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Increased expression of ADAM12 and ADAM17 genes in laser-capture microdissected breast cancers and correlations with clinical and pathological characteristics

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ABSTRACT

ADAMs (a desintegrin and metalloprotease) are transmembrane glycoproteins involved in cell growth, differentiation, motility, and respectively, tumor growth and progression. Our aim was to evaluate ADAM12 spliced variants (ADAM12L – long membrane-bound and ADAM12S – secreted-short variant) and ADAM17 genes expression in breast cancers and to correlate their level of expression with clinical and pathological characteristics.

Expression of ADAMs was analyzed using quantitative reverse-transcription polymerase chain reaction in laser-capture microdissected specimens of breast cancers and corresponding non-neoplastic breast tissues from 92 patients. The proteins' expression was confirmed by immunohistochemistry. Significantly elevated amounts of ADAM12L, ADAM12S and ADAM17 transcripts were found in malignant breast cells compared with normal breast tissue and both ADAMs proteins showed moderate to strong immunoexpression in tumor cells and peritumoral fibroblasts. ADAM12L and ADAM12S expressions were correlated with age, younger patients having higher expression of ADAM12L and ADAM12S; ductal cancers had higher expression of ADAM12L compared with lobular types, whereas ADAM12S was higher expressed in lobular cancers; higher expressions were found for both ADAM12 and ADAM17 in HER2/neu positive and highly proliferative cancers. High-grade cancers showed significantly increased expression of ADAM17. Our study on laser-capture microdissected specimens confers motivation for future work on development of ADAM-selective inhibitors for treatment of breast cancers.

Introduction

Proteolytic processing of transmembrane proteins, a process termed ectodomain shedding, releases their soluble extracellular domains from the membrane and is an important post-translational mechanism to regulate the function of membrane proteins (Blobel, 2005; Arribas et al., 2006). A variety of structurally and functionally distinct cell surface proteins are subjected to protein ectodomain shedding, including: cytokines, chemokines and their receptors, growth factors, such as transforming growth factor alpha (TGF-α), epidermal growth factor (EGF) and their receptors, adhesion molecules. The functional consequences of ectodomain shedding include regulation of availability or active range of membrane-bound growth factors and the activation or inactivation of their receptors (Peschon et al., 1998; Ohtsu et al., 2006; Mochizuki and Okada, 2007; Murphy, 2008; Edwards et al., 2008).

The ADAMs (a desintegrin and metalloprotease) sheddases are a large family of more than 30 proteins that belong to the metzincin family of zinc-dependent proteases and are multidomain proteins with protease, adhesion, fusion and signaling activities.

Abbreviations: ADAM, a desintegrin and metalloprotease (meltrin-alpha); ADAM12L, a desintegrin and metalloprotease 12 long membrane-bound variant; ADAM12S, a desintegrin and metalloprotease 12 short secreted variant; ADAM17, a desintegrin and metalloprotease17; CDI, invasive ductal carcinoma; CLI, invasive lobular carcinoma; CT, crossing points cycle number where the fluorescence crossed the threshold; ΔCT, CT (target gene) – CT (housekeeping gene); ΔΔCT, ΔCT patients – ΔCT normal controls the comparative level of expression: $2^{-\Delta\Delta CT}$; DCIS, *in situ* ductal carcinoma; DNA, deoxyribonucleic acid; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ER, estrogen receptor; FFPE, formalin-fixed paraffin embedded; G, tumor grade; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPI, glucose-6-phosphate-isomerase; HB-EGF, heparin-binding-epidermal growth factor; HER2/neu, human epidermal growth factor receptor 2; IGF, insulin-like growth factor; IHC, immunohistochemistry; LCM, aser-capture microdissection; mRNA, messenger ribonucleic acid; PR, progesterone receptor; Q-RT-PCR, quantitative-reverse transcription polymerase chain reaction; RIN, RNA integrity number; S.D., standard deviation; TACE, TGF-α converting enzyme; TGF-α, tumor growth factor alpha; TGF-β, tumor growth factor beta.

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These multiple functions are reflected in the structure of the protein, which can be divided into head, body and tail. From the N terminus, the head of the protein, consisting of the pro and catalytic domains mediates processing of growth factors and cytokines by ectodomain shedding and has been implicated in epidermal growth factor (EGF) and insulin-like growth factor (IGF) receptor signaling. The body of the protein, consisting of the desintegrin, cysteine-rich, and EGF-like domains is involved in contacts with the extracellular matrix and other cells, through interactions with integrins and syndecans. The tail of the protein (cytoplasmic domain) is involved in interactions with intracellular signaling molecules. In addition, splice forms exist for several ADAMs, for example for ADAM9, ADAM12 and ADAM28, shorter secreted and soluble forms have been described (Seals et al., 2003; Blobel, 2005; Wewer et al., 2006; Duffy et al., 2009).

The human ADAM12 gene resides on chromosome 10q26 and encodes two different forms of proteins: a transmembrane form, ADAM12L, and a spliced secreted form, ADAM12S. ADAM12S has all the extracellular domains, but lacks the transmembrane and cytoplasmic domains. Instead, the EGF-like domain is followed by a stretch of 33 amino acids. ADAM12 is expressed in high amounts in tissues characterized by excessive growth, including human placenta and tumors (Wewer et al., 2006; Kveiborg et al., 2008; Jacobsen and Wewer, 2009). ADAM12 is expressed at low levels in most normal adult tissues, but it is expressed at higher level by malignant tumor cells (Iba et al., 2000). It is associated with the progression and spread of human cancers (Fröhlich, 2006). Recently, it was shown that ADAM12 regulates tumor progression in gene-modified mice models (Kveiborg et al., 2005; Peduto et al., 2006; Sorensen et al., 2008). Proteomic approaches detected ADAM12S in body fluids, including serum (Laigaard et al., 2003; Shi et al., 2000) and urine and appears to be an important non-invasive biomarker of disease involving tissue growth (Roy et al., 2004). It was demonstrated that the urinary levels of ADAM12 were correlated with breast cancer status and stage (Roy et al., 2004; Pories et al., 2008). Kveiborg et al. (2005) suggested that ADAM12 facilitates breast cancer progression by conferring both increased stromal cell apoptosis and decreased tumor cell apoptosis.

ADAM17 gene resides on chromosome 2p25 and ADAM17 protein, a 70 kDa enzyme, is responsible for the proteolytic release of several cell-surface proteins, including: TNF-receptor, interleukin 1 receptor type II, transforming growth factor-alpha, L-selectin, growth hormone receptor, MUC1 and the amyloid precursor protein. It is also involved in the activation of Notch pathway. ADAM17 (also known as TGF-α converting enzyme or TACE) is the major sheddase for TGF-α, amphiregulin, HB-EGF and epiregulin (Black et al., 1997; Borrell-Pages et al., 2003; Zheng et al., 2004; Kawaguchi et al., 2007; Horiuchi et al., 2007; Edwards et al., 2008; Duffy et al., 2009). It is overexpressed in human breast cancers and plays an important role in the progression of breast tumors *in vivo* (Borrell-Pages et al., 2003; McGowan et al., 2008). Knowing that ADAM17 is a key modulator of EGFR signaling, ADAM17 could have a role in identifying patients likely to be resistant to therapies directed against EGFR and HER2/neu, for example tyrosine kinase inhibitors and trastuzumab, respectively (Zucker et al., 2000; Zhou et al., 2006; Liu et al., 2006; Fridman et al., 2007).

Only few studies with a relative small number of subjects have been performed regarding ADAMs gene expression *in vivo*, at mRNA level and, to the best of our knowledge, ADAMs genes expressions were not analyzed so far in laser-capture microdissected samples. On the other hand, few studies have made a distinction between the two spliced variants of ADAM12 and, therefore, their specific biological functions are still not precisely established. In this context, our purpose was to evaluate ADAM12L, ADAM12S and ADAM17 genes expression in homogenous, laser-capture microdissected breast cancers. We confirmed their expressions at translational level using immunohistochemistry, and correlated the levels of expression with the clinical and pathological characteristics of the breast cancer patients.

Table 1
Characteristics of breast cancer patients.

Characteristic	Breast cancers ($n = 92$)	
	n	Percent
Age (range between 38 and 90, mean: 61, median: 60)		
≤50	18	19.56
>50	74	80.44
Tumor size (cm)		
<5	56	60.86
≥5	36	39.14
Nodal status		
Positive	57	61.95
Negative	35	38.05
Histology		
Invasive ductal	62	67.39
Invasive lobular	19	20.65
Other types[a]	11	11.96
Histological grade (G)		
G1	4	4.34
G2	76	82.60
G3	12	13.06
Stage		
I, IIA	30	32.60
IIB, IIIA	29	31.52
IIIB, IV	33	35.88
Estrogen receptor status		
Positive	54	58.70
Negative	26	28.26
Unknown	12	13.04
Progesterone receptor status		
Positive	53	57.60
Negative	27	29.36
Unknown	12	13.04
HER2/neu status		
Negative (0)	28	30.43
Negative (+1)	46	50.00
Positive (+2, +3)	6	6.53
Unknown	12	13.04
Ki67 (%)		
≤10%	30	32.60
>10%	51	55.43
Unknown	11	11.97

[a] Atypical medullary (3), papillary *in situ* (1), invasive papillary (1), colloid (3), metaplastic squamous (2), anaplastic (1), DCIS (1).

Patients and methods

Patients and tumor characteristics

We evaluated 92 specimens of breast cancers from patients who underwent surgery at the University Clinic of Surgical Oncology, Timisoara, during 2009–2010. Corresponding normal tissues remote from the cancer in the same patients were taken as controls. Informed consent was obtained from all the patients before surgery, and the study was approved by the ethical committee of our University. Table 1 summarizes the characteristics of the breast cancer patients included in our study.

Preparation of samples

Following surgical resection and macroscopic pathological assessment, prelevated tissues (0.5–1/0.5 cm) were preserved in tubes with RNAlater solution (Ambion, Applied Biosystems, Darmstadt, Germany) for 24 h at +4 °C and then frozen at −80 °C. Corresponding available non-lesional tissues remote from the same patients served as normal controls and were treated in similar manner.