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Research Article

Immunohistochemical features of partial regression in cutaneous melanoma – E-cadherin key molecule

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Abstract. Background and objectives: Tumor regression in cutaneous melanoma remains a subject of both intense studies and scientific contradictions. While some groups acknowledge it as a marker of good prognosis, others associate it with a worse prognosis. The aim of our study was to depict the immunohistological pattern of partially regressed cutaneous melanomas linking the known general markers (AE1-AE3, vimentin, S100) with the expression of melanosomal proteins (tyrosinase, TRP-2, MART, HMB45), intra/intercellular signaling proteins (c-Kit), adhesion molecules (E-Cadherin) and cell cycle control factors (p16, p21, p53, cyclin D1, bcl2, cerb2, cox2). Material and methods: 102 tumor cutaneous melanoma samples were selected for which patients' gave their informed consent. The following clinical data were correlated with the investigated tissue markers: gender, age, tumor localization, size, familial history, sun exposure, lesion evolution prior to surgical excision, dermatoscopy, and in vivo confocal microscopy evaluation, follow-up after surgical excision. Results: The regressed areas were evaluated as both in surface and in depth, in relation with gold standard parameters: Breslow index, Clark score, invasion in various normal adjacent structures, presence of ulceration, vascular and/or perineural invasion, intratumoral inflammatory infiltrate cellular pleomorphism, mitotic index, intratumoral vascularization. Among all the mentioned investigated parameters, we found that the loss of E-cadherin is frequent in advanced melanomas and in those without regression, while the areas with regression presented high E-cadherin expression. E-cadherin can thus be considered a tissue parameter associated with regression in cutaneous melanoma.

Key Words: Cutaneous melanoma, regression, E-cadherin

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Introduction

Melanoma is one of the most aggressive cancers in humans; the majority of the cases have infaust prognosis due to its capacity of developing lympho-vascular and perineural metastases very early in the disease progression; its biologic behavior qualifies melanoma as one of the most important medical challenges in current dermato-oncologic practice. (Boda et al 2012). Several clinical and pathological types of melanoma are described: superficial spreading melanoma (SSM), nodular melanoma (NM), acral melanoma (ALM), lentigo maligna melanoma (LMM) and few rare types: spitzoid melanoma, blue nevus melanoma, nevoid melanoma etc (Boda et al 2012).

Regression in melanoma represents a complex process which consists in tumor destruction, replacement by inflammatory infiltrate (lymphocytes and/or melanophages) and fibrosis with vascular hyperplasia. The disappearance of tumor can be partial or complete to the point of complete absence of tumor cells in routine histopathologic stains and even in immunohistochemical tests (Zurac et al 2012). Several phenotypic variants of regression can be identified: complete (no tumor is present), segmentary (complete disappearance of the tumor in one/multiple parts of the tumor) and partial (preservation of few tumor cells in the area of regression) (Boda et al 2012; Zurac et al 2012).

Tumor regression in cutaneous melanoma (CM) is quite frequent, and, in relation to the stage, the phenomenon was identified in 10-35% of the cases (Zurac et al 2012). Partial tumour regression can have even a higher incidence, registered in up to 60% of the thin CM with a Breslow index under 0.75 mm (McGovern et al 1983, Abramova et al 2002, Emanuel et al. There is no general consensus regarding the significance and the prognostic power of CM regression. The general dogma in dermatopathology associates a less favorable disease evolution with a completely regressed CM. The arguments that sustain this assertion are based on few reported cases in which the complete CM regression has led to extensive metastasis in lymph nodes and/or in other organs. Actually there are only 38 reported cases with histopathologically confirmed complete regression (High et al 2005). Other groups have shown statistic association

Table 1. Primary antibodies

ANTIBODY	CLONE	PRETREATMENT*	DILUTION	SOURCE
AE1-AE3	AE1-AE3	HIER pH 6	2/200	Leica Biosystems
VIMENTINA	V9	HIER pH 6	0.6/200	Leica Biosystems
S100 protein	-	None	0.4/200	Leica Biosystems
HMB 45	HMB 45	HIER pH 9	2/200	Cell Marque
Melan A/ MART 1	A103	HIER pH 6	2/200	Cell Marque
TRP2	-	HIER pH 9	2/200	LSBIO
Tyrosinase	T311	HIER pH 9	2/200	Cell Marque
Calponin	26A11	HIER pH 9	Ready-to-use	Leica Biosystems
Caldesmon	h-CALD	HIER pH 9	Ready-to-use	ThermoFischer
E-cadherin	EP700Y	HIER pH 9	2/200	Cell Marque
CYCLIN D1	P2D11F11	HIER pH 9	4/200	Leica Biosystems
P16	E6H4	HIER pH 9	Ready-to-use CINtec Kit	Roche
P21	DCS-60.2	HIER pH 9	1/200	Cell Marque
P53	DO7	HIER pH 9	2/200	Cell Marque
cerb2/HER2	SP3	HIER pH 9	2/200	Cell Marque
C-KIT	YR145	HIER pH 9	2/200	Cell Marque
BCL 2	BCL-2/100/D5	HIER pH 6	2/200	Leica Biosystems

*HIER – heat induced epitope retrieval

between regression and unfavorable prognosis. Thus, earlier reports showed that 40% of thin CM with regression will develop metastasis (Slingluff et al 1988) and more recent studies have shown that CM with more than 50% regression have a high risk of regional relapse (Maurichi et al 2014). Other groups did not find any correlation with the disease prognosis (Briggs et al 1984, Fontaine et al 2003, Kaur et al 2008). Recent studies showed in meta-analytic investigations that regression proved by histopatological examination represents a favorable factor for survival (Gualano et al 2017); also, stage III melanoma with positive lymph node has a favorable prognosis when it associates regression (Zugna et al 2017); moreover, the risk of sentinel lymph node positivity is inversely associated with histologically present regression (Ribero et al 2015).

The existing paradox that a regressed CM has an unfavorable prognosis resides probably in the complex interactions of immune system elements, like tumor infiltrating lymphocytes, and other molecules that comprise the tumor milieu.

Our study focuses on the evaluation of several biomarkers expression in CM with and without regression using immunohistochemical (IHC) methods. We analyzed several classes of markers: general markers (vimentin, AE1-AE3, S100 protein), melanosomal/ melanosomal-related markers (tyrosinase T311, HMB45, melan A/MART, TRP2), stromal / tumor-to-stromal transition (caldesmon, calponin), markers involved in proliferation, apoptosis and intra/extracellular signaling (cyclin D1, p16, p21, p53, cerb2/HER2, c-kit, bcl2), cellular adhesion markers (E-Cadherin).

Material and Method

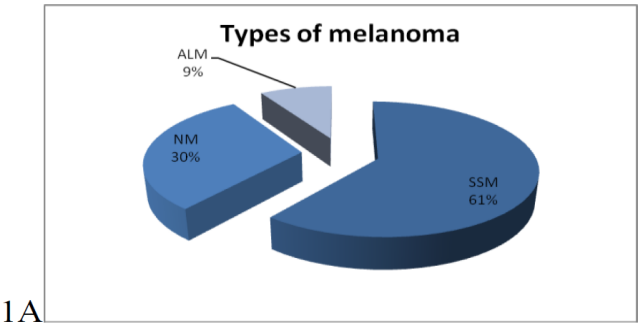
Samples

102 tumor CM samples were selected from the archives of the Department of Pathology, Colentina University Hospital between 2011-2013, for which patients' informed consent was obtained (according to annex 4, HG 451/2004). Cases with at least two paraffin blocks of tumor tissue were included; histopathologic slides were reviewed and several areas of interest were identified: in case of melanoma with regression, one area of regression (regressed component RC) and one area without regression (non-regressed component NRC) were selected; in case of melanoma without regression one area of tumor without necrosis (absence of regression – AR) was selected. 13 tissue multi-array (TMA) blocks were performed and further analyzed. Several clinical data were correlated with the tissue markers: gender, age, tumor localization, In each case, several histopathologic gold standard parameters were recorded: Breslow index (maximum tumor thickness – pT stage), Clark score, mitotic index, presence of ulceration, vascular and/or perineural invasion, intratumoral inflammatory infiltrate (TIL), prior detected nevi, status of surgical excision. Cases with regression were separated as segmentary regression and partial regression based on previously published criteria (Zurac et al, 2012). In areas of regression, the density of TILs (mild, moderate, marked), the number of melanophages per mm^3 and the grade of fibroplasia (mild, moderate, marked) were recorded.

Tissue markers

Immunohistochemical tests (on all 13 TMAs) were performed using several primary antibodies (see Table 1) and the Novolink Max DAB (Polymer, Leica, Nussloch, Germany). Light microscopy examination of slides was performed using a Nikon Eclipse 80i microscope, and pictures were acquired using a digital camera attached to a computer. The IHC tests were performed either manually or using an automat immunostainer Bond 3 (Leica, Nussloch, Germany).

The level of expression recorded for each marker was compared between NRC versus RC in the same tumor and in NRC



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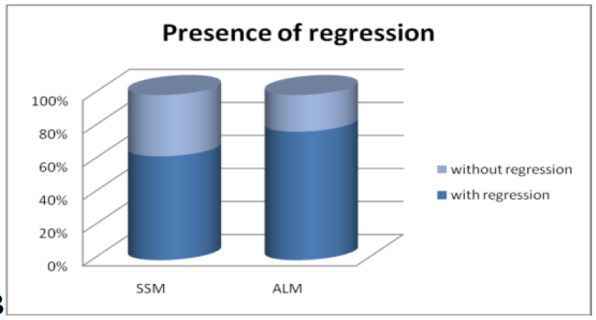

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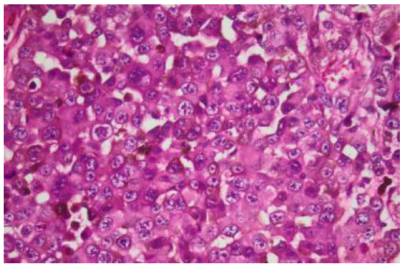

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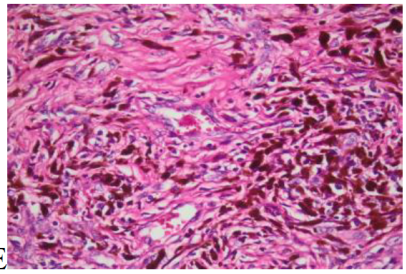

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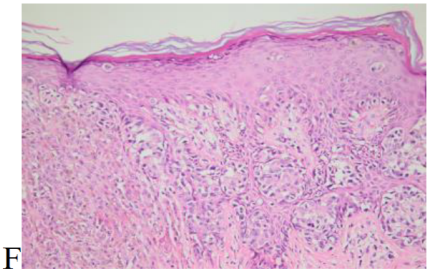

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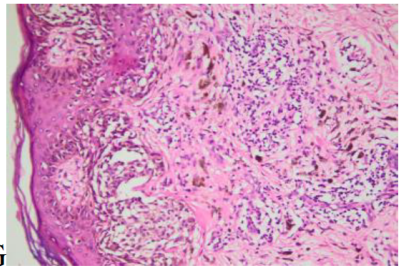

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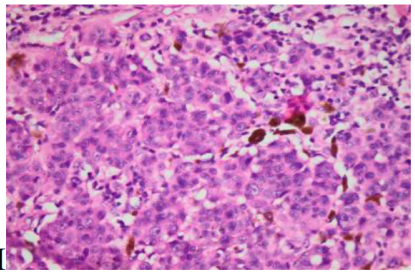

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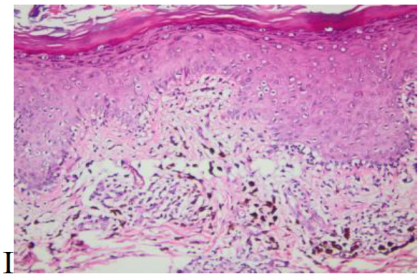

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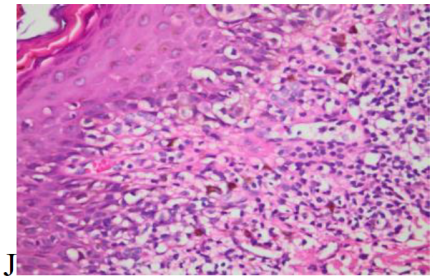

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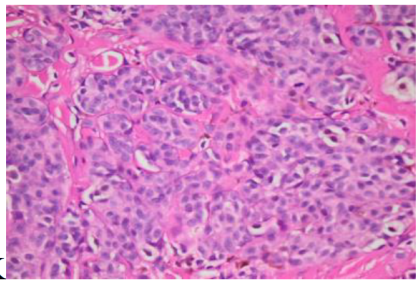

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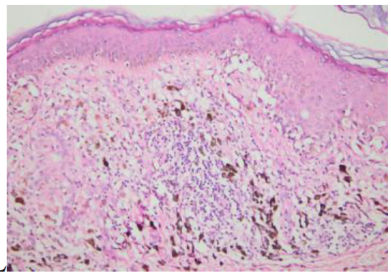

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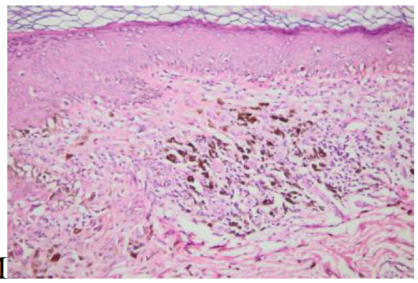
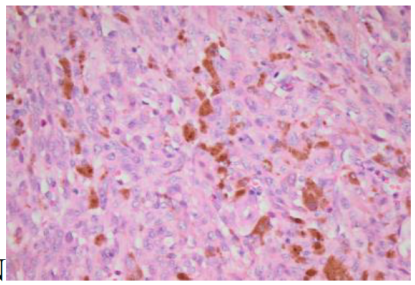


1M



1N

Figure 1. 1A-B: Distribution of investigated samples according to clinical-pathological type (1A) and regression (1B). 1C-E. Superficial spreading melanoma, pT2a stage (1C macroscopic picture) with nonregressed areas (1D. HE x 400) and areas of segmental regression (1E. HE x 400). 1F-H. Superficial spreading melanoma, pT3b stage with nonregressed areas of both radial and vertical growth (1F. HE x 200) and areas of partial regression (1G. HE x 200). Details of tumor proliferation in vertical growth phase HE x 400. 1I-K. Superficial spreading melanoma, pT2a, with partial regression (1I-1J. HE x 200); nonregressed component with epithelioid cells. 1K. HE x 200. 1L-N. Superficial spreading melanoma, pT4b, partial regression (1L-1M. HE x 200); nonregressed component with epithelioid-to-spindle cells with numerous melanophages within the tumor (1N. HE x 200).

Table 2. Distribution of cases according to the presence, type of regression and pT stage

Type of MM	Number of cases	Total cases with regression	Cases with segmented regression	Cases with partial regression
pT1 (<1mm thickness)	46	25	14	11
pT2 (1-2mm thickness)	29	12	9	3
pT3 (2-4mm thickness)	29	20	16	4
pT4 (>4mm thickness)	24	10	3	7

versus AR using a four-grade scale: negative (-), faint positive (+), moderate positive (++) and strongly positive (+++), irrespective of the actual number of positive cells. For better appreciation of the differences between NRC and RC component we have subtracted the immunohistochemical score for RC from that of NRC (i.e.: NRC +++ and RC ++: NRC/RC = +1; NRC ++ and RC +++: NRC/RC = -1).

Statistics

Comparison for all investigated markers was done between regressed and non-regressed zone and with non-regressed samples using Microsoft EXCEL and EPIINFO, P under 0.05 was considered statistically significant.

Results

Analyzed cases

From a total of 102 CM samples, 62 where superficial spreading melanoma (SSM), 31 nodular melanomas (NM) and 9 acrallentiginous melanoma (ALM); 46 cases (39 SSM and 7 ALM) presented regression; 56 cases (23 SSM, 2 ALM, 31 NM) did not present any regression (Figure 1A-B). A typical case with areas of segmental regression is presented in Figure 1C-E and a typical case of partial regression is presented in Figure 1F-H. The analyzed CM samples were grouped in thin melanomas (pT1), pT2, pT3 and pT4, respectively and further presented as distinct sub-groups (Table 2).

In the pT1 group, 46 cases were included, out of which 25 cases had regression areas (14 segmented, 11 partial). Analyzing the relation with gender or age, no statistical correlation was found. Age distribution shows that partial regression is encountered at 30-49 years age group in comparison to the 50-69 years group where the segmentary regression prevails (P = 0.0198). Tumor localization was not statistically correlated with presence of regression, but we noticed the presence of fewer cases with regression located in limbs comparing with other sites. There is no correlation between the same localization and the type of regression (partial or segmented regression).

We did not find any statistical correlations between Breslow index and regression, although there is a tendency of regression to be present in thin CMs. The same lack of correlation was found between regression and Clark index. We did not find any statistical correlation between the presence of regression and the mitotic index, but it should be noted that none of the samples with high mitotic index had regression areas. No correlations were found between regression presence and ulceration, vascular invasion, TIL presence, prior nevi or the partial/total surgical resection.

The sub-group of CMs with 1-2mm thickness (Breslow index 1-2 mm, pT2 stage) included 29 cases, out of which 12 with regression (9 segmentary, 3 partial) (typical case presented in Figure 1I-K). For this group no correlation of regression presence with gender or age was found.

Regarding the correlation of regression with tumor localization we did not find statistical significant values, but there is a small percentage of regression tumors with limbs localization. Also in this group, there is a tendency of lower regression percentage when the percentage of fusiform cells from the tumor area increases (P = 0.22). No correlation was found between the regression presence and the following investigated parameters: Clark index, mitotic index, presence of ulceration, vascular/lymph nodes invasion, presence of nevi, surgical resection. No correlation was found between regression type, regression extension and regression thickness. The morphologic analyses of the pT1 CMs shows that in the two types of regression, the density of TILs, the number of melanophages, the fibroplasia grade and the vascular density does not differ.

The sub-group of CMs with 2-4mm thickness (Breslow index 2-4 mm, pT3 stage) included 29 cases, out of which 20 with regression (16 segmentary, 4 partial) (typical case presented in Figure 1L-N). As in the prior sub-group, also in this one no correlation was found between regression presence and gender or age. In this group, there is a lower percentage of regression in the patients with trunk and limbs localization of the tumor. There is a tendency for this sub-group, to associate the higher percentage of spindle cells from the tumor area, with lower percentage of regression area.

Clark index and the presence of regression is not significantly correlated (with the exception of two Clark IV cases). There is no correlation of regression with the mitotic index, ulceration presence, lymphatic/vascular invasion, TIL or plasma cells presence.

In the group with over 4 mm thickness (Breslow index over 4 mm, MM stage pT4) 24 cases were included. 10 cases presented regression (3 segmentary, 7 partial), without differences regarding gender, age, localization, Clark index, mitotic index, ulceration, TIL, plasma cells and so on.

Tumor cells markers

All 102 cases (included in 13 TMA blocks) were analyzed for several IHC markers: general markers (vimentin, AE1-AE3, S100 protein), melanosomal/ melanosomal-related markers (tyrosinase T311, HMB45, melan A/MART, TRP2), stromal / tumor-to-stromal transition (caldesmon, calponin), markers involved in proliferation, apoptosis and intra/extracellular signaling (cyclin D1, p16, p21, p53, cerb2/HER2, c-kit, bcl2) and cellular adhesion markers (E-cadherin).

Pan-citokeratin AE1-AE3 was found positive in epidermis and in the epithelia of cutaneous annexes and negative in tumor cells. All CMs, regardless of the type and/or of the regression presence were found positive for vimentin and S100 protein.

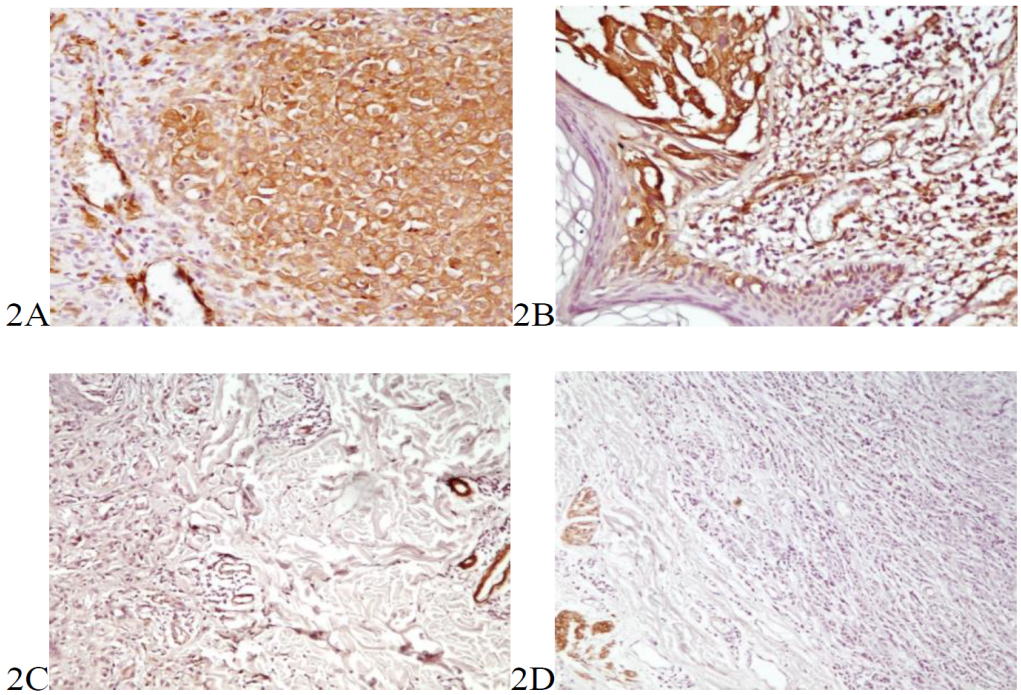


Figure 2. 2A-B. Caldesmon expression. 2A. Tumor cells positive for caldesmon. Smooth muscle fibers from vascular walls positive (internal control). Nodular melanoma with epithelioid cells, no regression. Caldesmon x 200. 2B. Tumor cells positive for caldesmon. Smooth muscle fibers from vascular walls positive (internal control). Superficial spreading melanoma with epithelioid cells, segmental regression. Caldesmon x 200. 2C-D. Calponin expression. 2C. Tumor cells negative for calponin. Smooth muscle fibers from vascular walls positive (internal control). Superficial spreading melanoma with epithelioid cells, segmental regression. Calponin x 100. 2D. Tumor cells negative for calponin. Smooth muscle fibers from vascular walls positive (internal control). Superficial spreading melanoma with spindle cells, segmental regression Calponin x 100.

Melanosomal markers (tyrozinase, TRP-2, MART, HMB45) were expressed in all CMs regardless of the type and/or the presence of regression. We registered some expression differences intratumor (between RC and NRC areas) and inter-tumoral (between NRC and AR), but without statistical significance.

We did not register significant differences between caldesmon and calponin expression and the CM type and/or regression presence (Figure 2).

c-kit expression varied amidst each investigated category without any clear trend within (Figure 3A-C).

From the proteins that are known to be involved in cell cycle control we have tested p16, p21, p53, cyclin D1, bcl2, cerb2, cox2 expressions. We did not register statistically different expression of these proteins associated with the regression existence. For bcl2 expression we found both bcl2 negative tumor cells types (Figure 3D-F) and positive tumor cell types (Figure 3G-I). p16 marker is presented in Figure 4A-D and p53 in Figure 4E-F.

E-cadherin expression was analyzed in 59 cases: 43 SSM cases (including 27 cases of CMs with regression and 16 AR cases) and 16 cases of NMs (without regression - AR). In 19 SSMs, 9 ALMs and 15 NMs IHC tests for E-cadherin were suboptimal. E-cadherin expression is completely lost or diminished in several cases (27,89% of all cases), more often in advanced melanomas (75% of melanomas with lost/diminished E-cadherin expression were thick tumors – Breslow index over 2 mm) and in those without regression (41,66% of melanomas with lost/diminished E-cadherin expression had no regression – AR). E-cadherin is over-expressed in NRC in comparison to AR, especially in advanced (pT3-pT4 cases) (44% of NRC versus 31% of AR in pT3-pT4 cases) (Figure 5) but the difference has no statistical significance.

E-cadherin expression in RC compared to the NRC is presented in Table 3 and Figure 6.

We obtained E-cadherin over-expression in RC versus NRC in the cases where Breslow index > 2 mm (pT3 and pT4).

Among all the mentioned investigated parameters, we found that the loss of E-cadherin is frequent in advanced melanomas and in those without regression, while the areas with regression presented high E-cadherin expression.

Discussion

Analyzing the relation between presence of regression and gender or age, localization, Breslow index, Clark index, mitotic index, presence of ulceration, vascular invasion, TIL presence, prior nevi or the partial/total surgical resection no statistical correlation were found, nor for the whole lot, nor for each tumor stage separately. There was an overall tendency for less frequent melanomas with regression in limbs, but no statistic significant differences were recorded. Also, melanomas with predominant spindle cells phenotype tend to have less frequently regression.

An interesting finding link age with type of regression – melanoma patients of 30-49 years age develop more often melanomas with partial regression in comparison with patients of 50-69 years that develop more often segmented regression ($P = 0.0198$); it is debatable if those types of regression have different prognostic significance or represent evolutive phase in biology of regression (Zurac et al 2012); irrespective of their significance, this finding may be related with age-related differences in

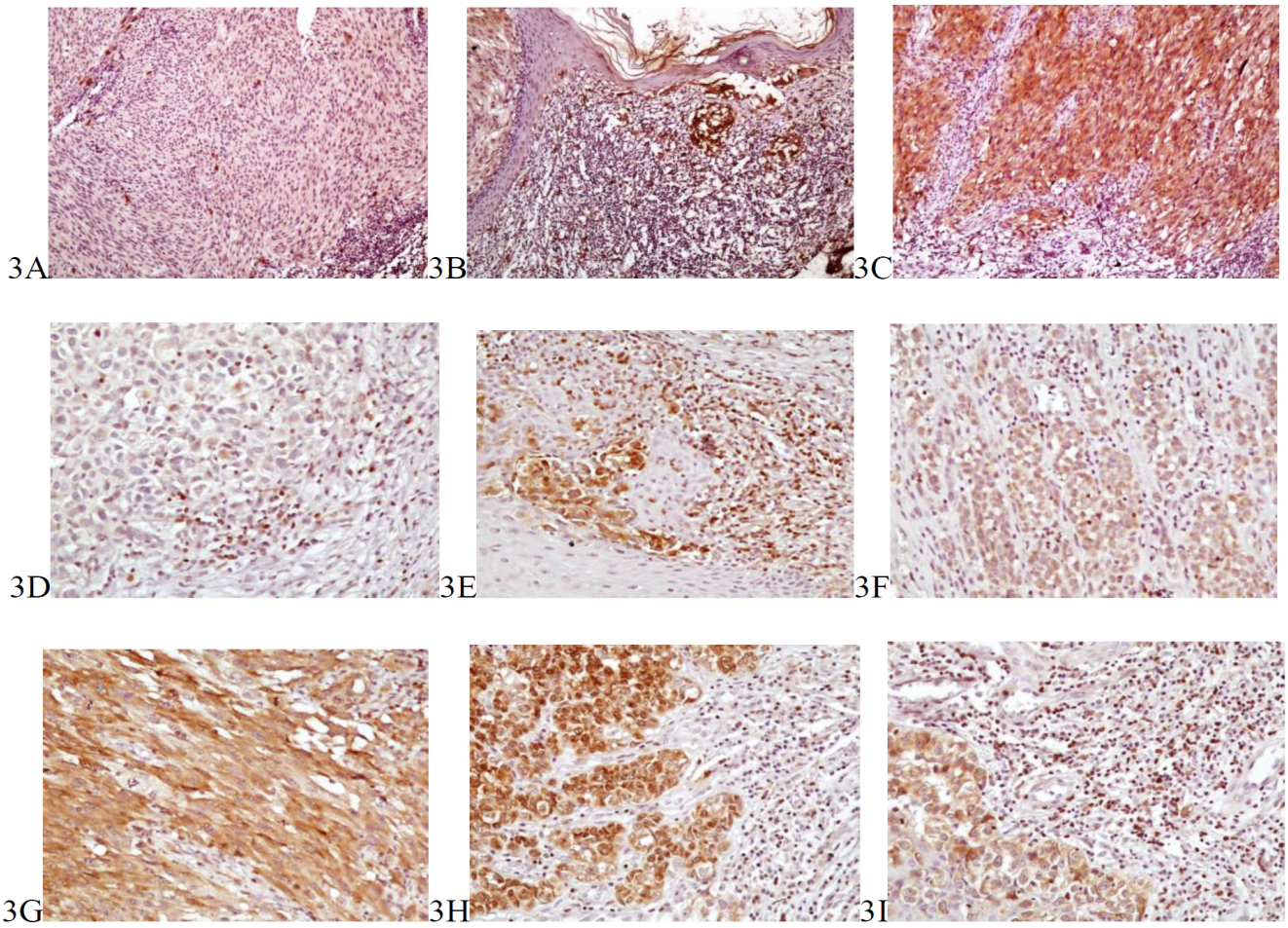


Figure 3. 3A-C. C-kit (CD117) expression: 3A. Tumor cells negative for c-kit (CD117) in aria of tumor progression. Superficial spreading melanoma with regression. CD117 x100. 3B. Tumor cells positive for c-kit (CD117) in aria of tumor regression. Superficial spreading melanoma with regression. CD117 x100 3C. Tumor cells positive for c-kit (CD117) in superficial spreading melanoma without regression. CD117 x100. 3D-F. bcl2 positive tumor cells 3D. Tumor cells negative for bcl2. Intra-tumor lymphocytes positive for bcl2 (internal control). Melanoma without regression. Bcl2 x 200. 3E. Tumor cells positive for bcl2. Intratumor lymphocytes positive for bcl2 (internal control). Nonregressed area in a melanoma with regression. Bcl2 x 200. 3F. Tumor cells faint positive for bcl2. Intratumor lymphocytes positive for bcl2 (internal control). Melanoma without regression. 3G-I. bcl2 negative tumor cells 3G. Tumor cells intense positive for bcl2. Intra-tumor lymphocytes positive for bcl2 (internal control). Melanoma with spindle cells without regression. Bcl2 x 200. 3H. Tumor cells intense positive for bcl2. Intra-tumor lymphocytes positive for bcl2 (internal control). Melanoma with epithelioid cells with partial regression. Bcl2 x 200. 3I. Tumor cells focally positive for bcl2. Intra-tumor lymphocytes positive for bcl2 (internal control). Melanoma with epithelioid cells with partial regression. Bcl2 x 200.

prognosis in melanoma. To our knowledge this is the first time such an association (age and type of regression) was reported. Interesting results were obtained analyzing the expression of different IHC markers.

Vimentin is an intermediary filament positive in all mesenchymal cells; all CMs are positive for vimentin – in fact vimentin negativity in metastases excludes the diagnostic of melanoma (Boda et al 2012). AE1-AE3 is another intermediate filament found positive in epithelial cells; very seldom melanomas are positive for AE1-AE3 and only in isolated cells (Riddel et al 2012). S100 protein (former "gold marker" for melanoma in IHC) is positive in almost all melanomas, in some cases (desmoplastic melanomas) being the only positive marker beside vimentin used in melanocytic lineage demonstration; several other tumors arising from neural crests are also positive for S100 protein, thus imposing a low specificity (a S100 protein negative tumor is unlikely to be a melanoma while a S100 positive one is not necessary melanoma) (Boda et al 2012). Our data confirmed the positivity for vimentin and S100 protein of all melanomas, irrespective of type or presence of regression and negativity for AE1-AE3.

Tyrosinase is an enzyme involved in melanin synthesis with hormone-like activity. Melanocytic tumors are more often positive for tyrosinase than standard melanosomal markers (Boda et al 2012). TRP-2 (tyrosinase-related protein 2, dopachrom tautomerasis) due to its partial homology with epidermal growth factor, regulates melanocytic functions; its expression precedes tyrosinase expression during melanocytic differentiation (Boda et al 2012). HMB 45 is a cytoplasmic glycoprotein expressed by stage 1 and 2 premelanosoms; stage 3 premelanosoms also express HMB45 in their non-melanised area; HMB45 stains immature and activated melanocytes and is negative in mature

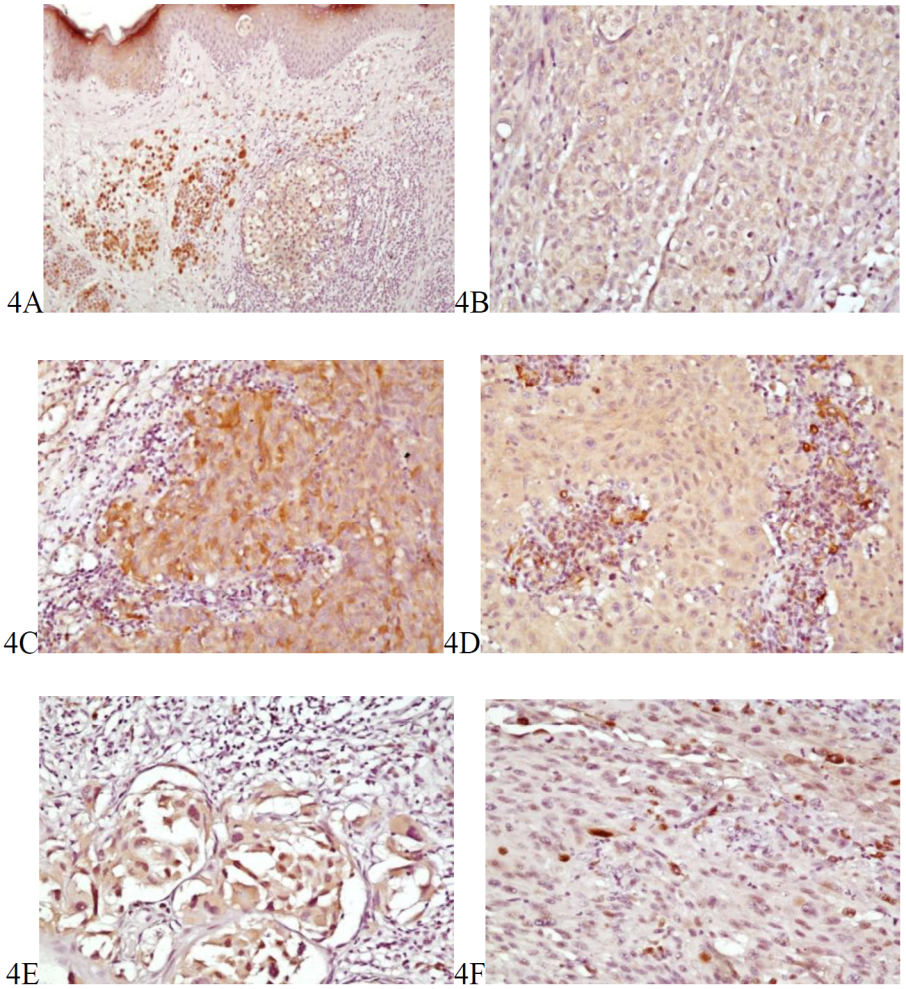
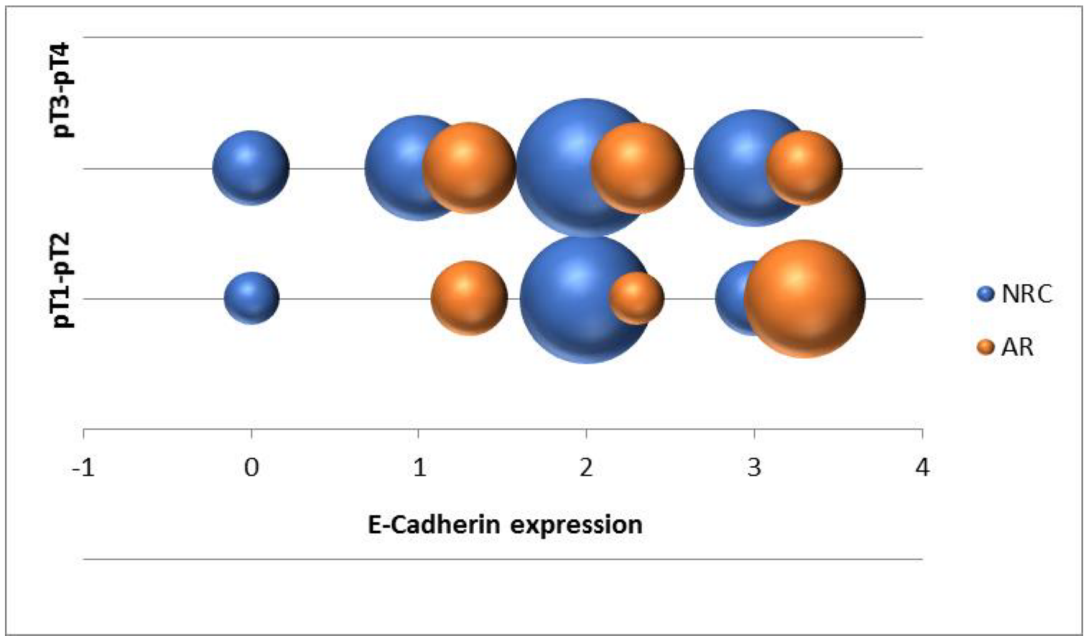


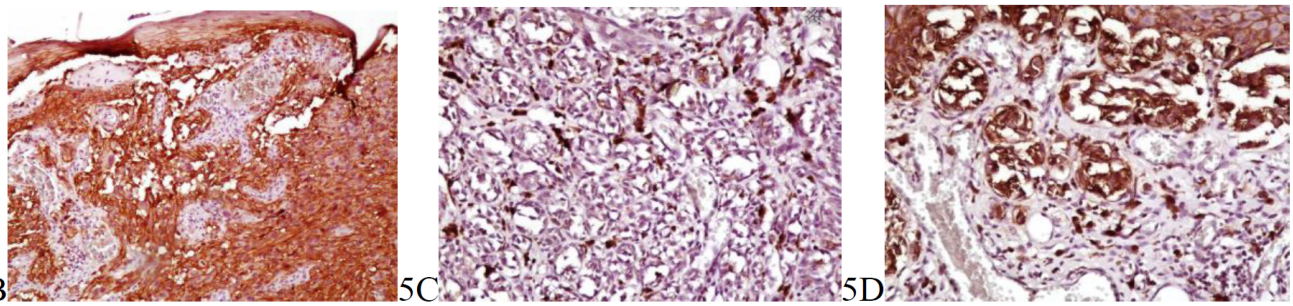
Figure 4. 4A. Malignant tumor cells negative for p16. Nevoid cells (pre-existing nevocellular nevus) positive for p16. Melanoma with epithelioid cells with partial regression. p16 x 200. 4B. Tumor cells negative for p16. Melanoma with epithelioid cells without regression. p16 x 200. 4C. Tumor cells focally positive for p16. Melanoma with epithelioid cells with partial regression. p16 x 200. 4D. Tumor cells negative for p16. Melanoma with epithelioid cells with partial regression. p16 x 200. 4E. Tumor cells focally positive for p53. Melanoma with epithelioid cells with partial regression. p53x200. 4F. Tumor cells focally positive for p53. Melanoma with spindle cells with partial regression. p53x200.

ones (Boda et al 2012). Melan A/MART1 is another melanosomal marker positive in most melanomas, special nevi (i.e. Spitz nevus, cellular blue nevus, deep penetrating nevus) but also in steroid hormone-producing tumors, angiomyolipomas, clear cell sugar tumours, PEC-omas (Boda et al 2012). The differences in expression of melanosomal markers present between different tumors or in the same tumor had no statistical significance in our study; they reflect the large variability of immunophenotipic expression in melanoma, sometimes occurring in the settings of the same tumor as consequence of intratumor heterogeneity. Caldesmon (calmodulin binding protein) and calponin (calcium binding protein) inhibit the ATPase activity of myosin in smooth muscle cells. They regulate smooth muscle contraction. Caldesmon binds to calcium, calmodulin, tropomyosin and actin and calponin to actin, filamin, calmodulin and other actin binding proteins and phospholipids. Caldesmon has two isoforms, a high molecular weight one (h-caldesmon) specific for smooth muscle cells and a low molecular weight one (l-caldesmon) present in non-muscle cells (Hayashi et al 1991). Calponin was reported as expressed in sinonasal melanomas with spindle cell morphology, thus predisposing to diagnostic errors (Lee et al 2011). Caldesmon was not reported as being expressed by melanoma cells (not even spindled variants) but very few studies were performed. One study identified a possible prognostic significance of caldesmon and calponin expression in the walls of intratumor vessels – diminished expression determines blood vessels frailty thus being associated with higher frequency of metastasis in melanoma (Koganehira et al 2003). Our study failed to identify statistically significant differences between caldesmon and calponin expression and the CM type and/or regression presence.

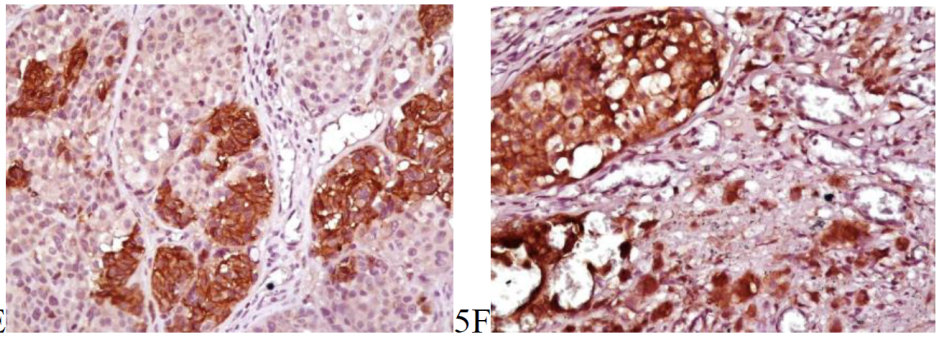
p53 is the product of a tumor suppressor gene usually mutated in human neoplastic lesions (Liu et al 2014). p16 is also a tumor suppressor protein encoded by CDKN2A gene (primarily linked to familial melanoma) controlling the progress from G1 to S phase of cell cycle (Potrony et al 2015). p21 is a cyclin-dependent kinase inhibitor encoded by the CDKN1A; it represents one of the most important targets of p53, its activation ending in cell cycle arrest after DNA damage (Engeland et al 2017). HER2/neu (cerbB2) is a transmembranary tyrosine kinase belonging to



5A



5B 5C 5D



5E 5F

Figure 5. 5A. E-cadherin expression in NRC in comparison to AR cases in thin (pT1-pT2) and thick (pT3-pT4) cases. 5B. E-cadherin expression in tumor cells. Melanoma without regression. E-Cad x 100. 5C. Tumor cells negative for E-cadherin. NRC in a melanoma with epithelioid cells with partial regression. E-Cad x200. 5D. Tumor cells from area of partial regression positive for E-cadherin. RC in a melanoma with epithelioid cells with partial regression. E-Cad x200. 5E. Tumor clone positive for E-cadherin. NRC in a melanoma with epithelioid cells with partial regression. E-Cad x200. 5F. Tumor cells from area of partial regression positive for E-cadherin. RC in a melanoma with epithelioid cells with partial regression. E-Cad x200.

the human epidermal growth factor receptor family. It can form dimers or heterodimers with any of the remaining three members of ErbB family. As a consequence the phosphorylation of the tyrosine residues takes place in the intracellular (cytoplasmic) domain (ligand-independent activity) and further interacts with numerous signaling biomolecules (ligand-dependent activity). Cyclin D1 (member of cyclin family) is a regulator of cyclin-dependant kinases (CDKs), especially CDK4 and CDK6 involved in G1/S transition in cell cycle; cyclin D1 massively accumulates within the nucleus in G1 phase, being degraded after G1/S transition (Fu et al 2004). Alteration of either structure or quantity of cyclin D1 is involved in tumorigenesis in several types of tumors, including melanoma (Alekseenko et al 2010, Coppock et al 1995, Gerogieva et al 2001, Sauter et al 2002, Stefanaki et al 2007); its over-expression is associated with higher risk of metastases and shorter survival (Gammon et al 2012, Hawryluk et al 2013, Pouryazdanparast et al 2009), but it can be used as therapeutic target as well (Arioka et al 2017,

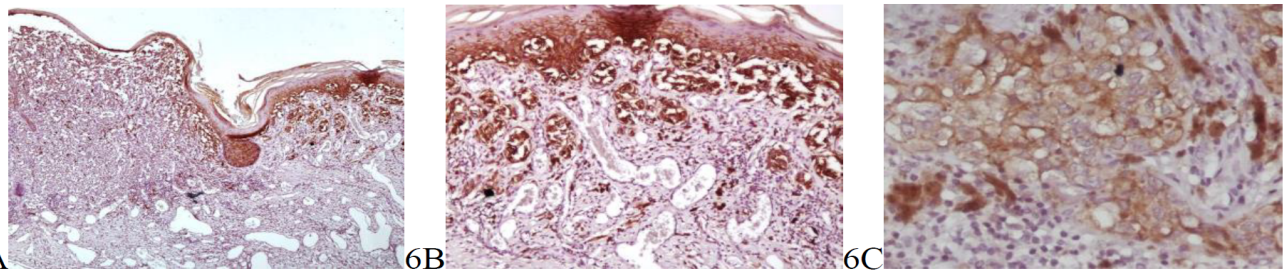


Figure 6. 6A. Over-expression of E-cadherin in RC versus NRC in a superficial spreading melanoma with partial regression. E-cad x 40. 6B. Over-expression of E-cadherin in RC in a superficial spreading melanoma with partial regression. E-cad x 100. 6C. Reduced expression of E-cadherin in NRC in a superficial spreading melanoma with partial regression. E-cad x200 (Note: 6C and 6D pictures belong to the same tumor).

Table 3. E-cadherin expression in RC compared to the NRC

E cadherin	.-. RC < NRC	RC = NRC	.+. RC > NRC
pT1	3	6	1
pT2	3	0	1
pT3	2	4	1
pT4	-	4	1

Georgantas et al 2014, Yadav et al 2014). c-Kit (Mast/stem cell growth factor receptor (SCFR), CD 117) is a receptor tyrosine kinase protein with different isoforms located on the cell surface of normal hematopoietic stem cells, mast cells, Cajal interstitial cells and melanocytes. c-Kit activation interferes in cell proliferation and survival. Activatory mutation of c-kit gene is associated with several tumors including melanoma. In Caucasian population cohorts, c-kit mutations were identified mostly in acral lentiginous and mucosal melanomas (Bourillon et al, 2013; Schoenewolf et al, 2012) while in Chinese population, almost 10% of melanoma patients showed c-kit mutations disregard of their specific clinico-pathologic type (Lin et al, 2013; Si et al, 2013). c-kit mutation occurs more often in melanomas and may represent a marker of tumor progression in local tumors and it is absent or diminished in metastases (Gonzalez et al, 2011; Nazarian et al, 2010; Pilloni et al, 2011; Ponti et al, 2017). Their presence may predict favorable response to KIT inhibitors therapeutic regimens (Luo et al, 2017; Najem et al, 2017; Zhan et al, 2017). Bcl-2 is an anti-apoptotic protein present in two isoforms with differences in anti-apoptotic activity. It is located on mitochondrial membrane on the outer surface where inhibits action of pro-apoptotic proteins such as Bax and Bak. It is involved in the pathogenesis of several types of cancer, including melanoma; however, its over-expression is not sufficient per se to determine neoplastic proliferation of cells; it has to be correlated with over-expression of a proto-oncogene (with biologic function of growth signal transducer). Bcl2 over-expression also interferes in the chemotherapeutic resistance in melanoma and may represent a target for novel drugs (Goraczniak et al, 2013; Mukherjee et al, 2015; Serasinghe et al 2015; Watanabe et al, 2013). Our findings reveal similar expression of all these markers in CMs, irrespective of tumor type, pT and/or regression.

Cadherins are calcium-dependent transmembranary proteins having a complex biological role as both receptors and ligands at adherens junctions between epithelial cells. These proteins sustain the intercellular adhesion through their cis-homodimers while their cytoplasmic component is anchored to the intermediary filaments of actin through catenins (Thiery and Sleeman 2006, Takai and Nakanishi 2003). Loosing progressively E-cadherin expression is a crucial event in the initiation and progression of melanoma bearing a prognostic value (Kreizenbeck et al 2008, Tucci et al 2007). Also, several studies showed that loss of e-cadherine expression is an unfavorable prognostic event in melanoma biology (Bønnelykke-Behrndtz et al 2015; Kreizenbeck et al 2008; Liang et al 2015; Mitchell et al 2016; Spatz et al 2010). In our study, the loss/diminishing of E-cadherin expression is frequently found in advanced melanomas and in those without regression. We also identified differences between RC and NRC in the same tumor (E-cadherin expression preservation in RC with diminished/ abolished expression in NRC) and between NRC and AR (E-cadherin overexpression in NRC compared with AR).

Tumor regression is a complex process, the result of the balance between the proliferating tumor cell and the cells of the immune system that are counter-acting these abnormal cells. Different expression of several markers was shown in the same tumor for the regressed and non-regressed areas. Thus, we have identified the loss of E-cadherin in NRC areas of melanomas with regression as being associated with the progression of the non-regressed area (thick tumors – highly invasive tumors with unfavorable prognosis), while the areas with regression still have high E-cadherin expression; luckily this is a rather unfrequent event (16.66% of melanomas with regression), the remaining cases preserving an increased E-cadherin expression, in any case superior to that identified in melanomas without regression at all. We have previously published that MMP3, MMP11, MMP13, TIMP1, TIMP2 and TIMP3 is diminished in the regressed areas in comparison to the non-regressed ones (Andrei et al 2015, Zurac et al 2016) sustaining the fact that regression is the expression of tumor heterogeneity (Neagu et al 2013).

Conclusion

E-cadherin can be considered a tissue parameter that is associated with regression in cutaneous melanoma and can thus open

new therapeutical possibilities to over-expression inducement of a molecule that hinders the metastatic process.

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