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RESEARCH

Molecular Profiling of *ADAM12* and *ADAM17* Genes in Human Malignant Melanoma

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Abstract ADAM12 and ADAM17 proteins belong to a family of transmembrane disintegrin-containing metalloproteinases (ADAMs) involved in the proteins ectodomain shedding and cell-cell and cell-matrix interactions. However, the specific biological functions of ADAMs are still unclear and, until now, these proteins were not investigated yet in melanoma. The aim of this study was to analyze the splicing variants of *ADAM12* (*L* and *S*) and *ADAM17* gene expression in melanoma at transcriptional and translational level in comparison with control (non-tumor) tissues. Taking in account that ADAM17 sheddase is involved in the modulation of TNF-α (tumor necrosis factor alpha), we analyzed also this cytokine in the plasma of the same patients before any treatment, and we compared the results with healthy controls. Quantitative-RT-PCR and immunohistochemistry were used to analyze *ADAM12* and *ADAM17* genes expression and the analysis of TNF-α expression was carried out in the plasma using ELISA. We demonstrated that *ADAM12L* splicing variant together with *ADAM17* gene are strongly overexpressed in melanomas, whereas *ADAM12S*, although up-regulated when compared with the non-tumor controls, the difference was not statistically significant. When we compared the levels of expression for the ADAMs genes according to the tumor stage, we observed that all three investigated genes were significantly overexpressed in advanced stage in comparison with early stage melanomas. In the plasma of the same patients, the expression of TNF-α was up-regulated and significantly correlated with the expression of ADAM17 and respectively, with the advanced tumor stage.

Keywords ADAM12 · ADAM17 · Melanoma · Gene expression · Cytokines

Abbreviations

ADAM	A disintegrin and metalloprotease (meltrin-alpha)
ADAM12L	A disintegrin and metalloprotease 12 long membrane-bound variant
ADAM12S	A disintegrin and metalloprotease 12 short secreted variant
ADAM17	A disintegrin and metalloprotease17
CDI	Invasive ductal carcinoma
CLI	Invasive lobular carcinoma
C_T	Crossing points cycle number where the fluorescence crossed the threshold
DCIS	In situ ductal carcinoma
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
FFPE	Formalin fixed paraffin embedded
G	Tumor grade
GPI	Glucose-6-phosphate-isomerase
HB-EGF	Heparin-bindingepidermal growth factor
HER2/neu	Human epidermal growth factor receptor 2
IHC	Immunohistochemistry
LCM	Laser-capture microdissection
mRNA	Messenger ribonucleic acid
PR	Progesterone receptor
Q-RT-PCR	Quantitative-reverse transcription polymerase chain reaction

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RIN	RNA integrity number
S.D.	Standard deviation
TACE	TNF-α converting enzyme
TNF-α	Tumor necrosis factor alpha

Introduction

Malignant melanoma is the second most common cancer in people aged between 15 and 29 years. The incidence of malignant melanoma is increasing more rapidly than other cancers at the rate of 4 % per year. Early diagnosis and surgical excision of thin primary cutaneous melanoma could prevent successfully metastatic progression. However, the response rates of patients with advanced metastatic disease remain low to both standard chemotherapy and immunologically based strategies [1]. Further understanding of the biological behavior of melanoma cells is necessary for more successful and personalized treatment approaches.

An important feature of the human tumor cells is their ability to invade the extracellular matrix of the host tissue using a variety of different proteases. These proteases include ADAM (A Disintegrin and Metalloproteinase) family of proteases. The ADAMs are comprising of a large family of multidomain glycoproteins, highly homologous to the class PIII snake venom metalloproteinase-disintegrins that include more than 30 proteins zinc-dependent proteases [2–6]. Apart of their proteolytic activities, ADAMs family is involved in cell 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 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 [7–10]. ADAMs family members are involved in the cell-matrix interaction by shedding of integrins and also by directly binding of integrin receptors, syndecans and TGF-β type II receptor through cysteine-rich domain [11–14]. Through the cytoplasmic domain, they influence downstream signaling cascades, especially the EGFR signaling pathway through processing of EGFR-ligands. This is of particular interest, as EGFR is a well-established drug target for cancers [15, 16]. It was established before that ADAMs family members (ADAM 9, 12, 15, 17 and 28) could be differentially expressed between normal and tumoral tissues, but their pattern of expression and the intimate mechanism of action are not precisely established [17–24].

The human *ADAM12* gene 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 unique amino acids. *ADAM12* is expressed at low levels in most normal adult tissues, but in high amounts in tissues characterized by excessive growth, including human placenta and tumors [9, 25, 26]. *ADAM12* is associated with the progression and spread of human cancers [11, 27]. Recently, it was shown that *ADAM12* regulates the tumor progression in gene-modified mice models [28–30].

ADAM17 (also known as TNF-α converting enzyme or TACE) is the major sheddase for TNF-α, amphiregulin, HB-EGF and epiregulin [6, 10, 15, 31–34]. Given the involvement of the EGFR in the development of tumors of epithelial origin and the importance of ADAM17 in its control, several studies have focused on this ADAM protein. For example, *ADAM17* overexpression was found in breast cancer [15], renal carcinoma [35] and ovarian cancer [36]. Overexpression of *ADAM17* increases invasion and proliferation of MCF-7 (human breast adenocarcinoma cell line) cells, whereas down-regulation of *ADAM17* expression decreases invasion and proliferation of MDA-MB-435 (a melanoma cell line) cells [16]. *ADAM17* expression was significantly up-regulated in breast cancer at both mRNA and protein levels compared with those of normal breast tissue [21]. It has been found that cell proliferation was decreased when breast cancer cells were treated with anti-ADAM17 antibodies [23].

Because in malignant melanoma the expression of *ADAM12* and *ADAM17* genes has not been evaluated yet, our purpose was to analyze the expression of *ADAM12* and *ADAM17* genes in melanomas, at both transcriptional and translational level. Given that ADAM17 sheddase is involved in the modulation of TNF-α, we analyzed also this cytokine in the plasma of the same patients before any treatment using ELISA, and we compared the results with healthy controls. We observed that *ADAM12* splicing variants and *ADAM17* were overexpressed in melanomas. In the plasma of the same patients, the expression of TNF-α was up-regulated and significantly correlated with the expression of ADAM17 and respectively, with the advanced tumor stage. To the best of our knowledge this is the first study that reports the expression of *ADAM12* and *ADAM17* genes in melanomas.

Methods

Patients and Tumor Characteristics

In the present study were included patients who underwent surgery at the Surgical Oncology Clinic of University of

Medicine from Timisoara during 2010. Informed consent was obtained from all patients and the study was approved by the ethical committee of our University. In order to evaluate the ADAMs and TNF-α levels of expression according to the tumor stage, the group of patients was divided in "early stage" (I-IIA) and "advanced stage" (IIB-IV) patients. Table 1 summarizes the characteristics of the melanoma patients included in this 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, Germany) for 24 h at +4 °C and then frozen at −80 °C. Corresponding non-lesional tissues remote from the same patients served as normal controls and were treated in similar manner.

Plasma samples were obtained from 17 patients before treatment and from 20 healthy volunteers (matched by gender and age). Peripheral venous blood was collected using EDTA as anticoagulant. Within 30 min after collection, samples were centrifuged for 5 min at 3,000×*g*; plasma was immediately separated, aliquoted and stored at −80 °C until further analyses. Freeze-thaw cycles were avoided.

RNA Extraction

RNA was extracted using AllPrep DNA/RNA Micro Kit (Qiagen, Germany) following the manufacturer's protocol. RNA concentration and purity were quantified spectrophotometrically (NanoDrop ND1000) and the quality of RNA was evaluated on RNA electrophoregrams generated by the Agilent 2100 Bioanalyzer (Agilent Technologies, France). RNA was stored at −80 °C until further gene expression analyses.

Q-RT-PCR

The genes expression was quantified using the Q – RT – PCR on LightCycler® 1.5, software version 5.3 (Roche, Germany) and the SYBR Green method. Each investigated gene was normalized against 2 reference genes: *β-actin* and *GPI* (Glucose-6-Phosphate-Isomerase). Commercial available validated primer sets from Qiagen, QuantiTect Primer Assays (Hs_ACTB _1_SG; Hs_GPI_1_SG; Hs_ADAM12_va.1_SG; Hs_ADAM12_vb.1_SG; Hs_ADAM17_1_SG; sequences not available from the Qiagen Company) were used, together with the recommended QuantiTect SYBR Green one-step RT-PCR master-mix (Qiagen, Germany). RNA was diluted in RNase free water, in order to obtain the same input template concentration (0.5 ng/μl) for each reaction. The manufacturer's protocol adapted for LightCycler® 1.5. was followed. Briefly, in LightCycler Capillaries (Roche, USA) were mixed: 5 μl master-mix (RT SYBRGreen Buffer), with 1 μl specific primers, 0.1 μl RT-PCR enzymes, 5 ng RNA/tube and RNase free water for a total volume of 10 μl. The real-time PCR device was programmed following the QuantiTect Primer Assay kit protocol: reverse transcription at 50 °C for 20 min, initial polymerase activation step at 95 °C for 15 min followed

Table 1 Patients' and tumor characteristics

Age	Sex	Location	Histology	TNM	Stage	Clark	Breslow (mm)	Ulceration
51	F	Trunk	Nodular	T3bN0M0	IIB	III	4	Present
24	F	Upper limb	Superficial spreading	T1aN0M0	IA	III	0.9	Absent
43	F	Thigh	Nodular	T4bN2aM0	IIIB	V	>3.5	Present
57	F	Leg	Superficial spreading	T1aN0M0	IA	III	1	Absent
46	M	Abdomen	Superficial spreading	T2aN0M0	IB	III	1.5	Present
59	F	Head	Nodular	T2bN0M0	IIA	V	1.5	Present
53	F	Thigh	Nodular	T2aN0M0	IB	III	1.5	Absent
51	F	Upper limb	Superficial spreading	T1aN0M0	IA	III	1	Absent
54	F	Halluces	Acral lentiginous	T3aN0M0	IIA	IV	2.3	Present
30	M	Head	Nodular	T4bN0M0	IIC	V	>3	Present
54	F	Leg	Nodular	T3aN2M0	IIIA	III	3.5	Absent
52	M	Head	Nodular	T3bN0M0	IIB	IV	>3	Present
36	M	Trunk	Lentigo malignant	T3bN0M0	IIB	IV	≥2.5	Present
69	F	Head	Nodular	T3bN0M0	IIB	IV	<3	Present
54	M	Trunk	Nodular	T4aN2bM0	IIIB	V	>4	Present
60	F	Trunk	Superficial spreading	T4bN0M0	IIC	V	>4	Present
59	M	Trunk	Nodular	T4bN3M0	IIIC	V	6	Present

by 3-steps amplification cycles (denaturation at 94 °C for 15s, annealing at 55 °C for 20s and elongation at 72 °C for 20 s). 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.

The relative quantification level for the genes expression was 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 (reference gene); $\Delta\Delta C_T = \Delta C_T$ patients - ΔC_T controls. Using this method, the expression comparative level will be: $2^{-\Delta\Delta CT}$ [37].

Immunohistochemistry

Serial sections from corresponding FFPE tissues were cut at 3 μm and placed on FLEX IHC microscope coated glass slides (DAKO, Denmark). Sections were deparaffinized in xylene and rehydrated in alcohol series. For antigen retrieval, specimens were pretreated for 30 min with sodium citrate buffer (pH=6) in a microwave oven; endogenous peroxidase was blocked using 3 % hydrogen peroxide. Immunostaining was performed using primary antibodies directed against ADAM12 (goat polyclonal, from Abcam, Cambridge, UK, dilution 1:25) or ADAM17 (mouse monoclonal from Abcam, Cambridge, UK, dilution 1:333). After 30 min of incubation with the primary antibodies, the biotinylated anti-rabbit/mouse/goat IgG secondary antibody from Universal LSAB+kit/HRP (DAKO, Glostrup, Denmark) were applied for 30 min; between steps, the sections were washed in Tris-buffered saline solution. Immune complexes were visualized with 3,3′-diaminobenzidine (DAB) (DAKO, Denmark) and the sections were counterstained with Lillie's modified hematoxylin. The specificity of the immunostaining was controlled using prostate tissue as positive control (recommended by the vendor company) or by omitting the primary antibodies as negative control.

Cytokine Analysis

TNF-α analysis was performed using a standard ELISA technique (Multi-analyte ELISArray kits SABiosciences, Qiagen, Germany). Briefly, 50 μl of samples and controls were incubated for 2 h into the appropriate wells of the ELISA plate to allow the proteins of interest to bind to their specific capture antibodies. After three times washing to remove the unbound protein, 100 μl solution of biotinylated detection antibodies were added to the wells to bind the capture complex. After washing to remove the unbound material, 100 μl avidin–horseradish peroxidase conjugate were added and incubated for 30 min. After four times washing, 100 μl colorimetric substrate solution was added and incubated for 15 min in dark. The color development was stopped by adding 100 μl of stop solution and finally, the absorbance, directly proportional to the amount of the target protein, was read at 450 nm using an automatic ELISA microplate reader, Stat Fax 2100 (Awareness Technology Inc, Palm City, USA).

Statistical Analysis

Due to skewed distributions, gene expressions had large standard deviations relative to their means, so the two-sample, rank sum Wilcoxon (Mann - Whitney) test was used to compare groups. Data for all genes expression normalized each against 2 reference genes are reported as summary statistics (mean ± S.D. and median). The threshold for significance was set at $p<0.05$.

Results

Gene Expression Analysis

Using the AllPrep DNA/RNA Micro Kit, we obtained a quantity of RNA ranged between 0.55 and 44.4 μg/μl with an average of 12.38 and median of 5.6 μg/μl total RNA. The purity reflected by the absorbance ratio $A_{260/280}$ was between 1.86 and 2.11, with an average of 1.95 and median of 1.96. The RIN (RNA integrity number) generated by Agilent 2100 Bioanalyzer (Agilent Technologies, USA) was between 8 and 9 and showed a good RNA quality with a close homogeneity between samples. Gene amplification was successful in all the samples.

ADAM12L and *ADAM17* genes revealed a statistically significant up-regulation in the malignant melanoma tissues ($p<10^{-3}$) when compared to non-tumor controls, irrespective of the reference gene that was used. Regarding *ADAM12S* gene, although up-regulated in malignant melanoma tissue, when it was compared with the non-tumor tissues remote from the same patients, the difference was not statistically significant (Table 2). Regarding the comparative level of expression ($2^{-\Delta\Delta CT}$) for the investigated genes, *ADAM12L* was overexpressed with 14.62 fold, ADAM12S with 3.20 fold, whereas *ADAM17* presented a median comparative level of expression of 1.03. The levels of expression for the three genes were compared and it was observed that *ADAM12L* presented the highest expression, followed by *ADAM12S*. Compared with the other two investigated genes, *ADAM17* had the lowest level of expression in melanoma. The differences between the expressions of *ADAM12L*, *ADAM12S* and respectively *ADAM17* were statistically significant (p between 0.01 and < 0.0001). When we compared the levels of expression for the ADAMs genes according to the tumor stage, we observed that all

Table 2 Expression patterns (ΔCT) of *ADAM12L, ADAM12S* and *ADAM17* in malignant melanoma vs. non-tumor adjacent tissue normalized against two reference genes *(β-actin* and *GPI)*

ΔCT = CT (target gene) – CT (reference gene). Smaller ΔCT, less difference between the reference and interest gene expression meaning an increased expression of the target gene

Marker	*β-actin*			*GPI*		
	Mean ± s.d	Median	*P*	Mean ± s.d	Median	*P*
ADAM12L						
Normal	12.09±1.68	12.00		6.65±1.85	6.43	
Malign	8.25±1.98	8.26	$<10^{-4}$	4.33±1.87	3.92	0.0007
ADAM12S						
Normal	14.42±1.88	14.61		8.98±1.55	9.02	
Malign	14.11±2.05	14.00	0.63	9.04±1.65	8.45	0.91
ADAM17						
Normal	10.11±0.33	10.08		5.04±0.93	4.53	
Malign	9.27±0.42	9.35	$<10^{-4}$	3.83±0.98	3.95	0.0006

three investigated genes were significantly overexpressed in advanced stage melanomas (Table 3).

Immunohistochemistry

The distribution and expression pattern of ADAM12 and ADAM17 proteins in malignant melanoma and adjacent non-tumor tissues was investigated using immunohistochemistry for all investigated patients. The ADAMs proteins showed moderate to strong heterogeneous cytoplasmic expression in the tumor cells of all investigated melanomas, irrespective of their histological type. We observed a tendency for the advanced stages melanoma (T4, ulcerated and metastasized melanomas) to express ADAM12 and ADAM17 with a higher intensity, suggesting a role for ADAM12 and ADAM17 in tumor progression. Both ADAM proteins but especially ADAM17 protein was expressed also with a low intensity in the normal epithelial cells, the fibroblast of the stroma, smooth muscle cells of vessel walls, adipocytes and some inflammatory cells (Fig. 1a–f).

Table 3 Comparative median level of expression ($2^{-\Delta\Delta CT}$) in malignant melanoma for the three investigated genes – *ADAM12L, ADAM12S* and *ADAM17* – each normalized against *β-actin,* according to the tumor stage. For all three investigated genes, the difference of expression according to the tumor stage was statistically significant ($p<0.01$)

Marker	Median expression ($2^{-\Delta\Delta CT}$)	*p*
ADAM12L		
Early stage	92.84	$p<0.01$
Advanced stage	96.73	
ADAM12S		
Early stage	85.81	$p<0.01$
Advanced stage	89.23	
ADAM17		
Early stage	90.65	$p<0.01$
Advanced stage	93.71	

9 cases of advanced melanomas and 8 cases of melanomas in early stage were investigated

Cytokine Analysis

In order to analyze the concentration of TNF-α in the plasma of malignant melanoma patients and healthy controls, plasma samples from patients and healthy volunteers were assessed using ELISA. Our results showed that the plasma levels of TNF-α were significantly increased ($p<0.001$) in the plasma of malignant melanoma patients (327.09 ng/ml) in comparison with healthy controls (27.37 ng/ml) and correlated with the tumor stage. Moreover, we noticed that TNF-α levels were significantly correlated with the ADAM17 mRNA expression ($p<0.01$) (Table 4).

Discussions

Overexpression of ADAM12 mRNA and protein has been reported in breast, colon, bladder, lung carcinoma tissue and glioblastomas [28, 39, 40]. Several lines of evidence have implicated TACE/ADAM-17 as a major TNF-α sheddase [2]. ADAM10 and 17 are involved in the shedding of most EGFR ligands [41, 42]. It has been proposed that inhibitors of the shedding of EGFR ligands could be used to treat cancer by blocking the EGFR pathway. Thus, inhibitors of these ADAMs could be developed as anti-EGFR drugs [43]. In this context, our purpose was to evaluate the expression of *ADAM12* splicing variants and *ADAM17* gene expression in melanoma. Our study provides the first report regarding the expression of *ADAM12* and *ADAM17* genes in melanomas.

Our results showed that *ADAM12L* and *ADAM17* genes are strongly up-regulated in melanoma. *ADAM12S* gene was also up-regulated in melanoma, although not statistically significant. When the levels of expression for the three genes were compared, it was noticed that *ADAM12L* presented the highest, whereas *ADAM17* had the lowest level of expression. The expression at the transcriptional level was confirmed at the translational level by immunohistochemistry. The finding that ADAM17 protein had an

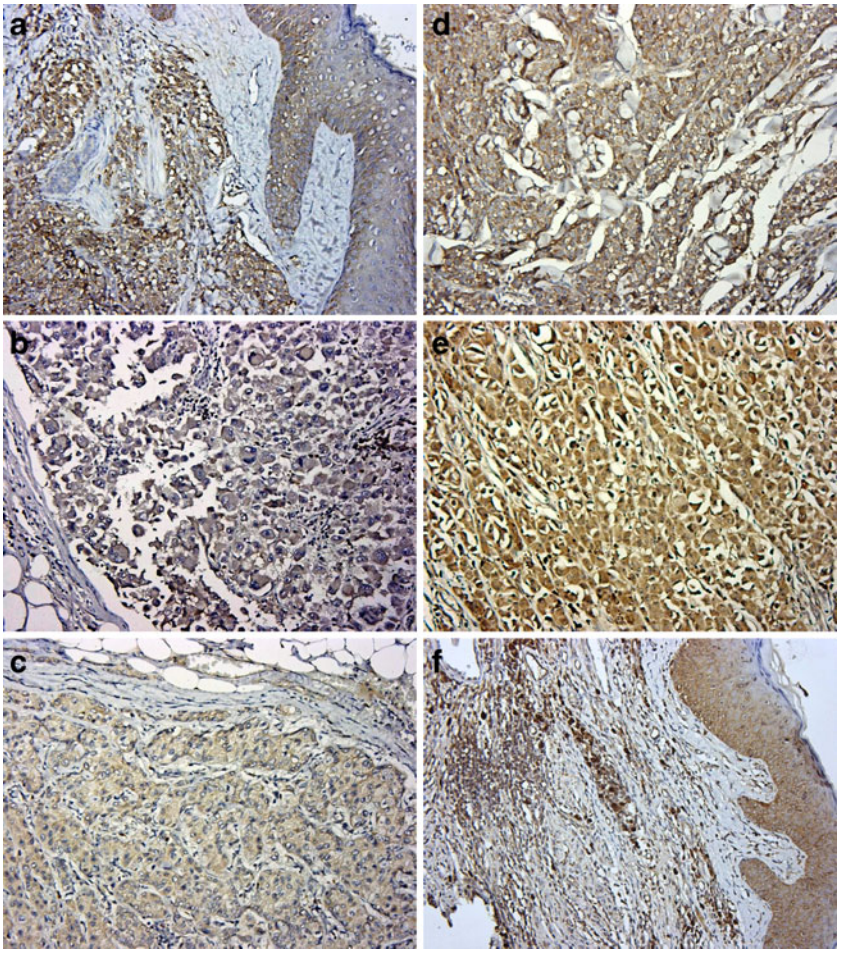

Fig. 1 **a** Immunohistochemical expression of ADAM12 and **b** ADAM17 in melanoma in comparison with non-tumor adjacent tissues. Both ADAM12 and ADAM17 were expressed also with low intensity in the normal epithelial cells, the fibroblast of the stroma, smooth muscle cells of vessel walls, adipocytes and some inflammatory cells. In these structures, ADAM17 was expressed with a higher intensity than ADAM12; **c** Immunohistochemical expression of ADAM12 and **d** ADAM17 in a primary melanoma (stage IIA, Breslow 2.3 mm). It can be noticed a heterogeneous, moderate to strong expression of ADAM12 and ADAM17 in the cytoplasm of the tumor cells; **e** Immunohistochemical expression of ADAM12 and **f** ADAM17 in an advanced stage melanoma (Breslow 6 mm, T4bN3M0). Both ADAMs proteins showed a strong cytoplasmic expression in the tumor cells, although ADAM17 was stronger expressed than ADAM12. *Immunostaining with primary antibodies anti-ADAM12 (goat polyclonal, dilution 1:25, Abcam) or ADAM17 (mouse monoclonal, dilution 1:333, Abcam). Hematoxylin counterstaining, magnification 200X*

intense immunoexpression, whereas using Q-RT-PCR, compared with the other two investigated genes, *ADAM17* had the lowest level of expression, can be explained by the fact that ADAM17 protein was also expressed with a low to moderate intensity by the normal epithelial cells. We noticed the tendency for the advanced stages melanoma (T4, ulcerated and metastasized melanomas) to express ADAM12 and ADAM17 proteins with a higher intensity, suggesting a role for ADAM12 and ADAM17 proteins in tumor progression and invasion.

Table 4 Comparison between the median expression of ADAM17 and TNF-α in melanomas according to the tumor stage. TNF-α levels were significantly increased in advanced stage carcinomas and correlated with the ADAM17 gene expression ($p<0.01$)

Melanomas	TNF-α (ng/ml)	ADAM17 mRNA ($2^{-\Delta\Delta CT}$)	*P*
Early stage	366.14	90.65	$p<0.01$
Advanced stage	1359.23	93.71	

9 cases of advanced melanomas and 8 cases of melanomas in early stage were investigated

When we compared the expression of ADAM12 with the expression of TNF-α, we did not obtain significant correlations (the data are not displayed). These results are in agreement with previous studies that found ADAM17 (TACE) as the major sheddase for TNF-α [2, 32, 33]

In one of our previous work, we investigated the expression of *ADAM12L*, *ADAM12S* and *ADAM17* genes in breast cancers and concluded that *ADAM12L*, *ADAM12S* and *ADAM17* transcripts were statistically significant elevated in breast cancers compared with controls, and *ADAM17* was higher expressed than *ADAM12* in breast cancers [38]. Regarding melanoma vs. breast cancer comparison, we noticed that in melanoma, *ADAM12L* presented a higher expression, whereas *ADAM12S* exhibited a lower expression in

comparison with breast cancers. Interestingly, *ADAM17* was statistically significant lower expressed in melanomas when compared to breast cancers. These results could suggest that the role played by these genes in tumor formation and progression is dependent on tumor type.

Our results are in agreement with some previous studies; for instance, the study of Ziober and his co-workers reported that during melanoma progression, the acquisition of a highly tumorigenic and metastatic melanoma phenotype is associated with the loss of α7β1 [44]. The α7β1 integrin, a laminin-binding receptor interacts with ADAM12 and was originally identified in melanoma [45]. In the present study, as it was observed using immunohistochemistry, both ADAM12 and ADAM17 were expressed also with lower intensity in the normal epithelial cells, the fibroblast of the stroma, smooth muscle cells of vessel walls, adipocytes and some inflammatory cells. In line with our study, it was observed that in normal human epidermis, ADAM12 displayed a homogeneous, increased cytoplasmic immunoreactivity in the upper epidermal cell layer keratinocytes [46]. Harsha and colleagues reported that the expression of *ADAM12* is fivefold up-regulated in the non-healing edge of chronic ulcers compared to healthy skin. They hypothesize that increased expression of *ADAM12* in chronic wounds impairs wound healing through the inhibition of keratinocyte migration, and that topical ADAM12 inhibitors may therefore prove useful for the treatment of chronic wounds [47]. Another ADAM protein, ADAM9 was detected by Zigrino et al. in melanoma cells and in peritumoral stromal fibroblasts [48].

Invasion and metastasis of human tumors requires cell-cell and cell-matrix interactions within the host tissues that are partly mediated by these ADAMs family members. The result of these interactions is the production, release and activation of a variety of cytokines and chemokines [49, 50]. In this regard and given that ADAM17 (TACE) is the major sheddase for TNF-α [2], we extended our study to the analysis of this cytokine in the plasma of the same melanoma patients that were compared with healthy controls. Our results showed that the levels of TNF-α were significantly up-regulated in the plasma of melanoma patients in comparison with healthy controls. Moreover, TNF-α was significantly increased in advanced stage melanomas when compared with early stage carcinomas. These levels were also significantly correlated with the expression of ADAM17. In accordance with our results, TNF-α was found to be involved in melanoma progression in some previous studies [51, 52]. Moreover, when we compared the expression of ADAM12 with the expression of TNF-α, we did not obtain significant correlations (data are not represented). These results are in agreement with the previous studies that found ADAM17 (TACE) as the major sheddase for TNF-α [2, 31–33]. Whereas ADAM17 sheds TNF-α and is required for the activation of the EGFR by TGF-alpha in tumors, ADAM12 interacts with TGF-β type II receptor, to enhance TGF-β signaling and modulates the Notch signaling by sheding DII1, a ligand for Notch receptor [12–15].

In summary, we have shown that *ADAM12L* and A*DAM17* genes are strongly up-regulated in melanoma, whereas *ADAM12S*, although overexpressed in melanoma in comparison with the non-tumor tissues, its level of expression did not achieve a statistical significance. Advanced stage melanomas expressed ADAM12 and ADAM17 proteins with a higher intensity, thus suggesting the role exerted by these proteins in the tumor invasion and metastasis. In comparison with breast cancer, *ADAM12L* was higher expressed, whereas *ADAM12S* and *ADAM17* exhibited a lower expression, suggesting that the role played by these genes in tumor formation and progression is dependent on the tumor type. TNF-α was significantly increased in advanced stage melanomas when compared with early stage carcinomas and their levels of expression were significantly correlated with the expression of ADAM17. Further studies will be needed to elucidate the role carried by *ADAM12* and *ADAM17* in melanoma progression and metastasis as well as the relationships between these ADAMs sheddases and the cytokines expression.

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Conflict of Interest The authors declare no conflict of interest.

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