ORIGINAL PAPER

Molecular profiling of ADAM12 gene in breast cancers

DIANA NARIŢA[1)], A. ANGHEL[1)], E. SECLAMAN[1)], R. ILINA[2)], NATALIA CIREAP[2)], S. URSONIU[3)]

[1)]*Department of Biochemistry*
[2)]*Department of Surgical Oncology*
[3)]*Department of Public Health*
"Victor Babeş" University of Medicine and Pharmacy, Timisoara

Abstract

ADAMs (a disintegrin and metalloproteinase) family have been associated with the process of proteolytic "shedding" of membrane-associated proteins ectodomain and hence the rapid modulation of key cell signaling pathways in tissues microenvironment. A variety of cytokines, chemokines and growth factors which are initially produced as transmembrane proforms are activated by these sheddase activities. ADAM12 is highly expressed in rapidly growing tissues such as placenta and malignant tumors and it was found as one of the Candidate Cancer Genes in a comprehensive mutational analysis of human breast cancers. Our aim was to determine the gene expression profile of ADAM12 in breast cancers in comparison with normal breast and to correlate their level of expression with the clinical and pathological characteristics of breast cancers. Gene expression of ADAM12 spliced variants (12L and 12S) was evaluated using quantitative reverse-transcription PCR in samples obtained by laser capture microdissection from 38 patients with breast cancers and compared with adjacent healthy breast tissues. Both ADAM12L and 12S expression were significantly up-regulated in breast cancers, while in the normal breast, we found a very low expression. ADAM12L expression was significantly correlated with the histopathological types and, although not statistically significant, ADAM12 both variants were up-regulated in high-grade, highly-proliferative and HER2/neu positive tumors. From these preliminary results, we found that ADAM12 could be an interesting marker and eventually a therapeutic target for breast cancer.

Keywords: ADAM12, breast cancers, laser-capture microdissection, gene expression.

Introduction

ADAMs roles in cancers

The ADAMs (a disintegrin and metalloproteinase) comprise of a large family of more than 30 proteins that belong to the metzincin family of matrix zinc-dependent proteases and together with the snake venom metallo-proteases and ADAMs containing thrombospondin sequences (ADAMTS) they constitute the adamalysin subfamily [1–3]. They are multidomain, transmembrane and secreted proteins with protease, adhesion, fusion and signaling activities. 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 disintegrin, 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 ADAM 9, 12 and 28, shorter secreted and soluble forms have been described [4–9].

The gene for human ADAM12 resides on chromosome 10q26 and encodes two different forms: 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 unique amino acids [10, 11].

ADAM12 is expressed in high amounts in tissues characterized by excessive growth, including human placenta and tumors. ADAM12 is expressed at low levels in most normal adult tissues, but it is expressed at higher levels by tumor cells and is associated with the progression and spread of human cancers [12]. Most recently, it was showed that ADAM12 regulates tumor progression in gene-modified mice models [13–15]. Breast cancers are often associated with elevated levels of ADAM 9, 12, 15, 17 and 28 [16–18].

Recently, ADAM12 has been identified as one of the candidate cancer genes in a comprehensive mutational analysis of human breast cancer. Among 122 genes found to be mutated with high frequencies in breast cancers, there was only one ADAM, namely ADAM12 and furthermore, only 14 genes had a higher cancer mutation prevalence score than ADAM12. High mutation frequency, together with a strongly up-regulated expression of ADAM12 in breast cancer suggests that ADAM12 may play an important role in breast cancer progression [19].

Gene expression profiling using laser capture microdissection

Molecular profiling of single cell population is essential for correlating molecular signatures in diseased and disease-free cells from the complexes, heterogeneous tissues. The heterogeneous tumor microenvironment may hamper molecular analysis because it is difficult to discern which cells contribute to the signal. Laser capture microdissection (LCM) is to date one of the most suitable methods that enable researchers to isolate specific cells of interest under direct microscopic visualization, without contamination from surrounding cells (95% purity). LCM in combination with gene expression analyses have the potential to provide expression profiles from highly homogeneous clusters of cells, giving the opportunity to understand the process of tumor genesis. On the other hand, there are still debates regarding the impact of successive steps of LCM procedure on RNA quality [20–23].

In this context, our aim was to confirm the up-regulation of ADAM12 gene splicing variants in homogenous laser-capture microdissected breast malignant cells compared to healthy adjacent breast acini and to correlate the ADAM12 level of expression with significant clinicopathological characteristics of investigated breast cancers.

Material and Methods

Patients and tumor characteristics

We have evaluated a total of 38 malignant breast tumors from patients (range between 38 and 74; mean: 56; median: 58) who underwent surgery at the Surgical Oncology Department of the "Victor Babeş" University of Medicine and Pharmacy, Timişoara, during 2009–2010. Corresponding normal tissues remote from 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 patients that were included in our study.

Table 1 – ***Characteristics of breast cancer patients***

Characteristic	Breast cancers	
	n=38	***Percent***
Age [years]		
≤50	11	29
>50	27	71
Tumor size [cm]		
<5	25	65.79
≥5	13	34.21
Nodal status		
Positive	22	57.89
Negative	17	42.11
Histology		
Invasive ductal	25	65.79
Invasive lobular	8	21.05
Other types	5	13.16
Histological grade (G)		
G1	4	10.53
G2	30	78.94
G3	4	10.53
Stage		
I, IIA	16	42.11
IIB, IIIA	10	26.31
IIIB, IV	12	31.58
Estrogen receptor status		
Positive	28	73.68
Negative	10	26.32
Progesterone receptor status		
Positive	29	76.315
Negative	9	23.685
HER2/neu status		
Negative (0)	6	15.79
Negative (+1)	28	73.68
Positive (+2, +3)	4	10.53
Unknown	–	–
Ki67 [%]		
≤10	15	39.47
>10	23	50.53
Unknown	–	–
Inflammatory infiltrate		
Moderate/Abundant	19	50
Absent/Low	19	50
BMI (Body Mass Index) [kg/m^2]		
<25	11	29
≥25	27	71

Samples preparation

Following surgical resection and macroscopic pathological assessment, prelevated tissues (0.5–1/0.5 cm) were preserved in 2 mL tubes with RNAlater solution (Ambion, Applied Biosystems, Germany) at +4^{0}C for 24 hours and then frozen at -80^{0}C. Corresponding non-lesional tissues served as normal controls and were treated in similar manner.

Laser captured microdissection (LCM)

We used laser-capture microdissection to select and procure only the desired cell types (malignant groups of cells/normal mammary acini), under direct microscopic visualization. We used the UV cutting system mmi SmartCut Plus (MMI Molecular Machines & Industries, Glattburg, Switzerland) with Olympus microscope. Following the manufacturer protocol, frozen tissues were embedded in TissueTek medium and cut at -30^{0}C (Leica CM1850 cryostat, Leica Microsystems GmbH, Wetzlar, Germany). The 4-µm cryosections were mounted on RNase free membrane slides (mmi MembranSlides, MMI, Glattburg, Switzerland), which were immediately processed or stored at -80^{0}C. Consecutive cryosections from each specimen were mounted also on silanized glass slides and, after standard Hematoxylin–Eosin staining, the sections were evaluated by an experienced pathologist. The membrane slides for LCM were stained using either HE staining kit for LCM (MMI, Switzerland), following the manufacturer protocol or cresyl violet acetate staining (Acros Organics, Geel, Belgium). For cresyl violet acetate staining, the slides were thawed at room temperature for 30 seconds, and then covered for 1 minute with cresyl

violet 1% solution in absolute ethanol, followed by 1 minute washing in 70% ethanol, dehydration for 1 minute in 100% ethanol and air dried for maximum 5 minutes. LCM was performed immediately after staining. The polyethylene tetraphthalate (PET) membrane slide was protected with an RNase free normal glass slide (15×49 mm) and placed on microscope. The selected cells were cut using adequate power and focus for UV laser shots. The cut area was captured together with the membrane on which it was cleaved to, by automatically placing down the adhesive lid of an RNase free microcentrifuge tube (mmi IsolationCaps 500 µL tube with adhesive lid and diffuser, MMI Switzerland) onto the cut area.

RNA extraction

When all selected groups of cells were removed, 100 µL lyses buffer (RNAqueous-Micro kit, Ambion, Applied Biosystems, Germany) was added in the microcentrifuge tube and vortexed in order to remove the cells from the lid. We proceeded further with the RNA extraction using RNAqueous-Micro kit, following exactly the manufacturer protocol for microdissected cells. The RNA was eluted in 20 µL elution solution provided with the kit. RNA concentration and purity were spectrophotometrically quantified (NanoDrop ND1000). Extracted RNA was stored at -80^{0}C until further gene expression analyses.

Real-time RT–PCR

We quantified the gene expression for the investigated genes normalized against β-actin housekeeping gene, using the Q–RT–PCR on LightCycler 1.5, software version 5.3 (Roche, Germany) and SYBRGreen method. Because small, precious quantity of RNA extracted from LCM samples do not allow normalization with multiple housekeeping genes, the most appropriate normalizer gene (β-actin) was selected from literature [24]. We used QuantiTect SYBR Green one-step RT-PCR kit and QuantiTect Primer Assays (Hs_ADAM12_va.1_SG; Hs_ADAM12_vb.1_SG and Hs_ACTB_1_SG; the manufacturer do not provides the sequences) (Qiagen, Germany). We diluted the RNA in RNase free water in order to obtain an input template concentration of 0.5 ng/µL for each reaction. We followed further the manufacturer protocol adapted for LightCycler 1.5. for a total volume of 10 µl. Briefly, we pipetted in 20 µL LightCycler Capillaries: 5 µL of master-mix (RT SYBRGreen Buffer) with 1 µL specific primers, 0.1 µL RT-PCR enzymes, 5 ng RNA/tube (usually 1 µL RNA containing 5 ng RNA) and RNase free water (usually 2.9 µL). We programmed the real-time device following the QuantiTect Primer Assay kit protocol: reverse transcription at 50^{0}C for 20 minutes, initial polymerase ctivation step at 95^{0}C for 15 minutes followed by three-steps amplification cycles (denaturation at 94^{0}C for 15 seconds, annealing at 55^{0}C for 20 seconds and elongation at 72^{0}C for 20 seconds). The fluorescence intensity reflecting the amount of actually double-stranded formed PCR-product was read in real-time at the end of each elongation step. All samples were run in duplicate together with appropriate non-template controls; the coefficient of variation was <2% for all replicates. In parallel, in order to verify the results, we used also the two-step RT–PCR method. We synthesized cDNA using QuantiTect Reverse Transcription kit and performed amplification with QuantiTect SYBR Green PCR kit (Qiagen, Germany) following exactly the manufacturer protocols. The results that we obtained with both, two-step and one-step methods were quite similar. The relative quantification levels for the genes expression were calculated using the $2^{-\Delta\Delta CT}$ method (C_T = crossing points, cycle number where the fluorescence crossed the threshold): $\Delta C_T = C_T$ (target gene) – C_T (housekeeping gene, β-actin); $\Delta\Delta C_T = \Delta C_T$ patients - ΔC_T normal controls. The expression comparative level will be $2^{-\Delta\Delta CT}$ [25].

Statistical analysis

Data analyses were carried out using the two-sample, rank sum Wilcoxon (Mann–Whitney) test and equality of populations (Kruskal–Wallis) test. Data for all gene expressions normalized to β-actin are reported as summary statistics (mean ± SD and median). The threshold for significance was set at $p<0.05$.

Results

Laser-capture microdissection

The statistics regarding the areas and number of cells that were captured and corresponding RNA quantity and purity are represented in Table 2.

Table 2 – ***Statistics for the LCM samples regarding the areas and number of cells that were captured and corresponding RNA quantity and purity***

Parameter	Laser shots (N)	Captured area [µm^2]	Captured cells (N)	Correspondent total RNA [ng]	$A_{260/280}$
Average	48.307	3660570	13972	139.72	1.82
Median	56	2569720	9808	98.08	1.85
Min.	11	1178654	4499	44.99	1.75
Max.	116	9274800	35400	354.00	1.98

Because we observed that microdissection of a large number of cells (>40000 cells) affected the proportionality with the quantity of the obtained RNA, we assumed that the LCM duration affects the RNA quality. In order to minimize this effect, we tried to reduce the LCM duration at 30 minutes. The number of cells that were microdissected/sample was dependent on the abundance of malignant cells that were available on each slide. We did not observed variations in RNA quality related to staining that was used, but the HE staining procedure permitted a better visualization of the morphology of cells (Figures 1 and 2).

Gene expression analyses

Gene amplification was successful in the large majority of samples except two LCM samples that were excluded because of the low quantity of RNA.

Gene expression variability between normal and malignant breast tissues

Both investigated genes (normalized to β-actin) revealed significantly elevated mRNA amounts (ΔC_T

mean ± SD and median for ADAM12L: 5.833±1.839, median=6.243 *vs.* 8.849±0.116, median=8.865, $p<10^{-3}$ and for ADAM12S: 7.625±3.188, median=8.165 *vs.* 10.141±0.121, median=10.112, p=0.0021) in breast cancers *vs.* adjacent paired healthy laser-capture microdissected breast samples. Regarding the differences of expression between the two genes, in both healthy adjacent tissue and malignant tissues, ADAM12L was overexpressed in comparison with ADAM12S (p=1.943e-10 for healthy and p=3.058e-15 for malignancies) (Figure 3).

Correlations between gene expression of ADAM12 splicing variants (12L and 12S) and breast cancers characteristics

ADAM12L (membrane-bound long variant) was significantly correlated only with the histopathological types, being higher expressed in ductal invasive carcinomas (p=0.01). Although not statistically significant, both ADAM12 variants had a higher level of expression in HER2/neu positive, highly-proliferative (Ki67>10%), high-grade (G3) and in tumors with by an abundant inflammatory infiltrate. ADAM12L had a higher expression in younger patients with ductal invasive carcinomas, while ADAM12S was overexpressed in older patients with lobular invasive carcinomas. No significant differences were found regarding the expression levels of ADAM12L and 12S when considering the different TNM stages, the tumor size, nodal or ER/PR status but interesting the two ADAM12 gene variants were up-regulated in smaller, node negative tumors. These data are summarized in Figures 4–7.

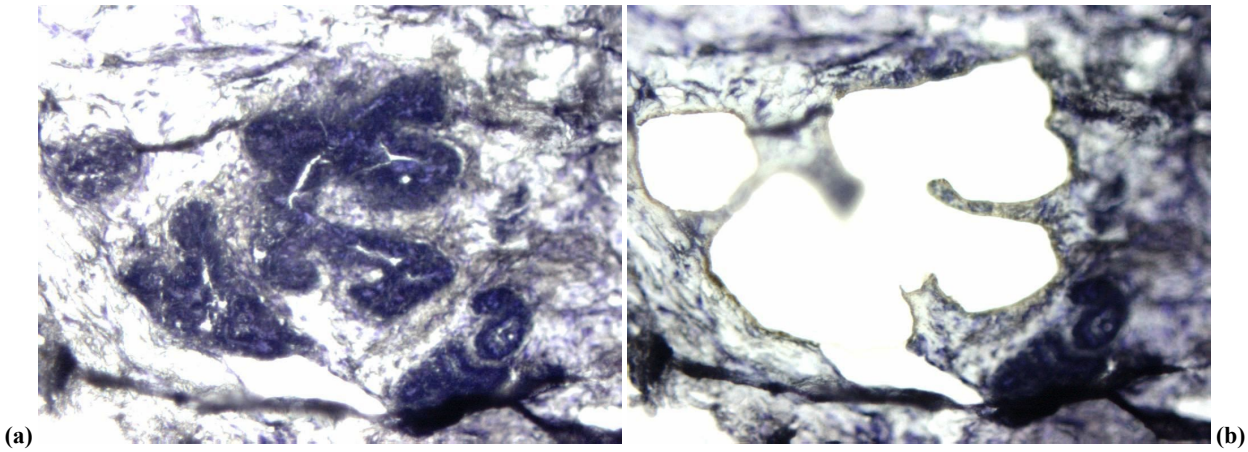
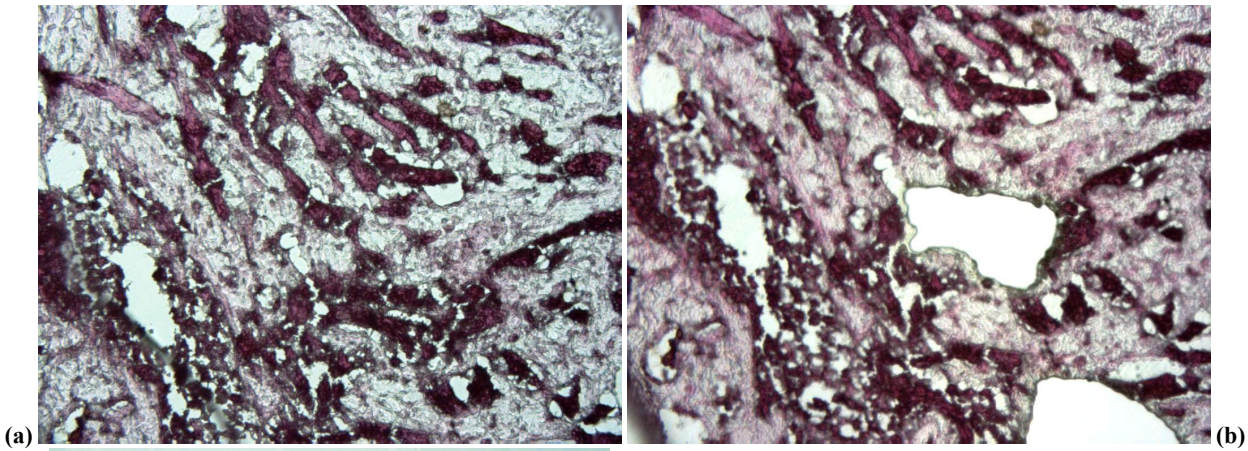


Figure 1 – ***Normal mammary gland acini, ob. 20×, cresyl violet acetate staining; (a) before, and (b) after laser-capture microdissection.***



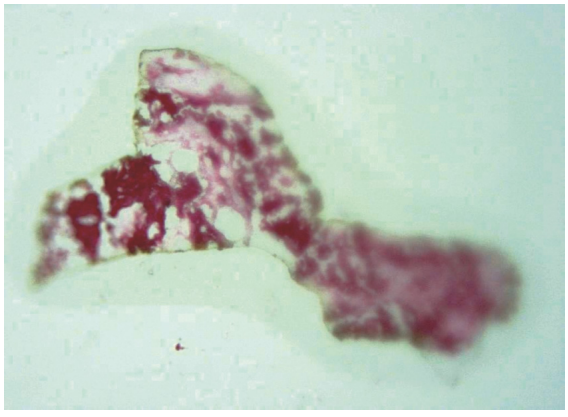


Figure 2 – ***Invasive ductal carcinoma, ob. 20×, HE staining; (a) before, (b) after laser-capture microdissection, and (c) the cap with the captured groups of cells.***

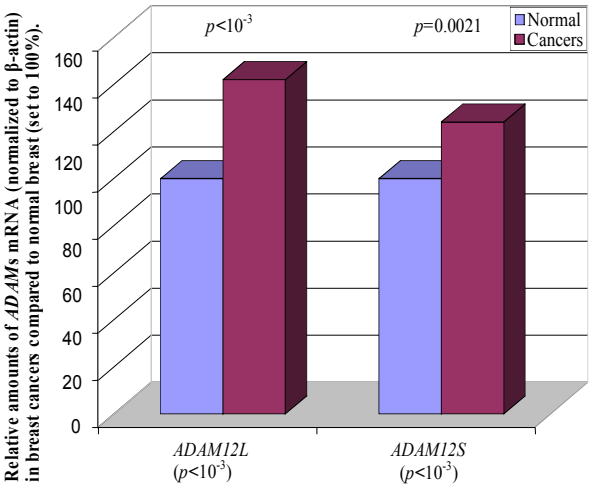


Figure 3 – ***ADAM12 gene variants up-regulation in breast cancers vs. normal breast tissues.***

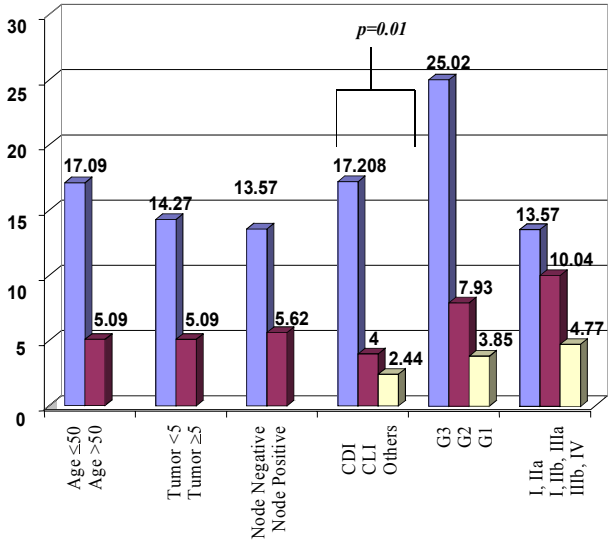


Figure 4 – ***Correlation of ADAM12L expression with clinicopathological characteristics (age at diagnostic, tumor size, nodal status, histological type and grade, TNM stage).***

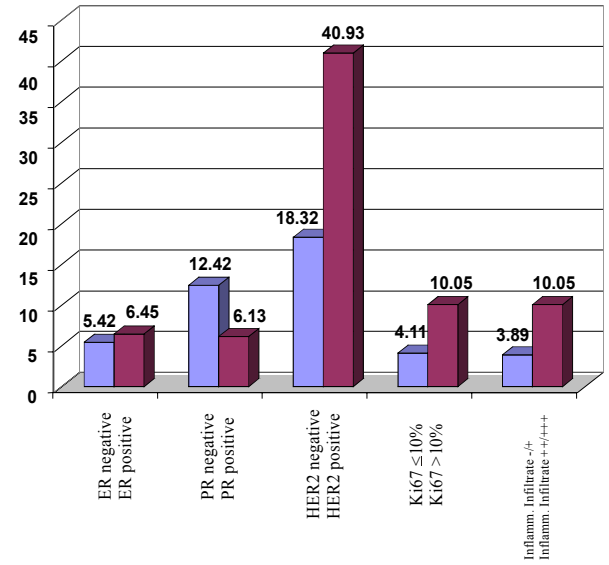


Figure 5 – ***Correlation of ADAM12L expression with clinicopathological characteristics (ER/PR/HER2 status, Ki67, tumor inflammatory infiltrate).***

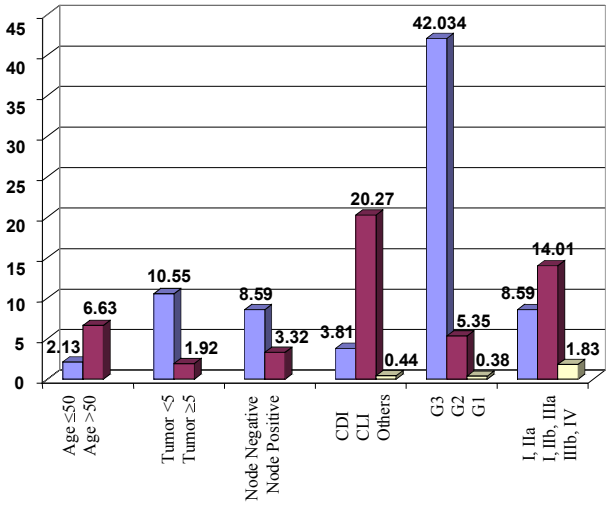


Figure 6 – ***Correlation of ADAM12S expression with clinicopathological characteristics (age at diagnostic, tumor size, nodal status, histological type and grade, TNM stage).***

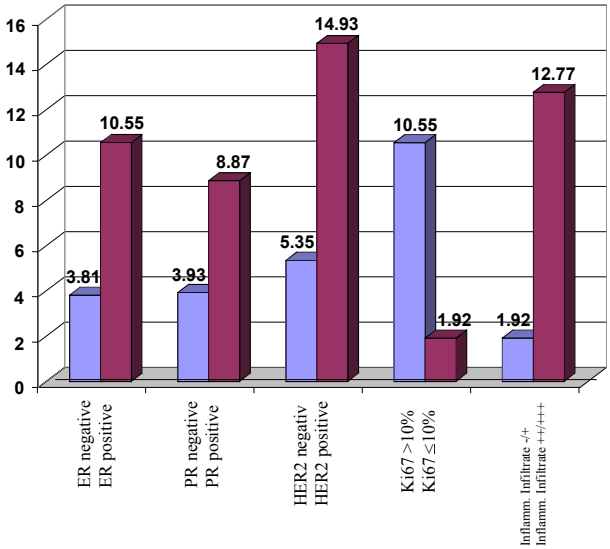


Figure 7 – ***Correlation of ADAM12S expression with clinicopathological characteristics (ER/PR/HER2 status, Ki67, tumor inflammatory infiltrate).***

Discussion

It was established before that ADAMs family members and especially ADAM 9, 12, 15, 17 and 28 could be differentially expressed between normal and pathological mammary gland, but their pattern of expression and the intimate mechanism of action are not precisely established yet [16–18, 26–30]. Few studies regarding ADAMs gene expression were performed in cancers at mRNA level and, after our knowledge, ADAM genes expression was not analyzed yet in laser-capture microdissected samples. In this context, the aim of this study was to compare the expressions of ADAM12 gene splicing variants (ADAM12L, ADAM12S) in breast cancers paired with normal adjacent tissues as controls and to compare their level of expression with significant clinical and pathological breast cancer characteristics. As novelty, this is the first study addressed to monitor some of the ADAMs gene family member's expression

at mRNA level in LCM-processed breast samples. We showed that although there was a bias in monitoring gene expression in LCM samples induced especially by the time required for processing LCM samples, this bias was circumvented by limiting the time for microdissection to 30 minutes. The microdissection of about 4000 cells was enough to monitor the expression of three genes, all in duplicate (with a starting RNA template concentration of 0.5 ng/μL). Our results regarding the amount and the purity of the RNA (measured spectrophotometrically) obtained from LCM samples were similar with that from other studies [23, 31, 32]. Using two different staining (MMI HE staining kit for LCM and an in-house rapid cresyl violet acetate staining) we did not observed differences in the RNA quality, but the HE staining confers a better estimation of cells morphology.

We observed a significant higher expression for ADAM12L ($p<10^{-4}$) and ADAM12S ($p=0018$) in cancers compared to normal breast. Our findings regarding ADAM12L and 12S pattern of expression are in accordance with some previous studies [12, 33], whereas others have demonstrated that only the long transmembrane variant was up-regulated in breast cancers [3, 34]. In a study performed by Lendeckel U *et al.* [17] on 24 breast cancer specimens and corresponding non-neoplastic tissue, mRNA expression of ADAM 9, 12 and 17 were increased, whereas ADAM 10 and 15 were not differently expressed. Application of anti-ADAM15 and anti-ADAM17 antibodies significantly inhibited the proliferation of both MCF-7 and MDA–MB453 breast cancer cell lines, whereas the growth of MCF-7 cells appeared to be stimulated after the administration of anti-ADAM12 antibodies [17].

Recent proteomic approaches detected ADAM12S in body fluids, including serum [27, 28] and urine [29] that appears to be an important noninvasive biomarker of disease involving tissue growth. Moreover, it was suggested that urinary levels of ADAM12 correlate with breast cancer status and stage [29, 30]. In the present study, performed at mRNA transcriptional level, we did not find correlations with the TNM stages, however, although not statistically significant, both ADAM12 variants showed an increased expression in high-grade cancers, in cancers with high degree of proliferation and in HER2/neu positive tumors. ADAM12L (membrane-bound long variant) expression was increased in invasive ductal carcinomas compared to lobular invasive; in contrast, ADAM12S seemed to be higher expressed in lobular carcinomas. This finding regarding ADAM12 overexpression in ductal carcinomas was not in accordance with a previous microarray study, where ADAM12 showed an up-regulation in invasive lobular carcinomas, but that study was performed only on invasive lobular carcinomas from five patients and made no distinction between the ADAM12 variants [35].

For ADAM12 variants, the variability between individual tumors was significant, suggesting a high dependence of gene expression of tumor characteristics. Because our tumors were collected consecutively and had different characteristics regarding histopathological type, stage, grade or proliferation rate, the variability regarding the gene expression is not unexpected.

Kveiborg M *et al.* [13] proposed that ADAM12 increased tumor aggressiveness by decreasing time for tumor onset, increasing tumor burden and metastasis and increasing the degree of malignancy by conferring both increased stromal cell apoptosis and decreased tumor cell apoptosis especially through interactions with β-integrin and syndecans and this effect could be independent of the protease activity of ADAM12. The protease function is attributable especially to ADAM12S. Peduto L *et al.* [14] describe the identification of ADAM12 as a novel marker for a subpopulation of stromal cells that are adjacent to epithelial tumor cells in three mouse carcinoma models (models for prostate, breast and colon cancer) and they show that ADAM12 is essential for crosstalk between stromal and tumor cell.

Our findings regarding the overexpression of ADAM12 in smaller, node negative tumors could be in accordance with the study performed by Dyczynska E *et al.* [36]. They investigated the effects of cancer-associated mutations on ADAM12 function and showed that two mutations that are classified as cancer-causing by bioinformatics approach block the generation of the mature, active form of ADAM12, interfere with the intracellular trafficking of ADAM12, leading to the retention of ADAM12 in the endoplasmic reticulum and result in loss of ADAM12 function at the cell surface. The aforementioned study suggests that ADAM12 has a dual effect on breast cancer progression. This effect would be similar to the effect of TGF-β, which has tumor growth-inhibiting activity at the early stage of tumor development and tumor-promoting activity during later stages of tumor progression and invasion [37, 38]. This explanation is sustained by the fact that ADAM12 interact with TGF-β type II receptor, to enhance TGF-β signaling and this interaction may represent a tumor-suppressing aspect of ADAM12. Furthermore, ADAM12 sheds DII1, a ligand for Notch receptor and modulates the Notch signaling. DII1, similar to ADAM12, is highly expressed in breast tumors. Although Notch signaling stimulates mammary tumorigenesis, the Notch pathway has also been reported to have tumor-suppressive functions [39]. On other hand, in the study of Lendeckel U *et al.* [17], application of anti-ADAM12 antibodies on MCF-7 cells appeared to stimulate their growth. In consequence, the effect of ADAM12 in breast cancer progression may be dual and dependent on tumor stage.

Conclusions

In summary, our study, performed at transcriptional level, in homogenous laser-capture microdissected samples supports the previous studies regarding the overexpression of ADAM12 both splicing variants in breast cancers. Between the two spliced variants expression, ADAM12L was significantly higher expressed than ADAM12S in either normal breast tissues or breast cancers. ADAM12L and 12S were higher expressed in high-grade, highly-proliferative and HER2/neu positive, but interestingly, node negative and smaller tumors. ADAM12L was higher expressed in younger patients

with ductal invasive carcinomas, while ADAM12S was higher expressed in older patients with lobular invasive carcinomas. Our study confers further evidence that ADAM12 is implicated in breast cancers tumorigenesis and progression and could be an interesting marker and eventually a therapeutic target for breast cancer.

Acknowledgements

This work was supported by CNCSIS–UEFISCSU, Project No. 1197/2009, PN II – IDEI, PCE, Code 1750/2008.

References

[1] BLOBEL CP, *ADAMs: key components in EGFR signalling and development*, Nat Rev Mol Cell Biol, 2005, 6(1):32–43.

[2] ARRIBAS J, BECH-SERRA JJ, SANTIAGO-JOSEFAT B, *ADAMs, cell migration and cancer*, Cancer Metastasis Rev, 2006, 25(1):57–68.

[3] IBA K, ALBRECHTSEN R, GILPIN B, FRÖHLICH C, LOECHEL F, ZOLKIEWSKA A, ISHIGURO K, KOJIMA T, LIU W, LANGFORD JK, SANDERSON RD, BRAKEBUSCH C, FÄSSLER R, WEWER UM, *The cysteine-rich domain of human ADAM 12 supports cell adhesion through syndecans and triggers signaling events that lead to β1 integrin-dependent cell spreading*, J Cell Biol, 2000, 149(5):1143–1156.

[4] OHTSU H, DEMPSEY PJ, EGUCHI S, *ADAMs as mediators of EGF receptor transactivation by G protein-coupled receptors*, Am J Physiol Cell Physiol, 2006, 291(1):C1–C10.

[5] MOCHIZUKI A, OKADA Y, *ADAMs in cancer cell proliferation and progression*, Cancer Sci, 2007, 98(5):621–628.

[6] MURPHY G, *The ADAMs: signalling scissors in the tumour microenvironment*, Nat Rev Cancer, 2008, 8(12):929–941.

[7] EDWARDS DR, HANDSLEY MM, PENNINGTON CJ, *The ADAM metalloproteinases*, Mol Aspects Med, 2008, 29(5):258–289.

[8] DUFFY MJ, MCKIERNAN E, O'DONOVAN N, MCGOWAN PM, *Role of ADAMs in cancer formation and progression*, Clin Cancer Res, 2009, 15(4):1140–1144.

[9] JACOBSEN J, WEWER UM, *Targeting ADAM12 in human disease: head, body or tail?*, Curr Pharm Des, 2009, 15(20):2300–2310.

[10] KVEIBORG M, ALBRECHTSEN R, COUCHMAN JR, WEWER UM, *Cellular roles of ADAM12 in health and disease*, Int J Biochem Cell Biol, 2008, 40(9):1685–1702.

[11] WEWER UM, MÖRGELIN M, HOLCK P, JACOBSEN J, LYDOLPH MC, JOHNSEN AH, KVEIBORG M, ALBRECHTSEN R, *ADAM12 is a four-leafed clover: the excised prodomain remains bound to the mature enzyme*, J Biol Chem, 2006, 281(14):9418–9422.

[12] FRÖHLICH C, ALBRECHTSEN R, DYRSKJØT L, RUDKJAER L, ØRNTOFT TF, WEWER UM, *Molecular profiling of ADAM12 in human bladder cancer*, Clin Cancer Res, 2006, 12(24):7359–7368.

[13] KVEIBORG M, FRÖHLICH C, ALBRECHTSEN R, TISCHLER V, DIETRICH N, HOLCK P, KRONQVIST P, RANK F, MERCURIO AM, WEWER UM, *A role for ADAM12 in breast tumor progression and stromal cell apoptosis*, Cancer Res, 2005, 65(11):4754–4761.

[14] PEDUTO L, REUTER VE, SCHARA-FUJISAWA A, SHAFFER DR, SCHER HI, BLOBEL CP, *ADAM12 is highly expressed in carcinoma-associated stroma and is required for mouse prostate tumor progression*, Oncogene, 2006, 25(39):5462–5466.

[15] SØRENSEN HP, VIVÈS RR, MANETOPOULOS C, ALBRECHTSEN R, LYDOLPH MC, JACOBSEN J, COUCHMAN JR, WEWER UM, *Heparan sulfate regulates ADAM12 through a molecular switch mechanism*, J Biol Chem, 2008, 283(46):31920–31932.

[16] O'SHEA C, MCKIE N, BUGGY Y, DUGGAN C, HILL AD, MCDERMOTT E, O'HIGGINS N, DUFFY MJ, *Expression of ADAM-9 mRNA and protein in human breast cancer*, Int J Cancer, 2003, 105(6):754–761.

[17] LENDECKEL U, KOHL J, ARNDT M, CARL-MCGRATH S, DONAT H, RÖCKEN C, *Increased expression of ADAM family members in human breast cancer and breast cancer cell lines*, J Cancer Res Clin Oncol, 2005, 131(1):41–48.

[18] KUEFER R, DAY KC, KLEER CG, SABEL MS, HOFER MD, VARAMBALLY S, ZORN CS, CHINNAIYAN AM, RUBIN MA, DAY ML, *ADAM15 disintegrin is associated with aggressive prostate and breast cancer diseases*, Neoplasia, 2006, 8(4):319–329.

[19] SJÖBLOM T, JONES S, WOOD LD, PARSONS DW, LIN J, BARBER TD, MANDELKER D, LEARY RJ, PTAK J, SILLIMAN N, SZABO S, BUCKHAULTS P, FARRELL C, MEEH P, MARKOWITZ SD, WILLIS J, DAWSON D, WILLSON JK, GAZDAR AF, HARTIGAN J, WU L, LIU C, PARMIGIANI G, PARK BH, BACHMAN KE, PAPADOPOULOS N, VOGELSTEIN B, KINZLER KW, VELCULESCU VE, *The consensus coding sequences of human breast and colorectal cancers*, Science, 2006, 314(5797):268–274.

[20] EMMERT-BUCK MR, BONNER RF, SMITH PD, CHUAQUI RF, ZHUANG Z, GOLDSTEIN SR, WEISS RA, LIOTTA LA, *Laser capture microdissection*, Science, 1996, 274(5289):998–1001.

[21] FEND F, RAFFELD M, *Laser capture microdissection in pathology*, J Clin Pathol, 2000, 53(9):666–672.

[22] OKUDUCU AF, JANZEN V, HAHNE JC, WERNERT N, *Influence of histochemical stains on quantitative gene expression analysis after laser-assisted microdissection*, Int J Mol Med, 2003, 11(4):449–453.

[23] ESPINA V, WULFKUHLE JD, CALVERT VS, VANMETER A, ZHOU W, COUKOS G, GEHO DH, PETRICOIN III EF, LIOTTA LA, *Laser-capture microdissection*, Nature Protocols, 2006, 2(1):586–602.

[24] DE KOK JB, ROELOFS RW, GIESENDORF BA, PENNINGS JL, WAAS ET, FEUTH T, SWINKELS DW, SPAN PN, *Normalization of gene expression measurements in tumor tissues: comparison of 13 endogeneous control genes*, Lab Invest, 2005, 85(1):154–159.

[25] LIVAK KJ, SCHMITTGEN TD, *Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method*, Methods San Diego (Calif), 2001, 25(4):402–408.

[26] MCGOWAN PM, MCKIERNAN E, BOLSTER F, RYAN BM, HILL ADK, MCDERMOTT EW, EVOY D, O'HIGGINS N, CROWN J, DUFFY MJ, *ADAM-17 predicts adverse outcome in patients with breast cancer*, Ann Oncol, 2008, 19(6):1075–1081.

[27] SHI Z, XU W, LOECHEL F, WEWER UM, MURPHY LJ, *ADAM12, a disintegrin and metalloprotease, interacts with insulin-like growth factor-binding protein-3*, J Biol Chem, 2000, 275(24):18574–18580.

[28] LAIGAARD J, SØRENSEN T, FRÖHLICH C, PEDERSEN BN, CHRISTIANSEN M, SCHIØTT K, ULDBJERG N, ALBRECHTSEN R, CLAUSEN HV, OTTESEN B, WEWER UM, *ADAM12: a novel first-trimester maternal serum marker for Down syndrome*, Prenat Diagn, 2003, 23(13):1086–1091.

[29] ROY R, WEWER MU, ZURAKOWSKI D, PORIES SE, MOSES MA, *ADAM 12 cleaves extracellular matrix proteins and correlates with cancer status and stage*, J Biol Chem, 2004, 279(49):51323–51330.

[30] PORIES SE, ZURAKOWSKI D, ROY R, LAMB CC, RAZA S, EXARHOPOULOS A, SCHEIB RG, SCHUMER S, LENAHAN C, BORGES V, LOUIS GW, ANAND A, ISAKOVICH N, HIRSHFIELD-BARTEK J, WEWER U, LOTZ MM, MOSES MA, *Urinary metalloproteinases: noninvasive biomarkers for breast cancer risk assessment*, Cancer Epidemiol Biomarkers Prev, 2008, 17(5):1034–1042.

[31] MICHEL C, DESDOUETS C, SACRE-SALEM B, GAUTIER JC, ROBERTS R, BOITIER E, *Liver gene expression profiles of rats treated with clofibric acid: comparison of whole liver and laser capture microdissected liver*, Am J Pathol, 2003, 163(6):2191–2199.

[32] ERICKSON HS, ALBERT PS, GILLESPIE JW, WALLIS BS, RODRIGUEZ-CANALES J, LINEHAN WM, GONZALES S, VELASCO A, CHUAQUI RF, EMMERT-BUCK MR, *Normalization strategies for quantitative RT–PCR in microdissected tissue*, Lab Invest, 2007, 87(9):951–962.

[33] LE PABIC H, BONNIER D, WEWER UM, COUTAND A, MUSSO O, BAFFET G, CLÉMENT B, THÉRET N, *ADAM12 in human liver cancers: TGF-β-regulated expression in stellate cells is associated with matrix remodeling*, Hepatology, 2003, 37(5):1056–1066.

[34] KODAMA T, IKEDA E, OKADA A, OHTSUKA T, SHIMODA M, SHIOMI T, YOSHIDA K, NAKADA M, OHUCHI E, OKADA Y, *ADAM12 is selectively overexpressed in human glioblastomas and is associated with glioblastoma cell proliferation and shedding of heparin-binding epidermal growth factor*, Am J Pathol, 2004, 165(5):1743–1753.

[35] TURASHVILI G, BOUCHAL J, BAUMFORTH K, WEI W, DZIECHCIARKOVA M, EHRMANN J, KLEIN J, FRIDMAN E, SKARDA J, SROVNAL J, HAJDUCH M, MURRAY P, KOLAR Z, *Novel markers for differentiation of lobular and ductal invasive breast carcinomas by laser microdissection and microarray analysis*, BMC Cancer, 2007, 7:55.

[36] DYCZYNSKA E, SYTA E, SUN D, ZOLKIEWSKA A, *Breast cancer-associated mutations in metalloprotease disintegrin ADAM12 interfere with the intracellular trafficking and processing of the protein*, Int J Cancer, 2008, 122(11):2634–2640.

[37] ATFI A, DUMONT E, COLLAND F, BONNIER D, L'HELGOUALC'H A, PRUNIER C, FERRAND N, CLÉMENT B, WEWER UM, THÉRET N, *The disintegrin and metalloproteinase ADAM12 contributes to TGF-β signaling through interaction with the type II receptor*, J Cell Biol, 2007, 178(2):201–208.

[38] BIERIE B, MOSES HL, *Tumour microenvironment: TGFβ: the molecular Jekyll and Hyde of cancer*, Nat Rev Cancer, 2006, 6(7):506–520.

[39] NIIMI H, PARDALI K, VANLANDEWIJCK M, HELDIN CH, MOUSTAKAS A, *Notch signaling is necessary for epithelial growth arrest by TGF-β*, J Cell Biol, 2007, 176(5):695–707.

Corresponding author
Diana Narița, Assistant Professor, MD, PhD, Department of Biochemistry, "Victor Babeş" University of Medicine and Pharmacy, 2A Eftimie Murgu Square, 300041 Timişoara, Romania; Phone/Fax +40256–220 479, e-mail: diana_narita@yahoo.com

Received: August 10th, 2010

Accepted: October 25th, 2010