancer Res **70**(5): 1825-34.

Seo, D. W., H. Li, L. Guedez, P. T. Wingfield, T. Diaz, R. Salloum, B. Y. Wei and W. G. Stetler-Stevenson (2003). "TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism." Cell **114**(2): 171-80.

Serrano, M. (1997). "The tumor suppressor protein p16INK4a." Exp Cell Res **237**(1): 7-13.

Sharpless, E. and L. Chin (2003). "The INK4a/ARF locus and melanoma." Oncogene **22**(20): 3092-8.

Shaw, H. M., V. J. McGovern, G. W. Milton and G. A. Farago (1981). "Cutaneous malignant melanoma: occupational and prognosis." Med J Aust **1**(1): 37-8.

Shayakhmetov, D. M. and A. Lieber (2000). "Dependence of adenovirus infectivity on length of the fiber shaft domain." J Virol **74**(22): 10274-86.

Short, J. J., A. V. Pereboev, Y. Kawakami, C. Vasu, M. J. Holterman and D. T. Curiel (2004). "Adenovirus serotype 3 utilizes CD80 (B7.1) and CD86 (B7.2) as cellular attachment receptors." Virology **322**(2): 349-59.

Skak, K., M. Kragh, D. Hausman, M. J. Smyth and P. V. Sivakumar (2008). "Interleukin 21: combination strategies for cancer therapy." Nat Rev Drug Discov.

Skelton, N. J., Y. M. Chen, N. Dubree, C. Quan, D. Y. Jackson, A. Cochran, K. Zobel, K. Deshayes, M. Baca, M. T. Pisabarro and H. B. Lowman (2001). "Structure-function analysis of a phage display-derived peptide that binds to insulin-like growth factor binding protein 1." Biochemistry **40**(29): 8487-98.

Smith, A. E. and A. Helenius (2004). "How viruses enter animal cells." Science **304**(5668): 237-42.

Smith, G. P. (1985). "Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface." Science **228**(4705): 1315-7.

Smith, G. P. and J. K. Scott (1993). "Libraries of peptides and proteins displayed on filamentous phage." Methods Enzymol **217**: 228-57.

Smith, T., N. Idamakanti, H. Kylefjord, M. Rollence, L. King, M. Kaloss, M. Kaleko and S. C. Stevenson (2002). "In vivo hepatic adenoviral gene delivery occurs independently of the coxsackievirus-adenovirus receptor." Mol Ther **5**(6): 770-9.

Soengas, M. S., P. Capodieci, D. Polsky, J. Mora, M. Esteller, X. Opitz-Araya, R. McCombie, J. G. Herman, W. L. Gerald, Y. A. Lazebnik, C. Cordon-Cardo and S. W. Lowe (2001). "Inactivation of the apoptosis effector Apaf-1 in malignant melanoma." Nature **409**(6817): 207-11.

Soengas, M. S. and S. W. Lowe (2003). "Apoptosis and melanoma chemoresistance." Oncogene **22**(20): 3138-51.

Soiffer, R., F. S. Hodi, F. Haluska, K. Jung, S. Gillessen, S. Singer, K. Tanabe, R. Duda, S. Mentzer, M. Jaklitsch, R. Bueno, S. Clift, S. Hardy, D. Neuberg, R. Mulligan, I. Webb, M. Mihm and G. Dranoff (2003). "Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma." J Clin Oncol **21**(17): 3343-50.

Spurbeck, W. W., C. Y. Ng, T. S. Strom, E. F. Vanin and A. M. Davidoff (2002). "Enforced expression of tissue inhibitor of matrix metalloproteinase-3 affects functional capillary morphogenesis and inhibits tumor growth in a murine tumor model." Blood **100**(9): 3361-8.

Spurbeck, W. W., C. Y. Ng, E. F. Vanin and A. M. Davidoff (2003). "Retroviral vector-producer cell-mediated in vivo gene transfer of TIMP-3 restricts angiogenesis and neuroblastoma growth in mice." Cancer Gene Ther **10**(3): 161-7.

Stamenkovic, I. (2000). "Matrix metalloproteinases in tumor invasion and metastasis." Semin Cancer Biol **10**(6): 415-33.

Stamenkovic, I. (2003). "Extracellular matrix remodelling: the role of matrix metalloproteinases." J Pathol **200**(4): 448-64.

Starritt, E. C., R. F. Uren, R. A. Scolyer, M. J. Quinn and J. F. Thompson (2005). "Ultrasound examination of sentinel nodes in the initial assessment of patients with primary cutaneous melanoma." Ann Surg Oncol **12**(1): 18-23.

Sternlicht, M. D. and Z. Werb (2001). "How matrix metalloproteinases regulate cell behavior." Annu Rev Cell Dev Biol **17**: 463-516.

Stetler-Stevenson, W. G., N. Bersch and D. W. Golde (1992). "Tissue inhibitor of metalloproteinase-2 (TIMP-2) has erythroid-potentiating activity." FEBS Lett **296**(2): 231-4.

Stetler-Stevenson, W. G., H. C. Krutzsch and L. A. Liotta (1989). "Tissue inhibitor of metalloproteinase (TIMP-2). A new member of the metalloproteinase inhibitor family." J Biol Chem **264**(29): 17374-8.

Stewart, P. L., R. M. Burnett, M. Cyrklaff and S. D. Fuller (1991). "Image reconstruction reveals the complex molecular organization of adenovirus." Cell **67**(1): 145-54.

Stewart, P. L., S. D. Fuller and R. M. Burnett (1993). "Difference imaging of adenovirus: bridging the resolution gap between X-ray crystallography and electron microscopy." Embo J **12**(7): 2589-99.

Stevenson, S. C., M. Rollence, B. White, L. Weaver and A. McClelland (1995). "Human adenovirus serotypes 3 and 5 bind to two different cellular receptors via the fiber head domain." J Virol **69**(5): 2850-7.

Strongin, A. Y., I. Collier, G. Bannikov, B. L. Marmer, G. A. Grant and G. I. Goldberg (1995). "Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease." J Biol Chem **270**(10): 5331-8.

Suckling, A. J., S. Pathak, S. Jagelman and H. E. Webb (1978). "Virus-associated demyelination. A model using avirulent Semliki Forest virus infection of mice." J Neurol Sci **39**(1): 147-54.

Sundquist, B., E. Everitt, L. Philipson and S. Hoglund (1973). "Assembly of adenoviruses." J Virol **11**(3): 449-59.

Sundstedt, A., M. Celander and G. Hedlund (2008). "Combining tumor-targeted superantigens with interferon-alpha results in synergistic anti-tumor effects." Int Immunopharmacol **8**(3): 442-52.

Suojanen, J., T. Salo, E. Koivunen, T. Sorsa and E. Pirila (2009). "A novel and selective membrane type-1 matrix metalloproteinase (MT1-MMP) inhibitor reduces cancer cell motility and tumor growth." Cancer Biol Ther **8**(24): 2362-70.

Suominen, E.-. (2005). " www.kaypahoito.fi." Duodecim **121**(24): 2726-2740.

Suominen, E., R. Toivonen, R. Grenman and M. Savontaus (2006). "Head and neck cancer cells are efficiently infected by Ad5/35 hybrid virus." J Gene Med **8**(10): 1223-31.

Tang, Y., T. Han, M. Everts, Z. B. Zhu, G. Y. Gillespie, D. T. Curiel and H. Wu (2007). "Directing adenovirus across the blood-brain barrier via melanotransferrin (P97) transcytosis pathway in an in vitro model." Gene Ther **14**(6): 523-32.

Tawbi, H. A. and J. M. Kirkwood (2007). "Management of metastatic melanoma." Semin Oncol **34**(6): 532-45.

Terhune, M. H., N. Swanson and T. M. Johnson (1998). "Use of chest radiography in the initial evaluation of patients with localized melanoma." Arch Dermatol **134**(5): 569-72.

Thomas, C. E., A. Ehrhardt and M. A. Kay (2003). "Progress and problems with the use of viral vectors for gene therapy." Nat Rev Genet **4**(5): 346-58.

Thompson, J. F., H. M. Shaw, P. Hersey and R. A. Scolyer (2004). "The history and future of melanoma staging." J Surg Oncol **86**(4): 224-35.

Toivonen, R., E. Suominen, R. Grenman and M. Savontaus (2009). "Retargeting improves the efficacy of a telomerase-dependent oncolytic adenovirus for head and neck cancer." Oncol Rep **21**(1): 165-71.

Toriseva, M. and V. M. Kahari (2009). "Proteinases in cutaneous wound healing." Cell Mol Life Sci **66**(2): 203-24.

Tran, P. L., J. P. Vigneron, D. Pericat, S. Dubois, D. Cazals, M. Hervy, Y. A. DeClerck, C. Degott and C. Auclair (2003). "Gene therapy for hepatocellular carcinoma using non-viral vectors composed of bis guanidinium-tren-cholesterol and plasmids encoding the tissue inhibitors of metalloproteinases TIMP-2 and TIMP-3." Cancer Gene Ther **10**(6): 435-44.

Trotman, L. C., N. Mosberger, M. Fornerod, R. P. Stidwill and U. F. Greber (2001). "Import of adenovirus DNA involves the nuclear pore complex receptor CAN/Nup214 and histone H1." Nat Cell Biol **3**(12): 1092-100.

Tsao, H., X. Zhang, E. Benoit and F. G. Haluska (1998). "Identification of PTEN/MMAC1 alterations in uncultured melanomas and melanoma cell lines." Oncogene **16**(26): 3397-402.

Tucker, M. A., J. D. Boice, Jr. and D. A. Hoffman (1985). "Second cancer following cutaneous melanoma and cancers of the brain, thyroid, connective tissue, bone, and eye in Connecticut, 1935-82." Natl Cancer Inst Monogr **68**: 161-89.

Tuittila, M., P. Nygardas and A. Hinkkanen (2004). "mRNA expression of proinflammatory cytokines in mouse CNS correlates with replication rate of semliki forest virus but not with the strain of viral proteins." Viral Immunol **17**(2): 287-97.

Tuittila, M. T., M. G. Santagati, M. Roytta, J. A. Maatta and A. E. Hinkkanen (2000). "Replicase complex genes of Semliki Forest virus confer lethal neurovirulence." J Virol **74**(10): 4579-89.

Tuve, S., H. Wang, C. Ware, Y. Liu, A. Gaggar, K. Bernt, D. Shayakhmetov, Z. Li, R. Strauss, D. Stone and A. Lieber (2006). "A new group B adenovirus receptor is expressed at high levels on human stem and tumor cells." J Virol **80**(24): 12109-20.

Tzukert, K., N. Shimony, L. Krasny, S. Urieli-Shoval, R. Gorodetsky, I. Avrahami, D. M. Nettelbeck and Y. S. Haviv (2010). "Human melanoma cells expressing the alphavbeta3 integrin are partially protected from necrotic cell death induced by dynamic matrix detachment." Cancer Lett **290**(2): 174-81.

Urbanelli, L., C. Ronchini, L. Fontana, S. Menard, R. Orlandi and P. Monaci (2001). "Targeted gene transduction of mammalian cells expressing the HER2/neu receptor by filamentous phage." J Mol Biol **313**(5): 965-76.

Uren, R. F., J. F. Thompson, R. Howman-Giles and D. K. Chung (2006). "The role of lymphoscintigraphy in the detection of lymph node drainage in melanoma." Surg Oncol Clin N Am **15**(2): 285-300.

Waddington, S. N., J. H. McVey, D. Bhella, A. L. Parker, K. Barker, H. Atoda, R. Pink, S. M. Buckley, J. A. Greig, L. Denby, J. Custers, T. Morita, I. M. Francischetti, R. Q. Monteiro, D. H. Barouch, N. van Rooijen, C. Napoli, M. J. Havenga, S. A. Nicklin and A. H. Baker (2008). "Adenovirus serotype 5 hexon mediates liver gene transfer." Cell **132**(3): 397-409.

Wahlfors, J. J., S. A. Zullo, S. Loimas, D. M. Nelson and R. A. Morgan (2000). "Evaluation of recombinant alphaviruses as vectors in gene therapy." Gene Ther **7**(6): 472-80.

Valente, P., G. Fassina, A. Melchiori, L. Masiello, M. Cilli, A. Vacca, M. Onisto, L. Santi, W. G. Stetler-Stevenson and A. Albini (1998). "TIMP-2 over-expression reduces invasion and angiogenesis and protects B16F10 melanoma cells from apoptosis." Int J Cancer **75**(2): 246-53.

Valentine, R. C. and H. G. Pereira (1965). "Antigens and structure of the adenovirus." J Mol Biol **13**(1): 13-20.

Walterscheid, J. P., D. X. Nghiem, N. Kazimi, L. K. Nutt, D. J. McConkey, M. Norval and S. E. Ullrich (2006). "Cis-urocanic acid, a sunlight-induced immunosuppressive factor, activates immune suppression via the 5-HT2A receptor." Proc Natl Acad Sci U S A **103**(46): 17420-5.

Walther, S. E. and D. T. Denhardt (1996). "Directed mutagenesis reveals that two histidines in tissue inhibitor of metalloproteinase-1 are each essential for the suppression of cell migration, invasion, and tumorigenicity." Cell Growth Differ **7**(11): 1579-88.

van Beusechem, V. W., A. L. van Rijswijk, H. H. van Es, H. J. Haisma, H. M. Pinedo and W. R. Gerritsen (2000). "Recombinant adenovirus vectors with knobless fibers for targeted gene transfer." Gene Ther **7**(22): 1940-6.

van Oostrum, J. and R. M. Burnett (1985). "Molecular composition of the adenovirus type 2 virion." J Virol **56**(2): 439-48.

Wang, M., Y. E. Liu, J. Greene, S. Sheng, A. Fuchs, E. M. Rosen and Y. E. Shi (1997). "Inhibition of tumor growth and metastasis of human breast cancer cells transfected with tissue inhibitor of metalloproteinase 4." Oncogene **14**(23): 2767-74.

Wang, Z., R. Juttermann and P. D. Soloway (2000). "TIMP-2 is required for efficient activation of proMMP-2 in vivo." J Biol Chem **275**(34): 26411-5.

Watanabe-Fukunaga, R., C. I. Brannan, N. G. Copeland, N. A. Jenkins and S. Nagata (1992). "Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis." Nature **356**(6367): 314-7.

Weber, B. H., G. Vogt, R. C. Pruett, H. Stohr and U. Felbor (1994). "Mutations in the tissue inhibitor of metalloproteinases-3 (TIMP3) in patients with Sorsby's fundus dystrophy." Nat Genet **8**(4): 352-6.

Weedon, D. (2002). Skin Pathology. London, Churchill Livingston.

Vergani, V., A. Garofalo, M. R. Bani, P. Borsotti, M. P. Parker, T. Drudis, G. Mazzarol, G. Viale, R. Giavazzi, W. G. Stetler-Stevenson and G. Taraboletti (2001). "Inhibition of matrix metalloproteinases by over-expression of tissue inhibitor of metalloproteinase-2 inhibits the growth of experimental hemangiomas." Int J Cancer **91**(2): 241-7.

Whang, T. and G. Goldenberg (2009). "American Academy of Dermatology--Summer Meeting. 29 July-2 August 2009, Boston, MA, USA." IDrugs **12**(10): 617-9.

White, J. M. (2003). "ADAMs: modulators of cell-cell and cell-matrix interactions." Curr Opin Cell Biol **15**(5): 598-606.

White, N., G. E. Knight, P. E. Butler and G. Burnstock (2009). "An in vivo model of melanoma: treatment with ATP." Purinergic Signal **5**(3): 327-33.

White, S. J., S. A. Nicklin, H. Buning, M. J. Brosnan, K. Leike, E. D. Papadakis, M. Hallek and A. H. Baker (2004). "Targeted gene delivery to vascular tissue in vivo by tropism-modified adeno-associated virus vectors." Circulation **109**(4): 513-9.

Wickham, T. J., P. W. Roelvink, D. E. Brough and I. Kovesdi (1996). "Adenovirus targeted to heparan-containing receptors increases its gene delivery efficiency to multiple cell types." Nat Biotechnol **14**(11): 1570-3.

Wickham, T. J., D. M. Segal, P. W. Roelvink, M. E. Carrion, A. Lizonova, G. M. Lee and I. Kovesdi (1996). "Targeted adenovirus gene transfer to endothelial and smooth muscle cells by using bispecific antibodies." J Virol **70**(10): 6831-8.

Wickham, T. J., E. Tzeng, L. L. Shears, 2nd, P. W. Roelvink, Y. Li, G. M. Lee, D. E. Brough, A. Lizonova and I. Kovesdi (1997). "Increased in vitro and in vivo gene transfer by adenovirus vectors containing chimeric fiber proteins." J Virol **71**(11): 8221-9.

Wiethoff, C. M., H. Wodrich, L. Gerace and G. R. Nemerow (2005). "Adenovirus protein VI mediates membrane disruption following capsid disassembly." J Virol **79**(4): 1992-2000.

Vihinen, P. P., M. Hernberg, M. S. Vuoristo, K. Tyynela, M. Laukka, J. Lundin, J. Ivaska and S. Pyrhonen (2010). "A phase II trial of bevacizumab with dacarbazine and daily low-dose interferon-alpha2a as first line treatment in metastatic melanoma." Melanoma Res **20**(4): 318-25.

Wilkinson, G. W. and A. Akrigg (1992). "Constitutive and enhanced expression from the CMV major IE promoter in a defective adenovirus vector." Nucleic Acids Res **20**(9): 2233-9.

Villanueva, J. and M. Herlyn (2008). "Melanoma and the tumor microenvironment." Curr Oncol Rep **10**(5): 439-46.

Visse, R. and H. Nagase (2003). "Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry." Circ Res **92**(8): 827-39.

Wolf, J. K., D. C. Bodurka, J. B. Gano, M. Deavers, L. Ramondetta, P. T. Ramirez, C. Levenback and D. M. Gershenson (2004). "A phase I study of Adp53 (INGN 201; ADVEXIN) for patients with platinum- and paclitaxel-resistant epithelial ovarian cancer." Gynecol Oncol **94**(2): 442-8.

Volpers, C. and S. Kochanek (2004). "Adenoviral vectors for gene transfer and therapy." J Gene Med **6 Suppl 1**: S164-71.

Von Seggern, D. J., C. Y. Chiu, S. K. Fleck, P. L. Stewart and G. R. Nemerow (1999). "A helper-independent adenovirus vector with E1, E3, and fiber deleted: structure and infectivity of fiberless particles." J Virol **73**(2): 1601-8.

Von Seggern, D. J., S. Huang, S. K. Fleck, S. C. Stevenson and G. R. Nemerow (2000). "Adenovirus vector pseudotyping in fiber-expressing cell lines: improved transduction of Epstein-Barr virus-transformed B cells." J Virol **74**(1): 354-62.

Wood, T. F., L. A. DiFronzo, D. M. Rose, P. I. Haigh, S. L. Stern, L. Wanek, R. Essner and D. L. Morton (2001). "Does complete resection of melanoma metastatic to solid intra-abdominal organs improve survival?" Ann Surg Oncol **8**(8): 658-62.

Work, L. M., H. Buning, E. Hunt, S. A. Nicklin, L. Denby, N. Britton, K. Leike, M. Odenthal, U. Drebber, M. Hallek and A. H. Baker (2005). "Vascular bed-targeted in vivo gene delivery using tropism-modified adeno-associated viruses." Mol Ther.

Work, L. M., S. A. Nicklin, N. J. Brain, K. L. Dishart, D. J. Von Seggern, M. Hallek, H. Buning and A. H. Baker (2004). "Development of efficient viral vectors selective for vascular smooth muscle cells." Mol Ther **9**(2): 198-208.

Wrighton, N. C., F. X. Farrell, R. Chang, A. K. Kashyap, F. P. Barbone, L. S. Mulcahy, D. L. Johnson, R. W. Barrett, L. K. Jolliffe and W. J. Dower (1996). "Small peptides as potent mimetics of the protein hormone erythropoietin." Science **273**(5274): 458-64.

Wu, E., J. Fernandez, S. K. Fleck, D. J. Von Seggern, S. Huang and G. R. Nemerow (2001). "A 50-kDa membrane protein mediates sialic acid-independent binding and infection of conjunctival cells by adenovirus type 37." Virology **279**(1): 78-89.

Wu, E., L. Pache, D. J. Von Seggern, T. M. Mullen, Y. Mikyas, P. L. Stewart and G. R. Nemerow (2003). "Flexibility of the adenovirus fiber is required for efficient receptor interaction." J Virol **77**(13): 7225-35.

Vuoristo, M., P. Vihinen, T. Vlaykova, C. Nylund, J. Heino and S. Pyrhonen (2007). "Increased gene expression levels of collagen receptor integrins are associated with decreased survival parameters in patients with advanced melanoma." Melanoma Res **17**(4): 215-23.

Vähä-Koskela, M. J., M. T. Tuittila, P. T. Nygardas, J. K. Nyman, M. U. Ehrengruber, M. Renggli and A. E. Hinkkanen (2003). "A novel neurotropic expression vector based on the avirulent A7(74) strain of Semliki Forest virus." J Neurovirol **9**(1): 1-15.

Väisänen, A., M. Kallioinen, P. J. Taskinen and T. Turpeenniemi-Hujanen (1998). "Prognostic value of MMP-2 immunoreactive protein (72 kD type IV collagenase) in primary skin melanoma." J Pathol **186**(1): 51-8.

Väisänen, A., H. Tuominen, M. Kallioinen and T. Turpeenniemi-Hujanen (1996). "Matrix metalloproteinase-2 (72 kD type IV collagenase) expression occurs in the early stage of human melanocytic tumour progression and may have prognostic value." J Pathol **180**(3): 283-9.

Xie, X., N. Brunner, G. Jensen, J. Albrectsen, B. Gotthardsen and J. Rygaard (1992). "Comparative studies between nude and scid mice on the growth and metastatic behavior of xenografted human tumors." Clin Exp Metastasis **10**(3): 201-10.

Xiong, C., R. Levis, P. Shen, S. Schlesinger, C. M. Rice and H. V. Huang (1989). "Sindbis virus: an efficient, broad host range vector for gene expression in animal cells." Science **243**(4895): 1188-91.

Yang, T. T. and S. P. Hawkes (1992). "Role of the 21-kDa protein TIMP-3 in oncogenic transformation of cultured chicken embryo fibroblasts." Proc Natl Acad Sci U S A **89**(22): 10676-80.

Ylösmäki, E., T. Hakkarainen, A. Hemminki, T. Visakorpi, R. Andino and K. Saksela (2008). "Generation of a conditionally replicating adenovirus based on targeted destruction of E1A mRNA by a cell type-specific MicroRNA." J Virol **82**(22): 11009-15.

Yoon, S. O., S. J. Park, C. H. Yun and A. S. Chung (2003). "Roles of matrix metalloproteinases in tumor metastasis and angiogenesis." J Biochem Mol Biol **36**(1): 128-37.

Yoshimoto, Y., C. K. Augustine, J. S. Yoo, P. A. Zipfel, M. A. Selim, S. K. Pruitt, H. S. Friedman, F. Ali-Osman and D. S. Tyler (2007). "Defining regional infusion treatment strategies for extremity melanoma: comparative analysis of melphalan and temozolomide as regional chemotherapeutic agents." Mol Cancer Ther **6**(5): 1492-500.

Yoshino, Y., T. Kageshita, M. Nakajima, M. Funakubo and H. Ihn (2008). "Clinical relevance of serum levels of matrix metallopeptidase-2, and tissue inhibitor of metalloproteinase-1 and -2 in patients with malignant melanoma." J Dermatol **35**(4): 206-14.

Yu, W. H., S. Yu, Q. Meng, K. Brew and J. F. Woessner, Jr. (2000). "TIMP-3 binds to sulfated glycosaminoglycans of the extracellular matrix." J Biol Chem **275**(40): 31226-32.

Yuan, Z. Y., R. H. Xu, Y. J. He and Z. Z. Guan (2003). "[Clinical comparison of CHO regimen versus CHOP regimen for treatment of patients with intermediate-grade non-Hodgkin's lymphoma]." Ai Zheng **22**(4): 393-6.

Yuan, Z. Y., L. Zhang, S. Li, X. Z. Qian and Z. Z. Guan (2003). "[Safety of an E1B deleted adenovirus administered intratumorally to patients with cancer]." Ai Zheng **22**(3): 310-3.

Zaal, L. H., W. J. Mooi, J. H. Sillevis Smitt and C. M. van der Horst (2004). "Classification of congenital melanocytic naevi and malignant transformation: a review of the literature." Br J Plast Surg **57**(8): 707-19.

Zhu, Z., P. Rockwell, D. Lu, H. Kotanides, B. Pytowski, D. J. Hicklin, P. Bohlen and L. Witte (1998). "Inhibition of vascular endothelial growth factor-induced receptor activation with anti-kinase insert domain-containing receptor single-chain antibodies from a phage display library." Cancer Res **58**(15): 3209-14.

Zigrino, P., C. Mauch, J. W. Fox and R. Nischt (2005). "Adam-9 expression and regulation in human skin melanoma and melanoma cell lines." Int J Cancer **116**(6): 853-9.