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SECURIN, CDC20 AND CDC27 IN PREDICTING THE OUTCOME OF BREAST CANCER

Henna Karra

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To my son Emil

ABSTRACT

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Securin, Cdc20 and Cdc27 in predicting the outcome of human breast cancer

Department of Pathology and Forensic Medicine, University of Turku, Turku, Finland (2015)

Deregulated proliferation has been recognized among the most important factors promoting breast cancer development and progression. The aim of the project is to gain understanding of the role of specific cell cycle regulators of metaphase-anaphase transition and evaluate their potential in breast cancer prognostication and treatment decisions. Metaphase-anaphase transition is triggered by activation of anaphase promoting complex (APC) which is activated by a cascade of regulatory proteins, among them securin, Cdc20 and Cdc27. These proteins promote the metaphase–anaphase transition and participate in the timely separation of the chromatids.

This study is based on a patient material of approximately 600 breast cancer patients and up to 22 years of follow-up. As the main observation, based on DNA cytometric and immunohistochemical methods, securin, Cdc20 and Cdc27 protein expressions were associated with abnormal DNA content and outcome of breast cancer. In the studied patient material, high securin expression alone and in combination with Cdc20 and Cdc27 predicted up to 9.8-fold odds for aneuploid DNA content in human breast cancer. In Kaplan–Meier analyses, high expression of securin systematically indicated decrease in breast cancer survival as compared to low expression cases. The adverse effect of high securin expression was further strengthened by combining it with Cdc20 or Cdc27 expressions, resulting in up to 6.8-fold risk of breast cancer death. High securin and Cdc20 expression was also associated with triple-negative breast cancer type with high statistical significance.

Securin, Cdc20 or Cdc27 have not previously been investigated in a clinically relevant large breast cancer patient material or in association with DNA ploidy. The present findings suggest that the studied proteins may serve as potential biomarkers for identification of aggressive course of disease and unfavourable outcome of human breast cancer, and that they may provide a future research aim for understanding abnormal proliferation in malignant disease.

Key words: securin, Pttg1, Cdc20, Cdc27, breast cancer, ploidy, prognosis

TIIVISTELMÄ

Henna Karra

Securin, Cdc20 ja Cdc27 rintasyövän ennusteen arvioinnissa

Patologian ja oikeuslääketieteen oppiaine, Biolääketieteen laitos, Turun yliopisto, Turku (2015)

Hallitsematonta solunjakautumista pidetään yhtenä tärkeimpänä rintasyövän syntyä ja etenemistä edistävänä tekijänä. Tässä tutkimuksessa pyrittiin keräämään lisätietoa solusyklin metafaasi-anafaasi-siirtymän säätelijöiden osuudesta ja merkityksestä rintasyövän ennusteen arvioinnissa potilaiden hoitopäätösten tueksi. Metafaasi-anafaasi-siirtymä käynnistyy solunjakautumisessa anaphase promoting complex (APC) –proteiinin aktivoituessa laajan säätelyverkoston toiminnan seurauksena. Tähän säätelyverkostoon kuuluvat myös tutkimuksen kohteena olleet proteiinit, securin, Cdc20 ja Cdc27, joiden toiminta osaltaan edistää kromatiinimateriaalin täsmällistä ja oikea-aikaista jakautumista tytärsoluihin.

Tämä tutkimus perustuu noin 600 rintasyöpäpotilaan aineistoon, joista oli käytettävissä seurantatietoa pisimmillään 22 vuoden ajalta. Tutkimuksen keskeisimpänä havaintona todettiin DNA-sytometriaan ja immunohistokemiallisiin menetelmiin perustuen securin-, Cdc20- ja Cdc27-proteiinien ilmentymän ennustavan korkeimmillaan 9,8-kertaista poikkeavan DNA-määrän todennäköisyyttä potilaan rintasyöpäsolukossa. Kaplan-Meier-eloonjäämistarkasteluissa securinin korkea ilmentyminen rintasyöpäsolukossa liittyi systemaattisesti lyhyempään elinaikaan kuin alhainen ilmentyminen. Yhdessä Cdc20- ja Cdc27-proteiinien kanssa securin ennusti korkeimmillaan 6,8-kertaista kuolleisuutta koko potilasaineistossa. Cdc20- ja securin- proteiinien korkean ekspression perusteella voitiin myös löytää kolmoisnegatiivisia rintasyöpätapauksia, jotka tällä hetkellä muodostavat rintasyövän hoidollisen haasteen.

Securin-, Cdc20- tai Cdc27-proteiinien kliinistä merkitystä ei ole aiemmin tutkittu laajassa merkittävässä rintasyöpäpotilasaineistossa tai yhteydessä DNA-ploiditeettiin. Tämän tutkimuksen havainnot antavat viitteitä tutkittujen proteiinien käyttömahdollisuuksista rintasyövän aggressiivisen taudinkulun ja epäedullisen ennusteen tunnistamisessa ja voivat avata lisätutkimuskohteen syöpäsolukon epänormaalin solunjakautumisen mekanismien ymmärtämiseen.

Avainsanat: securin, Pttg1, Cdc20, Cdc27, rintasyöpä, ploiditeetti, ennuste

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ABBREVIATIONS

AJCC	American Joint Committee of Cancer
APC	anaphase promotic complex
bFGF	basic fibroblast growth factor
BRCA	breast cancer gene
Cdc20	cell division cycle protein 20
Cdc27	cell division cycle protein 27
CDK	cyclin dependent kinase
5cER	5c exceeding rate
16cER	16c exceeding rate
CHD1	*CDC20* homologue 1
CI	confidence interval
CIN	chromosomal instability
CV	coefficient of variation
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
EGFR	epidermal growth factor receptor
HE	haematoxylin and eosin
HER2	human epidermal growth factor receptor 2
HR	hazard ratio
ICM	image cytometry
IHC	immunohistochemistry
IOD	integrated optical density
ISH	*in situ* hybridization
MYC	V-Myc Avian Myelocytomatosis Viral Oncogene Homolog
NF1	neurofibromatosis type 1
NGS	next generation sequencing
NIH	National Institutes of Health
OR	odds ratio
PBP	*pttg1* binding protein
PCNA	proliferating cell nuclear antigen
PTEN	phosphatase and tensin homolog

PTTG1	pituitary tumor-transforming gene 1
RT	room temperature
SAC	spindle assembly checkpoint
SD	standard deviation
SLNB	sentinel lymph node biopsy
SNP	single nucleotide polymorphisms (SNPs)
SPF	S-phase fraction
STK11	serine/threonine kinase 11
TMA	tissue microarray
TP53	tumor protein p53
TRBC	triple-negative breast carcinoma
UICC	Union for International Cancer Control

LIST OF ORIGINAL PUBLICATIONS

The study is based on the following original communications and unpublished results.

The publications are referred to in the text by roman numerals (I-IV).

I Talvinen K, Karra H, Hurme S, Nykänen M, Nieminen A, Anttinen J, Kuopio T, Kronqvist P. Securin promotes the identification of favourable outcome in invasive breast cancer. Br J Cancer 101:1005-1010, 2009.

II Karra H, Pitkänen R, Nykänen M, Talvinen K, Kuopio T, Söderström M, Kronqvist P. Securin predicts aneuploidy and survival in breast cancer. Histopathology 60:586-96, 2012.

III Talvinen K, Karra H, Pitkänen R, Ahonen I, Nykänen M, Lintunen M, Söderström M, Kuopio T, Kronqvist P. Low cdc27 and high securin expression predict short survival for breast cancer patients. APMIS 121:945-53, 2013.

IV Karra H, Repo H, Ahonen I, Löyttyniemi E, Pitkänen R, Lintunen M, Kuopio T, Söderström M, Kronqvist P. Cdc20 and securin overexpression predict short-term breast cancer survival. Br J Cancer 10:2905-13, 2014.

1. INTRODUCTION

Deregulated proliferation has been recognized as one of the most important factors promoting breast cancer development and progression (Hanahan and Weinberg 2011, Desmedt and Sotiriou 2006). Metaphase-anaphase transition during chromosome segregation, in turn, is one of the tightly controlled essential events in cell division (Nasmyth 2002). In proliferating cell, chromatin material is monitored through positive and negative feed-back mechanisms at numerous checkpoints which secure genetic stability in cell division (Molinari *et al.* 2000). One crucial step in this process is timing and control of sister chromatid separation at the spindle assembly checkpoint (SAC) with the aim to detain anaphase initiation until all sister chromatids are correctly attached to the mitotic spindle by their kinetochores (Kops *et al.* 2005). The key element of SAC is inhibition of anaphase promoting complex (APC), an ubiquitin ligase that targets several regulatory proteins, eg. securin and cyclin B1, for degradation when activated by cell division protein Cdc20 (cell-division cycle protein 20) (Peters 2006). With several other proteins, like Cdc27 (cell-division cycle protein 27), Mad2, separase and cohesins, they form a complex signalling network aiming to maintain chromosomal cohesion by blocking mitosis and holding up the mitotic spindle until sister chromatid segregation is complete (Musacchio and Salmon 2007). Disruption of this intrigue interaction can result in incorrect DNA content and structure and consequently influence the development, progression and behaviour of cancer.

The study concentrates on a cascade of regulatory proteins involving securin, cdc20 and cdc27, which have been identified with roles in metaphase-anaphase transition of cell division (Vlotides *et al.* 2007, Zhang *et al.* 2013b, Izawa and Pines 2011). The study addresses the associations of the studied proteins with abnormal DNA content and prognosis of the patient material suggesting that securin alone and enforced by cdc20 and cdc27 has potential to identify specific aggressive patient subgroups and predicts survival of breast cancer patients.

2. REVIEW OF THE LITERATURE

2.1 Review of breast cancer

Invasive breast cancer is the most common malignancy among Western women with annual incidence rising constantly since the 1950's. In high incidence areas, such as Finland, every 8th woman develops invasive breast cancer during her lifetime (www.cancer.fi). At the same time, development of clinical diagnostic and treatment options has resulted in steadily improving breast cancer survival rates. Still, breast cancer continues to be the leading cause of cancer death among women in Finland. (Table I) (www.cancerregistry.fi, Finnish Cancer Registry 2014, Pukkala *et al.* 2011).

Table I Summary of statistics on incidence, mortality, prevalence and survival of new breast cancer cases among Finnish women. The table presents most recent data on cases of invasive breast cancer and all female cancer cases (in brackets).

	Incidence 2014	Mortality 2014	Time after diagnosis	Prevalence 2014	Survival
New cases	4694 (14965)	882 (5629)	1 year	4555 (12261)	1-year 97% (80%)
			5 year	20934 (49869)	5-year 89% (66%)
Per 100 000#	91,4 (258,9)	14,0 (74,8)	10 year	35937 (82700)	
			Any	61947 (145316)	

* Data from: www.cancerregistry.fi and from Finnish Cancer Registry – Institute for Statistical and Epidemiological Cancer Research Cancer in Finland 2008 and 2009. Cancer Statistics of the National Institute for Health and Welfare (THL) Cancer Society of Finland. Publication No. 84, Helsinki 2011.
# Age-specific age-adjusted

2.1.1 Etiology of breast cancer

According to present understanding, breast cancer is a multifactorial disease inflicted by a complex network of environmental factors and genetic predisposition (Lang *et al.* 2015). The known environmental factors affecting the risk of breast cancer development include reproductive lifestyle, bodyweight, dietary factors, physical activity, and exogenous and endogenous sex hormones. The majority of the predisposing factors can be defined by the cumulative life-long exposure to estrogens (Lakhani *et al.* 2012, Shah *et al.* 2014). Increased risk of breast cancer is also associated with age, personal history of breast cancer, radiation, proliferative breast disease and family history (Shah *et al.* 2014).

Breast cancer development has been described as a consequence of accumulating sequential genetic alterations, including activation (e.g. amplification) of oncogenes, such as HER2 and EGFR (Lakhani *et al.* 2012, Epstein *et al.* 2010), or inactivation of tumor suppressor genes, such as TP53, CHD1, STK11, PTEN or NF1 (McCubrey *et al.* 2014, Shah *et al.*

2014, Steelman *et al.* 2008, van der Groep *et al.* 2011, Sharif *et al.* 2007). A considerable part (20-25%) of breast carcinomas show familiar distribution (Colditz *et al.* 2012) but only 5-10% of the cases can be demonstrated with an autosomal dominant inheritance (van der Groep *et al.* 2011, Margolin *et al.* 2006). Defects in two high-penetrance genes, BRCA1 and 2, have established their clinical value in assessing increased risk of breast cancer (Honrado *et al.* 2005, Honrado *et al.* 2006). This far, a large number of low-risk mutations have been identified with the help of genome-wide association studies (Lalloo and Evans 2012) and the multi-genetic nature of the disease is under intense investigation through several high-throughput technologies, especially next generation sequencing (NGS) methods, aiming at personalized diagnosis and treatment in breast cancer (Pu *et al.* 2014). However, the clinical value of these mutations remains to be determined.

2.1.2 Classification of breast cancer

Traditionally, breast carcinomas have been classified into histological types according to their morphological characteristics. The ductal and lobular types are the two most common histological types comprising 40-75% and 5-15% of all invasive breast cancer cases, respectively. The most recent breast cancer classification by WHO recognizes 18 different morphological types of invasive breast carcinoma, the majority of which are very rare (Lakhani *et al.* 2012).

More recently, gene expression profiling has provided a molecular basis for breast cancer classification (Perou *et al.* 2000). Hierarchical cluster analyses of genes have revealed new molecular categories of breast cancer, the most established being luminal A (high estrogen [ER] and progesterone receptor [PR] expression, sensitivity to antiestrogens, favourable prognosis), luminal B (low ER expression, negative PR, less responsive to antiestrogens, high proliferative rate in Ki-67, less favourable prognosis), HER2- (human epidermal growth factor receptor 2, ERBB2) enriched (ER and PR negative, HER2 oncogene-amplification, responsive to trastuzumab), and basal-like (ER and PR negative, no HER2 oncogene-amplification, partly responsive to chemotherapy, high relapse rate and unfavourable prognosis) (Sorlie *et al.* 2001, Sorlie *et al.* 2003, Sotiriou *et al.* 2009). Basal-like carcinoma has an approximately 80% overlap between triple negative breast carcinomas but also other molecular subtypes display triple negative phenotype (e.g. claudin-low and molecular apocrine cancers) (Sorlie *et al.* 2001, Sorlie *et al.* 2003). Recently, the molecular triple-negative category has been divided into six further subtypes (basal-like 1 and 2, immunomodulatory, mesenchymal, mesenchymal stem-like and luminal androgen receptor subtype) (Lehmann *et al.* 2011). Also, apocrine (Farmer *et al.* 2005), claudin-low (Prat *et al.* 2010) and interferon-rich (Hu *et al.* 2006) molecular subtypes have been identified. The lack of standardization and reproducibility of the different intrinsic subtypes has been addressed by the development of PAM50,

a 50-gene set which allocates cases of invasive carcinoma into luminal A, luminal B, HER2-enriched and basal-like subgroups (Parker *et al.* 2009).

The use of the molecular subclassification is grounded by the observed significant survival difference between the intrinsic subtypes (Sorlie *et al.* 2001, Sorlie *et al.* 2003) but limited by relatively high costs and availability of the necessary methodology. Therefore, the 12th St Gallen International Breast Cancer Conference (2011) Expert Panel adopted for practical purposes surrogate intrinsic subtypes which may be approximated based on the expression of ER and PR, and HER2-amplification. However, the St Gallen Expert Panel did not support incorporation of tests for cytokeratin 5/6 or epidermal growth factor receptor for the determination of 'basal-like' tumors. Although classification based on molecular signatures has somewhat improved risk prediction of breast carcinomas, the genomic alterations and therapeutic implications of these subtypes have yet not been fully established.

2.1.3 Treatment of breast cancer

Commonly adopted treatment decisions of breast cancer are based on a consensus between experts of breast surgery, oncoplastic surgery, oncology, radiology and histopathology in a clinical meeting. The standard treatments for breast cancer include surgery, anti-hormonal therapy, anti-HER2 therapy, radiation therapy and cytotoxic chemotherapy. Treatment for each individual patient is based on the established predictive and prognostic parameters of breast cancer, as presented later.

Breast conserving surgery is the preferred surgical treatment and mastectomy indicated only in case of multicentric tumour, inflammatory breast cancer or reasons related to inability to receive radiation therapy (Early Breast Cancer Trialists' Collaborative Group 2005, Kaufmann *et al.* 2010). Negative surgical margins are a hallmark for successful local control of breast cancer. However, the definition of a negative margin is under considerable debate (Houssami *et al*. 2010).

Assessment of the regional lymph node status is an essential part of breast cancer treatment. In the last decade, sentinel lymph node biopsy (SLNB) has for the most part replaced the conventional axillary lymph node evacuation. Presently, sentinel node biopsy is the standard of care for patients with clinically negative axilla. According to the 13th St Gallen (Switzerland) Conference on the Primary Therapy of Early Breast Cancer in 2011 (Goldhirsch *et al.* 2011) axillary dissection could safely be omitted in cases with one or two positive sentinel nodes following breast-conserving surgery when whole breast radiation therapy is planned.

The selection of patients for chemoendocrine therapy, for the major part, applies classification principles based on recent molecular pathological understanding of the disease (EBCTCG

2005, Reis-Filho and Pusztai 2011). The 13th St Gallen Conference summarized new treatment selection criteria for the chemoendocrine management of breast cancer (Goldhirsh *et al.* 2011). Despite increasing information on surrogate definitions of intrinsic subtypes defined by gene expression arrays and their respective systemic treatment modalities (Tables II and III) treatment decisions of systemic adjuvant therapy are still, for most part, based on biological prognostic factors, such as hormone receptor and HER2 status.

Presently, the main clinical challenges in breast cancer treatment are, on one hand, the lack of individualized prognosticators for screen-detected early breast cancer patients and, on the other hand, targeted therapies for patients with the aggressive triple-negative breast cancer, ER-, PR- and HER2-negative (O'Toole *et al.* 2013). Despite the heterogeneous nature of breast cancer at the molecular level, the repertoires of systemic therapies available for breast cancer patients are defined on the basis of hormone receptors and HER2 status. For patients with triple-negative disease, the only systemic therapy currently available is chemotherapy. In order to develop efficient and reliable personalized breast cancer prognostication, prediction and treatment methods extensive research involving evidence from transitional studies are conducted.

Table II Surrogate definitions of intrinsic subtypes of breast cancer.*

Subtype	Clinicopathologic definition
Luminal A	ER and PR +
	HER2 –
	Ki-67 low#
	Multi-gene-expression indicates low risk of recurrence¤
Luminal B	ER +
	HER2 –
	and at least one of:
	Ki-67 high#
	PR – or low
	Multi-gene-expression indicates high risk of recurrence¤
	or
	ER +
	HER2 amplified
	Any Ki-67
	Any PR
HER2-enriched	HER2 amplified
	ER and PR –
"Basal-like"	ER and PR –
	HER2 –

* Modified from Goldhirsch *et al*. 2013.

The cut-point between high and low Ki-67 varies between laboratories. A level of <14% best correlates with the gene-expression based definition of Luminal A breast cancer.

¤ Although neither the 21-gene recurrence score nor the 70-gene signature was designed to define intrinsic subtypes, research has indicated over 90% and 80% concordance with the test, respectively.

Table III Systemic treatment recommendations.*

Subtype	Therapy
Luminal A-like	Endocrine therapy
	Cytotoxic therapy for selected patients
Luminal B-like – HER2 negative	Endocrine therapy for all patients
	Cytotoxic therapy for most patients
Luminal B-like – HER2 positive	Cytotoxic + HER2 + endocrine therapies
HER2 positive	Cytotoxic + endocrine therapies
Triple-negative	Cytotoxic therapy
Special histological types	
Endocrine responsive	Endocrine therapy
Endocrine non-responsive	Cytotoxic therapy

* Modified from Goldhirsch *et al*. 2013.

2.1.4 Prognostic factors in breast cancer

Despite the ongoing advances in identifying molecular markers and genetic alterations in breast cancer, in routine clinical work breast cancer treatment decisions are based on clinical information and on the established prognosticators of breast cancer. These include clinicopathological parameters, such as axillary lymph node status, tumor size, stage, histological grade and type. In addition, ER, PR and HER2 status, and proliferation activity expressed as Ki-67 index are routinely assessed for each individual tumor (Cuzick *et al.* 2011).

2.1.4.1 Clinical prognostic factors

Axillary lymph node status remains to be the most important single prognostic factor for breast carcinoma, the disease-specific survival decreasing with the increasing number of positive nodes (Marty *et al.* 2008). Positive lymph nodes can be seen as a marker for distant dissemination out of reach for surgical treatment and, therefore, a major adverse prognostic feature (Giuliano *et al.* 2011). SLNB has established its value in reducing breast cancer morbidity and acts as the main criteria in treatment decisions for surgical and oncological therapies (Ashikaga *et al.* 2010). Although negative lymph node status is a sign of very favorable prognosis in breast cancer, 10-30% of patients still develop distant metastases. Particularly, basal-like carcinomas may show an aggressive course of disease without nodal involvement (Weichmann *et al.* 2009). Lymphovascular invasion is also an adverse prognostic feature and in combination of nodal metastasis predicts a worse outcome that either features alone (Lee *et al.* 2010).

Axillary lymph node status and tumor size (Angele *et al.* 2004) are among the most powerful established prognostic factors in breast cancer and part of the TNM staging system, the gold standard of breast cancer prognostication managed by the American

Joint Committee of Cancer (AJCC)/Union for International Cancer Control (UICC) (Edge and Byrd 2010). Especially, the combination of node-negativity and tumor diameter smaller than 1.0 cm indicated a 10-year disease-free survival rate of about 90% (Seidman *et al.* 1995, Fitzgibbons *et al.* 1999).

Young age appears to be an independent risk factor for breast cancer recurrence and death. Also, recent reports suggest that probabilities for distant disease spread are higher for women aged 25-39 (Johnsson *et al.* 2013). Partly, the more sinister course of disease among young breast cancer patients may be explained by delays in diagnosis, but recent research also suggests that young women are more likely to develop more aggressive subtypes of breast cancer with unique biologic features (Johnsson *et al.* 2013, Freedman and Partridge 2013). The influence of pregnancy and lactation on breast cancer prognosis appears to be controversial (Krishna and Lindsay 2013). There is increasing evidence that unfavorable prognosis may also be related to postmenopausal status, especially among women with overweight and excessive alcohol consumption (Rodenhiser *et al.* 2011). In summary, age and pregnancy may independently influence breast cancer outcomes, although the effects seem to vary within tumor subtypes (Arvold *et al.* 2011).

2.1.4.2 Histopathological prognostic factors

Invasive breast carcinomas are routinely graded based on assessment of the extent of tubule formation and nuclear atypia, and mitotic count (Elston and Ellis 1991, Lakhani *et al.* 2012). Clear association of histological grade with breast cancer survival has been reported in many studies (Henson *et al.* 1991). Poorer survival related to high-grade cases has been verified independent of eg. lymph node status and tumor size (Lee *et al.* 2010, Manie *et al.* 2009, Marty *et al.* 2008). Elston and Ellis (1991) have attempted to improve the objectivity and prognostic power of the histological grading by implicating semi-quantitative criteria for histological grade, tumor size and axillary lymph node status which are included in the classification Nottingham Prognostic Index (Blamey *et al.* 2007).

Hormone receptor expression plays a crucial role in the development and behavior of breast cancer and is part of the standard prognostication regime of invasive breast cancer (Hammond *et al.* 2010). Approximately 80% of breast carcinomas show ER expression and 60-70% PR expression, higher expression correlating with high cellular differentiation (Harvey *et al.* 1999, Anderson 2002). In estrogen-sensitive tissues, ER and PR expressions are interrelated in the way that estrogen treatment induces PR while PR, in turn, surrogates for functionally active ER. Estrogen is a nuclear transcription factor that through activation of the hormone receptor acts as a growth factor(s) mediating proliferation stimulus to the cell nucleus (Clarke 2003). ER is a strong predictor of response to adjuvant hormone therapies (Sullivan *et al.* 2005) and independent

predictors of favorable outcome of the disease (Bundred 2001, Elston and Ellis 1991). Gene expression profiling studies have demonstrated that ER-positive and ER-negative breast cancers are fundamentally different diseases, with distinct risk factors, clinical presentation, natural history and response to therapy (Reis-Filho *et al.* 2011, Gruvberger *et al.* 2001, Weigelt *et al.* 2010).

The prognostic value of HER2 overexpression was first reported in 1987 and has been, thereafter, extensively investigated (Epstein *et al.* 2010). The HER2 gene is located on chromosome 17 and encodes a growth factor receptor on the surface of breast epithelial cells. HER2 gene is amplified in approximately 15% of breast carcinomas and results in increased protein expression. (Epstein *et al*. 2010). HER2 overexpression seems to be a weak to moderate independent predictor of breast cancer survival, but the importance of HER2 status lies in response of HER2 overexpressing invasive breast cancer to targeted treatment, such as the HER2-directed therapy (Bedard *et al.* 2009).

2.1.4.3 Proliferation as a prognostic factor

Proliferative activity is one of the most fundamental biological processes and has been recognized among the most important factors influencing breast cancer development and prognosis. Markers for proliferation have been extensively investigated in breast cancer and increased proliferation has been strongly associated with unfavorable outcome of disease, as evaluated by any detection method (Stuart-Harris *et al.* 2008, Baak *et al.* 2009, Desmedt and Sotiriou 2006, van Diest *et al.* 2004). Also, several other routine clinical prognostic factors for breast cancer are directly or indirectly related to proliferation. In addition, several multi-gene assays have provided prognostic information on proliferation-related genes (Wirapati *et al.* 2008). The role of individual genes participating in cell proliferation is under extensive investigations but, still, proliferation itself has remained clinically more important prognostic factor in invasive breast cancer (van Diest *et al*. 2004).

Mitotic count and immunohistochemical proliferation marker Ki-67/MIB-1 are in routine clinical use for breast cancer prognostication (Goldhirsch *et al*. 2011). Mitotic index is an accurate means of estimating tumor cell proliferation and included in the histologic grading system (Clayton 1991). Ki-67/MIB-1 is a non-histone nuclear protein expressed in all phases of the cell cycle except for the resting cells in G0 (Gerdes *et al*. 1984, Brown and Gatter 2002). Based on this expression profile, Ki-67 is used in histopathological diagnostic and prognostic evaluations to identify the fraction of dividing cells. Ki-67 is a predictor for high tumor grade and unfavorable outcome in malignant diseases, including breast cancer (Colozza *et al.* 2005, Stuart-Harris *et al.* 2008, Yerushalmi *et al*. 2010). Moreover, recent literature suggests that immunohistochemical analysis of ER, PR, HER2 and Ki-67 expression may provide equally reliable prognostic information as the multi-

gene signatures based on expression of proliferation-related genes (Cuzick *et al.* 2011). Several studies have also provided evidence that the combined immunohistochemical expression of ER and Ki-67 predicts response to neoadjuvant chemotherapy and strongly correlates with the outcome of breast cancer patients treated with conventional multi-drug chemotherapy regimens (Dowsett *et al.* 2011). At the same time, there have been concerns about the lacking standardization among laboratories and the varying intra- and inter-observer reproducibilities in assessing Ki-67 immunohistochemistry (Dowsett *et al*. 2011, Polley *et al.* 2013, Varga *et al.* 2012). Despite the prognostic utility, routine use of Ki-67 is not recommended e.g. by consensus guideline panel of American Society of Clinical Oncology, mainly because of concerns on analytic validity (Harris *et al.* 2006). An international working group has been assembled to agree the development and validation of Ki-67 as a prognostic and predictive marker for breast cancer patients (Dowsett *et al.* 2011).

2.1.4.4 Chromosomal instability and aneuploidy as prognostic factors

Correctly replicating and dividing the genetic material during cell proliferation is a tightly guarded fundamental cellular process, often distracted in malignancy. High rate of genetic alterations (genomic instability) is a common feature in malignant cells and can be present at a DNA sequence level, e.g. in microsatellite instability, or at the whole karyotype level leading to abnormal DNA content and aneuploidy. (Abbas *et al.* 2013, Draviam *et al.* 2004) The term CIN (chromosomal instability) is used to describe a phenotype in which cell division leads to abnormally high rate of chromosome loses or gains causing karyotypic heterogeneity between tumor cells (Geigl *et al.* 2008). Abnormal chromosome content (aneuploidy), often as a consequence of CIN (Thompson and Compton 2008), is a consistent character of human solid tumors and has been suggested to have properties to both promote and suppress tumor formation. The mechanisms of aneuploidy contributing to tumor progression, however, seem to be complex and not completely understood. (Kops *et al.* 2005, Duesberg *et al.* 2006, Weaver and Cleveland 2009, Silk *et al.* 2013, Holland and Cleveland 2009, Siegel and Amon 2012) The cause of CIN is postulated to be defects in the mechanisms that control chromosome segregation during mitosis (Kops *et al.* 2005, Holland and Cleveland. 2009, Draviam *et al.* 2004) and many chromosome segregation genes have shown mutated in human cancer (Draviam *et al.* 2004). It has been postulated that aneuploidy could be a consequence of CIN-promoting mutations and that dysregulation of the mitotic machinery could result in aneuploidy and cancer formation (Nowak *et al.* 2002, Duesberg *et al.* 2006). CIN is associated with poor prognosis in solid tumors (Carter *et al.* 2006), possibly reflecting increased genetic diversity and more efficient cell evolution leading to e.g. drug-resistance and adaptation to environmental stress than possible for the chromosomally stable cells (Duesberg *et al.* 2000, McClelland *et al.* 2009, Bakhoum and Compton 2012).

Aneuploidy is also a common feature in breast carcinoma but according to literature the results on association with behavior and outcome of invasive breast cancer have been contradictory (Ross *et al.* 2003). There is, however, clear indications on the prognostic significance of DNA ploidy in breast cancer (Li *et al.* 2008, Yanagawa *et al.* 2012, Yildrim-Assaf *et al.* 2007, Moureau-Zabotto *et al.* 2005). Also CIN status of breast carcinoma has been shown to be a determinant of breast cancer prognosis and capable to stratify patients in different risk groups (Szasz *et al.* 2013, Habermann *et al.* 2009). Measurements of DNA ploidy, however, are rarely used in clinical practice owing to the technical difficulties and lack of clear therapeutic implications.

2.1.4.5 Expression profiling as a prognostic factor in breast cancer

Extensive research efforts are directed towards detecting specific gene alterations or changes in the expression of genes or proteins in the tumor cells in order to achieve personalized prognostication and treatment options for breast cancer patients. As a common feature, many of the newly detected associations lack clinical significance, or those with significant prognostic value function through already known biomarkers, such as proliferation markers. Therefore, few analyses have been able to provide information beyond that already in clinical use in treatment of breast cancer.

Some approaches of high-throughput technologies are, however, promising for prognostic purposes. Genome-wide analyses may detect new, previously unknown prognostic and predictive factors of the disease (The Cancer Genome Atlas Network 2012, Stevens *et al.* 2011, Ellis *et al.* 2012). Micro-array analyses, in turn, have proven applicable for forecasting the outcome and to some extent treatment response of known gene expression changes. Expression signatures or "multigene predictors" are already in use to classify breast cancer on basis of information on expression levels achieved from multiple genes simultaneously (Reis-Filho and Pusztai 2011, Sotiriou and Pusztai 2009, Kim and Paik 2010, Sorlie *et al.* 2003, van't Veer *et al.* 2002, Paik *et al.* 2006, Wang *et al.* 2005, Filipits *et al.* 2011, van de Vijver *et al.* 2002). The so-called first generation signatures are capable of providing significant prognostic information, but their prognostic and predictive implications largely stem from the expression levels of proliferation-related genes. Also, the levels of expression of proliferation-related genes are only prognostic in ER-positive breast cancers, hence, their usefulness in ER-negative disease is minimal (Reis-Filho and Pusztai 2011, Wirapati *et al.* 2008). A single prognostic signature for all breast cancers is unlikely to be clinically useful and the second line of prognostic signatures for subgroups of breast cancer is emerging (Reis-Filho and Pusztai 2011). Several commercial multi-gene molecular assays deriving prognostic information also from proliferation-related genes, such as PAM50 (Parker *et al.* 2009), 70-gene signature (Drukker *et al.* 2013) and 21-gene recurrence

score (Paik *et al.* 2006), have gained a role in breast cancer classification in some institutes.

2.2 Review of the cell cycle and cell cycle control

Proliferation is a fundamental cellular phenomenon governed by a tightly regulated complex mechanism called the cell cycle. Cell cycle is an ordered series of events where cyclic production and degradation of regulatory proteins drives cell through multiple steps leading to replication of genome and, eventually, to production of two genetically identical daughter cells. Breaking free from the cell cycle control and exhibiting independent cell proliferation without regulation from internal or external stimulus is one of the hallmarks of cancer and prerequisites for cancer development (Hanahan and Weinberg 2011).

2.2.1 Cell cycle

Cell cycle is composed of two molecular processes, parental chromosome duplication to form two identical sister chromatids (synthesis, S phase) and distribution of the sister chromatids into each daughter cell (mitosis, M phase). These phases are interrupted with intervals (resting, G1 and G2 phases) and end in the quiescent (G0) stage. When cells in G1 phase have passed the specific initiation point they are committed to division without possibility to exit the cycle. Unless chromosome replication and segregation in daughter cells occurs according to the exact proper order and timing, the cell division will result in lost or incorrect genetic information or cell death (Nurse 2002, Murray 2004, Sanchez and Dynlacht 2005).

Mitosis is divided in multiple stages. First, during prophase, microtubules form the mitotic spindle, and chromatin condenses into chromosomes, each having two sister chromatids. In prometaphase, the nuclear envelope breaks down and chromosomes attach to their kinetochores through the microtubule framework of the mitotic spindle. At metaphase, the chromosomes align on an imaginary plane at the equator between the spindle poles and the kinetochore microtubules attach sister chromatids to opposite poles of the spindle. At the beginning of the anaphase, the cohesion between sister chromatids is released and they are synchronously separated from each other and rapidly travel to the opposite poles of the mitotic spindle. The kinetochore microtubules shorten and the spindle poles move apart as part of chromosome separation. During telophase, the last stage of mitosis, the actions of prophase and prometaphase are reversed, and finally after mitosis, cytokinesis follows (Musacchio and Salmon 2007, Nasmyth 2002, Peters 2006) (Fig 1).

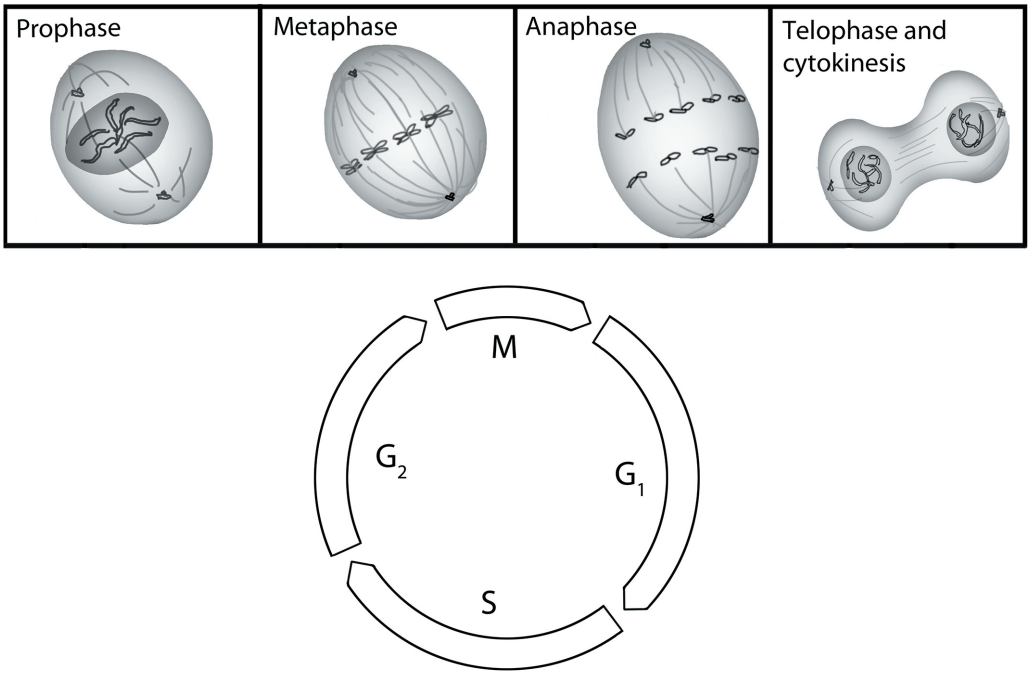

Figure 1. The cell cycle and phases of mitosis.

2.2.2 Cell cycle control

In normal dividing cells, high fidelity of DNA replication is achieved by a network of control mechanisms which ensure that the previous phase of cell cycle is fully completed before the next phase can proceed or, when necessary, can arrest the cell cycle at any specific cell-cycle checkpoint. Particularly, the DNA damage checkpoints control that the whole genome has been correctly replicated before entry into the M-phase (Harrison and Haber 2006), and the spindle assembly checkpoint (SAC) controls anaphase promoting complex (APC), the release of sister chromatids during metaphase and the exit from the mitosis (Musacchio and Salmon 2007, Murray 2011). Mutations that transform or inactivate the normal function of these pathways result in chromosomal rearrangements, abnormal chromosome content and gene expression changes contributing to malignant transformation (Kastan and Bartek 2004). For example, *BRCA1* and *2*, among their many roles in malignant transformation, have been suggested to participate in several cell-cycle checkpoints where their influence as tumor suppressors could be explained by their role in controlling chromosome duplication and segregation across the cell cycle (Venkitaraman 2014).

The path through the cell cycle is controlled by heterodimeric protein kinases comprising of catalytic and a regulatory subunits. The catalytic subunits, cyclin-dependent kinases

(CDKs), are a family of small (30-40kD) serine/threonine kinases, which are not active in the monomeric form. Bound to their activating regulatory cyclin subunit the CDKs, however, drive the whole process of cell division and act in each of the important transition points of cell cycle. Each CDK can function with a set of different cyclins that determine the proteins phosphorylated. The cyclins, on the other hand, are phase-specific, i.e. they can only present and function during specific phages of the cell cycle. Once activated, the mitotic CDKs, for example, drive the cell into mitosis by phosphorylating and activating hundreds of proteins to promote chromosome segregation and other events in mitosis (Nurse 2002, Murray 2004, Sanchez and Dynlacht 2005). Equally, cell-cycle control is dependent on the timely proteolysis of cyclins by an ubiquitin-dependent mechanism. In M phase, APC is responsible for the ubiquinylation and proteolysis of M-cyclins and other regulators of M-phase (Peters 2006). In all, a complex network of regulatory proteins function as the cell-cycle control system involving both the specific checkpoint pathways and an additional layer of regulation through proteins responding to various signals from both inside and outside the cell, ultimately, eliminating the unwanted cells by apoptosis.

2.2.2.1 Spindle assembly checkpoint

Cells usual spend about half of M phase in metaphase waiting for the signal to release sister chromatid separation and initiation of anaphase. During this period, the attachment of the chromosomes to the mitotic spindle is maintained by regulation of SAC (Musacchio and Salmon 2007, Kim and Yu 2011, Murray 2011). SAC functions by monitoring the state of the spindle so that any kinetochore that is not properly attached sends out a negative signal delaying entry to anaphase. The mechanisms of the signal generated by an unattached kinetochore are not clear although several proteins, like Mad2, have been suggested to participate in this regulation (Moyle *et al.* 2014). In mammalian cells, inactivation of SAC has shown to cause premature anaphase and result in genetic defects (Kops *et al.* 2005, Weaver and Cleveland 2006). Anti-mitotic cancer drugs, such as colchicine or cinblastine, that destabilize microtubules, trigger SAC leading to mitotic arrest in checkpoint-deficient cancer (Gascoigne and Taylor 2009, Weaver and Cleveland 2006).

2.2.2.2 Anaphase promoting complex

After SAC activation, anaphase begins abruptly by release of the cohesion linkage that holds the sister chromatids together at the metaphase plate. This metaphase-anaphase transition is triggered by activation of APC, also called the cyclosome or APC/C. Human APC is a highly regulated complex composed of 14 distinct proteins composing a complex of at least 19 subunits with a total molecular mass of ~1.2MD (Zhang *et al.* 2013b). Functionally, APC is an ubiquitin ligase that promotes destruction of several

mitotic regulatory proteins. The activated APC, first, cleaves and inactivates the M-phase cyclin and, next, cleaves the inhibitory protein, securin. Activation of securin and inactivation of M-phase cyclin results in a sequence of events where a protease called separase cleaves a subunit of cohesion complex to release the sister chromatids which, in an instant, separate and move to opposite poles. (Harper *et al.* 2002, Peters 2006).

The activation of APC requires a catalytic protein, which also directs the substrate specifity of APC complex. Cell division cycle 20 protein (Cdc20) catalyzes the metaphase-anaphase transition, and after anaphase APC swiftly switches to catalytic subunit Cdh1, leadin to mitotic exit. (Peters 2006). According to present knowledge, as the first phase of the APC activation, the synthesis of Cdc20 increases due to increased transcription of the gene. Next, phosphorylation of APC helps Cdc20 to bind and create an APC-Cdc20 complex. Activation of the APC–Cdc20 complex and degradation of securin and cyclin B1 then mark anaphase onset in normally regulated cell division (Schwab *et al.* 1997, Visintin *et al.* 1997, Irniger 2002). Cell division cycle 27 protein (cdc27) as a member of the APC-complex and numerous checkpoint proteins, such as Mad1, Mad2, BubR1, Bub1, Bub3, Mps1 and AuroraB form a complex signaling network that is suggested to regulate APC-Cdc20 although exact interactions and functions of the proteins are not completely resolved (Musacchio and Salmon 2007, Kim and Yu 2011). As literature has pointed out, there is a significant delay between M-Cdk activation and activation of the APC-Cdc20 complex, and that this is the phase where SAC takes it´s actions. The as yet unrevealed regulatory mechanisms taking part in suspending anaphase during this delay may eventually explain how anaphase is exactly regulated (Fig. 2).

2.3 Review of securin, Cdc20 and Cdc27

2.3.1 Securin

Human securin, a protein encoded by pituitary tumor transforming gene (*Pttg1*), is a multifunctional protein originally identified from rat pituitary cell lines (Pei and Melmed 1997). The human homolog was identified at the same time by different groups (Dominiguez *et al*. 1998, Kakar and Jennes 1999). In adult human tissue, abundant *Pttg1* mRNA expression has been seen in testis and thymus. Weaker expression has been observed in colon, small intestine, placenta, spleen, brain, pancreas, breast and lung, while in tissues like heart, liver, skeletal muscle, kidney or ovary, no expression has been reported (Zhang *et al.* 1999, Vlotides *et al.* 2007). *Pttg1* mRNA expression as well as protein expression levels are cell cycle dependent, peaking at the S-G2 transition (Zou *et al.* 1999, Vlotides *et al.* 2007, Yu *et al.* 2000a). Although transcription factors like Sp1 and nuclear factor Y regulate the transcriptional activity of the *Pttg1* promoter (Clem *et*

al. 2003, Zhou *et al.* 2003) and DNA damage induces p53-mediated inhibition of *Pttg1* transcription (Zhou *et al.* 2003), the mechanisms controlling the normal variation of securin transcription in proliferating cells and the pattern of dysregulation in transformed cells remain still largely unclear (Zhou *et al.* 2005, Hlubek *et al.* 2006).

Human securin localizes both in the cell cytoplasm and nucleus, although the ratio and distribution of the localization during the cell cycle are not settled (Zhang *et al.* 1999, Dominiguez *et al.* 1998, Saez *et al.* 1999, Yu *et al.* 2000b, Chien and Pei 2000, Stratford *et al.* 2005, Mu *et al.* 2003). Partly, the reported differences may be explained to different cellular systems or techniques used in the studies. Securin interacts with proteins including PTTG1 binding factor (PBF) that facilitates nuclear localization and plays an important role in the transcriptional activity of securin (Chien and Pei 2000). Thus, the localization of securin might also reflect the amount and activity of PBF (Chien and Pei 2000, Stratford *et al*. 2005).

2.3.1.1 Securin in cell division

During most of the cell cycle securin binds separase preventing its proteolytic activity and thus inhibiting anaphase (Waizenegger *et al.* 2002). In this purpose, securin participates in preventing cell cycle progression in metaphase-anaphase transition (Fig. 2). Inhibition or overexpression of securin blocks sister chromatid separation and results in cell cycle dysregulation (Peters 2002). Thus, securin is one of the important regulators to ensure that chromosome segregation is complete before sister chromatid separation. When APC catalyzed by Cdc20, targets degradation of securin, separase is liberated to mediate degradation of the cohesin complex, and to release the chromatids in the beginning of anaphase (Zou *et al.* 1999, Zur and Brandeis 2002, Nasmyth 2002). During metaphase, replicated sister chromatids are held together by the cohesin complex, a multisubunit complex including proteins Smc1, Smc3, Scc1 and Scc3 (Tanaka *et al.* 2000). In mammalian cells, the separation of chromosomes during mitosis is a two-step process where Scc3 subunits are first phosphorylated by Polo-like kinase PLK1 at prophase (Sumara *et al.* 2000, Hauf *et al.* 2005) and later, at the onset of anaphase, Scc1 is cleaved by separase (Uhlmann *et al.* 1999). Securin is, thus, degraded at the end of metaphase to allow properly timed sister chromatid separation (Zou *et al.* 1999). In addition, an anaphase-promoting role for securin has been described based on observations that securin is necessary for proper localization of separase during mitosis. Furthermore, separase activation is dependent on its prior interaction with securin (Zur and Brandeis 2002, Kumada *et al.* 1998, Jallepalli *et al.* 2001, Jensen *et al.* 2001).

Also a securin independent mechanism to control sister chromatid separation has been suggested. This theory has been based on the observation that cultured human cells

and mice survive without securin (Mei *et al.* 2001, Wang *et al.* 2001). One explaining mechanism elaborates the phosphorylation of separase and the subsequent association with CDK1-cyclin B1, which appears to be sufficient for separase inhibition in X laevis egg extracts (Stemmann *et al.* 2001, Gorr *et al.* 2005). Thus, APC/C-cdc20 might contribute to separase activation also by ubiquitylating cyclin B1 and not only by targeting securin for degradation. Additional evidence has been provided by embryonic fibroblasts from Pttg1-null mice, which show shortened G, prolonged G2-M phases, and abnormal nuclear and chromosome morphology although the mice are viable and fertile with tissue-specific defects (Wang *et al.* 2001). An *in vitro* study on human colorectal cancer HCT116 cells showed that knockout of hPttg1, initially, resulted in a persisting reduction of separase and inefficient cleavage of the cohesin subunit Scc1. After a few passages, however, hPttg1 knockout cells became chromosomally stable and executed normal mitoses (Pfleghaar *et al.* 2005). Based on these findings, it appears that properly functioning securin is not mandatory for cell division although it may be critical for the integrity of the genome.

2.3.1.2 Other known functions of securin

Several possible functions for securin both in normal and diseased cells have been described in the literature. In addition to its function as separase inhibitor, securin has been suggested with a role in regulating the G1/S transition by acting together with Sp1 as a transcription factor to localize the cyclin D3 promoter and driving cells towards S-phase (Tong *et al.* 2008). Furthermore, securin has been suggested to function in regulation of DNA damage checkpoint by blocking cell cycle progression while DNA damage repair takes place and, consequently, participating in maintaining chromosomal integrity (Romero *et al.* 2001).

In vitro experiments in cell cultures have suggested that securin may also regulate apoptosis. The findings are, however, controversial. Securin-overexpression has been shown to promote apoptosis both in p53 dependent and independent manner *in vitro* (Yu *et al.* 2000a, b and Hamid *et al.* 2005). On the other hand, both *in vitro* and *in vivo* experiments have suggested that securin may interact with p53 in preventing its pro-apoptotic function, thus proposing a tumorigenic role for securin (Bernal *et al.* 2002, Cho-Rok *et al.* 2006).

There are implications on securin having a role in the gene transcription regulation (Vlotides *et al.* 2007). The findings implicate for securin both a direct transcriptional activity and a role in inducing the expression of genes such as mitogenic and angiogenic fibroblast growth factor FGF-2 and c-myc oncoprotein (Tong *et al.* 2011, Boelaert *et al.* 2004, Pei *et al.* 2001).

Transfection experiments have also shed conflicting light on the role of securin in the cell proliferation since there are, on one hand, studies demonstrating that securin overexpression promotes proliferation (Pei 2001, Hamid *et al.* 2005, Heaney *et al.* 2002) and, on the other hand, that securin inhibits proliferation and accumulates cells in the G2/M phase (Mu *et al.* 2003, Yu *et al.* 2000a, b, Bernal *et al.* 2002). The results on the inhibitory functions of securin might well reflect the physiological role of securin as an anaphase inhibitor. Any definitive conclusions from the results are, however, hampered by the experiments applying several different cell lines and inconsistent study settings with varying securin expressions achieved in transfection. Also, the phosphorylation status of securin influences the cell proliferation, which may have caused the bias in the interpretation of the results, as one transfection study showed phosphorylated form to decrease and non-phosphorylated form to increase proliferation compared to wild-type securin expression (Boelaert *et al.* 2004).

2.3.1.3 Securin in cancer

While the complete role of securin in diseased cells is still not settled, multifactorial effects on proliferation, aneuploidy, apoptosis, tumour cell transformation, microenvironment regulation and DNA repair have been suggested (Tfelt-Hansen *et al.* 2006, Vlotides *et al.* 2007, Salehi *et al.* 2008). The ability of securin to transform cells has been shown both in *in vitro* and *in vivo* experiments. There are implications that an important mechanism for securin overexpression to mediate the transforming effect is through induction of chromosomal instability and aneuploidy (Yu *et al.* 2000a, b, Jallepalli *et al.* 2001). In previous literature, securin has been detected in human malignancies originating from the hematopoietic system (Dominguez *et al.* 1998), lung (Zhang *et al.* 1999), kidney (Ai *et al.* 2004), pancreas (Zhang *et al*. 2008), ovary (Panguluri *et al*. 2008, El-Naggar *et al*. 2007, Chen *et al.* 2004), esophagus (Zhang *et al.* 2013a, Yan *et al*. 2009, Zhou *et al.* 2005, Shibata *et al.* 2002), colorectum (Zhou *et al.* 2014) and prostate (Huang *et al.* 2012, Huang *et al.* 2014). Securin overexpression has been reported to promote genetic instability in human cell lines (Yu *et al.* 2000b, Christopoulou *et al.* 2003, Yu *et al.* 2003) as well as in thyroid (Kim *et al.* 2005) and colorectal (Kim *et al.* 2007b) carcinomas. Both overexpression and the lack of securin have been suggested to compromise chromosomal stability (Jallepalli *et al*. 2001, Bernal *et al.* 2002, Yu *et al.* 2003, Kim *et al.* 2005, 2007a). Recently, a single mutation in securin gene was shown to induce chromosomal instability (CIN) (Mora-Santos *et al.* 2013). Furthermore, there are studies suggesting that securin regulates the tumor microenviroment by induction of angiogenesis (Vlotides *et al.* 2007).

Based on its observed functions as, first, a regulator of cell division and initiation of genetic instability and, secondly, as a transactivator of growth factors, securin appears a potential initiator and promoter of tumorigenesis. Securin overexpression has been associated

with invasion, spread and metastasis of cancer cells, and the course of disease in several malignancies. Previously, the prognostic value of securin has reported for oesophageal (Shibata *et al.* 2002, Ito *et al.* 2008), thyroid (Saez *et al.* 2006), hepatocellular (Fujii *et al.* 2006), lung (Rehfeld *et al.* 2006) and colorectal (Talvinen *et al.* 2006) carcinomas, malignant melanoma (Winnepenninckx *et al.* 2006) and glioma (Genkai *et al.* 2006). There are, however, indications that, e.g. in squamous cell carcinoma, securin expression has no prognostic value or may even be a marker for favorable outcome (Ishitsuka *et al.* 2013, Rehfeld *et al.* 2006, Mu *et al.* 2003). More recently, evidence has accumulated that abnormally expressed securin may also affect response to chemotherapeutic treatments for example in prostate cancer (Castilla *et al.* 2014).

2.3.1.4 Securin in breast cancer

According to literature, Pttg1 has been suggested with oncogenic properties (Ramaswamy *et al.* 2003, Hunter *et al.* 2002, Abbud *et al.* 2005, Chesnokova *et al.* 2005). Recently, it has also been suggested that Pttg1 is required for morphogenesis of the mammary gland in mice and that in the absence of Pttg1, the mammary gland epithelial cells displayed an altered gene expression profile which lead to increased proliferation in mammary epithelial cells (Hatcher *et al.* 2014). Consistent with the observed developmental defects, Pttg1-null female mice developed spontaneous mammary gland tumors. The authors also found a significant correlation between the PTTG1 levels and the degree of malignancy in human breast tumors, indicating that PTTG1 might be a tumor suppressor in the mammary gland (Hatcher *et al.* 2014).

Earlier, overexpression of securin has been reported in human malignant mammary tumour cell lines and tissues (Kakar and Jennes 1999, Thompson and Kakar 2005, Solbach *et al.* 2004, Ogbagabriel *et al.* 2005). A number of studies have also demonstrated a correlation between securin expression and metastatic spread as well as disease recurrence in breast cancer (Solbach *et al.* 2004, Ogbagabriel *et al.* 2005). Furthermore, high mitotic index and pleomorphic phenotype has been associated to securin overexpression in breast cancer tissue (Solbach *et al.* 2004, Ogbagabriel *et al.* 2005). In a report on circulating tumor cells, securin mRNA was detected from blood of breast cancer patients (Chen *et al.* 2006). Hormone stimulus has been observed to increase the expression level of MAD2 and securin in association with aneuploidy in p53 null mammary cells (Pati *et al.* 2004). Recent evidence has also suggested that radiation therapy might induce senescence in human breast cancer cells with low securin expression (Tong *et al.* 2011, Liao *et al.* 2014). Moreover, Pttg1 expression has been associated with aggressivity, lymph node infiltration, and distant metastases in breast cancer suggesting that immunohistochemical securin expression might be a powerful biomarker of predicting breast cancer outcome and a potential target for therapeutic strategy for primary and metastatic breast cancer (Grizzi *et al.* 2013).

2.3.2 Cdc20

Human cell-division cycle protein 20 (Cdc20), a homolog of Saccharomyces cerevisiae cell division cycle 20 protein, is an essential regulator of cell division activating the APC (Weinstein *et al.* 1994, Weinstein 1997). Up to date, no thorough reports on the expression pattern of Cdc20 in normal human tissues are to be found in scientific literature. It has, however, been shown expressed in neonatal and embryonic human and rat tissues and placenta. Expression was also seen in proliferating adult tissues like hematopoietic tissue, but not in terminatelly differentiated cells like peripheral blood leucocytes. (Weinstein *et al.* 1994) Both nuclear and cytoplasmic physiological Cdc20 expression has been seen, and either low or negative expression has been reported in normal tissues, for example in the gastrointestinal (Kim *et al.* 2005, Wu *et al.* 2013), pancreatic (Chang *et al.* 2012) and squamous (Thirthagiri *et al.* 2007) epithelium. According to internet database most normal tissues are reported negative (www.proteinatlas.org).

2.3.2.1 Cdc20 in cell division

Cdc20, as an integral part of SAC, monitors the integrity of the genome ensuring that anaphase proceeds only when the centromeres of all sister chromatids are lined up in the metaphase plate and properly attached to microtubules (Fang *et al.* 1998) (Fig. 2). In this, Cdc20 functions in collaboration with APC. Dysregulation of APC by abnormal expression or dysfunction of Cdc20 may, therefore, induce premature anaphase resulting in aneuploidy (Rajagopalan and Lengauer 2004). Cdc20 is synthesized already during S- and G2- phases, but Cdc20 activation of APC occurs only in mitosis through phosphorylation of specific APC subunits by M-Cdk/Cdk1-cyclinB1 (Kraft *et al.* 2005). Also evidence from HeLa cells shows that Cdc20 protein level and APC-Cdc20 binding peaks in mitosis and decreases rapidly in early G1 phase (Weinstein *et al.* 1994, Chang *et al.* 2012).

Cdc20 is regulated by two spindle checkpoint proteins, Mad2 and BubR1, which inhibit its activity until all kinetochores achieve a bipolar attachment (Bardin and Amon 2001). In mitosis, active Cdc20 together with Cdh1 (Cdc20 homologue 1) sequentially binds to APC (Kramer *et al.* 2000, Kraft *et al.* 2005, Yang *et al.* 2014), which leads to securin and cyclin B1 degradation (Zur and Brandeis 2002). Cdc20 is active from metaphase through anaphase to promote separation of sister chromatids, while Cdh1 is active from the end of mitosis until the G1-to-S transition to prevent premature entry into S phase. However, the exact mechanisms explaining their functions are not settled, but it is believed that they enable the APC to bind to its specific substrates (Izawa and Pines 2011, Peters 2006, Kraft *et al.* 2005). At metaphase/anaphase transition through securin degradation, APC-Cdc20 complex activates separase which initiates break down of the cohesion between sister chromatids (Nasmyth 2002). Cdc20 is also needed for the exit from mitosis (Schwab *et al.* 1997, Visintin *et al.* 1997, Lim *et al.* 1998). Active APC-Cdc20

leads to cyclin B1 degradation, Cdk1 inactivation and activating subunit switch from Cdc20 to Cdh1 which, in turn, leads to Cdc20 degradation and, ultimately, to mitotic exit (Yang *et al*. 2014). Experimentally, the role of Cdc20 in sister chromatid separation has been demonstrated in Cdc20-depleted mice whose embryos were arrested in metaphase at the two-cell stage with high levels of cyclin B1 and securin (Li *et al.* 2007b). In an experiment involving Cdc20 and securin double mutant embryo, metaphase was not arrested but the loss of securin could not rescue the embryos from Cdc20 deficiency-induced lethality (Li and Yang 2007a).

2.3.2.2 Cdc20 in cancer

High cdc20 expression has been reported in several human cancer cell lines and tissues, where a number of studies have linked Cdc20 dysfunction to checkpoint defects, chromosomal instability and aneuploidy (Kim *et al.* 2005, Iacomino *et al.* 2006, Thirthagiri *et al.* 2007, Yuan *et al.* 2006, Jiang *et al.* 2011, Chang *et al.* 2012, Ouellet *et al.* 2006, Kidokoro *et al.* 2008, Jiang *et al.* 2011). In malignant disease, high Cdc20 expression has been linked to poor prognosis in cervical squamous cell carcinomas (Kim *et al.* 2014), hepatocellular carcinoma (Li *et al.* 2014), gastric carcinoma (Ding *et al.* 2014), lung (Kato *et al.* 2012), oral squamous cell (Moura *et al.* 2013), bladder (Choi *et al.* 2013), colon (Wu *et al.* 2013) and pancreatic (Chang *et al.* 2012) carcinomas. Recently, it has also been suggested that depleting endogenous Cdc20 suppresses tumorigenesis by triggering mitotic arrest and subsequent apoptosis (Wan *et al.* 2014). Cdc20 overexpression has also been associated with inappropriately functioning SAC and aneuploidization in oral cancer (Mondal *et al.* 2007). The function of Cdc20 in tumorigenesis and progression of malignant disease has been elaborated in *in vitro* experiments with mice carrying a mutated Cdc20 incabable in interaction with Mad2. The results suggest that mutant, SAC inhibition resistant, Cdc20 promotes tumor progression, providing direct evidence for the role of cdc20 in tumourigenesis (Li *et al.* 2009). Vice versa, knockdown of Cdc20 expression has resulted in growth suppression of tumour cells (Kidokoro *et al.* 2008, Taniguchi *et al.* 2008). Studies with siRNA have provided further evidence for the role of Cdc20 in tumor progression, while treatment with siRNA against Cdc20 has been shown to induce G2/M arrest and to suppress cell growth (Kidokoro *et al.* 2008, Taniguchi *et al*. 2008). Consequently, controlling Cdc20 has also been suggested a potential therapeutic strategy for cancer (Kim *et al.* 2014, Wang *et al.* 2013).

2.3.2.3 Cdc20 in breast cancer

While increased levels of Cdc20 have been observed in several human malignancies, little information is available on Cdc20 in breast cancer. In breast cancer cells down regulation of Cdc20 expression has been associated with inhibition of cell proliferation

in vitro (Jiang *et al.* 2011, 2012). Cdc20 was found overexpressed in cDNA microarray analyses based on a large set of human malignancies, including breast cancer (Kidokoro *et al.* 2008). In this study, Cdc20 expression was reported increased more than three-fold in 44% of all examined cancer tissues and in 60% of the breast cancer tissues, providing evidence that Cdc20 overexpression may have a role in the tumorigenesis of breast cancer (Kidokoro *et al.* 2008).

2.3.3 Cdc27

Cell division cycle protein 27, Cdc27, is one of the core components of APC responsible for destroying proteins involved in mitosis (Izawa and Pines 2011). According to internet databases, Cdc27 is expressed comprehensively in normal tissues, expression being particularly strong in the gastrointestinal tract, pancreas and gall bladder, uterine cervix, skin, lymph nodes, endocrine glands and bronchus (www.proteinatlas.org). In mammalian cells, Cdc27 has been reported to be expressed in the nucleus, where it predominantly localizes at centrosomes of interphase cells, spindle poles, spindle microtubules, kinetochores and along chromosome arms in mitotic cells (Tugendreich *et al.* 1995, Topper *et al.* 2002).

2.3.3.1 Cdc27 in cell division

Cdc27 has shown to contribute to the interactions between the mitotic checkpoint proteins, especially the substrates and co-activators of APC, thus, with a role in the timing of mitosis (Peters 2006, Izawa and Pines 2011) (Fig. 2). Phosphorylation of Cdc27 has been suggested one of the mechanisms by which the spindle checkpoint may regulate APC activity at mitosis (Topper *et al.* 2002). According to King and co-workers (1995), phosphorylation of Cdc27 serves as an important mechanism for activation of APC determining the affinity between APC and its substrate specificity activators, Cdc20 or Cdh1 and, thus, ensuring the activation of APC to destroy anaphase inhibitors and permitting chromatid separation (King *et al.* 1995). According to Kraft and coworkers (2005), under CDK-mediated phosphorylation, Cdh1 dissociates from Cdc27 which results in the inactivation of APC/C ubiquitin ligase. Particularly, TGF-β-induced phosphorylation of Cdc27 might be a mechanism that enhances APC to communicate with its activator Cdh1 (Zhang *et al.* 2011). Cdc27 is also suggested to catalyze the formation of cyclin B-ubiquitin conjugate, responsible for the ubiquitin-mediated proteolysis of B-type cyclins. According to Topper and coworkers (2002), microinjection of anti-Cdc27 antibody into cells resulted in arrest at metaphase. Co-injection of anti-Cdc27 antibody with anti-Mad2 antibody was also shown to result in metaphase arrest, without premature anaphase onset normally induced by anti-Mad2 antibody.

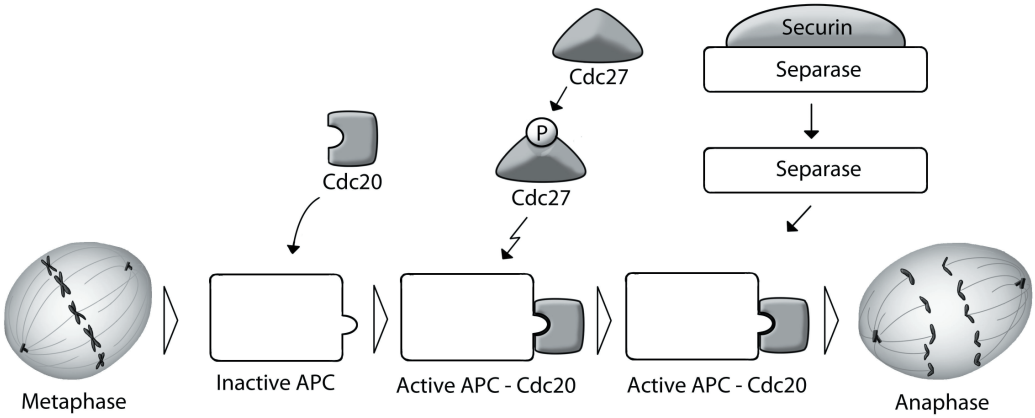

Figure 2. Metaphase-anaphase regulation with securin, Cdc20 and Cdc27.

2.3.3.2 Cdc27 in cancer

Published research on Cdc27 in cancer is very sparse and mostly composed of reports on the structural studies in relation to APC functions. On genetic level, though, Cdc27 has been reported with significant mutations in prostatic adenocarcinoma (Lindberg *et al.* 2013) and non-small cell lung carcinoma (Zhang *et al.* 2014). However, in literature there are no previous reports on protein expression patterns or prognostic associations of Cdc27 in human breast or other types of cancer. Cdc27 has been shown to be down-regulated in several breast carcinoma cell lines, suggesting that Cdc27 might have tumor suppressor qualities (Pawar *et al.* 2010). In addition, cdc27 has been shown one of the genes showing germline polymorphism associated with high grade breast cancer risk (Stevens *et al.* 2011).

Cdc27 has been proposed with a role in radiation therapy response, as down-regulation of Cdc27 was one of the gene expression changes predicting poor treatment response in cervix carcinomas (Rajkumar *et al.* 2005).

3. AIMS OF THE STUDY

The focus of this thesis is in the role of securin, Cdc20 and Cdc27, a cascade of metaphase-anaphase regulators, in human breast cancer. The protein expression of the regulators is detected with the help of immunohistochemistry and associations are identified in relation to DNA content and disease survival in a set of a maximum of 772 patients with up to 22 years of follow-up. In previous literature, the prognostic value of the studied proteins is largely unrevealed, with contradictory observations concerning the cellular and subcellular expression, and association to ploidy and outcome in human malignancies, including breast cancer.

The specific aims of this thesis are:

1. To characterize the protein expression of securin, Cdc20 and Cdc27 in human breast cancer. (I-IV)

2. To evaluate the associations of securin, Cdc20 and Cdc27 to DNA content in human breast cancer. (I-IV)

3. To evaluate the prognostic impact of securin, Cdc20 and Cdc27 in breast cancer patients. (I-IV).

4. MATERIALS AND METHODS

4.1 Patients and tissue material

4.1.1 Patients

Studies I – IV are based on a material comprising a total of 772 unselected patients diagnosed with invasive breast cancer between 1987-1998 in Jyväskylä Central Hospital, Jyväskylä, Finland. However, in the beginning of the project (Study I) only part of the material (n = 310) was collected and available. Also in Studies II – IV the number of patients varied considerably due to material lost during tissue processing, as explained later. The patients involved in each of the studies are summarized in Table IV.

The treatment of all patients was performed according to the international guidelines for breast cancer management at the time of diagnosis (Goldhirsch *et al.* 2009). The treatment included surgical resection or mastectomy with axillary evacuation, radiation and/or adjuvant treatment with anti-estrogenic or cytostatic drugs depending on patient's age, hormone receptor and lymph node status. No preoperative adjuvant treatment was administered. Clinical and follow-up data were available from patient files. Causes of death were collected from autopsy reports, death sertificates and from the Finnish Cancer Registry. Mean follow-up time in the whole patient material was 10.2 years (SD 5.8; maximum 22.4 years). Summary of survival information is presented in Table IV.

The clinico-pathological data of the material was collected according to the criteria presented by WHO (Lakhani and International Agency for Research on Cancer, World Health Organization, 2012). In addition, intrinsic breast cancer classification for the genetically identified breast cancer subtypes (Perou *et al*. 2000, Sorlie *et al.* 2001, Sotiriou *et al.* 2009) was performed as approximations recommended by the 12th St Gallen International Breast Cancer Conference Expert Panel (Hammond *et al.* 2010, Goldhirsch *et al.* 2011).

The presented studies have the approval of the Ethical Committee of Turku University Hospital, or Jyväskylä Central Hospital, and the National Authority for Medicolegal Affairs. The research was carried out in accordance with the Helsinki Declaration.

4.1.2 Tissue materials

Two types of tissue materials were applied in the studies. All studies (I – IV) involved archieval material of breast cancer blocks. In addition, studies II, III and IV included

fresh breast cancer material prepared as cell imprints. Both types of tissue materials were obtained from the Department of Pathology, Jyväskylä Central Hospital, Jyväskylä, Finland.

Archieval material processing was performed according to standard histopathology practice, i.e. fixation in buffered formalin (pH 7.0) and embedding in paraffin. TMA's were prepared from the paraffin block of each patient's breast cancer tissue and arranged into sets of 128 – 312 cores on each histological slide as presented by Kononen *et al.* (1998). In preparing the TMAs, special attention was placed on verification of the primary diagnosis and histological classification of the carcinomas. After identifying in HE stained slides the most representative tumor areas in the histological section, two tissue punches (diameter 0.6 mm; minimum height 5 mm) from each tumor were taken: the first core from the central tumor area, and the second core from the peripheral, theoretically most proliferative front of the tumor. From the total of 1544 cores representing 772 breast cancers, 119-293 cases were excluded because of inadequate tissue material in studies I - IV.

Cell imprints were prepared for image cytometry from fresh tissue material from a total of 331 breast cancer cases. The imprints were prepared by applying a freshly cut surface of the tumor on a glass slide, as described by Rosai *et al*. (2007). Simultaneously, a consecutive section of the tumor was taken and prepared for histological confirmation on H&E staining.

4.2 Methods

4.2.1 Immunohistochemistry

4.2.1.1 Immunohistochemical procedure

For immunohistochemistry of the TMAs, 3 µm-thick sections were cut from the blocks and treated according to standard immunohistochemistry procedure at the time of the study in the Department of Pathology, Turku University Hospital, Turku, Finland. Summary of antibodies and the applied immunohistochemical staining methods for securin, Cdc20 and Cdc27 are presented in Table IV. In short, after manually performed deparafinization, antigen retrieval was performed for securin and Cdc27 with citrate buffer pH 6 for 10-14 min in a microwave oven. After that the automated staining technology of LabVision Autostainer 480 (Thermo Fisher Scientific, Fremont, CA, USA) was used for antigen detection. Optimally diluted monoclonal antibodies (Abcam, Cambridge, UK) were incubated for 1h at RT with Power Vision detection kit (HRP conjugated polymeric secondary antibody and DAB chromogen). For Cdc20

antibody (Nordic BioSite AB, Täby, Sweden), fully automated staining technology of BenchMark XT (Roche Diagnostics/Ventana Medical Systems, Tucson, AZ, USA) was used. Deparafinization, antigen retrieval (heated in CC1-buffer for 60 min), incubation with optimally diluted primary antibody (32 min at 37 C) and detection with UltraVIew Universal DAB Detection Kit (HRP conjugate multimeric secondary antibody and DAB chromogen) were all performed on the platform.

Table IV Summary of patients and staining methods in Studies I-IV.

		Studies			
		I	**II**	**III**	**IV**
Patients					
	Total	310	603	429	445
	IHC	310	603	429	445
	ICM	0	331	229	229
IHC stainings					
	Antibody	**Securin**	**Securin**	**Cdc27**	**Cdc20**
	Type	Monoclonal	Monoclonal	Monoclonal	Polyclonal
	Clone	DCS-280	DCS-280	AF3.1	Q105
	Source	Abcam	Abcam	Abcam	Abcam
	Dilution	1:50	1:50	1:12000	1:100
	Antigen retrieval	MW* pH 6	MW* pH 6	MW* pH 6	sCC1**
	Incubation	1h RT***	1h RT***	1h RT***	32min 37C
Detection		Automated#	Automated#	Automated#	Automated##

* Micro-wave, citrate-buffer
** Standard CC1 pretreatment buffer (performed on platform)
*** Room temperature
# Labvision Autostainer, Thermo Fisher Scientific, Fremont CA USA. PowerVision + Poly. HRP IHC kit, Immunovision Technologies, Vision BioSystems, Norwell MA USA
BenchMark XT, Roche Diagnostics/Ventana Medical Systems, Tucson AZ USA. UltraView Universal DAB Detection Kit, Roche Diagnostics/Ventana Medical Systems, Tucson AZ USA

Immunohistochemical stainings for the established prognosticators of breast cancer were performed as part of the clinical immunohistochemical routine of the Departments of Pathology, Turku University Hospital, Turku, Finland. Summary of antibodies and the applied immunohistochemical staining methods is presented in Table V. In short, ER, PR, HER2 and Ki-67 immunostainings were performed using the fully automated immunostaining machine BenchMark XT (Roche Diagnostics/Ventana Medical Systems, Tucson, AZ, USA). Antigen retrieval and incubation times with ready-to-use antibodies were optimized for UltraView Universal DAB Detection Kit.

Table V Details for the used immunohistochemical methods for ER, PR, HER2 and Ki-67 stainings.

Antibody	Clone	Source	Dilution	Antigen retrieval	Incubation	Detection
ER	SP1	Roche	RTU*	sCC1[#]	24 min 37C	automated[¤]
PR	1E2	Roche	RTU	sCC1	32 min 37C	automated[¤]
HER2	4B5	Roche	RTU	sCC1	24 min 37C	automated[¤]
Ki-67	30-9	Roche	RTU	sCC1	12 min 37C	automated[¤]

* Ready-to-use
[#] Standard CC1 pretreatment buffer
¤ BenchMark XT, Roche Diagnostics/Ventana Medical Systems, Tucson, AZ, USA

Each experiment included negative controls where the primary antibody was omitted or substituted with an appropriate preimmune serum. With LabVision Autostainer 480 it was also possible to include negative controls where the secondary antibody was omitted. In case of ER, PR, Ki-67 and HER2 immunostainings positive controls were adopted from immunohistochemical routine procedure at the department.

4.2.1.2 Interpretation of immunohistochemistry

Securin, Cdc27 and Cdc20 immunopositivities were registered as positively stained cancer cell nuclei and/or cytoplasm. For each antibody, immunohistochemical expressions were evaluated in each tissue core as a fraction (%) of positively stained cancer cells. This fraction was calculated as an average of sets of 100 cancer cells (minimum of 100 cells, maximum of three sets of 100 cells). Tissue cores presenting fewer than 100 invasive cancer cells were excluded from the study.

The interpretation of securin immunostaining was preceded by a training session between at least two independent observers in order to achieve optimal standardization for the evaluation. During the training session, cases with over 5% difference between the two observers' registered immunopositivities were considered contradictory. These cases were revisited and settled as a consensus between the observers. After the training session, one observer performed the final evaluation of all cases. Interpretation of Cdc20 and Cdc27 immunostainings were also performed after training sessions between two observers.

Interpretations of ER, PR, HER2 and Ki-67 immunostainings were performed according to generally accepted international guidelines at the time of the study (Wolff *et al.* 2007, Wolff *et al.* 2013, Hammond *et al.* 2010).

4.2.2 Image cytometry analysis

4.2.2.1 Image cytometry procedure

For image cytometric analysis, the cell imprints were stained according to Feulgen (CAS DNA Staining Kit, Becton Dickinson Cellular Imaging Systems, Elmhurst, IL, USA) and analysed by determining the DNA content of each tumour quantitatively as the intensity of nuclear staining in light microscopy. Intensity of the nuclear staining is considered quantitatively proportional to the DNA content. The measurement of nuclear DNA content of the cancer cells was performed with the CAS 200 Image Analysis System (Cell Analysis Systems, Elmhurst, IL, USA), which automatically selects the cells enabling their visual control. Calibration and biological reference of the image analysis system was based on internal control of diploid cells and external control of rat tetraploid hepatocytes. An average of 184 (SD 58, range 35–350) non-overlapping and well-preserved cancer cells were analyzed in each specimen with quantitative DNA analysis software.

4.2.2.2 Interpretation of image cytometry

To begin the analysis, the integrated optical density (IOD) of the calibration cells was measured. Thereafter, the software calibrated the system by using the modal peak value of the IOD of the control cells. Often the analyzed tumor sample also contained benign diploid cells, which served as internal controls. Calibration was performed at the beginning of every measurement. Separate peaks were visually identified from the DNA histograms. For each imprint, mean, SD and coefficient of variation (CV) values of the visually identified peaks and calibration CV were determined to control the quality of the measurements.

Figure 3 summarizes examples of different types of histograms. Peaks with a DNA index between 1.6c and 2.4c were considered to represent diploid cell populations, whereas peaks between 3.6c and 4.4c were classified as tetraploid cell populations. Peaks outside these ranges were considered to represent aneuploid cell populations. In the first phase, the histograms were divided into diploid, tetraploid and aneuploid. Diploid cases showed only a G 0 / G 1 (2c) peak, and the number of cells in the G 2 (4c) peak did not exceed 10%. If the number of cells in the tetraploid or near-tetraploid (4c) peak exceeded 10% of the cells, the case was considered to be tetraploid. Cases with peaks outside these ranges were classified as aneuploid (>10% of the total amount of cells). Particularly aneuploid cases were further described by identifying cases harboring >5% of cancer cells with DNA content exceeding 5c [5c exceeding rate (5cER)]) and cases that showed cells with over 16c DNA content [16c exceeding rate (16cER)], considered to represent aneuploidy and high aneuploidy, respectively. Finally, the fraction of cells in S-phase (S-phase fraction, SPF) was evaluated separately for proliferating diploid and non-diploid cell populations.

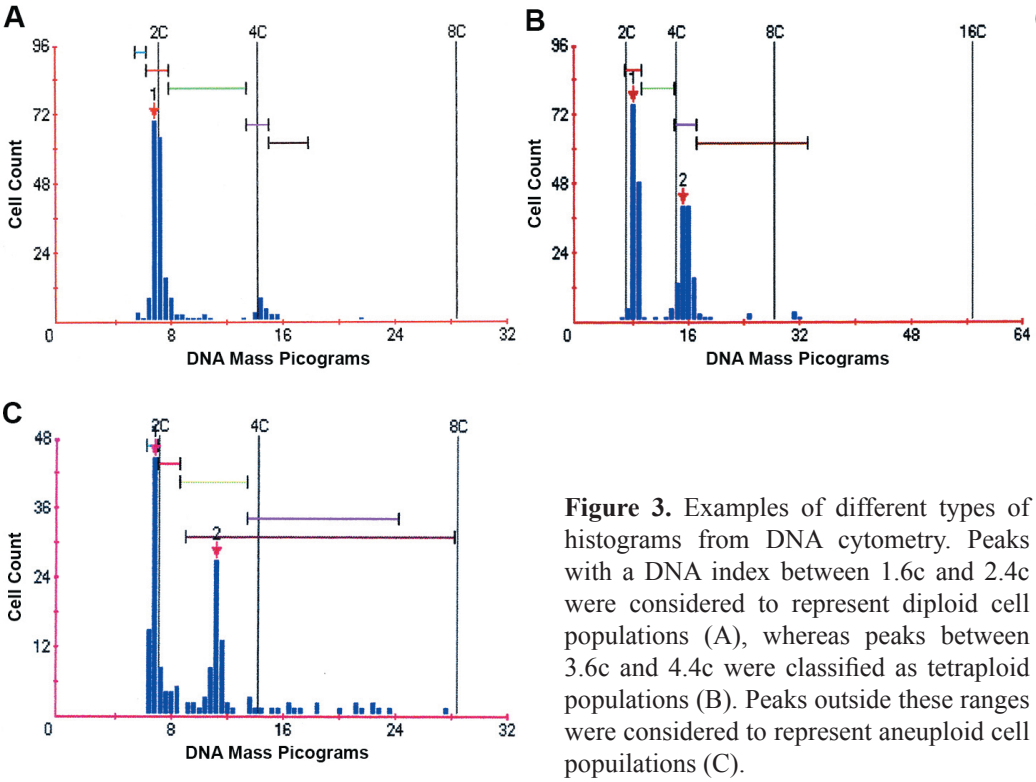

Figure 3. Examples of different types of histograms from DNA cytometry. Peaks with a DNA index between 1.6c and 2.4c were considered to represent diploid cell populations (A), whereas peaks between 3.6c and 4.4c were classified as tetraploid populations (B). Peaks outside these ranges were considered to represent aneuploid cell popuilations (C).

4.2.3 In situ hybridization

Interpretation of both HER2 immunohistochemistry and in situ hybridisation was performed according to generally accepted international guidelines (Wolff *et al.* 2007). Cases showing immunohistochemical HER2-positivity (2+ and 3+) were allocated into confirmation of amplification with the help of HER2/Chr17 double in situ hybridisation (BenchMark XT, Roche/Ventana, AZ, USA) using Ventana HER2 DNA probe and Inform Chromosome 17 probe (Roche/Ventana, AZ, USA) and detecting with ultraView SISH and Alkaline Prosphatase Red ISH detection kits (Roche/Ventana, Tucson, AZ, USA). Briefly, ISH Protease 3 (Roche/Ventana, Tucson, AZ, USA) for 8 min was used as a pretreatment step, and HER2 hybridisation was performed at 52˚C for 6 h and Chr17 hybridisation at 44˚C for 2 h.

4.2.4 Statistical analysis

4.2.4.1 Consistency of immunohistochemistry

Immunohistochemical interpretations showed two variation sources: variation between the two tissue cores on the TMAs representing different areas of the tumours (I) and variation between the different independent observers of immunohistochemistry (II).

Study I addresses the variation between evaluations of securin immunostaining in the TMAs comparing results from the two punched cores, the central core and the peripheral core from each tumour. Using intraclass correlation coefficient (ICC), McNemar's test for marginal homogeneity and kappa test a moderate consistency could be statistically shown between the results from the two tissue cores (ICC 0.737, P = 0.63 and κ = 0.59, respectively). On this basis, the consistency between the pair of tissue cores from each tumour were considered acceptable which allowed applying for final prognostic purposes either the mean of the results of two cores (65% of cases) or a single observation, in case of only one core representative of cancer cells. In Studies II, III and IV the highest score from the tissue cores of each tumor was chosen for statistical analysis.

The issue of intra- and interobserver reproducibility was examined in Study II with the help of a training session where two independent observers evaluated securin immunopositivity in the TMAs. In this paper, ICCs were calculated between 27 randomly chosen tissue cores as repeated observations of one observer, and as independent observations made by two observers. The resulting high reproducibilities (intraobserver 0.95 and interobserver 0.87) allowed applying, in the final analyses, one single evaluation from each patient's tumour.

4.2.4.2 Determination of cut-off values for prognostic evaluations

Cut-off values for prognostic analyses were determined on the basis of previous literature (Talvinen *et al.* 2008) and three statistical approaches involving receiver operating characteristics analysis (I), descriptive statistical characteristics of the material (II) and observed prognostic associations (II, III, IV). The cut-off points for securin were set at 1.5% (I) and 10% (I – IV) of positive cancer cells and for Cdc27 at 10% of cancer cells. Cdc20 (IV) was allocated into four expression groups on basis of the immunohistochemical staining properties. Based on the observed prognostic associations division in two groups was used in final analyses.

4.2.4.3 Prognostic associations

Survival analysis was performed to investigate the prognostic value of securin, Cdc27 and Cdc20 in relation to the features of DNA content (ploidy, 5cER, 16cER, and SFP) and the established prognosticators of breast cancer. For survival analyses patients were allocated into low-level and high-level expression groups according to the observed securin, Cdc27 and Cdc20 expression. In univariate analyses, the cumulative percentages of breast cancer-specific mortality were estimated using the Kaplan-Meier technique and the differences between categorized values were tested using the log-rank test. Differences between categories were quantified by calculating hazard ratios (HRs) with 95% confidence intervals (95% CIs) using Cox's proportional hazards

models. Associations between securin, Cdc27 and Cdc20 immunopositivity, features of DNA content and the established prognosticators of breast cancer were analysed by Fisher's exact and Wilcoxon rank sum tests, and the results were quantified as odds ratios with 95% CIs. Cox's regression analysis was applied to involve in the analyses the established prognosticators of breast cancer. Patients with missing data were forced to exclude from the analyses. The validity of proportional hazards assumption was assessed both visually and numerically, and no marked deviation for assumptions were observed. P-values less than 0.05 were considered statistically significant. Kaplan-Meier survival plots were generated using R 2.15.0. The statistical computations were performed using SAS Systems for Windows, Version 9.1.3, 9.2. or 9.3. and SAS Enterprise Guide 4.1. (SAS Institute Inc., Cary, NC).

5. RESULTS

5.1 Immunohistochemical expression patterns of securin, Cdc20 and Cdc27 in human breast cancer (I-IV)

Photomicrographs in Figure 4 present representative examples of the extent and localization of the studied immunohistochemical expressions for breast carcinomas with varying histological differentiation.

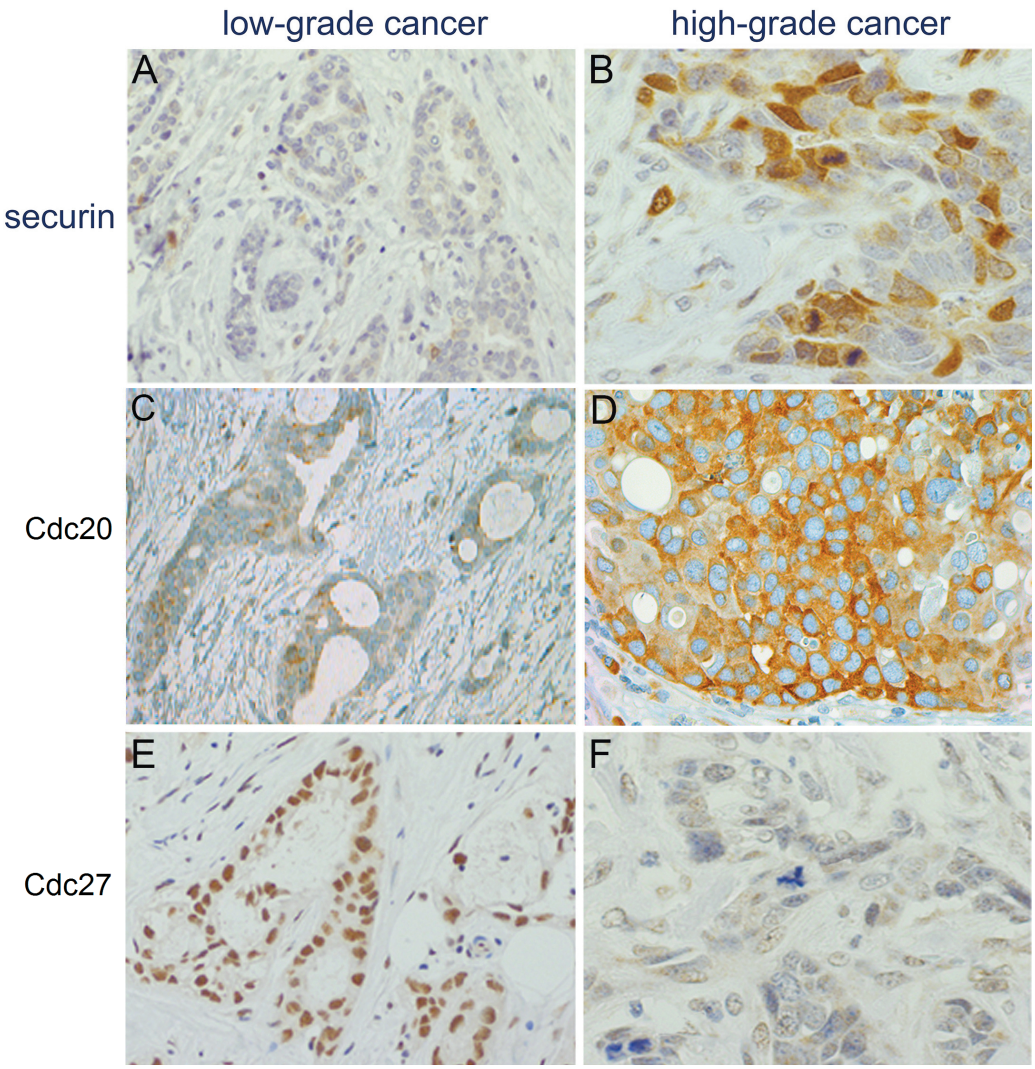

Figure 4. Immunohistochemical staining patterns of securin, Cdc20 and Cdc27 in well and poorly differentiated breast carcinoma (A, C, E x200, B, D, F x400)

Securin expression was absent or scarce in benign breast epithelium evaluated in normal reference specimen from surgical breast reductions. In malignant cells, securin expression was evaluated in TMAs comprising a total of 603 breast cancer cases. In malignant tissue, securin positivity was heterogeneous both between and within different cases. In summary, securin positivity was observed in the nucleus and cytoplasm. Some cases also showed concentration of perinuclear staining and in a small fraction of cases granular cytoplasmic staining was present. The different staining patterns were often present in different areas of the same sample. In interpreting the stainings, the different staining patterns were not separated but a total percentage of positively staining cells was registered. The extent and intensity of securin immunohistochemical expression appeared to strenghten along with increasing nuclear atypia and aggressive morphology of the malignant tumour. Also mitotic figures showed heterogenous immunohistochemical staining pattern. Cell divisions appeared to be predominantly securin-positive in early mitosis, i.e. prophase to metaphase, but mostly negative towards the conclusion of mitosis, in anaphase to telophase. In the patient material, the average fraction securin positive cells was 9.7% (median 7.5%, range 0 – 84.5%). In Study II, different cut-off points for securin immunohistochemical expression were tested and on basis of statistical analyses, the final threshold at 10% immunohistochemical positivity was set for dividing the material in low and high expression groups (low 62.9% and high 37.1% of cases).

Faint Cdc20 immunohistochemical expression was observed in normal breast epithelial cells. Cdc20 immunohistochemical positivity (Study IV, n=445) was observed in the cytoplasm of cancer cells. Cdc20 immunohistochemical positivity was allocated into four expression groups: negative in more than 95% of cancer cells (score 0), positive in more than 5% of single cancer cells (score 1+), weak diffuse staining (score 2+) and strong diffuse staining in more than 95% of cancer cells (score 3+). The majority of cases (61%) were classified negative while 12% and 23% of the cases scored 1+ and 2+, respectively. The cases with strong diffuse staining (3+) were clearly identifiable among all tissue cores. The high expression group comprised a small (n=19, 4.3% of the whole material) but distinct patient group clearly distinguished on basis of, first, pronounced Cdc20 expression and, secondly, the observed extremely poor prognosis as compared to the rest of the patients ($p<0.001$). Therefore, in further analyses, the 3+ cases were designated as the high expression group and evaluated separately from the rest of the cases (low expression group, scores 0 – 2+).

Cdc27 was evaluated in a total of 429 cases (Study III). Substantial Cdc27 expression (Study III) was present in the nucleus of benign breast epithelial cells and well differentiated carcinomas with mild nuclear atypia. Instead, moderate or faint Cdc27 immunohistochemical expression was observed in breast carcinoma cells with less

significant differentiation while clearly atypical cancer cells were totally negative. Mitotic figures seamed to be Cdc27 negative. In the patient material, Cdc27 expression was classified into 8 categories based on the extent of immunohistochemical positivity in malignant epithelial cells. For statistical analyses, the threshold at 10% immunohistochemical positivity was used for dividing the material in low and high expression groups (low 56.7% and high 43.3% of cases).

Correlations of the registered immunohistochemical expressions showed statistically significant - but inverse in case of Cdc27 - associations ($p=0.01$ for association between securin and Cdc27, $p<0.0001$ for association between securin and Cdc20).

5.2 Associations between securin, Cdc20 and Cdc27, and DNA content (II-III)

Table VI summarizes the associations of securin and Cdc20, and Cdc27, and DNA cytometry analyses from cell imprints of fresh breast carcinoma tissue.

Table VI Summary of associations between securin alone and in combination with Cdc27, Cdc20 and DNA content among breast cancer cases (n=229-331)*.

	Securin (n=331) (II)		
DNA parameters	**OR**	***p***	**95% CI**
Aneuploid	3,8	0,0002	1,9-7,5
5cER	3,5	<0,0001	2,1-5,9
16cER	3,6	0,0002	1,8-7,0
	Securin and Cdc27 (n=229) (III)		
Aneuploid	6,0	0,0006	2,2-16,7
5cER	5,3	<0,0001	2,3-11,9
16cER		ns	
	Securin and Cdc20 (n=229) (IV)		
Aneuploid	19,0	0,005	1,1-344,8
5cER	17,0	<0,001	2,1-135,1
16cER		ns	

* Cut-off points for low and high expression at 10% immunohistochemical positivity for securin and Cdc27.

Securin immunohistochemical expression (Study II) was significantly associated with the different parameters of DNA content in the tissue material of 291 breast cancer patients. Specifically, statistically significant association could be observed with securin expression and aneuploidy, tetraploidy, 5cER, 16cER and SPF of the aneuploidy cell population. In particular, high-level securin expression was associated

with 9.8-fold odds for aneuploid DNA content in the patient material (p=0.0007), when comparing cases showing over 15% of immunohistochemically positive cancer cells to those with under 10% of positive cancer cells. In the same analysis, tetraploid DNA content predicted high securin expression with up to 5.1-fold odds (p=0.004). Based on analyses involving 5cER and 16cER, high securin immunohistochemical expression systematically predicted the presence of a very aneuploid cell population among the studied cases.

Concerning Cdc20 (Study IV, n=229), Cdc20 sparsely failed to show statistical significant association with ploidy (p=0.059). Instead, high expression of both securin and Cdc20 predicted 19-fold odds (p=0.004, CI 1.1-344.8) for aneuploid DNA content. High Cdc20 expression in combination with high securin expression predicted the occurrence of 5cER cells (OR 17.0, CI 2.1-135.1).

Based on analysis of Cdc27 in a set of 229 breast cancer cases (Study III), the combined impact of securin and Cdc27 was a particularly strong predictor of the DNA content in analysis comparing cases with low Cdc27 and high securin to cases with high Cdc27 and low securin immunohistochemical expression ($p = 0.0006$ for aneuploidy, $p = 0.009$ for tetraploidy and $p < 0.0001$ for 5cER) (Table VII). In particular, the combination of low Cdc27 and high securin expression predicted 6.0-fold odds ratio (CI 2.1–16.7) for aneuploid DNA content and 5.3-fold odds ratio (CI 2.3– 11.9) for 5cER as compared with high Cdc27 and low securin expression. Cdc27 alone predicted inversely the occurrence of 5cER cells (p=0.03) and aneuploidy DNA content although the latter association was not statistically significant.

5.3 Associations between securin, Cdc20 and Cdc27, and the established prognostic factors of breast cancer (I-IV)

Statistically significant associations between the studied proteins and the established prognosticators of breast cancer are presented in Table VII. Particularly, all studied proteins showed a clear association with the proliferative activity of the breast cancer tissue as determined with Ki-67 expression ($p<0.001$, n=310) (Studies I-IV). In addition, tumor grade was associated with immunohistochemical expression of all three proteins. Securin showed significant association with all features except with HER2-amplification and nodal status. Cdc20 was also associated with ER and PR immunohistochemical positivity, and intrinsic classification reflecting the genetically identified breast cancer subtypes. Ploidy was statistically associated with all studied features.

Table VII Presentation of statistically significant associations between securin, Cdc20, Cdc27 and ploidy, and the established prognostic factors of invasive breast cancer.

Prognostic factor	Securin	Cdc20	Cdc27	Ploidy
Nodal status	ns	ns	ns	0,017 (IV)
Tumor size	<0.001 (I, IV)	ns	<0.001 (III)	0,008 (IV)
Grade	<0.001 (IV)	0,004 (IV)	<0.001 (III)	<0,001 (IV)
Histological type	<0.001 (I, IV)	ns	ns	0,001 (IV)
Ki-67	<0.001 (I, IV)	<0.001 (IV)	<0.001 (III)	<0,001 (IV)
ER	<0.001 (IV)	<0.001 (IV)	ns	<0,001 (IV)
PR	<0.001 (IV)	<0.005 (IV)	ns	<0,001 (IV)
HER2-amplification	ns	ns	ns	0,010 (IV)
Intrinsic classification	<0.001 (IV)	<0.001 (IV)	ns	<0,001 (IV)

5.4 Prognostic associations of securin, Cdc20 and Cdc27 (I-IV)

High securin expression was in the present patient material consistently associated with disease outcome (Table VIII). In the different publications (II-IV), all univariate Cox's survival analyses of securin expression systematically showed statistically significant correlations with breast cancer survival (HRs varying between 2.0 and 3.1, p<0,0001). The prognostic significance of securin could also be demonstrated in subgroup of invasive ductal subgroup (p=0.010), and in subgroups of small and large tumor size (p=0.034 and p=0.033, respectively) (I). High Cdc20 expression alone predicted 2-fold risk of breast cancer death (p=0.047, CI 1.0-3.9). Instead, univariate analyses involving Cdc27 alone showed no statistically significant prognostic associations in the present patient material. Still, combining Cdc20 and Cdc27 expressions with securin expression showed slightly intensified prognostic associations as compared to analyses involving securin alone. The combination of high Cdc20 and high securin expressions indicated 4.3-fold risk of breast cancer death (p<0.001, CI 2.0–8.9) as related to low Cdc20 and low securin expression. Combination of low Cdc27 and high securin, in turn, was associated with 2.9-fold risk of breast cancer death as compared to cases showing high Cdc27 and low securin expression (CI 1.7–5.0, p<0.0001). In addition, combining securin and Ki-67 predicted 5.8-fold risk of breast cancer death (p=0.004, CI 1.8–18.9) comparing patients with high securin and high Ki-67 to cases showing low securin and Ki-67 expression (I). High Ki-67 alone indicated a 2.4-fold risk of breast cancer death in our material (p=0.004, CI 1.3–4.5).

Table VIII Univariate prognostic analyses involving securin, Cdc27 and Cdc20 expressions in the whole-follow-up period.

	Whole follow-up period		
	HR	***p***	**95% CI**
Single proteins			
high securin (II)	2,05	<0,001	1,6-2,7
Cdc27 (III)			ns
high Cdc20 (IV)	2,0	0,047	1,0-3,9
Combinations of proteins			
high securin and low Cdc27 (III)	2,9	<0,001	1,7-5,0
high securin and high Cdc20 (IV)	4,3	<0,001	2,0-8,9

Survival analyses (Fig. 5) demonstrated the consistent and independent prognostic value of the studied proteins among the breast cancer patients (I-IV). Kaplan-Meier curves for securin expression alone exhibited the previous conclusions on the prognostic value of disease-specific breast cancer survival ($p<0.001$) (Fig 5a). Also Cdc20 expression could be used to allocate the patients into two distinct prognostic categories with a statistically significant survival difference ($p=0.047$) (Fig 5c). Especially the combination of Cdc20 and securin expression was efficient in prognostic evaluations, and in detecting a subgroup of patients (high Cdc20 and high securin) with a particularly sinister outcome of disease ($p<0.0001$) (IV). The survival differences detected with the help of the studied proteins could also be demonstrated in detailed Kaplan-Meier analysis of individual patients. This type of analysis revealed that the majority (75%) of patients with the most favorable combination of Cdc20 and securin (low expression for both) were alive at 13.3 years of survival. Instead, the majority (75%) of patients with the most unfavourable combination of Cdc20 and securin (high expression for both) could expect only 1.3-year breast cancer survival. In the same vein, 75% of the patients with the most favourable combination of securin and Cdc27 were alive after 18.3 years whereas 75% with the most unfavourable combination lived only for 3.2 years after primary diagnosis (Cdc27 > 10% and securin <10% of cancer cells vs. Cdc27 <10% and securin >10% of cancer cells, respectively).

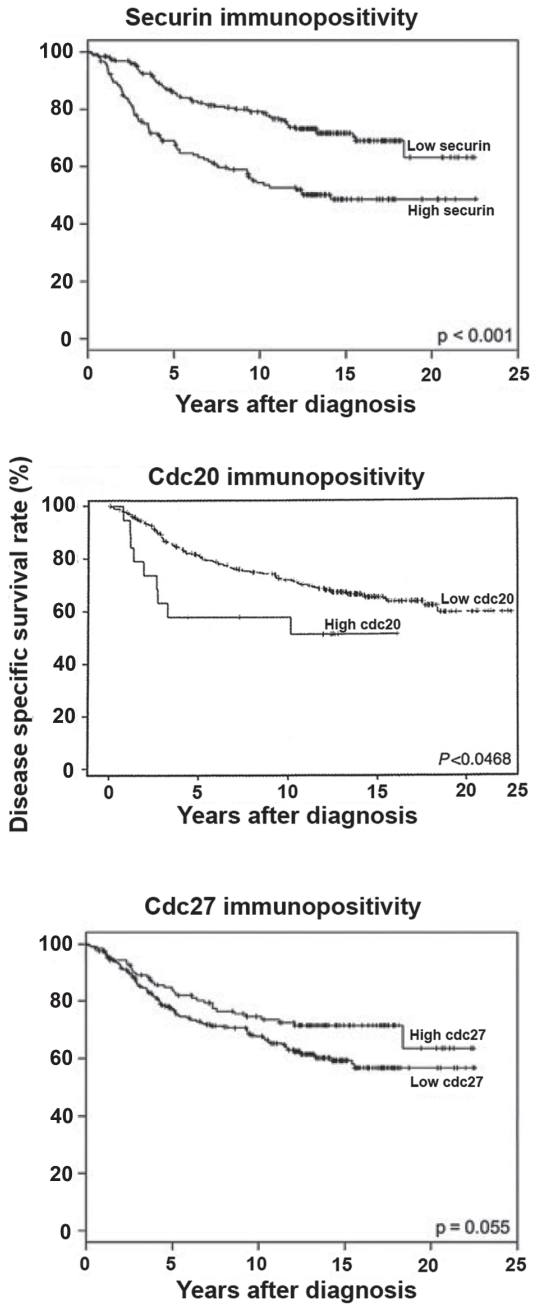

Figure 5. Summary of univariate Kaplan-Meier estimates to show disease-specific survival of patients divided into groups according to securin, Cdc27 and Cdc20 immunopositivity, and their combinations. The curves are based on the whole follow-up.

The survival differences demonstrated by the Kaplan-Meier curves of all studied proteins and their combinations were most significant in the beginning of the follow-up period. Towards the end of the follow-up, however, the gap between the survival curves of the

different patient groups gradually diminished. To illustrate this observation, the risk of breast cancer death was determined separately during the first 5 years after diagnosis and treatment. In these analyses comparing the cases with the most unfavourable protein expression (high securin in combination with high Cdc20 or low Cdc27) to cases with the most favourable expression (low securin in combination with low Cdc20 or high Cdc27) resulted in up to a 6.8-fold risk of breast cancer death ($p<0.001$, CI 3.2–14.9) (III, IV).

Finally, multivariate analyses were performed involving the studied proteins and the established prognosticators of breast cancer, i.e. axillary lymph node status, tumor size, histological grade and type, ER and PR status, HER2-amplification status and intrinsic classification. When the data of breast cancer cases were adjusted for different known prognosticators of breast cancer securin immunohistochemical positivity still appeared as a statistically significant predictor for breast cancer outcome with risk of breast cancer death ranging between 1.7- 2.3 –fold in different studies ($p<0.05$) (I-IV). In multivariate analyses, Cdc20 or Cdc27 expressions alone failed to show statistical significance among the studied prognosticators. However, in combination with securin both Cdc20 and Cdc27 increased the risk ratios associated with securin alone. In all studies, axillary lymph node status was the most significant prognosticator, with HRs of breast cancer death for node-positive patients ranging from 2.9 to 3.8, compared to node negative patients. Along with securin, axillary lymph node status and tumor size and in some analyses also histological grade were shown statistically significant prognosticators of breast cancer death in our material.

6. DISCUSSION

This summary is based on four original studies, in which the expression of securin oncoprotein and related proteins in cell cycle regulation, Cdc20 and Cdc27, were examined in a clinical material containing a maximum of 603 primary breast cancer patients with up to 22 years of follow-up. Immunohistochemical expression levels of the proteins were compared with breast cancer survival, ploidity, proliferation and clinico-pathological parameters of the patient material, in order to analyze their role in breast cancer progression and prognosis.

6.1 Securin, Cdc20 and Cdc27 in breast cancer prognosis

6.1.1 Securin

The oncoprotein securin has been suggested with multiple functions in tumorigenesis and progression of malignant diseases (Kim *et al.* 2007a, b, Hamid *et al.* 2005). Securin has been associated with various functions in cell metabolism, signal transduction and control of the cell cycle, especially in the regulation of chromosomal integrity in cell proliferation (Vlotides *et al.* 2007, Jallepalli *et al.* 2001). In mitosis, securin has been characterized as an anaphase-inhibitor, in which role it inhibits the catalytic activity of separase until the onset of anaphase (Waizenegger *et al.* 2002). The exact sequence of activation and degradation of regulatory proteins during the cell cycle are not sufficiently known at the moment. Still, the common understanding is that regulatory processes involving securin are critical for the timely separation of the chromatids and even distribution of chromosomes in the two daughter cells. According to present knowledge, particularly APC-Cdc20 catalyzed ubiquitylation and related Cdc27-dependent regulation of securin participates in initiation of sister chromosome segregation to promote the metaphase–anaphase transition (Peters 2006, Hagting *et al.* 2002). This mitotic check-point has been suggested as one of the main events to prevent premature cell division resulting in abnormal DNA content and aneuploidy, which are known as main characteristics in tumorigenesis, and feature of poor outcome in several malignancies (Bannon and McGee 2009, Weaver and Cleveland 2009, Draviam *et al.* 2004, Kops *et al.* 2005, Bharadwaj and Yu 2004, Hagting *et al.* 2002). In recent years, research on securin in normal and malignant cells and tissues, including breast cancer, has steadily started to accumulate. Still, the exact functional defects of securin in cancer cells are largely unknown. Also, the prognostic associations are not previously reported in relevant clinical materials of human breast cancer.

The present investigation suggests that immunohistochemical expression for securin is an independent prognosticator of invasive breast cancer and can be used to identify both favourable (I) and unfavourable (II-IV) outcome of disease, depending on the chosen viewpoint. In our analyses, high securin immunohistochemical expression was associated with disease-specific survival and unfavourable outcome in the whole material of invasive breast carcinoma cases, and in patient subgroups divided according to standard clinical prognosticators, axillary lymph node status, tumor size and histological type. Also in terms of patient survival time, the prognosis of patients with high securin immunohistochemical expression was considerably less favourable than of patients with low securin expression (mean survival time after diagnosis 15.8 years and 9.4 years, respectively, $p<0.0001$, $n=603$) (II). Previously, securin expression has been documented in invasive (Saez *et al.* 1999, Solbach *et al.* 2004, Ogbagabriel *et al.* 2005) and in situ carcinomas of the breast (Puri *et al.* 2001). In a material of 90 breast tumors including human tissues and cell cultures, Ogbagabriel and coworkers (2005) have reported a strong securin immunohistochemical expression in breast cancer cells as compared to normal breast epithelium. In this report, the most prominent securin positivity was observed in the most aggressive, mitotically active and morphologically pleomorphic invasive ductal carcinomas, in metastatic disease, and in breast carcinoma cell cultures (Ogbagabriel *et al.* 2005). Solbach and collaborators (2004) analyzed Pttg1 mRNA expression in 72 breast carcinomas and benign breast tissue specimen and found a correlation between Pttg1 mRNA overexpression and lymph node involvement, and tumour recurrence during a 5-year follow-up period. Despite the accumulating evidence on the role of securin in breast cancer progression and metastasis, also controversial results have been published suggesting that securin might, instead, be a tumour suppressor (Hatcher *et al.* 2014). This interpretation was based on observations from *in vitro* experiments where mutant mice lacking Pttg1 developed spontaneous mammary tumors, and from 239 human breast tumors, where securin levels were down-regulated in the studied patient material (Hatcher *et al.* 2014). The bulk of information, still, supports the tumor-promoting role of securin in breast cancer (Salehi *et al*. 2008). Securin has also been associated with lymph node infiltration and distant metastasis (Grizzi *et al.* 2013). The role of securin in metastatic behavior has been explained by its involvement in activating known target genes involved in the metastatic process (Liao *et al.* 2012). *Pttg1* has also been associated with specific marker genes detected in circulating tumor cells in the blood of women with breast cancer, presenting a potential method for early detection of breast cancer and tool for monitoring breast cancer progression (Chen *et al.* 2006).

In literature, data on the interactions of securin in breast cancer tumorigenesis and progression is accumulating, especially concerning the association of securin with TP53, one of the main breast cancer susceptibility genes. *Pttg1* has been associated with

modulation of TP53-mediated transcriptional activity and apoptosis, suggesting that *Pttg1* might have a role among the mitotic checkpoint genes (Bernal *et al.* 2002, Lo *et al.* 2007). Results from MCF7 breast cancer cells have indicated that *Pttg-1* overexpression may participate in both p53-dependent and p53-independent apoptosis, whereas, in the absence of p53, *Pttg1* inhibits apoptosis leading to aneuploidy (Yu *et al.* 2000b). These rapports appear to support the interpretation that *Pttg1* is among the regulatory pathways through which aneuploidy and malignant transformation are mediated. There have also been attempts to explain invasive and metastatic growth in breast cancer with the involvement of securin in the epithelial-mesenchymal transition and the role of cancer stem cells (Yoon *et al.* 2012). Importantly, *BRCA1* and *2* have been suggested with a role in regulation of several mitotic spindle checkpoint proteins, including securin. This conclusion is suggested by study of Bae and coworkers (2005) who showed in microarray analysis downregulation of *Pttg1* following *BRCA* siRNA knockout in MCF-7 breast carcinoma cell lines. This finding seems to support the conclusion that *BRCA* positively regulates *Pttg1* levels, but the exact mechanisms of interaction between *BRCA* and *Pttg1* are still unknown. According to some reports, the function of securin may also be mediated by estrogen levels and, consequently, overexpression of securin has been related to endocrine therapy resistance in breast cancer (Heaney *et al.* 1999, Ghayad *et al.* 2009).

In the present investigations, securin immunohistochemical expression was detected as both nuclear and cytoplasmic staining (I-IV). Similar expression pattern has been reported by others in breast carcinoma (Ogbagabriel *et al.* 2005) and other malignancies (Ishitsuka *et al.* 2013, Salehi *et al.* 2013, Talvinen *et al.* 2006, Zhu *et al.* 2006). Corresponding nuclear and cytoplasmic expression patterns apply also for Cdc20 and Cdc27 immunostainings in the present investigations (III, IV) and in literature (Choi *et al.* 2013, Moura *et al.* 2013). In our studies, the majority of securin, Cdc20 and Cdc27 immunopositivities were seen in non-mitotic cancer cells, and mitotic cells were variably positive. Immunohistochemical expression of the studied proteins was not either systematically present or absent in the morphologically identified separate phases of cell division. Based on the known roles of securin, Cdc20 and Cdc27, one would expect that the function and prognostic role of the proteins would be dependent on expression in the early phases of the cell division and on the fraction of nuclear expression in the malignant cells. However, the separate prognostic value of nuclear and cytoplasmic securin expression is not sufficiently evaluated in literature. Up to date only one paper reporting on endometrial carcinoma suggests that nuclear, and not cytoplasmic, securin expression is associated with prognostic value (Kim *et al.* 2008). It appears probable that the subcellular localization of securin is relevant to its mechanisms in cell proliferation and cancer but further investigations are needed to verify this. Also the role of pituitary tumor transforming gene binding protein (PBP)

needs to be evaluated before the role of nuclear and cytoplasmic securin expression in breast cancer progression and prognosis is settled (Watkins *et al.* 2010, Smith *et al.* 2010).

6.1.2 Securin and Cdc20

As an activating subunit of APC, Cdc20 is known to drive mitosis from metaphase to anaphase, where the APC-Cdc20-mediated degradation of securin is one of the critical steps of cell division (Peters 2006). Increase in Cdc20 expression has been reported in many human cancers, often with associated less favorable prognosis (Chang *et al.* 2012, Choi *et al*. 2013, Kato *et al.* 2012, Moura *et al.* 2013, Wu *et al.* 2013). In the present prognostic analyses, substantially increased risk of breast cancer death was observed for patients with high Cdc20 immunohistochemical expression (HR 2.0, p=0.047). The prognostic associations were, however, still emphasized in combination with high securin immunohistochemical expression (HR 4.3, p<0.001). Previous literature available on Cdc20 expression and particularly on its role in progression or prognosis in breast cancer is very sparse. Kidokoro and coworkers (2008) detected Cdc20 overexpression in a large set of human malignancies, including breast cancer based on cDNA microarray analyses. In this study, Cdc20 expression was increased more than threefold in the majority (60%) of the breast cancer cases examined. Down-regulation of Cdc20 in breast cancer cells has been associated with inhibition of cell proliferation *in vitro* (Jiang *et al*, 2011, Jiang *et al*, 2012). Yuan and coworkers (2006) have detected Cdc20 among the checkpoint proteins overexpressed in breast cancer cells with chromosomal instability compared to chromosomally stable cancer cell lines and normal breast tissue. Based on their observations, in breast cancer, defective mitotic checkpoint seems not to be caused by mutations in checkpoint genes, but increased expression of checkpoint genes might be a marker for chromosomally instable breast cancer. Also, treatment with siRNA against Cdc20 has been shown to induce G2/M arrest and suppress cell growth (Kidokoro *et al*. 2008, Taniguchi *et al*. 2008). Cdc20 has, moreover, emerged in studies attempting to identify gene expression effects resulting from a nutritional intervention (Shike *et al.* 2014).

The present results emphasize the value of the combination of securin and Cdc20 expression in identification of triple-negative breast carcinoma (TNBC). TNBCs were strongly over-presented among the patients with high securin and Cdc20 expression (HR 68.0, p<0.001) but the patient subgroup (n=14) is obviously too small for any conclusions. Also, expression of basal cytokeratins was observed in all but one of the cases with high securin and Cdc20 expression. Currently, the main clinical problem is the lack of targeted therapies for TNBC patients (O'Toole *et al*. 2013). According to different sources, TNBCs account for approximately 12-

17% of all breast cancers and constitute a heterogeneous subgroup of breast cancer with specific molecular signatures and therapeutic challenges (Chiorean *et al.* 2013, Stagg and Allard 2013, Foulkes *et al.* 2010, Carey *et al.* 2010, Metzger-Filho *et al.* 2012). TNBCs are concentrated among younger women and show more aggressive course of disease than ER-positive/HER2 negative cancers, tendency towards hematogenous spread, and higher risk of recurrence and death than breast carcinoma cases in average (Blows *et al.* 2010, Tischkowitz *et al.* 2007, Fulford *et al.* 2007). These tumors are enriched in the intrinsic breast cancer group of basal-like cancers, which are most commonly diagnosed in association with *BRCA1* mutation (Dent *et al.* 2007, Anders and Carey 2008, Billar *et al.* 2010, Foulkes *et al.* 2010). Both basal like breast cancers and cancers arising in *BRCA1* mutation carriers show a specific pattern of cell cycle protein expression (Schneider *et al.* 2008, Wolff *et al.* 2007, Hammond *et al.* 2010, Lehmann *et al.* 2011). According to the present observations, combined high Cdc20 and securin expression might be useful in identifying patients with a particularly aggressive triple-negative breast cancer but more investigations in a larger patient material are needed for definitive conclusions. In the present patient material, though, TNBCs showing high Cdc20 and securin expression were associated with 3.2-fold risk of breast cancer death as compared to the rest of the triple-negative cases, although the association in the small patient subgroup sparsely failed to show statistical significance (p=0.0683).

6.1.3 Securin and Cdc27

Cdc27 as part of regulation of APC is critical for controlling the timing of mitosis, particularly, the onset of anaphase and arrest of the cell cycle in case of unattached kinetochore or disruption of the mitotic spindle (Peters 2006, Topper *et al.* 2002). The APC is responsible for the degradation of anaphase inhibitors, such as securin, and may also have a role in the control of spindle checkpoint signaling (Topper *et al.* 2002). In the present analyses, high Cdc27 was inversely associated with breast cancer outcome. This finding is in line with previous *in vitro* experiments where Cdc27 was found downregulated in most breast cancer cell lines, but detectable in human untransformed breast epithelial cells (Pawar *et al.* 2010). However, Cdc27 alone did not show independent prognostic value in the studied breast cancer material. Instead, the combination of securin and Cdc27 predicted 2.9-fold risk of breast cancer death (95% CI 1.7–5.0, p<0.0001) in univariate and Kaplan–Meier analyses. In multivariate analysis involving also the traditional prognosticators of breast cancer, the combination of securin and Cdc27 immunohistochemistry was the strongest predictor of breast cancer death (HR 2.7, 95% CI 1.4–5.0, p=0.0018) after axillary lymph node status.

6.2 Securin and proliferation in breast cancer

As the main functions of securin involve participation in regulation of the cell cycle and balance between cell proliferation and apoptosis, it is not surprising that a correlation between securin and proliferation marker Ki-67 expression was found in our analyses (I). Previous literature is, however, controversial concerning the relation between securin and proliferation, as securin has been associated with both pro-proliferative (Kakar and Jennes 1999, Hamid *et al.* 2005, Pei 2001) and inhibitory effects in cell division (Yu *et al.* 2000a, Yu *et al.* 2000b, Cho-Rok *et al.* 2006, Mu *et al.* 2003). Some investigations suggest that securin overexpression could reduce cell proliferation by arresting mitosis (Yu *et al.* 2000a,b) while others have speculated that securin could function in cell proliferation through the induction of apoptosis and delay of mitosis (Akino *et al*. 2005). Available clinical studies, however, suggest a correlation between securin expression and cell proliferation in different malignancies (Heaney *et al*. 2002, Tsai *et al*. 2005, Filippella *et al*. 2006, Genkai *et al*. 2006, Zhu *et al*. 2006). The present and previous analyses suggest for securin prognostic value beyond the routine proliferation marker (I) (Talvinen *et al.* 2008) since high securin expression identified a subgroup of patients with high risk of breast cancer death (HR 13.1, p=0.02, 95% CI 1.4 – 121.3, n=310) among tumors with low proliferative activity (Ki-67 less than 10%). In our material, the combination of high immunohistochemical positivity for both securin and Ki-67 indicated a 4.3-fold risk of breast cancer death as compared with the prognostic value of low securin and Ki-67 ($p<0.05$, 95% CI 1.3 – 14.2), which is higher than the risk associated with either separately. To conclude, our results suggest that the application of securin alone or in combination with the traditional proliferation markers could contribute to the prognostication of invasive breast cancer.

Also based on literature, securin clearly differs from the established markers of cell proliferation used in routine clinical pathology. Ki-67 and proliferating cell nuclear antigen (PCNA) are known to be expressed in all phases of the cell cycle (Takasaki *et al.* 1981, Guillaud *et al.* 1989, van Oijen *et al.* 1998). In contrast, securin concentrates on a specific phase of the cell cycle, the expression gradually increasing during the S phase with a peak at G2/M (Yu *et al.* 2000a, Chen *et al*. 2005). Based on this transient expression in dividing cells, one would expect securin protein expression in prophase cells, expression or absence of expression in metaphase cells, and absence in anaphase/telophase cells. To our knowledge, there is no previous literature available which would systematically report on securin expression in benign or malignant mitotic cells. Results from cell cultures suggest that conclusions on securin expression in mitosis may not be straightforward since securin expression in dividing malignant cells might only last for some hours (Yu *et al.* 2000b). Our observations from securin immunohistochemistry on TMAs were also equivocal since mitotic figures in different stages of cell division

did not stain systematically for securin. These findings emphasize the need for more thorough biological understanding of the function and subcellular localization of securin during a cell cycle in normal and malignant proliferating cells.

6.3 Securin and aneuploidy in breast cancer

As securin overexpression disrupts normal sister chromatid separation, dysfunctional securin may be one explanation for abnormal DNA content in breast carcinoma. Experiments in several human cell lines have shown that securin may have a role in cell transformation, chromosomal instability, and aneuploidy (Yu *et al.* 2000b, Kim *et al.* 2005, Jallepalli *et al.* 2001, Wang and Melmed 2000). Lo and coworkers (2007) studied single nucleotide polymorphisms (SNPs) in breast cancer and suggested that *Pttg1* SNPs were associated with increased risk of breast cancer development in women, indicating that the loss of chromosomal integrity may underlie breast tumorigenesis. Inhibition of sister chromatid separation also suggests that securin could partly explain for uneven chromatid separation and induction of aneuploidy in tumor progression (Tfelt-Hansen *et al.* 2006, Vlotides *et al.* 2007, Salehi *et al.* 2008). Also in the present study detected with static cytometry in cell imprints of fresh breast cancer tissue material, a strong correlation was observed between abnormal DNA content and high securin and Cdc20 and low Cdc27 expressions in single analyses and in analyses combining the studied proteins. For example, high securin expression (>10% of breast cancer cells) was related to 9.8-fold odds for aneuploid DNA content as compared with low securin expression ($p=0.0007$). The present analyses also seem to indicate that the prognostic value of securin is independent rather than mediated through ploidy (IV). In literature, it has been further suggested that high-level securin expression in breast cancer cells could restrict tumor growth by favoring extinction of the most pleomorphic cancer cells, possibly through apoptosis (Thompson and Compton 2008). In the present study, this observation may be supported from the unexpectedly favorable outcome of patients showing a significant fraction of cells exceeding 16x DNA content, although this correlation sparsely failed to show statistical significance in the small patient subgroup. In summary, aneuploidy and consequent abnormal gene expression are both common features of breast cancer, and decisive for the course of the malignant disease. According to present knowledge, aneuploidy promotes malignant tumors, presumably as a consequence of unstable genome, which may cause gain of oncogenes and / or loss of tumor suppressor genes (Weaver and Cleveland 2009).

7. CONCLUSIONS

To date, breast cancer treatment decisions are based on prognostic clinico-pathological parameters, ie. tumor size, presence of lymph node metastases and histological grade, and on three predictive markers of response to therapy, namely presence of estrogen and progesterone receptors and HER2 amplification. Despite the ongoing developments in diagnostics and treatment, breast cancer continues to be the major cause of morbidity and mortality among western women. The knowledge of breast cancer as a heterogeneous group of disease is constantly increasing, calling for cancer-specific individually designed therapies. Especially the treatment of triple-negative carcinoma still is a challenge and relies on non-specific therapeutic agents with heavy side effects. Thus, despite advances in diagnostics, treatment and the overall prognosis of breast cancer, the outcome of individual breast cancer patients may still be unpredictable and relapses may occur over a decade after the primary treatment (Thompson and Compton 2008). Clinico-pathological observations, molecular analyses and transcriptomic profiles are needed to establish new treatments and evaluate response to therapy (Reis-Filho and Pusztai 2011, Weigelt and Reis-Filho 2009, Weigelt *et al.* 2010).

This investigation is based on a patient material of approximately 600 breast cancer patients and up to 22 years of follow-up. The study reports on associations of specific metaphase-anaphase regulators, securin, Cdc20, and Cdc27 with DNA content and outcome of disease. To our knowledge, this is, to date, the only prognostic investigation of the studied proteins in a clinically relevant patient material. The study emphasizes the value of securin immunohistochemistry in detecting abnormal DNA content and different prognostic subgroups of patients with invasive breast cancer. Also, immunohistochemical expression of Cdc20 and Cdc27 show prognostic value in the patient material but their role is emphasized in combination with securin where they enforce the prognostic correlations of securin alone. As the main results, high securin expression alone and in combination with Cdc20 and Cdc27 predict up to 9.8-fold odds for aneuploid DNA content in human breast cancer ($p=0.0007$). In prognostic studies, securin is a strong and independent prognosticator for disease-specific survival in human breast cancer and associated in specific subgroups with 13-fold risk of breast cancer death ($p=0.024$). In Kaplan–Meier analyses, the combination of the studied cell cycle regulators indicated a 9-year decrease in breast cancer survival. Of special interest is the observation that the challenging subgroup of triple-negative and basal-type breast cancers is strongly overrepresented among cases with high securin and high Cdc20 (>90% of patients).

In recent years, data on the regulators of the metaphase-anaphase transition, securin, Cdc20 and Cdc27, has accumulated and been associated with breast cancer progression

and stage of disease. Also, the therapeutic potential of the proteins has been addressed, and e.g. securin has been associated with resistance to gefitinib (tyrosine kinase inhibitor of the epidermal growth factor receptor, EGFR) induced apoptosis and anticancer treatment (Yu *et al.* 2013). The present study introduces the prognostic value of securin, possibly enforced by Cdc20 and Cdc27, as potential clinical markers for advanced disease and unfavourable prognosis in breast cancer. The findings suggest that securin, Cdc20 and Cdc27 are justified among the myriad of biomarkers to be further investigated for potential applications in prognostication and development of immunotherapeutic strategies for primary and metastatic breast cancer.

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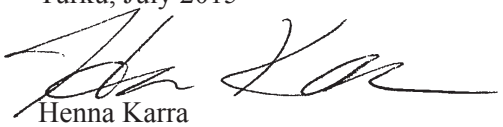
Turku, July 2015



Henna Karra

REFERENCES

Abbas T, Keaton MA, Dutta A. (2013). Genomic Instability in Cancer. Cold Spring Harb Perspect Biol *5*, a012914.

Abbud RA, Takumi I, Barker EM, Ren SG, Chen DY, Wawrowsky K, Melmed S. (2005). Early multipotential pituitary focal hyperplasia in the alphasubunit of glycoprotein hormone-driven pituitary tumor-transforming gene transgenic mice. Mol Endocrinol *19*, 1383–1391.

Ai J, Zhang Z, Xin D, Zhu H, Yan Q, Xin Z, Na Y, Guo Y. (2004). Identification of over-expressed genes in human renal cell carcinoma by combining suppression subtractive hybridization and cDNA library array. Sci China C Life Sci *47*, 148–157.

Akino K, Akita S, Mizuguchi T, Takumi I, Yu R, Wang XY, Rozga J, Demetriou AA, Melmed S, Ohtsuru A, Yamashita S. (2005). A novel molecular marker of pituitary tumor transforming gene involves in a rat liver regeneration. J Surg Res *129,* 142–146.

Anders C, Carey LA. (2008). Understanding and treating triple-negative breast cancer. Oncology (Williston Park) *22,* 1233-1239.

Anderson E. (2002).The role of oestrogen and progesterone receptors in human mammary development and tumorigenesis. Breast Cancer Res *4*, 197-201.

Angèle S, Jones C, Reis Filho JS, Fulford LG, Treilleux I, Lakhani SR, Hall J. (2004). Expression of ATM, p53, and the MRE11-Rad50-NBS1 complex in myoepithelial cells from benign and malignant proliferations of the breast. J Clin Pathol *57,* 1179-1184.

Arvold ND, Taghian AG, Niemierko A, Abi Raad RF, Sreedhara M, Nguyen PL, Bellon JR, Wong JS, Smith BL, Harris JR. (2011). Age, breast cancer subtype approximation, and local recurrence after breast-conserving therapy. J Clin Oncol *29*, 3885e91.

Ashikaga T, Krag DN, Land SR, Julian TB, Anderson SJ, Brown AM, Skelly JM, Harlow SP, Weaver DL, Mamounas EP, Costantino JP, Wolmark N; National Surgical Adjuvant Breast, Bowel Project. (2010). Morbidity results from the NSABP B-32 trial comparing sentinel lymph node dissection versus axillary dissection. J Surg Oncol *102*, 111-118.

Baak JP, Gudlaugsson E, Skaland I, Guo LH, Klos J, Lende TH, Søiland H, Janssen EA, Zur Hausen A. (2009). Proliferation is the strongest prognosticator in node-negative breast cancer: significance, error sources, alternatives and comparison with molecular prognostic markers. Breast Cancer Res Treat *115*, 241-254.

Bae I, Rih JK, Kim HJ, Kang HJ, Haddad B, Kirilyuk A, Fan S, Avantaggiati ML, Rosen EM. (2005). BRCA1 regulates gene expression for orderly mitotic progression. Cell Cycle *4*, 1641-1666.

Bakhoum SF, Compton DA. (2012). Chromosomal instability and cancer: a complex relationship with therapeutic potential. J Clin Invest *122*, 1138-1143.

Bannon JH, Mc Gee MM. (2009). Understanding the role of aneuploidy in tumorigenesis. Biochem Soc Trans *37,* 910–913.

Bardin AJ, Amon A. (2001). Men and sin: what is the difference. Nature Rev Mol Cell Biol 2, 815-826.

Bedard PL, Cardoso F, Piccart-Gebhart MJ. (2009). Stemming resistance to HER-2 targeted therapy. J Mammary Gland Biol Neoplasia *14,* 55-66.

Bernal JA, Luna R, Espina A, Lázaro I, Ramos-Morales F, Romero F, Arias C, Silva A, Tortolero M, Pintor-Toro JA. (2002). Human securin interacts with p53 and modulates p53-mediated transcriptional activity and apoptosis. Nat Genet *32*, 306-311.

Bharadwaj R, Yu H. (2004). The spindle checkpoint, aneuploidy, and cancer. Oncogene *23,* 2016-2027.

Billar JA, Dueck AC, Stucky CC, Gray RJ, Wasif N, Northfelt DW, McCullough AE, Pockaj BA. (2010). Triple-negative breast cancers: unique clinical presentations and outcomes. Ann Surg Oncol. 17 *Suppl 3,* 384-390.

Blamey RW, Pinder SE, Ball GR, Ellis IO, Elston CW, Mitchell MJ, Haybittle JL. (2007). Reading the prognosis of the individual with breast cancer. Eur J Cancer *43*, 1545-1547.

Blows FM, Driver KE, Schmidt MK, Broeks A, van Leeuwen FE, Wesseling J, Cheang MC, Gelmon K, Nielsen TO, Blomqvist C, Heikkilä P, Heikkinen T, Nevanlinna H, Akslen LA, Bégin LR, Foulkes WD, Couch FJ, Wang X, Cafourek V, Olson JE, Baglietto L, Giles GG, Severi G, McLean CA, Southey MC, Rakha E, Green AR, Ellis IO, Sherman ME, Lissowska J, Anderson WF, Cox A, Cross SS, Reed MW, Provenzano E, Dawson SJ, Dunning AM, Humphreys M, Easton DF, García-Closas M, Caldas C, Pharoah PD, Huntsman D. (2010). Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. PLoS Med *7*, e1000279.

Boelaert K, Yu R, Tannahill LA, Stratford AL, Khanim FL, Eggo MC, Moore JS, Young LS, Gittoes NJ, Franklyn JA, Melmed S, McCabe CJ. (2004). PTTG's c-terminal PXXP motifs modulate critical

cellular processes in vitro. J Mol Endocrinol *33*, 663-677.

Brown DC, Gatter KC. (2002). Ki67 protein: the immaculate deception? Histopathology *40*, 2-11.

Bundred NJ. (2001). Prognostic and predictive factors in breast cancer. Cancer Treat Rev *27*:137-142.

The Cancer Genome Atlas Network. (2012). Comprehensive molecular portraits of human breast tumours. Nature *490*, 61-70.

Carey L, Winer E, Viale G, Cameron D, Gianni L. (2010). Triple-negative breast cancer.Disease entity or title of convenience? Natl Rev Clin Oncol *7,* 683-692.

Carter SL, Eklund AC, Kohane IS, Harris LN, Szallasi Z. (2006). A signature of chromosomal instability inferred from gene expression prolifes predicts clinical outcome in multiple human cancers. Nat Genet *38*, 1043-1048.

Castilla C, Flores ML, Medina R, Pérez-Valderrama B, Romero F, Tortolero M, Japón MA, Sáez C. (2014). Prostate Cancer Cell Response to Paclitaxel Is Affected by Abnormally Expressed Securin PTTG1. Mol Cancer Ther *13*, 2372-2383.

Chang DZ, Ma Y, Ji B, Liu Y, Hwu P, Abbruzzese JL, Logsdon C, Wang H. (2012). Increased CDC20 expression is associated with pancreatic ductal adenocarcinoma differentiation and progression. J Hematol Oncol *5,* 15.

Chao JI, Hsu HS, Tsou TC. (2006). Depletion of securin increases arsenite-induced chromosome instability and apoptosis via a p53-independent pathway. Toxicol Sci *90,* 73–86.

Chen CC, Chang TW, Chen FM, Hou MF, Hung SY, Chong IW, Lee SC, Zhou TH, Lin S. (2006). Combination of multiple mRNA markers (PTTG1, Survivin, UbcH10 and TK1) in the diagnosis of Taiwanese patients with breast cancer by membrane array. Oncology *70,* 438–446.

Chen G, Li J, Li F, Li X, Zhou J, Lu Y, Ma D. (2004). Inhibitory effects of anti-sense PTTG on malignant phenotype of human ovarian carcinoma cell line SK-OV-3. J Huazhong Univ Sci Technolog Med Sci *24*, 369–372.

Chen Z, Merta PJ, Lin NH, Tahir SK, Kovar P, Sham HL, Zhang H. (2005). A-432411, a novel indolinone compound that disrupts spindle pole formation and inhibits human cancer cell growth. Mol Cancer Ther *4,* 562–568.

Chesnokova V, Kovacs K, Castro AV, Zonis S, Melmed S. (2005). Pituitary hypoplasia in Pttg−/− mice is protective for Rb+/− pituitary tumorigenesis. Mol Endocrinol *19*, 2371–2379.

Chien W, Pei L. (2000). A novel binding factor facilitates nuclear translocation and transcriptional activation function of the pituitary tumor-transforming gene product. J Biol Chem *275*, 19422-19427.

Chiorean R, Braicu C, Berindan-Neagoe I. (2013). Another review on triple negative breast cancer. Are we on the right way towards the exit from the labyrinth? Breast *22,* 1026-1033.

Choi JW, Kim Y, Lee JH, Kim YS. (2013). High expression of spindle assembly checkpoint proteins CDC20 and MAD2 is associated with poor prognosis in urothelial bladder cancer. *Virchows Arch*, *463,* 681-687.

Cho-Rok J, Yoo J, Jang YJ, Kim S, Chu IS, Yeom YI, Choi JY, Im DS. (2006). Adenovirus-mediated transfer of siRNA against PTTG1 inhibits liver cancer cell growth in vitro and in vivo. Hepatology *43,* 1042–1052.

Christopoulou L, Moore JD, Tyler-Smith C. (2003). Over-expression of wild-type securin leads to aneuploidy in human cells. Cancer Lett *202*, 213-218.

Clarke RB. (2003). Steroid receptors and proliferation in the human breast. Steroids *68*, 789-794.

Clayton F. (1991). Pathologic correlates of survival in 378 lymph node-negative infiltrating ductal breast carcinomas: mitotic count is the best single predictor. Cancer *68*, 1309–1317.

Clem AL, Hamid T, Kakar SS. (2003). Characterization of the role of Sp1 and NF-Y in differential regulation of PTTG/securin expression in tumor cells. Gene *322*, 113-21.

Colditz GA, Kaphingst KA, Hankinson SE, Rosner B. (2012). Family history and risk of breast cancer: nurses' health study. Breast Cancer Res Treat *133,* 1097-1104.

Colozza M, Azambuja E, Cardoso F, Sotiriou C, Larsimont D, Piccart MJ. (2005). Proliferative markers as prognostic and predictive tools in early breast cancer: where are we now? Ann Oncol *16*, 1723-1739.

Cuzick J, Dowsett M, Pineda S, Wale C, Salter J, Quinn E, Zabaglo L, Mallon E, Green AR, Ellis IO, Howell A, Buzdar AU, Forbes JF. (2011). Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. J Clin Oncol *29,* 4273-4278.

Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P, Narod SA. (2007). Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res *13,* 4429-4434.

Desmedt C, Sotiriou C. (2006). Proliferation: the most prominent predictor of clinical outcome in breast cancer. Cell Cycle *5*, 2198–2202.

Ding ZY, Wu HR, Zhang JM, Huang GR, Ji DD. (2014). Expression characteristics of CDC20 in gastric cancer and its correlation with poor prognosis. Int J Clin Exp Pathol *7*, 722-727.

Dominguez A, Ramos-Morales F, Romero F, Rios RM, Dreyfus F, Tortolero M, Pintor-Toro JA. (1998). hPTTG, a human homologue of rat PTTG, is overexpressed in hematopoietic neoplasms. Evidence for a transcriptionalactivation function of hPTTG. Oncogene *17*, 2187–2193.

Dowsett M, Nielsen ON, A'Herrn R, Bartlett J, Coombes C, Cuzick J, Ellis M, Henry NL, Hugh JC, Lively T, McShane L, Paik S, Penault-Llorca F, Prudkin L, Regan M, Salter J, Sotiriou C, Smith IA, Viale G, Zujewski JA, Hayes DF. (2011). Assessment of Ki67 in breast cancer: Recommendations from the International Ki67 in Breast Cancer Working Group. J Natl Cancer Inst *103*, 1656-1664.

Draviam VM, Xie S, Sorger PK. (2004). Chromosome segregation and genomic stability. Curr Opin Genet Dev *14,* 120-125.

Drukker CA, Bueno-de-Mesquita JM, Retèl VP, van Harten WH, van Tinteren H, Wesseling J, Roumen RM, Knauer M, van 't Veer LJ, Sonke GS, Rutgers EJ, van de Vijver MJ, Linn SC. (2013). A prospective evaluation of a breast cancer prognosis signature in the observational RASTER study. Int J Cancer *131*, 929–936.

Duesberg P, Li R, Fabarius A, Hehlmann R. (2006). Aneuploidy and cancer: from correlation to causation. Contrib Microbiol *13,* 16-44.

Duesberg P, Stindl R, Hehlmann R. (2000). Explaining the high mutation rates of cancer cells to drug and multidrug resistance by chromosome reassortments that are catalysed by aneuploidy. Proc Natl Acad Sci USA *97*:14295–14300.

Early Breast Cancer Trialists' Collaborative Group (EBCTCG). (2005). Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. Lancet *366*, 2087–2106.

Edge SB, Byrd DR, eds. AJCC cancer staging manual. 7th Edition. Springer: New York 2010.

Ellis MJ, Ding L, Shen D, Luo J, Suman VJ, Wallis JW, Van Tine BA, Hoog J, Goiffon RJ, Goldstein TC, Ng S, Lin L, Crowder R, Snider J, Ballman K, Weber J, Chen K, Koboldt DC, Kandoth C, Schierding WS, McMichael JF, Miller CA, Lu C, Harris CC, McLellan MD, Wendl MC, DeSchryver K, Allred DC, Esserman L, Unzeitig G, Margenthaler J, Babiera GV, KP Marcom, Guenther JM, Leitch M, Hunt K, Olson J, Tao Y, Maher CA, Fulton LL, Fulton RS, Harrison M, Oberkfell B, Du F, Demeter R, Vickery TL, Elhammali A, Piwnica-Worms H, McDonald S, Watson M, Dooling DJ, Ota D, Chang LW, Bose R, Ley TJ, Piwnica-Worms D, Stuart JM, Wilson RK, Mardis ER. (2012). Whole-genome analysis informs breast cancer response to aromatase inhibition. Nature *486*, 353-360.

El-Naggar SM, Malik MT, Kakar SS. (2007) Small interfering RNA against PTTG: a novel therapy for ovarian cancer. Int J Oncol *31*, 137–143.

Elston CW, Ellis IO. (1991). Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology *19*, 403-410.

Epstein M, Ma Y, Press MF. ERBB2 testing: assessment of status for targeted therapies. In: Disease of the breast, 4th edition. Harris JR, Lippman ME, Morrow M, Osbourne CK, eds. Wolter Kluwer Lippincott Williams & Wilkins: Philadelphia. pp 431-442, 2010.

Faber AC, Wong KK, Engelman JA. (2010). Differencies underlying EGFR and HER2 oncogene addition. Cell Cycle *9*, 851-852.

Fang G, Yu H, Kirschner MW. (1998). Direct binding of CDC20 protein family members activates the anaphase-promoting complex in mitosis and G1. Mol Cell *2*, 163-171.

Farmer P, Bonnefoi H, Becette V, Tubiana-Hulin M, Fumoleau P, Larsimont D, Macgrogan G, Bergh J, Cameron D, Goldstein D, Duss S, Nicoulaz AL, Brisken C, Fiche M, Delorenzi M, Iggo R. (2005). Identification of molecular apocrine breast tumours by microarray analysis. Oncogene *7,* 4660-4671.

Filippella M, Galland F, Kujas M, Young J, Faggiano A, Lombardi G, Colao A, Meduri G, Chanson P. (2006). Pituitary tumour transforming gene (PTTG) expression correlates with the proliferative activity and recurrence status of pituitary adenomas: a clinical and immunohistochemical study. Clin Endocrinol (Oxf) *65,* 536–543.

Filipits M, Rudas M, Jakesz R, Dubsky P, Fitzal F, Singer CF, Dietze O, Greil R, Jelen A, Sevelda P, Freibauer C, Müller V, Jänicke F, Schmidt M, Kölbl H, Rody A, Kaufmann M, Schroth W, Brauch H, Schwab M, Fritz P, Weber KE, Feder IS, Hennig G, Kronenwett R, Gehrmann M, Gnant M; EP Investigators. (2011). A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. Clin Cancer Res *17,* 6012-6020.

Finnish Cancer Registry – Institute for Statistical and Epidemiological Cancer Research Cancer in Finland 2008 and 2009. Cancer Statistics of the National Institute for Health and Welfare (THL) Cancer Society of Finland. Publication No. 84, Helsinki 2011.

Fitzgibbons PL, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, Ruby SG, O'Malley F, Simpson

JF, Connolly JL, Hayes DF, Edge SB, Lichter A, Schnitt SJ. (2000). Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med *124*, 966-978.

Foulkes WD, Smith IE, Reis-Filho JS. (2010). Triple-negative breast cancer. N Engl J Med *363,*1938-1948.

Freedman RA, Partridge AH. (2013). Management of breast cancer in very young women. Breast *22 Suppl 2*, S176-179.

Fujii T, Nomoto S, Koshikawa K, Yatabe Y, Teshigawara O, Mori T, Inoue S, Takeda S, Nakao A. (2006). Overexpression of pituitary tumor transforming gene 1 in HCC is associated with angiogenesis and poor prognosis. Hepatology *43*, 1267-1275.

Fulford LG, Reis-Filho JS, Ryder K, Jones C, Gillett CE, Hanby A, Easton D, Lakhani SR. (2007). Basal-like grade III invasive ductal carcinoma of the breast: patterns of metastasis and long-term survival. Breast Cancer Res *9,* R4.

Gascoigne KE, Taylor SS. (2009). How do anti-mitotic drugs kill cancer cells? J Cell Science *122,* 2579-2585.

Geigl JB, Obenauf AC, Schwarzbraun T, Speicher MR. (2008). Defining 'chromosomal instability'. Trends Genet *24*, 64–69.

Genkai N, Homma J, Sano M, Tanaka R, Yamanaka R. (2006). Increased expression of pituitary tumor-transforming gene (PTTG)-1 is correlated with poor prognosis in glioma patients. Oncol Rep *15*, 1569-1574.

Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H. (1984). Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol *133*, 1710-1715.

Ghayad SE, Vendrell JA, Bieche I, Spyratos F, Dumontet C, Treilleux I, Lidereau R, Cohen PA. (2009). Identification of TACC1, NOV, and PTTG1 as new candidate genes associated with endocrine therapy resistance in breast cancer. J Mol Endocrinol *42*, 87-103.

Giuliano AE, Hunt KK, Ballman KV, Leitsch PD, Whitworth PW, Blumencranz PW, Leith AM, Saha S, McCall LM, Morrow M. (2011). Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. JAMA *305*, 569-575.

Goldhirsch A. (2013). Personalized adjuvant therapies: lessons from the past: the opening address by the St. Gallen 2013 award recipient. Breast *22 Suppl 2*, S3-7.

Goldhirsch A, Ingle JN, Gelber RD, Coates AS, Thürlimann B, Senn HJ; Panel members. (2009). Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. Ann Oncol *20*, 1319-1329.

Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ & Panel members. (2011). Strategies for subtypes dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol *22*, 1736-1747.

Gorr IH, Boos D, Stemmann O. (2005). Mutual inhibition of separase and Cdk1 by two-step complex formation. Mol Cell *19*, 135-141.

Grizzi F, Di Biccari S, Fiamengo B, Štifter S, Colombo P. (2013). Pituitary Tumor-Transforming Gene 1 Is Expressed in Primary Ductal Breast Carcinoma, Lymph Node Infiltration, and Distant Metastases. Dis Markers *35*, 267–272.

Gruvberger S, Ringnér M, Chen Y, Panavally S, Saal LH, Borg A, Fernö M, Peterson C, Meltzer PS. (2001). Estrogen receptor status in breast cancer is associated with remarkably distinct gene expression patterns. Cancer Res *61*, 5979-5984.

Guillaud P, du Manoir S, Seigneurin D. (1989). Quantification and topographical description of Ki-67 antibody labelling during the cell cycle of normal fibroblastic (MRC-5) and mammary tumour cell lines (MCF-7). Anal Cell Pathol *1,* 25–39.

Habermann JK, Doering J, Hautaniemi S, Roblick UJ, Bundgen NK, Nicorici D, Kronenwett U, Rathnagiriswaran S, Mettu RKR, Ma Y, Kruger S, Bruch HP, Auer G, Guo NL, Ried T. (2009). The gene expression signature of genomic instability in breast cancer is an independent predictor of clinical outcome. Int J Cancer *124*, 1552–1564.

Hagting A, Den Elzen N, Vodermaier HC, Waizenegger IC, Peters JM, Pines J. (2002). Human securin proteolysis is controlled by the spindle checkpoint and reveals when the APC/C switches from activation by Cdc20 to Cdh1. J Cell Biol *157,* 1125–1137.

Hamid T, Kakar SS. (2004). PTTG/securin activates expression of p53 and modulates its function. Mol Cancer *3*, 18.

Hamid T, Malik MT, Kakar SS. (2005). Ectopic expression of PTTG1/securin promotes tumorigenesis in human embryonic kidney cells. Mol Cancer *4,* 3.

Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M, Hicks DG, Lester S, Love R, Mangu PB, McShane L, Miller K, Osborne CK, Paik S, Perlmutter J, Rhodes A, Sasano H, Schwartz JN, Sweep FC, Taube S, Torlakovic EE, Valenstein P, Viale G, Visscher D, Wheeler T, Williams RB, Wittliff JL, Wolff AC. (2010).

American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol *28,* 2784-2795.

Hanahan D, Weinberg RA. (2011). Hallmarks of cancer: the next generation. Cell *144*, 646-674.

Harper JW, Burton JL, Solomon MJ. (2002). The anaphase-promoting complex: it's not just for mitosis anymore. Genes Dev *16*, 2179-2206.

Harris LN, Broadwater G, Lin Nu, Miron A, Schnitt SJ, Cowan D, Lara J, Bleiweiss I, Berry D, Ellis M, Hayes DF, Winer EP, Dressler L. (2006). Molecular subtypes of breast cancer in relation to paclitaxel response ad outcomes in women with metastatic disease. Results for calgb 9342. Breast cancer Res *8,* R66.

Harrison JC, Haber JE. (2006). Surviving the Breakup: The DNA Damage Checkpoint. Annu Rev Genet *40*, 209–235.

Harvey JM, Clark GM, Osborne CK, Allred DC. (1999). Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant chemotherapy in breast cancer. J Clin Oncol *17*, 1474-1481.

Hatcher RJ, Dong J, Liua S, Biand G, Contreras A, Wang T, Hilsenbeck SG, Lib Y, Zhang P. (2014). Pttg1/securin is required for the branching morphogenesis of the mammary gland and suppresses mammary tumorigenesis. Proc Natl Acad Sci U S A *111*, 1008–1013.

Hauf S, Roitinger E, Koch B, Dittrich CM, Mechtler K, Peters JM. (2005). Dissociation of cohesion from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2. PLoS Biol *3*, e69.

Heaney AP, Fernando M, Melmed S. (2002). Functional role of estrogen in pituitary tumor pathogenesis. J Clin Invest *109,* 277–283.

Heaney AP, Horwitz GA, Wang Z. (1999). Early involvement of estrogen-induced PTTG and FGF expression in prolactinoma pathogenesis. Nat Med *5,* 1317–1321.

Henson DE, Ries L, Freedman LS, Carriaga M. (1991). Relationship among outcome, stage of disease, and histologic grade for 22,616 cases of breast cancer. The basis for a prognostic index. Cancer *68*, 2142-2149.

Hlubek F, Pfeiffer S, Budczies J, Spaderna S, Jung A, Kirchner T, Brabletz T. (2006). Securin (hPTTG1) expression is regulated by beta-catenin/TCF in human colorectal carcinoma. Br J Cancer *94*, 1672-1677.

Holland AJ, Cleveland DW. (2009). Boveri revisited: Chromosomal instability, aneuploidy and tumorigenesis. Nat Rev Mol Cell Biol *10*, 478–487.

Honrado E, Benítez J, Palacios J. (2005). The molecular pathology of hereditary breast cancer: genetic testing and therapeutic implications. Mod Pathol *18*, 1305-1320.

Honrado E, Benítez J, Palacios J. (2006). Histopathology of BRCA1- and BRCA2-associated breast cancer. Crit Rev Oncol Hematol *59*, 27-39.

Houssami N, Macaskill P, Marinovich ML, Dixon JM, Irwig L, Brennan ME, Solin LJ. (2010). Meta-analysis of the impact of surgical margins on local recurrence in women with early-stage invasive breast cancer treated with breast-conserving therapy. Eur J Cancer *46*, 3219-32.

Hu Z, Fan C, Oh DS, Marron JS, He X, Qaqish BF, Livasy C, Carey LA, Reynolds E, Dressler L, Nobel A, Parker J, Ewend MG, Sawyer LR, Wu J, Liu Y, Nanda R, Tretiakova M, Ruiz Orrico A, Dreher D, Palazzo JP, Perreard L, Nelson E, Mone M, Hansen H, Mullins M, Quackenbush JF, Ellis MJ, Olopade OI, Bernard PS, Perou CM. (2006). The molecular portraits of breast tumors are conserved across microarray platforms. BMC Genomics *27;7,* 96.

Huang S, Liao Q, Li L, Xin D. (2014). PTTG1 inhibits SMAD3 in prostate cancer cells to promote their proliferation. Tumor Biol *35*, 6265–6270.

Huang SQ, Liao QJ, Wang XW, Xin DQ, Chen SX, Wu QJ, Ye G. (2012). RNAi-mediated knockdown of pituitary tumortransforming gene-1 (PTTG1) suppresses the proliferation and invasive potential of PC3 human prostate cancer cells. Braz J Med Biol Res *45*, 995–1001.

Hunter K, Welch DR, Liu ET. (2002). Genetic background is an important determinant of metastatic potential. Nat Genet *34*, 23–24.

Iacomino G, Medici MC, Napoli D, Russo GL. (2006). Effects of histone deacetylase inhibitors on p55CDC/Cdc20 expression in HT29 cell line. J Cell Biochem *99,* 1122-1131.

Irniger S. (2002). Cyclin destruction in mitosis: a crucial task of Cdc20. FEBS Letters *532*:7-11.

Ishitsuka Y, Kawachi Y, Taguchi S, Maruyama H, Nakamura Y, Fujisawa Y, Furuta J, Nakamura Y, Ishii Y, Otsuka F. (2013). Pituitary tumor-transforming gene 1 as a proliferation marker lacking prognostic value in cutaneous squamous cell carcinoma. Exp Dermatol 22, 318-322.

Ito T, Shimada Y, Kan T, David S, Cheng Y, Mori Y, Agarwal R, Paun B, Jin Z, Olaru A, Hamilton JP, Yang J, Abraham JM, Meltzer SJ, Sato F. (2008). Pituitary tumor-transforming 1 increases cell motility and promotes lymph node metastasis in

esophageal squamous cell carcinoma. Cancer Res *68*, 3214-3224.

Izawa D, Pines J. (2011). Evidence for how APC-C-Cdc20 changes its substrate specificity in mitosis. Nat Cell Biol *13*, 223–233.

Jallepalli PV, Waizenegger IC, Bunz F, Langer S, Speicher MR, Peters JM, Kinzler KW, Vogelstein B, Lengauer C. (2001). Securin is required for chromosomal stability in human cells. Cell *105,* 445–457.

Jensen S, Segal M, Clarke DJ, Reed SI. (2001). A novel role of the budding yeast separin Esp1 in anaphase spindle elongation: evidence that proper spindle association of Esp1 is regulated by Pds1. J Cell Biol *152*, 27-40.

Jiang J, Jedinak A, Sliva D. (2011) Ganodermanontriol (GDNT) exerts its effect on growth and invasiveness of breast cancer cells through the down-regulation of CDC20 and uPA. Biochem Biophys Res Commun *415,* 325-329.

Jiang J, Thyagarajan-Sahu A, Krchňák V, Jedinak A, Sandusky GE, Sliva D. (2012). NAHA, a novel hydroxamic acid-derivative, inhibits growth and angiogenesis of breast cancer in vitro and in vivo. PLoS One *7*, e34283.

Johnson RH, Chien FL, Bleyer A. (2013). Incidence of breast cancer with distant involvement among women in the United States, 1976 to 2009. JAMA *309*, 800e5.

Kakar SS, Jennes L. (1999). Molecular cloning and characterization of the tumor transforming gene (TUTR1): a novel gene in human tumorigenesis. Cytogenet Cell Genet *84,* 211–216.

Kato T, Daigo Y, Aragaki M, Ishikawa K, Sato M, Kaji M. (2012). Overexpression of CDC20 predicts poor prognosis in primary non-small cell lung cancer patients. J Surg Oncol *106,* 423-430.

Kaufmann M, Morrow M, von Minckwitz G, Harris J and the Biedenkopf Expert Panel Members. (2010). Logoregional Treatment of primary breast cancer. Consensus recommendations from an international expert panel. Cancer *116*, 1184–1191.

Kastan1 & Jiri Bartek. (2004). Cell-cycle checkpoints and cancer. Nature *432*, 316-323.

Kidokoro T, Tanikawa C, Furukawa Y, Katagiri T, Nakamura Y, Matsuda K. (2008). CDC20, a potential cancer therapeutic target, is negatively regulated by p53. Oncogene *27,* 1562-1571.

Kim DS, Fong J, Read ML, McCabe CJ. (2007a). The emerging role of pituitary tumour transforming gene (PTTG) in endocrine tumourigenesis. Mol Cell Endocrinol *278,* 1-6.

Kim DS, Franklyn JA, Smith VE, Stratford AL, Pemberton HN, Warfield A, Watkinson JC, Ishmail T, Wakelam MJ, McCabe CJ. (2007b). Securin induces genetic instability in colorectal cancer by inhibiting double-stranded DNA repair activity. Carcinogenesis *28*, 749–759.

Kim Y, Jung-Woo C, Lee J-H, Kim Y-S. (2014). MAD2 and CDC20 are upregulated in high-grade squamous intraepithelial lesions and squamous cell carcinomas of the unterine cervix. Int J Gyn Pathol *33*, 517-523.

Kim C, Paik S. (2010). Gene-expression-based prognostic assays for breast cancer. Nat Rev Clin Oncol *7,* 340-347.

Kim JW, Song JY, Lee JM, Lee JK, Lee NW, Yeom BW, Lee KW. (2008). Expression of pituitary tumor-transforming gene in endometrial cancer as a prognostic marker. Int J Gynecol Cancer *18*, 1352-1359.

Kim JM, Sohn HY, Yoon SY, Oh JH, Yang JO, Kim JH, Song KS, Rho SM, Yoo HS, Kim YS, Kim JG, Kim NS. (2005). Identification of gastric cancer-related genes using a cDNA microarray containing novel expressed sequence tags expressed in gastric cancer cells. Clin Cancer Res *11,* 473-482.

Kim S, Yu H. (2011). Mutual regulation between the spindle checkpoint and APC/C. Semin Cell Dev Biol *22*, 551–558.

King RW, Peters JM, Tugendreich S, Rolfe M, Hieter P, Kirschner MW. (1995). A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. Cell *81*, 279–288.

Kononen J, Bubendorf L, Kallioniemi A, Bärlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP. (1998). Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med *4*, 844-847.

Kops GJ, Weaver BA, Cleveland DW. (2005). On the road to cancer: aneuploidy and the mitotic checkpoint. Nat Rev Cancer *5,* 773-785.

Kraft C, Vodermaier HC, Maurer-Stroh S, Eisenhaber F, Peters JM. (2005). The WD40 propeller domain of Cdh1 functions as a destruction box receptor for APC/C substrates. Mol Cell *18*, 543–553.

Kramer ER, Scheuringer N, Podtelejnikov AV, Mann M, Peters JM. (2000). Mitotic regulation of the APC activator proteins CDC20 and CDH1.Mol Biol Cell *11*, 1555-1569.

Krishna I, Lindsay M. (2013). Breast cancer in pregnancy. Obstet Gynecol Clin North Am *40*, 559-571.

Kumada K, Nakamura T, Nagao K, Funabiki H, Nakagawa T, Yanagida M. (1998). Cut1 is loaded onto the spindle by binding to Cut2 and promotes anaphase spindle movement upon Cut2 proteolysis. Curr Biol *8*, 633-641.

Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ (Eds.): WHO Classification of Tumours of the Breast. IARC: Lyon 2012.

Lalloo F, Evans DG. (2012). Familial breast cancer. Clin Genet *82*, 105-114.

Lang JE, Wecsler JS, Press MF, Tripathy D. (2015). Molecular markers for breast cancer diagnosis, prognosis and targeted therapy. J Surg Oncol *111*, 81-90.

Lee SK, Kim WW, Kim SH, Hur Sm, Kim S, Choi JH, Cho EY, Han SY, Hahn BK, Choe JH, Kim JH, Kim JS, Lee JE, Nam SJ, Yang JH. (2010). Characteristics of metastasis in the breast from extramammary malignancies. J Surg Oncol *101,* 137-140.

Lee AH, Pinder SE, Macmillan RD, Mitchell M, Ellis IO, Elston CW, Blamey RW. (2006). Prognostic value of lymphovascular invasion in women with lymph node negative invasive breast carcinoma. Eur J Cancer *42*, 357-62.

Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA. (2011). Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest *121,* 2750-67.

Li M, Fang X, Wei Z, York JP, Zhang P. (2009). Loss of spindle assembly checkpoint-mediated inhibition of Cdc20 promotes tumorigenesis in mice. J Cell Biol *185*, 983-994.

Li J, Gao J-Z, Du J-L, Huang Z-X, Wei L-X. (2014). Increased CDc20 expression is associated with development and progression of hepatocellular carcinoma. Int J Oncol *45*, 1547-1555.

Li L, Mu K, Zhou G, Lan L, Auer G, Zetterberg A. (2008). Genomic instability and proliferative activity as risk factors for distant metastases in breast cancer. Br J Cancer *99,* 513 – 519.

Li JP, Yang JL. (2007a). Cyclin B1 proteolysis via p38 MAPK signaling participates in G2 checkpoint elicited by arsenite. J Cell Physiol *212*, 481-488.

Li M, York JP, Zhang P. (2007b). Loss of Cdc20 causes a securin-dependent metaphase arrest in two-cell mouse embryos. Mol Cell Biol *27*, 3481-3488.

Liao EC, Hsu YT, Chuah QY, Lee YJ, Hu JY, Huang TC, Yang PM, Chiu SJ. (2014). Radiation induces senescence and a bystander effect through metabolic alterations. Cell Death Dis *5*, e1255.

Liao YC, Ruan JW, Lua I, Li MH, Chen WL, Wang JR, Kao RH, Chen JH. (2012). Overexpressed hPTTG1 promotes breast cancer cell invasion and metastasis by regulating GEF-H1/RhoA signalling. Oncogene *31*, 3086-3097.

Lim HH, Goh PY, Surana U.(1998). Cdc20 is essential for the cyclosome-mediated proteolysis of both Pds1 and Clb2 during M-phase in budding yeast. Curr Biol *8*, 231-234.

Lindberg J, Mills IG, Klevebring D, Liu W, Neiman M, Xu J, Wikström P, Wiklund P, Wiklund F, Egevad L, Grönberg H. (2013). The mitochondrial and autosomal mutation landscapes of prostate cancer. Eur Urol *63*, 702-708.

Lo YL, Yu JC, Chen ST, Hsu GC, Mau YC, Yang SL, Wu PE, Shen CY. (2007). Breast cancer risk associated with genotypic polymorphism of the mitotic checkpoint genes: a multigenic study on cancer susceptibility. *Carcinogenesis 28,* 1079-1086.

Manié E, Vincent-Salomon A, Lehmann-Che J, Pierron G, Turpin E, Warcoin M, Gruel N, Lebigot I, Sastre-Garau X, Lidereau R, Remenieras A, Feunteun J, Delattre O, de Thé H, Stoppa-Lyonnet D, Stern MH. (2009). High frequency of TP53 mutation in BRCA1 and sporadic basal-like carcinomas but not in BRCA1 luminal breast tumors. Cancer Res *69,* 663-671.

Margolin S, Johansson H, Rutqvist LE, Lindblom A, Fornander T. (2006). Family history, and impact on clinical presentation and prognosis, in a population-based breast cancer cohort from the Stockholm County. Fam Cancer *5*, 309-321.

Marty B, Maire V, Gravier E, Rigaill G, Vincent-Salomon A, Kappler M, Lebigot I, Djelti F, Tourdès A, Gestraud P, Hupé P, Barillot E, Cruzalegui F, Tucker GC, Stern MH, Thiery JP, Hickman JA, Dubois T. (2008). Frequent PTEN genomic alterations and activated phosphatidylinositol 3-kinase pathway in basal-like breast cancer cells. Breast Cancer Res *10,* R101.

McClelland SE, Burrell RA, Swanton C. (2009). Chromosomal instability: A composite phenotype that influences sensitivity to chemotherapy. Cell Cycle *8*, 3262–3266.

McCubrey JA, Davis NM, Abrans SL, Montalto G, Cervello M, Libra M, Nicoletti F, D'Assoro AB, Cocco L, Martelli AM, Steelman LS. (2014). Targeting breast cancer initiating cells: Advances in breast cancer research and therapy. Advances Biol Regul *56*, 81-107.

Mei J, Huang X, Zhang P. (2001). Securin is not required for cellular viability, but is required for normal growth of mouse embryonic fibroblasts. Curr Biol *11*, 1197-1201.

Metzger-Filho O, Tutt A, de Azambuja E, Saini KS, Viale G, Loi S, Bradbury I, Bliss JM, Azim HA Jr, Ellis P, Di Leo A, Baselga J, Sotiriou C, Piccart-Gebhart M. (2012). Dissecting the heterogeneity of triple-negative breast cancer. J Clin Oncol *30,* 1879-1887.

Molinari M. Cell cycle checkpoints and their inactivation in human cancer. (2000). Cell Prolif *33*, 261-74.

Mondal G, Sengupta S, Panda CK, Gollin SM, Saunders WS, Roychoudhury S.(2007). Overexpression of CDC20 leads to impairment of the spindle assembly checkpoint and aneuploidization in oral cancer. Carcinogenesis *28,* 81-92.

Mora-Santos M, Castilla C, Herrero-Ruiz J, Giráldez S, Limón-Mortés MC, Sáez C, Japón MÁ, Tortolero M, Romero F. (2013). A single mutation in securin induces chromosomal instability and enhances cell invasion. Eur J Cancer *49*, 500-510.

Moura IM, Delgado ML, Silva PM, Lopes CA, do Amaral JB, Monteiro LS, Bousbaa H. (2013). High CDC20 expression is associated with poor prognosis in oral squamous cell carcinoma. J Oral Pathol Med *43*, 225-231.

Moureau-Zabotto L, Bouchet C, Cesari D, Uzan S, Lefranc JP, Antoine M, Genestie C, Deniaud-Alexandre E, Bernaudin JF, Touboul E, Fleury-Feith J. (2005). Combined flow cytometry determination of S-phase fraction and DNA ploidy is an independent prognostic factor in node-negative invasive breast carcinoma: analysis of a series of 271 patients with stage I and II breast cancer. Breast Cancer Res Treat *91*, 61–71.

Moyle MW, Kim T, Hattersley N, Espeu J, Cheerambathur DK, Oegema K, Desai A. (2014). A Bub1–Mad1 interaction targets the Mad1–Mad2 complex to unattached kinetochores to initiate the spindle checkpoint. J Cell Biol *204*, 647–657.

Mu YM, Oba K, Yanase T, Ito T, Ashida K, Goto K, Morinaga H, Ikuyama S, Takayanagi R, Nawata H. (2003). Human pituitary tumor transforming gene (hPTTG) inhibits human lung cancer A549 cell growth through activation of p21(WAF1 ⁄ CIP1). Endocr J *50,* 771–781.

Murray AW. (2004). Recycling the Cell Cycle: Cyclins Revisited. Cell *116*, 221–234.

Murray AW. (2011). A brief history of error. Nature Cell Biol *13*, 1178-1182.

Musacchio A, Salmon ED. (2007). The spindle-assembly checkpoint in space and time. Nat Rev Mol Cell Biol *8*, 379-393.

Nasmyth K. (2002). Segregating sister genomes: the molecular biology of chromosome separation. Science *297*, 559-565.

Nowak MA, Komarova NL, Sengupta A, Jallepalli PV, Shih IM, Vogelstein B, Lengauer C. (2002). The role of chromosomal instability in tumor initiation. PNAS *10*, 16226–16231 .

Nurse P. (2002). Cyclin Dependent Kinases and Cell Cycle Control (Nobel Lecture). Chem BioChem *3*, 596-603.

Ogbagabriel S, Fernando M, Waldman FM, Bose S, Heaney AP. (2005). Securin in overexpressed in breast cancer. Mod Pathol *18*, 985-990.

Ouellet S, Vigneault F, Lessard M, Leclerc S, Drouin R, Guérin SL. (2006). Transcriptional regulation of the cyclin-dependent kinase inhibitor 1A (p21) gene by NFI in proliferating human cells. Nucleic Acids Res *34,* 6472-6487.

O'Toole SA, Beith JM, Millar EK, West R, McLean A, Cazet A, Swarbrick A, Oakes SR. (2013). Therapeutic targets in triple negative breast cancer. J Clin Pathol *66,* 530-542.

Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, Cronin M, Baehner FL, Watson D, Bryant J, Costantino JP, Geyer CE Jr, Wickerham DL, Wolmark N. (2006). Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J Clin Oncol *24*, 3726-3734.

Panguluri SK, Yeakel C, Kakar SS. (2008). PTTG: an important target gene for ovarian cancer therapy. J Ovarian Res *1*, 6.

Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z, Quackenbush JF, Stijleman IJ, Palazzo J, Marron JS, Nobel AB, Mardis E, Nielsen TO, Ellis MJ, Perou CM, Bernard PS. (2009). Supervised risk predictor of breat cancer based on intrinsic subtypes. J Clin Oncol *27*:1160-1167.

Pati D, Haddad BR, Haegele A, Thompson H, Kittrell FS, Shepard A, Montagna C, Zhang N, Ge G, Otta SK, McCarthy M, Ullrich RL, Medina D. (2004). Hormone-induced chromosomal instability in p53-null mammary epithelium. Cancer Res *64*, 5608-5616.

Pawar SA, Sarkar TR, Balamurugan K, Sharan S, Wang J, Zhang Y, *et al.* C/EBP{delta} targets cyclin D1 for proteasome-mediated degradation via induction of CDC27/APC3 expression. (2010). Proc Natl Acad Sci U S A *107,* 9210–9215.

Pei L. (2001). Identification of c-myc as a down-stream target for pituitary tumor-transforming gene. J Biol Chem *276,* 8484–8491.

Pei L, Melmed S. (1997). Isolation and characterization of a pituitary tumor-transforming gene (PTTG). Mol Endocrinol *11*, 433-441.

Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Phillips TM, McBride WH. (2000). Molecular portraits of human breast tumours. Nature *406*, 747–752.

Peters JM. (2002). The anaphase-promoting complex: proteolysis in mitosis and beyond. Mol Cell *9*, 931-943.

Peters JM. (2006). The anaphase promoting complex/ cyclosome: a machine designed to destroy.Nat Rev Mol Cell Biol *7,* 644-56.

Pfleghaar K, Heubes S, Cox J, Stemmann O, Speicher MR. (2005). Securin is not required for chromosomal stability in human cells. PLoS Biol *3*, e416.

Polley MYC, Leung SCY, McShane LM, Gao D, Hugh JC, Mastropasqua MG, Viale G, Zabaglo LA, Penault-Llorca F, Bartlett JMS, Gown AM, Symmans WF, Piper T, Mehl E, Enos RA, Hayes DF, Dowsett M, Nielsen TO, on behalf of the International Ki67 in Breast Cancer Working Group of the Breast International Group and North American Breast Cancer Group. (2013). An International Ki67 Reproducibility Study. J Natl Cancer Inst *105*, 1897–1906.

Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, He X, Perou CM. (2010). Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res *12,* R68

Pu X, Ye Y, Wu X. (2014). Development and validation of risk models and molecular diagnostics to permit personalized management of cancer. Cancer *120*, 11-19.

Pukkala E, Sankila R, Rautalahti M. (2011). Syöpä Suomessa 2011. Suomen Syöpäyhdistyksen julkaisuja nro 82. Suomen Syöpäyhdistys, Helsinki.

Puri R, Tousson A, Chen L, Kakar SS. (2001). Molecular cloning of pituitary tumor transforming gene 1 from ovarian tumors and its expression in tumors. Cancer Lett *163*, 131-139.

Rajagopalan H, Lengauer C. (2004). Aneuploidy and cancer. Nature *432*, 338-341.

Rajkumar T, Gopal G, Selvaluxmi G, Rajalekshmy KR. (2005). CDC27 protein is involved in radiation response in squamous cell cervix carcinoma. Indian J Biochem Biophys *42*, 271-278.

Ramaswamy S, Ross KN, Lander ES, Golub TR. (2003). A molecular signature of metastasis in primary solid tumors. Nat Genet *33*, 49–54.

Rehfeld N, Geddert H, Atamna A, Rohrbeck A, Garcia G, Kliszewski S, Neukirchen J, Bruns I, Steidl U, Fenk R, Gabbert HE, Kronenwett R, Haas R, Rohr UP. (2006). The influence of the pituitary tumor transforming gene-1 (PTTG-1) on survival of patients with small cell lung cancer and non-small cell lung cancer. J Carcinog *5*, 4.

Reis-Filho JS, Pusztai L. (2011). Gene expression profiling in breast cancer: classification, prognostication, and prediction. Lancet *378,* 1812-23.

Rodenhiser DI, Andrews JD, Vandenberg TA, Chambers AF. (2011). Gene signatures of breast cancer progression and metastasis. Breast Cancer Res *13*, 201.

Romero F, Multon MC, Ramos-Morales F, Domínguez A, Bernal JA, Pintor-Toro JA, Tortolero M. (2001). Human securin, hPTTG, is associated with ku heterodimer, the regulatory subunit of the DNA-dependent protein kinase. Nucleic Acids Res *29*, 1300-1307.

Rosai J, Damjanov I, Nola M. (2007). Rosai and Ackerman's Textbook of Surgical Pathology, Vol. 2, Mosby Elsevier, Philadelphia, p. 503.

Ross JS, Linette GP, Stec J, Ross MS, Anwar S, Boguniewicz A. (2003). DNA Ploidy and Cell Cycle Analysis in Breast Cancer. Am J Clin Pathol *120(Suppl 1),* S72-S84.

Sáez C, Japón MA, Ramos-Morales F, Romero F, Segura DI, Tortolero M, Pintor-Toro JA. (1999). hPTTG1 is over-expressed in pituitary adenomas and other primary epithelial neoplasias. Oncogene *18*, 5473-5476.

Saez C, Martínez-Brocca MA, Castilla C, Soto A, Navarro E, Tortolero M, Pintor-Toro JA, Japón MA. (2006). Prognostic significance of human pituitary tumor-transforming gene immunohistochemical expression in differentiated thyroid cancer. J Clin Endocrinol Metab *91*, 1404-1409.

Salehi F, Kovacs K, Scheithauer BW, Lloyd RV, Cusimano M. (2008). Pituitary tumor-transforming gene in endocrine and other neoplasms: a review and update. Endocr Relat Cancer *15,* 721-743.

Salehi F, Scheithauer BW, Sharma S, Kovacs K, Lloyd RV, Cusimano MD, Munoz DG. (2013). Immunohistochemical expression of PTTG in brain tumors. Anticancer Res *33*, 119-122.

Sanchez I, Dynlacht BD. (2005). New insights into cyclins, CDKs, and cell cycle control. Sem Cell Dev Biol *16*, 311–321.

Schneider BP, Winer EP, Foulkes WD Garber J, Perou CM, Richardson A, Sledge GW, Carey LA. (2008). Triple-negative breast cancer. Risk factors to potential targets. Clin Cancer Res *14,* 8010-8018.

Schwab M, Lutum AS, Seufert W. (1997). Yeast Hct1 is a regulator of Clb2 cyclin proteolysis. Cell *90*, 683-693.

Seidman JD, Schnaper LA, Aisner SC. (1995). Relationship of the size of the invasive component of the primary breast carcinoma to axillary lymph node metastasis. Cancer *75*, 65–71.

Shah R, Rosso K, Nathanson SD. (2014). Pathogenesis, prevention, diagnosis and treatment of breast cancer. World J Clin Oncol *10*, 283-298.

Sharif S, Moran A, Huson SM, Iddenden R, Shenton A. Howard E, Evans DG. (2007). Women with neurofibromatosis I are at a moderately increased

risk of developing breast cancer and should be considered for early screening. J Med Genet *44*, 481-484.

Shibata Y, Haruki N, Kuwabara Y, Nishiwaki T, Kato J, Shinoda N, Sato A, Kimura M, Koyama H, Toyama T, Ishiguro H, Kudo J, Terashita Y, Konishi S, Fujii Y. (2002). Expression of PTTG (pituitary tumor transforming gene) in esophageal cancer. Jpn J Clin Oncol *32*, 233–237.

Shike M, Doane AS, Russo L, Cabal R, Reis-Filho JS, Gerald W, Cody H, Khanin R, Bromberg J, Norton L. (2014). The Effects of Soy Supplementation on Gene Expression in Breast Cancer: A Randomized Placebo-Controlled Study. *JNCI J Natl Cancer Inst 106,* 9.

Siegel JJ, Amon A. (2012). New insights into the troubles of aneuploidy. Annu Rev Cell Dev Biol *28*, 189–214.

Silk AD, Zasadil LM, Holland AJ, Vitre B, Cleveland DW, Weaver BA. (2013). Chromosome missegregation rate predicts whether aneuploidy will promote or suppress tumors.PNAS *16,* E4134–E4141.

Smith VE, Franklyn JA, McCabe CJ. (2010). Pituitary tumor-transforming gene and its binding factor in endocrine cancer. Expert Rev Mol Med *3,* 12:e38.

Solbach C, Roller M, Fellbaum C, Nicoletti M, Kaufmann M. (2004). PTTG mRNA expression in primary breast cancer: a prognostic marker for lymph node invasion and tumor recurrence. Breast *13*, 80-81.

Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE, Børresen-Dale AL. (2001). Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA *98*, 10869-10874.

Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lønning PE, Brown PO, Børresen-Dale AL, Botstein D. (2003). Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA *100*, 8418-8423.

Sotiriou C, Pusztai L. (2009). Gene-expression signatures in breast cancer. N Engl J Med *360,* 790-800.

Stagg J, Allard B. (2013). Immunotherapeutic approaches in triple-negative breast cancer: latest research and clinical prospects. Ther Adv Med Oncol *5,* 169-181.

Steelman LS, Navolanic PN, Sokolosky M, Taylor JR, Lehmann BD, Chappell WH. (2008). Suppression of PTEN functions increase breast cancer chemotherapeutic drug resistance while conferring sensitivity of mTOR inhibitors. Oncogene *27*, 4086-95.

Stemmann O, Zou H, Gerber SA, Gygi SP, Kirschner MW. (2001). Dual inhibition of sister chromatid separation at metaphase. Cell *107,* 715–726.

Stevens KN, Wang X, Fredericksen Z, Pankratz VS, Cerhan J, Vachon CM, Olson JE, Couch FJ. (2011). Evaluation of associations between common variation in mitotic regulatory pathways and risk of overall and high grade breast cancer. Breast Cancer Res Treat *129*, 617–622.

Stratford AL, Boelaert K, Tannahill LA, Kim DS, Warfield A, Eggo MC, Gittoes NJ, Young LS, Franklyn JA, McCabe CJ. (2005). Pituitary tumor transforming gene binding factor: a novel transforming gene in thyroid tumorigenesis. J Clin Endocrinol Metab *90*, 4341-4349.

Stuart-Harris R, Caldas C, Pinder SE, Pharoah P. (2008). Proliferation markers and survival in early breast cancer: a systematic and meta-analysis of 85 studies in 32,825 patients. Breast *17*, 323-334.

Sullivan T, Raad RA, Goldberg S, Assaad SI, Gadd M, Smith BL, Powell SN, Taghian AG. (2005). Tubular carcinoma of the breast: a retrospective analysis and review of the literature. Breast Cancer Res Treat *93,* 199-205.

Sumara I, Vorlaufer E, Gieffers C, Peters BH, Peters JM. (2000). Characterization of vertebrate cohesion complexes and their regulation in prophase. J Cell Biol *151*, 749-762.

Szasz AM, Li Q, Eklund AC, Sztupinszki Z, Rowan A, Tokes AM, Szekely B, Kiss A, Szendroi M, Gyorffy B, Szallasi Z, Swanton C, Kulka J. (2013). The CIN4 Chromosomal Instability qPCR Classifier Defines Tumor Aneuploidy and Stratifies Outcome in Grade 2 Breast Cancer. PLOS *8,* e56707.

Takasaki Y, Deng JS, Tan EM. (1981). A nuclear antigen associated with cell proliferation and blast transformation. J Exp Med *154,* 1899–1909.

Talvinen K, Tuikkala J, Grönroos J, Huhtinen H, Kronqvist P, Aittokallio T, Nevalainen O, Hiekkanen H, Nevalainen T, Sundström J. (2006). Biochemical and clinical approaches in evaluating the prognosis of colon cancer. Anticancer Res *26*, 4745-4751.

Talvinen K, Tuikkala J, Nevalainen O, Rantanen A, Hirsimäki P, Sundström J, Kronqvist P. (2008). Proliferation marker securin identifies favourable outcome in invasive ductal breast cancer. Br J Cancer *99,* 335–340.

Tanaka T, Fuchs J, Loidl J, Nasmyth K. (2000). Cohesin ensures bipolar attachment of microtubules to sister centromeres and resists their precocious separation. Nat Cell Biol *2*, 492-499.

Taniguchi K, Momiyama N, Ueda M, Matsuyama R, Mori R, Fujii Y, Ichikawa Y, Endo I, Togo S, Shimada H. (2008). Targeting of CDC20 via small interfering RNA causes enhancement of the cytotoxicity of chemoradiation. Anticancer Res *28*, 1559-1563.

Tfelt-Hansen J, Kanuparthi D, Chattopadhyay N. (2006). The emerging role of pituitary tumor transforming gene in tumorigenesis. Clin Med Res *4,* 130-137.

Thirthagiri E, Robinson CM, Huntley S, Davies M, Yap LF, Prime SS, Paterson IC. (2007). Spindle assembly checkpoint and centrosome abnormalities in oral cancer. Cancer Lett *258,* 276-285.

Thompson AD 3rd, Kakar SS. (2005). Insulin ja IGF-1 regulate the expression of the pituitary tumor transforming gene (PTTG) in breast tumor cells. FEBS Lett 579, 3195-3200.

Tischkowitz M, Brunet JS, Bégin LR, Huntsman DG, Cheang MC, Akslen LA, Nielsen TO, Foulkes WD. (2007). Use of immunohistochemical markers can refine prognosis in triple negative breast cancer. BMC Cancer *7,* 134.

Thompson SL, Compton DA. (2008). Examining the link between chromosomal instability and aneuploidy in human cells. J Cell Biol *180,* 665-672.

Tong Y, Ben-Shlomo A, Zhou C, Wawrowsky K, Melmed S. (2008). Pituitary tumor transforming gene 1 regulates Aurora kinase A activity. Oncogene *27*, 6385-6395.

Tong Y, Zhao W, Zhou C, Wawrowsky K, Melmed S. (2011). PTTG1 attenuates drug-induced cellular senescence. PLoS One *6*, e23754.

Topper LM, Campbell MS, Tugendreich S, Daum JR, Burke DJ, Hieter P, Gorbsky GJ. (2002). The dephosphorylated form of the anaphase-promoting complex protein Cdc27/Apc3 concentrates on kinetochores and chromosome arms in mitosis. Cell Cycle *1*, 282-292.

Tsai SJ, Lin SJ, Cheng YM, Chen HM, Wing LY. (2005). Expression and functional analysis of pituitary tumor transforming gene-1 [corrected] in uterine leiomyomas. J Clin Endocrinol Metab *90,* 3715–3723.

Tugendreich S, Tomkiel J, Earnshaw W, Hieter P. (1995). CDC27Hs colocalizes with CDC16Hs to the centrosome and mitotic spindle and is essential for the metaphase to anaphase transition. Cell *81*, 261-268.

Uhlmann F, Lottspeich F, Nasmyth K. (1999). Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. Nature *400*, 37-42.

van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ, Parrish M, Atsma D, Witteveen A, Glas A, Delahaye L, van der Velde T, Bartelink H, Rodenhuis S, Rutgers ET, Friend SH, Bernards R. (2002). A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med *347*, 1999-2009.

van Diest PJ, van der Wall E, Baak JP. (2004). Prognostic value of proliferation in invasive breast cancer: a review. J Clin Pathol *57,* 675-681.

van der Groep P, van der Wall E, van Diest PJ. (2011). Pathology of hereditary breast cancer. Cell Oncol *34*, 71–88.

van Oijen MG, Medema RH, Slootweg PJ, Rijksen G. (1998). Positivity of the proliferation marker Ki-67 in noncycling cells. Am J Clin Pathol *110,* 24–31.

van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH. (2002). Gene expression profiling predicts clinical outcome of breast cancer. Nature *415*, 530–536.

Varga Z, Diebold J, Dommann-Scherrer C, Frick H, Kaup D, Noske A, Obermann E, Ohlschlegel C, Padberg B, Rakozy C, Sancho Oliver S, Schobinger-Clement S, Schreiber-Facklam H, Singer G, Tapia C, Wagner U, Mastropasqua MG, Viale G, Lehr HA. (2012). How reliable is Ki-67 immunohistochemistry in grade 2 breast carcinomas? A QA study of the Swiss Working Group of Breast- and Gynecopathologists. PLoS One *7,* e37379.

Venkitaraman AR. (2014). Cancer Suppression by the Chromosome Custodians, BRCA1 and BRCA2. Science *343*,1470.

Visintin R, Prinz S, Amon A. (1997). CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. Science *278*, 460-463.

Vlotides G, Eigler T, Melmed S. (2007). Pituitary tumor-transforming gene: physiology and implications for tumorigenesis. Endocr Rev *28,* 165-186.

Waizenegger I, Giménez-Abián JF, Wernic D, Peters JM. (2002). Regulation of human separase by securin binding and autocleavage. Curr Biol *12*, 1368-1378.

Wan L, Tan M, Yang J, Inuzuka H, Dai X, Wu T, Liu J, Shaik S, Chen G, Deng J, Malumbres M, Letai A, Kirschner MW, Sun Y, Wei W. (2014). APC(Cdc20) suppresses apoptosis through targeting Bim for ubiquitination and destruction. Dev Cell *29*, 377-391.

Wang Y, Klijn JG, Zhang Y, Sieuwerts AM, Look MP, Yang F, Talantov D, Timmermans M, Meijer-van Gelder ME, Yu J, Jatkoe T, Berns EM, Atkins D, Foekens JA. (2005). Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. Lancet *365,* 671-679.

Wang Z, Melmed S. (2000). Characterization of the murine pituitary tumor transforming gene (PTTG) and its promoter. Endocrinology *141,* 763-771.

Wang Z, Christman MF. (2001). Replication-related activities establish cohesion between sister chromatids. Cell Biochem Biophys *35*, 289-301.

Wang Z, Wan L, Zhong J, Inuzuka H, Liu P, Sarkar FH, Wei W. (2013). Cdc20: a potential novel therapeutic target for cancer treatment. Curr Pharm Des *19*, 3210-3214.

Wang Z, Yu R, Melmed S. (2001). Mice lacking pituitary tumor transforming gene show testicular and splenic hypoplasia, thymic hyperplasia, thrombocytopenia, aberrant cell cycle progression, and Premature Centromere Division. Molecular Endocrinology *15*, 1870–1879.

Watkins RJ, Read ML, Smith VE, Sharma N, Reynolds GM, Buckley L, Doig C, Campbell MJ, Lewy G, Eggo MC, Loubiere LS, Franklyn JA, Boelaert K, McCabe CJ. (2010). Pituitary tumor transforming gene binding factor: a new gene in breast cancer. Cancer Res *70*, 3739-3749.

Weaver BA, Cleveland DW. (2006). Does aneuploidy cause cancer? Curr Opin Cell Biol *18,* 658-667.

Weaver BA, Cleveland DW. (2009). The role of aneuploidy in promoting and suppressing tumors. J Cell Biol *185,* 935–937.

Weichmann L, Sampson M, Stempel M, Jacks LM, Patil SM, King T, Morrow M. (2009). Presenting features of breast cancer differ by molecular subtype. Ann Surg Oncol *16*, 2705-2710.

Weigelt B, Baehner FL, Reis-Filho JS. (2010). The contribution of gene expression profiling to breast cancer classification, prognostication and prediction: a retrospective of the last decade. J Pathol *220,* 263-280.

Weigelt B, Reis-Filho JS. (2009). Histological and molecular types of breast cancer: is there a unifying taxonomy? Nat Rev Clin Oncol *6*, 718-730.

Weinstein J. (1997). Cell cycle-regulated expression, phosphorylation, and degradation of p55Cdc. A mammalian homolog of CDC20/Fizzy/slp1. J Biol Chem *272*, 28501-28511.

Weinstein J, Jacobsen FW, Hsu-Chen J, Wu T, Baum LG. (1994). A novel mammalian protein, p55CDC, present in dividing cells is associated with protein kinase activity and has homology to the Saccharomyces cerevisiae cell division cycle proteins Cdc20 and Cdc4. Mol Cell Biol *14*, 3350-3363.

Winnepenninckx V, Debiec-Rychter M, Beliën JA, Fiten P, Michiels S, Lazar V, Opdenakker G, Meijer GA, Spatz A, van den Oord JJ. (2006). Expression and possible role of hPTTG1/securin in cutaneous malignant melanoma. Mod Pathol *19*, 1170-1180.

Wirapati P, Sotiriou C, Kunkel S, Farmer P, Pradervand S, Haibe-Kains B, Desmedt C, Ignatiadis M, Sengstag T, Schütz F, Goldstein DR, Piccart M, Delorenzi M. (2008). Meta-analysis of gene expression profiles in breast cancer: toward a unified understanding of breast cancer subtyping and prognosis signatures. Breast Cancer Res *10,* R65.

Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF, American Society of Clinical Oncology/College of American Pathologists. (2007). American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med *131:* 18-43.

Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JM, Bilous M, Fitzgibbons P, Hanna W, Jenkins RB, Mangu PB, Paik S, Perez EA, Press MF, Spears PA, Vance GH, Viale G, Hayes DF; American Society of Clinical Oncology; College of American Pathologists. (2013). Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/ College of American Pathologists clinical practice guideline update. J Clin Oncol *31,* 3997-4013.

Wu WJ, Hu KS, Wang DS, Zeng ZL, Zhang DS, Chen DL, Bai L, Xu RH. (2013). CDC20 overexpression predicts a poor prognosis for patients with colorectal cancer. J Transl Med *11,* 142.

Yan S, Zhou C, Lou X, Xiao Z, Zhu H, Wang Q, Wang Y, Lu N, He S, Zhan Q, Liu S, Xu N. (2009). PTTG overexpression promotes lymph node metastasis in human esophageal squamous cell carcinoma. Cancer Res *69*, 3283–3290.

Yanagawa m, Ikemoto K, Kawauchi S, Furuya T, Yamamoto S, Oka M, Oga A, Nagashima Y, Sasaki K. (2012). Luminal A and luminal B (HER2 negative) subtypes of breast cancer consist of a mixture of tumors with different genotype. BMC Research Notes *5,* 376-384.

Yang X, Xu W, Hu Z, Zhang Y, Xu N. (2014). Chk1 is required for the metaphase-anaphase transition via regulating the expression and localization of Cdc20 and Mad2. Life Sci *106*,12-18.

Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA. (2010). Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol *11*, 174–183.

Yildirim-Assaf S, Coumbos A, Hopfenmuller W, Foss HD, Stein H, Kuhn W. (2007). The prognostic significance of determining DNA content in breast cancer by DNA image cytometry: the role of high

grade aneuploidy in node negative breast cancer. J Clin Pathol *60,* 649–655.

Yoon CH, Kim MJ, Lee H, Kim RK, Lim EJ, Yoo KC, Lee GH, Cui YH, Oh YS, Gye MC, Lee YY, Park IC, An S, Hwang SG, Park MJ,Suh Y, Lee SJ. (2012). *PTTG1* Oncogene Promotes Tumor Malignancy via Epithelial to Mesenchymal Transition and Expansion of Cancer Stem Cell Population. J Biol Chem *287,* 19516–19527.

Yu SY, Liu HF, Wang SP, Chang CC, Tsai CM, Chao JI. (2013). Evidence of securin-mediated resistance to gefitinib-induced apoptosis in human cancer cells. Chem Biol Interact *203,* 412-22.

Yu R, Lu W, Chen J, McCabe CJ, Melmed S. (2003). Overexpressed pituitary tumor-transforming gene causes aneuploidy in live human cells. Endocrinology *144*, 4991-4998.

Yu R, Ren SG, Horwitz GA, Wang Z, Melmed S. (2000a). Pituitary tumor transforming gene (PTTG) regulates placental JEG-3 cell division and survival: evidence from live cell imaging. Mol Endocrinol *14,* 1137–1146.

Yu R, Heaney AP, Lu W, Chen J, Melmed S. (2000b). Pituitary tumor transforming gene causes aneuploidy and p53-dependent and p53-independent apoptosis. J Biol Chem *275,* 36502-3655.

Yuan B, Xu Y, Woo JH, Wang Y, Bae YK, Yoon DS, Wersto RP, Tully E, Wilsbach K, Gabrielson E. (2006). Increased expression of mitotic checkpoint genes in breast cancer cells with chromosomal instability. Clin Cancer Res *12*, 405-410.

Zhang H, Chen X, Wang J, Guang W, Han W, Zhang H, Tan X, Gu Y. (2014). EGR1 decreases the malignancy of human non-small cell lung carcinoma by regulating KRT18 expression. Sci Rep *4*, 5416.

Zhang L, Fujita T, Wu G, Xiao X, Wan Y. (2011). Phosphorylation of the anaphase-promoting complex/Cdc27 is involved in TGF-beta signaling. J Biol Chem *286*, 10041-10050.

Zhang X, Horwitz GA, Prezant TR, Valentini A, Nakashima M, Bronstein MD, Melmed S. (1999). Structure, expression, and function of human pituitary tumortransforming gene (PTTG). Mol Endocrinol *13*, 156–166.

Zhang ML, Lu S, Zheng SS. (2008). Epigenetic changes of pituitary tumorderived transforming gene 1 in pancreatic cancer. Hepatobiliary Pancreat Dis Int *7*, 313–317.

Zhang J, Yang Y, Chen L, Zheng D, Ma J. (2013a). Overexpression of pituitary tumor transforming gene (PTTG) is associated with tumor progression and poor prognosis in patients with esophageal squamous cell carcinoma. Acta Histochem *116*, 435-439.

Zhang Z, Yang J, Kong EH, Chao WC, Morris EP, da Fonseca PC, Barford D. (2013b). Recombinant expression, reconstitution and structure of human anaphase-promoting complex (APC/C). Biochem J *449*, 365-71.

Zhou C, Liu S, Zhou X, Xue L, Quan L, Lu N, Zhang G, Bai J, Wang Y, Liu Z, Zhan Q, Zhu H, Xu N. (2005). Overexpression of human pituitary tumor transforming gene (hPTTG), is regulated by beta-catenin /TCF pathway in human esophageal squamous cell carcinoma. Int J Cancer *113*, 891–898.

Zhou Y, Mehta KR, Choi AP, Scolavino S, Zhang X. (2003). DNA damage-induced inhibition of securin expression is mediated by p53. J Biol Chem *278*, 462-470.

Zhou C, Tong Y, Wawrowsky K, Melmed S. (2014). PTTG acts as a STAT3 target gene for colorectal cancer cell growth and motility. Oncogene *33*, 851–861.

Zhu X, Mao Z, Na Y, Guo Y, Wang X, Xin D. (2006). Significance of pituitary tumor transforming gene 1 (PTTG1) in prostate cancer. Anticancer Res *26*, 1253–1259.

Zou H, McGarry TJ, Bernal T, Kirschner MW. (1999). Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis, Science *285,* 418–422.

Zur A, Brandeis M. (2002). Timing of APC/C substrate degradation is determined by fzy/fzr specificity of destruction boxes. EMBO J *21*, 4500-4510.

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www.cancerregistry.fi

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www.proteinatlas.org