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Chapter 7

Matrix Metalloproteinases in Melanoma with and without Regression

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Abstract

Cutaneous melanoma is an aggressive tumor with increasing incidence worldwide. Recent development of promising treatments based on immune checkpoints blockade in cancer immunotherapy or signal transduction inhibitors (B-Raf enzyme inhibitor and MEK inhibitor) requires identification of new biomarkers predictive of either prognosis and/or therapeutic response. Dynamic interaction between melanoma and normal host cells influences tumor progression; proteins regulating connections between melanoma cells and extracellular matrix facilitate tumor invasion and dissemination. We discuss the various functions of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) in melanoma and their possible role as prognostic and/or predictive biomarkers. We also studied the correlation with regression of expression of several MMPs and TIMPs in melanoma; regressed and nonregressed components are in fact different tumor subclones; in some cases of melanoma with regression (with a specific morphology), the biologic aggressiveness of the tumor and implicitly the overall prognosis may be more favorable than that of melanoma without regression thus offering the possibility of a supplemental stratification of these patients beyond AJCC staging.

Keywords: melanoma, regression, matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases, biomarkers

1. Introduction

Cutaneous melanoma represents one of the most important challenges in routine dermato-oncologic practice due to its increasing incidence worldwide. Its unfavorable prognosis with the increasing number of annual deaths and impressive death toll even in incipient melanoma cases [1] indicate that current stratification of melanoma patients staging system (American Joint Committee on Cancer—AJCC) based on certain morphologic parameters—Breslow

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thickness, ulceration, nodal and distant metastases—and a serum one—lactate dehydrogenase, is unsatisfactory for both tumor biologic behavior assessment and predictive value of the systemic treatment [2].

The most problematic melanoma patients fall in two main categories: patients with advanced disease (highly invasive thick lesions and/or regional lymph node metastasis) and patients with progressive disease despite incipient stage.

In case of patients with advanced disease, despite major efforts to improve treatment, no significant advance was obtained in the last two decades; lately, development of promising treatments based on immune checkpoints blockade in cancer immunotherapy (nivolumab and pembrolizumab as PD-1 inhibitors; ipilimumab as anti-CTLA4 monoclonal antibody) or signal transduction inhibitors such as B-Raf enzyme inhibitor (vemurafenib and dabrafenib) and MEK inhibitor (trametinib) has been attained [3–8]. Even so, most patients develop acquired resistance with subsequent evolution *in faust*; potential years of life lost due to cutaneous melanoma remain as an epidemiologic indicator without significant improvement despite the extremely expensive costs of the therapy [9].

The other type of patients, those with progressive disease despite incipient stage belongs to pT1 melanomas (less than 1 mm in maximum thickness, i.e., thin melanomas). Prognosis is highly favorable if the tumor is localized (without metastases, either local or distant), surgical resection with 1 cm healthy tissue being curative but there are few patients that eventually die due to disease progression. For this incipient stage, we must look to the dark side of the statistical data: 5 years survival rate for pT1 melanomas is 97.7% with 2.3% mortality due to disease; 10 years survival rate for pT1 melanomas is 95.1% with 4.9% mortality due to disease [10]. Moreover, in case of patients with even thinner lesions (less than 0.75 mm), 10 years survival rate is 97% with a mortality rate due to disease of 3% [11, 12]. These data highlight the need for a supplementary stratification of patients with thin melanoma in "low risk" and "high risk" groups with subsequent more aggressive therapeutic approach for patients identified as "high risk" [13].

Identification of prognostic and/or predictive biomarkers is particularly difficult in cutaneous melanoma due to its complex biologic evolution, encumbered by myriad of different events caused by deregulations of several pathways [14]. Dynamic interaction between melanoma and normal host cells influences tumor progression; proteins regulating connections between melanoma cells and extracellular matrix facilitate tumor invasion and dissemination [15]. Cell adhesion molecules known to facilitate the metastatic potential of many cancers are also altered in melanoma when progressing from the non-invasive to the invasive growth phase and associate increased melanoma thickness and decreased survival [16, 17].

It becomes an imperious task that enzymes involved in degrading extracellular matrix should be investigated in relation with cancer invasion and metastases; these biomolecules belong to the metalloproteinases group that includes several classes of protease enzymes: matrix metalloproteinases (MMPs), a disintegrin and metalloproteinases (ADAMs) and ADAMs with thrombospondin motifs (ADAMTS). All of them are zinc-containing endopeptidases of metzincins family, some extracellular/soluble (most of MMPs and ADAMTS), the others membrane-bounded biomolecules (membrane-type MMPs (MT-MMPs) and ADAMs).

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Matrix metalloproteinases (MMPs) represent a complex family of biomolecules accomplishing a myriad of activities with equally physiological and pathological inferences. MMPs are involved in embryologic development and in wound healing as key players in epithelial to mesenchymal transition by enabling cell-cell detachment with subsequent basement membrane perforation; they also initiate Snail positive mechanisms of MMPs expression stimulation *via* increasing reactive oxygen species production [18–22]. MMPs are classified based on their primary function: collagenases (MMP-1, MMP-8, MMP-13, MMP-18), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10), matrilysins (MMP-7, MMP-26); also, MMPs include six forms of MT-MMPs (four transmembranary proteins of which two are anchored to the membrane *via* glycosylphosphatidylinositol) and a group of seven MMPs (metalloelastase (MMP-12), MMP-19, enamelysin (MMP-20), MMP-22, MMP-23, epilysin (MMP-28)), some with still unknown biological function in humans [23, 24].

MMPs dysfunctions contribute to various diseases: degenerative diseases of the brain, atherosclerosis, aortic aneurysm, arthritis, and cirrhosis [25, 26]. In cancer, MMPs facilitate invasion and metastasis and participate as regulators of tumor cells proliferation and apoptosis; also, they intervene in tumor differentiation, tumor immune-resistance, and tumor angiogenesis [27, 28].

In tumor invasion, they mediate adhesion of tumor cells to extracellular matrix components and concomitant proteolysis of extracellular matrix, thus favoring migration of tumor cells into the areas of matrix degradation [29]. Another important role of MMPs in cancer is represented by creation of a local environment able to host and provide specific conditions required by metastatic cells to survive in a distant organ—"metastatic niche" [30].

MMP-1 is a collagenase correlated with enhanced invasiveness and metastasis in melanoma. It is overexpressed in invasive melanoma comparing with *in situ* or microinvasive melanomas where MMP-1 is absent [31]; its knocking down in melanoma cell line diminished the tumor cell capacities for metastasis after implanting in nude mice [32] and, conversely, its introduction in noninvasive melanoma cells induces a metastatic phenotype *in vivo* [33]. MMP1 production is noticed especially in stromal fibroblasts being also involved in PAR1 thrombin receptor activation with subsequent role in increasing tumor progression and metastatic capability of tumor cells [34–36].

MMP-2 (gelatinase A) has an important role in tumor progression due to its ability to degrade collagen IV present in basement membrane; its presence is of outmost importance for tumor invasion, fact demonstrated by the significant reduction of invasion through basement membrane by treating A2058 melanoma cell line culture with an antibody anti human type-IV collagenase [37]. Moreover, in cell culture, MMP-2 is present as activated biomolecules when highly invasive melanoma cells lines are cultured in collagen lattices; by contrast its activity is inhibited by antibodies against MT1-MMP, TIMP-2, and through MMP-2 cleavage by MT1-MMP [38]. It is expressed in melanomatous cells and stromal cells especially in peritumoral areas where its proteolytic activity is present [39]. Using the B16-melanoma cell line mouse model it was shown that MMP-2 expression was predominantly present at the tumor-stroma border indicating stromal cells as primary source for this protease [40]. In another cell model,

using human melanoma cell lines M3 Da and M1Dor cocultured with dermal fibroblasts, it was shown that MMP-2 expression in membrane extracts was enhanced. Since stromal and cancer cell contacts have been shown to occur after disruption of the ECM, it is assumed that fibroblasts may influence melanoma cell invasion after the beginning of tumor progression through the dermis [41]. MMP-2 binds to $\alpha v\beta 3$ integrin and regulates $\alpha v\beta 3$ integrin-dependent tumor cell migration most likely by facilitating $\alpha v\beta 3$ integrin binding to previously cleaved extracellular matrix components; one such component, fibronectin, is cleaved by MMP-2 thus facilitating $\alpha v\beta 3$ integrin binding and subsequently migration of tumor cells [42].

MMP-2 expression is correlated with prognosis being proposed as an independent prognostic factor [43]. MMP-2 expression is variable in melanocytic tumors, according to their biologic aggressiveness. Thus, in benign melanocytic tumors (common nevocellular nevi), junctional nevi and melanoma, MMP-2 is present with differences in number according to cytonuclear and architectural features (more numerous cells as cell atypia and architectural disarray increase) [44–46]; MMP-2 was found over-expressed in lesional keratinocytes and enhanced by UVB-irradiation, but not found in melanocytic cells [47]; moreover, occurring of distant metastases is more frequent in melanomas with MMP-2 overexpression in primary tumor [44] but MMP-2 is not present in metastatic melanoma [43]. Also, due to the relation between p-Akt and MMP2, MMP2 could be used as a predictive biomarker for vemurafenib resistance as vemurafenib-treated patients with overexpression of MMP-2 might be more prone to develop resistance [43, 48].

MMP-3 (stromelysin-1) is a metalloproteinase with double role in tumor development, both in tumor progression and tumor suppressing. It activates MMP-1, thus increasing the invasion capabilities of the tumor cells [49]. MMP-3 is overexpressed in metastases of melanoma and associates significantly shorter disease-free survival than patients with lower levels of MMP-3 expression shorter [50]. Other data show that MMP-3 has antitumor effects in squamous cell carcinoma, thus tumors in mmp3 null mice are more aggressive than in control, most likely due to its pro-apoptotic effect in neoplastic cells [51].

MMP-7 (matrilysin) triggers proteolytic cleavage of HB-EGF, a biomolecule with pro-tumor activity in melanoma by both EGFR ligand role and MAPK and PI3K/Akt pathways activator [52–54]. MMP-7 expression was demonstrated in melanomas both primary (up to 80% of primary tumors) and metastatic ones (all the metastatic tumors) while it was absent in both common and Spitz nevi; MMP-7 was identified in tumor cells (both immunohistochemically and by *in situ* hybridization); Western blotting revealed the presence of active MMP-7 in all melanomas (both primary and metastatic); moreover, the MMP-7 immunohistochemical staining score was correlated with Breslow index (higher the MMP-7 positivity score, higher the Breslow thickness of the tumor) and 5-year survival (100% 5-year survival in case of MMP-7 negative melanomas and significant lower percentage—26.3% 5-year survival in case of MMP-7 positive cases), thus raising the issue of its selection as prognostic marker [55].

MMP-8 is a collagenase with anti-tumor attributes. MMP-8 expression is reverse correlated with metastatic potential of breast cancer tumor cells [56, 57]. Several different stable clones of murine B16F10 melanoma cells (normally MMP-8 negative) transfected with murine MMP-8 cDNA develop significantly less numerous and smaller lung metastases after injection

in *Mmp8*$^{-/-}$ C57BL/6 mice strain mice; moreover, these MMP-8+ clones obtained from B16F10 melanoma cells or B16F10 melanoma cells incubated with recombinant MMP-8 showed significant *in vitro* diminishing of invasive abilities compared with B16F10 controls due to increased tumor cells adherence to type I collagen and laminin-1 from extracellular matrix [58]. MMP-8 gene is often mutated in melanoma and MMP8-null mice have significantly increased incidence of skin tumors while bone-marrow transplantation (offering neutrophil-derived MMP8) restore MMP-8 antitumor protection [59, 60]. The antitumor properties of MMP-8 are related rather to its capacity to process proinflammatory biomolecules (MMP-8 absence allows the development of an important inflammatory response elicited by chemical substances with carcinogenetic properties) than to its collagenase function [61].

MMP-9 (gelatinase B), similar to MMP-2, is present in melanoma both in tumor cells and stroma with enhance activity at tumor border [39]. However, by contrast to MMP-2 that has tumor progression effects, MMP-9 has dual role, in some cases with anti-tumor activities. Evidences of such effects are both experimental demonstrated as HPV16 related carcinomas have more aggressive biologic behavior in mmp-9 null mice [62], and clinical confirmed as MMP-9 overexpression in colonic and breast carcinoma bears a positive significance—more favorable prognosis [63, 64]. A mechanism for tumor suppressing activity may be represented by inhibition of angiogenesis; collagen IV degradation by MMP-9 releases tumstatin, a biomolecule that inhibits endothelial cells activity and subsequently tumor angiogenesis [65, 66].

MMP-10 (stromelysin-2) over-expression in melanoma was recorded mainly in the extracellular matrix, adjacent both tumor cells and blood vessels, being more likely involved in tumor growth [67]. Its expression is rapidly increased after UV exposure in SCL-1 squamous cell carcinoma cell line [68] is present in stromal cells in squamous cell carcinoma [69] and it seems to be correlated with unfavorable prognosis when it is expressed in both tumor tissue and serum in patients with gastric cancer [70]. It also intervenes in other MMPs function by up-regulating tumor-progression favorable ones such as MMP-1, MMP-7, and MMP-13 [71].

MMP-12 (matrix metalloelastase) is another metalloproteinase with dual role, more likely a protective one; it may be secreted by macrophages or by tumor cells, the biologic role being related with the type of secreting cell, thus it appears that macrophage-secreted MMP-12 has antitumor role, while tumor-secreted one has pro-tumor activity [72]. It was shown that MMP-12 knock-out mice grafted with B16 melanoma cells were more susceptible to develop TNF/IFN-induced inflammation than their wild-type counterparts [73] and its overexpression determines slower tumoral growth in experiments in mice [74]. As other elastases, it contributes to the releasing of angiogenic inhibitors from collagen fibers and angiostatin from plasminogen, thus exercising an anti-angiogenic activity [75–77]. Also, its anti-angiogenic effects may rely to uPAR cleavage [78] being a possible anti-tumoral therapeutic agent [79].

MMP-13 is involved in tumor progression most probable by increasing VEGF production in the tumor stroma. It is expressed both by tumor cells in invasive tumors and by tumor stroma. In experimental models of knocked-down MMP13 gene transgenic mice, it was showed that implants of melanoma cells (intradermal injection with B16F1 cell-line) develop smaller local tumors with less prominent vascularity after a longer period of time comparing with injected littermate controls (MMP-13+ mice); moreover, the incidence of metastases in mmp13−/− mice

decreased dramatically in lung, liver, and brain being absent in the hearth as melanoma is known as one of the few tumors able to develop cardiac and spleen metastases; up to 40% of the control animal had cardiac metastases while none of the transgenic mice had such lesions [80, 81].

TIMPs (TIMP-1 to -4) are nodal biomolecules involved in epithelial-mesenchymal transition; since they are involved in several biological pathways their functions are more complex than simply modeling the extracellular matrix. Mostly, TIMPs regulates the activity of MMPs by binding to the catalytic sites of MMPs by their N-terminal domains forming a stoichiometric inhibitor complex; there is demonstrated selectivity of different types of TIMPs for specific type of MMPs. In case of MMP-2 and MMP-9, the C-terminal domain of TIMPs binds to the hemopexin-like domain of pro-MMP-2 and pro-MMP-9; replacement of C-terminal domain of TIMP-1 with either TIMP-2, TIMP-3 or TIMP-4 C-terminal domains improve TIMP-1 affinity for different MMPs (MMP14 and MMP19) and ADAMs (ADAM10 and ADAM17), inhibits TNF-α and HB-EGF shedding, inhibits cell migration in wound healing and eliminates the tumor growth effects of TIMP-1 [82–85].

TIMPs directly interact with cell adhesion molecules or directly intervene on cytoskeletal components, processes that alter both intercellular adhesion and cell growth. In addition, cellular proliferation is modulated by TIMPs direct interference with components of extracellular matrix [83, 86].

TIMPs are involved in angiogenesis, mostly with anti-angiogenic effects due to the modulation of MMPs activity. However, TIMP2 and TIMP-3 have supplemental effects in inhibiting angiogenesis. TIMP-2 inhibits proliferation and migration of endothelial cells and (either by interacting with α3β receptor or by inducing the RECK expression which subsequent inhibition of MMP-2, MMP-9, MT1-MMP, ADAM10). TIMP-3 blocks VEGF-A mitogenic actions and regulates VEGFR2 expression [23, 83, 86, 87].

Tumor growth effects of TIMPs are also related to their intervention in apoptosis, either pro- or anti-apoptotic molecules. TIMP-1 and TIMP-2 have antiapoptotic activity modulating PI3-kinase and JNK pathways (unrelated to TIMP-1 inhibition of MMP functions) while TIMP-3 stabilizes Fas and TNF-cell receptor 1 (proapoptotic effects) [23, 83, 86, 88].

2. MMPs and TIMPs role in response to treatment and resistance to therapeutic agents in melanoma

In melanoma treatment with acute BRAF inhibition, it was reported that active MMP-2, MT1-MMP, and MMP-9 are decreased, but it did not modulate TIMP-2 or RECK. Using cell models, it was shown that resistance to vemurafenib induces significant changes in the tumor microenvironment mainly by MMP-2 upregulation, but not upon TIMP expression, MMP up-regulation corresponding to an increase in cell invasiveness [89]. Another research group using experimental cell models by transfection of miR-21 and inducing over-expression in the melanoma cell lines WM1552c, WM793b, A375, and MEL 39 has shown that miR-21 decreases TIMP3 expression and enhances the invasiveness of melanoma cells. In an animal model, using 01B74 Athymic NCr-nu/nu mice, treatment with a miR-21 antagomir inhibited tumor growth and increased tumor expression of TIMP-3 [90].

3. MMPs and TIMPs expression in melanoma with regression

There are very few tumors that can present spontaneous regression up to the point of complete clinical and histopathologic vanishing. Melanoma is such a tumor, its disappearance reaching the point of impossibility of tumor cell identification even by immunohistochemical test; the indirect proof of previous existence of the tumor is represented by the presence of so-called tumor melanosis [91]. There different types of regression in melanoma are described: *complete regression* with total disappearance of tumor cells (immunohistochemistry fails to identified any neoplastic melanocyte, tumor being superseded by an area of fibrosis with hyperplastic blood vessels, and dense inflammatory infiltrate with numerous melanophages); *segmentary regression* (part of the tumor suffered complete regression); and *partial regression* (the regression is present but few tumor cells can be identified in the area, either morphologically or immunohistochemically) [92].

Regression is a phenomenon that occurs naturally relatively frequent in melanoma—up to 10–35% of cases; an even higher incidence was reported in thin melanomas—up to 60% of cases with a Breslow index of <0.75 mm [10, 92–94]. Despite these data, complete regression is reported in very few cases (mostly, as case reports and about 0.25% in large studies [95]); the real incidence of this phenomenon cannot be established since patients with completely regressed melanoma are not aware of the disease unless they develop distant metastasis. The biologic significance and prognosis of regression in melanoma is a matter of debate, various opinion being published [91, 96–105]. Mechanisms involved in occurring of regression in melanoma are yet to be deciphered; considering the importance of host response in this process, investigation of tumor microenvironment may offer some responses.

Study of MMPs and TIMPs expression in melanoma with regression was performed by our group by analyzing 93 melanomas (62 superficial spreading melanomas (SSM) and 31 nodular melanoma (NM)), 39 cases of SSM showing regression. Regression was present as both segmental and partial type, either pure form or combined (segmental regression (SR)—33.33%, partial regression (PR)—43.58%, and SR-PR in the same tumor—23.07%). Five MMPs and three TIMPs were analyzed (MMP-1, MMP-3, MMP-9, MMP-11, MMP-13, TIMP-1, TIMP-2, and TIMP-3) in both regressed and non-regressed areas of the tumors, the results being compared with those recorded in melanomas without regression (absence of regression—AR). A semiquantitative score with four levels based on the level of staining intensity, namely "absent" (−), "mild positive" (+), "moderate positive" (++), and "intense positive" (+++) was used to assess the immunohistochemical expression of each marker in either tumor or stromal cells.

Two types of results were obtained: (a) differences in MMPs and TIMPs expression between non-regressed component of melanoma with regression (NRC) and melanoma without regression (AR) and (b) differences in MMPs and TIMPs expression between regressed component (RC) and NRC in melanoma with regression [106].

3.1. Differences in MMPs and TIMPs expression in tumor cells between NRC and AR

MMP-1 was intense positive (+++) in all AR melanomas; also, most of the NRC were intense positive (+++) for MMP-1, only 15.84% of them being moderately positive (++) (**Figure 1a–c**).

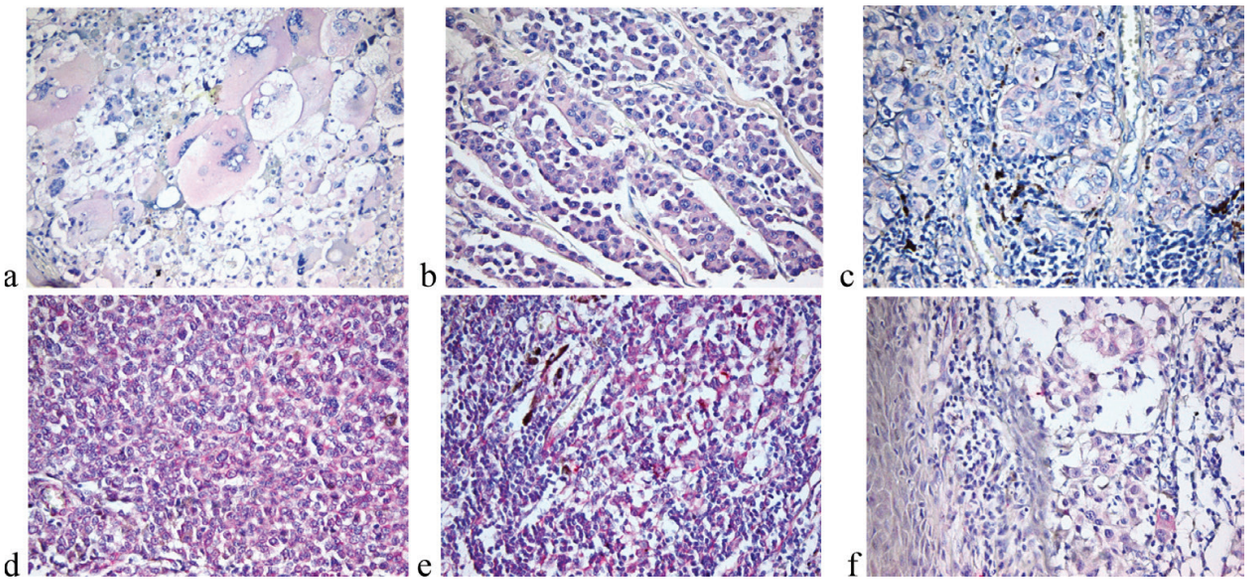

Figure 1. MMP-1 and MMP-11 (fast red detection): (a) melanoma without regression, faint diffuse positivity for MMP-1; monstrous tumor cells are more intense positive than main tumoral mass. MMP-1 × 200. (b) Melanoma with partial regression, faint diffuse positivity for MMP-1 in non-regressed component. MMP-1 × 200. (c) Melanoma with partial regression, faint diffuse positivity for MMP-1 in regressed component. MMP-1 × 400. (d) Melanoma with partial regression, faint diffuse positivity for MMP-11 in non-regressed component in both tumor and stromal cells. MMP-11 × 400. (e) Melanoma with partial regression, diffuse positivity for MMP-11 in regressed component in both tumor cells, endothelial cells and fibroblasts. MMP-11 × 400. (f) Melanoma with partial regression, faint positivity for MMP-11 in regressed component in both tumor cells. MMP-11 × 400.

Despite the small percentage of less positive cases for MMP-1 in melanomas with regression, this feature was statistically significant.

Similar findings as in case of MMP-1 were present for MMP-11 (**Figure 1d–f**): all AR cases were intense positive, while 74.35% of NRCs were intense positive, the rest (25.65%) being moderate positive; also, the tendency of a diminished expression of MMP-11 in NRC than in AR cases was statistically significant.

MMP-2 (**Figure 2a–c**), MMP-3 (**Figure 2d–f**), MMP-7 (**Figure 2g–i**), MMP-9 (**Figure 2j–l**), and MMP-13 (**Figure 2m–o**) showed similar features of diminished expression in NRC than in AR but the data were not statistically significant; data for MMP-13 expression had a level of statistical significance of 0.07.

No significant differences for TIMP-1 (**Figure 3a–c**), TIMP-2 (**Figure 3d–f**), and TIMP-3 (**Figure 3g–i**) expression were recorded between NRC and AR tumor cells.

Our study identified an overall diminished expression of MMP-1, MMP-3, MMP-9, MMP-11, and MMP-13 in NRC comparing to AR as control; since most of these biomolecules have pro-tumor activities, it is possible to speculate that they favor a less aggressive biologic behavior melanoma with regression.

There were differences when the specific type of regression was considered. Expression of MMP-1 in NRC of tumors with SR (either SR or combined SR-PR) was statistically significant diminished comparing with both AR cases or with NRC in PR cases. No such differences were

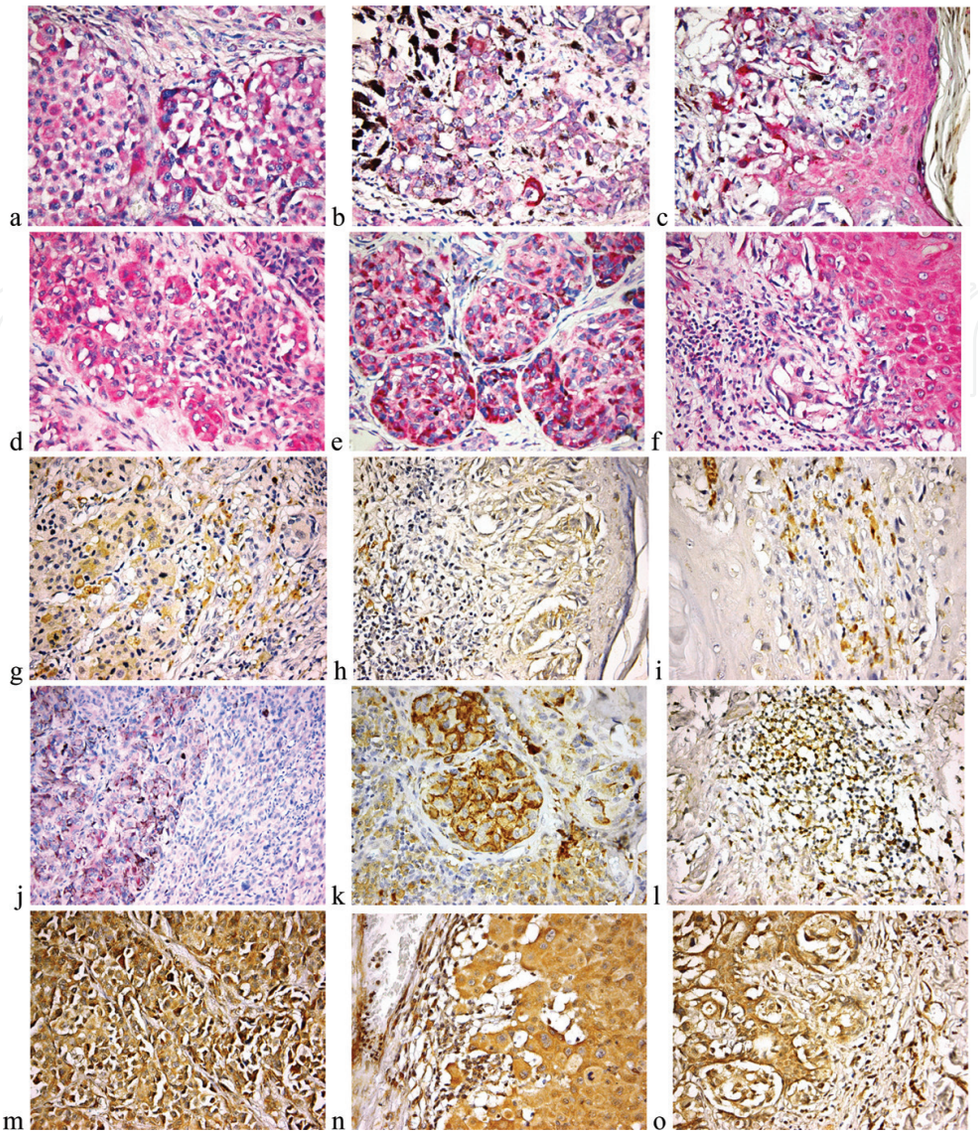

Figure 2. MMP-2, MMP-3, and MMP-7 (fast red detection), MMP-9 (fast red and DAB detection), MMP-13 (DAB detection): (a) melanoma without regression, diffuse positivity for MMP-2. MMP-2 × 400. (b) Melanoma with partial regression, diffuse positivity for MMP-2 in non-regressed component. MMP-2 × 400. (c) Melanoma with partial regression, diffuse positivity for MMP-2 in regressed component. MMP-2 × 400. (d) Melanoma without regression, diffuse positivity for MMP-3 in both tumor and stromal cells. MMP-3 × 400. (e) Melanoma with partial regression, diffuse positivity for MMP-3 in non-regressed component. MMP-3 × 400. (f) Melanoma with partial regression, diffuse positivity for MMP-3 in regressed component in both tumor and stromal cells. MMP-3 × 400. (g) Melanoma with partial regression, diffuse positivity for MMP-7 in non-regressed component in both tumor and stromal cells. MMP-7 × 400. (h) Melanoma with partial regression, diffuse positivity for MMP-7 in regressed component in both tumor and stromal cells. MMP-7 × 400. (i) Melanoma with segmental regression, intense positivity for MMP-7 in regressed component in stromal cells; no tumor cells are present. MMP-7 × 400. (j) Melanoma without regression, diffuse positivity for MMP-9; pigmented tumor cells are slightly more intense positive than nonpigmented ones. MMP-9 × 400 (fast red detection). (k) Melanoma with partial regression, diffuse positivity for MMP-9 in regressed component in tumor cells; few fibroblasts from area of regression are also positive; faint cytoplasmic positivity of plasma cells. MMP-9 × 400 (DAB detection). (l) Melanoma with segmental regression, positivity for MMP-9 in regressed component in inflammatory cells and fibroblasts; no tumor cells are present. MMP-9 × 400 (DAB detection). (m) Melanoma without regression, diffuse positivity for MMP-13; MMP-13 × 400. (n) Melanoma with partial regression, diffuse positivity for MMP-13 in non-regressed component in tumor cells, fibroblasts, endothelial and inflammatory cells. MMP-13 × 400. (o) Melanoma with partial regression, diffuse positivity for MMP-13 in regressed component in tumor cells; fibroblasts, endothelial and inflammatory cells from area of regression are also positive. MMP-13 × 400.

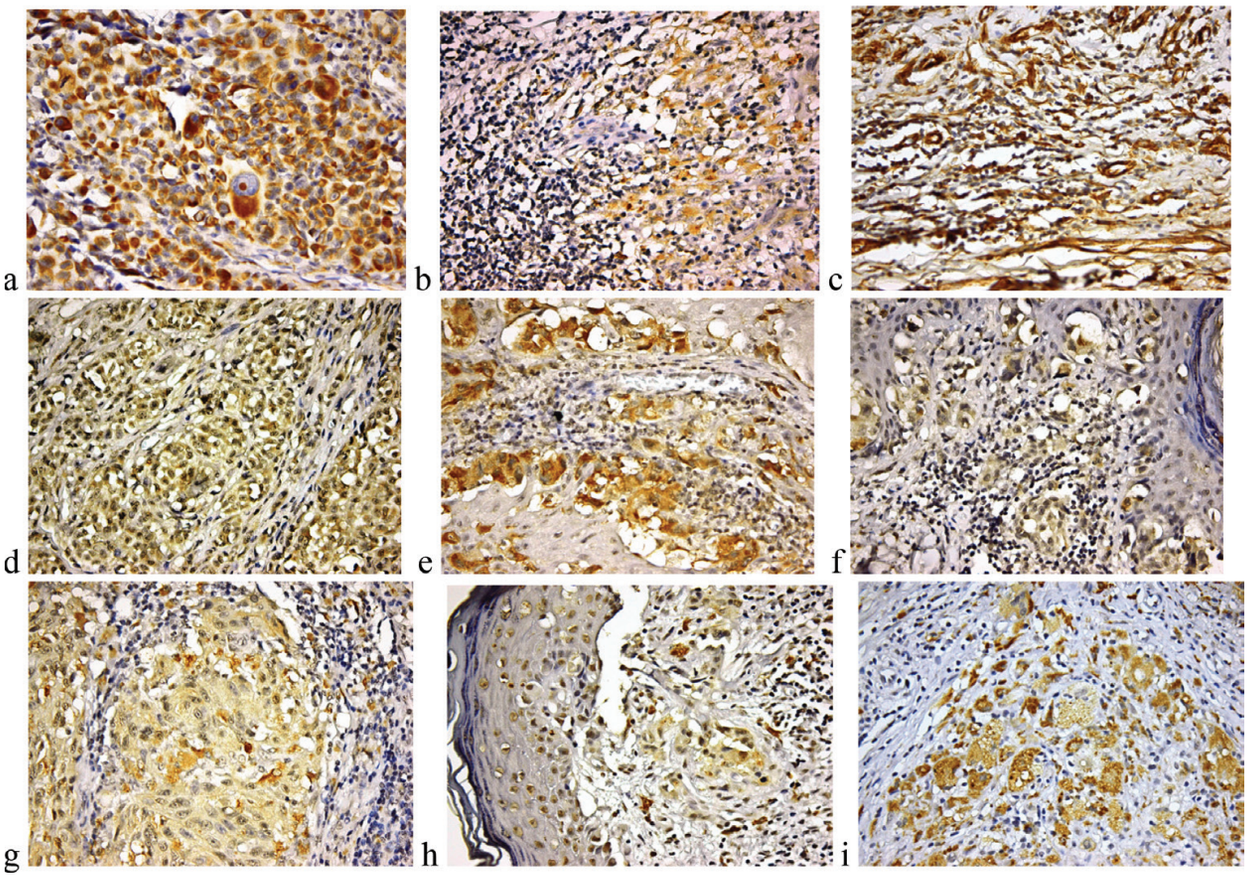

Figure 3. TIMP-1, TIMP-2, and TIMP-3 (DAB detection): (a) melanoma without regression, diffuse positivity for TIMP-1; TIMP-1 × 400. (b) Melanoma with partial regression, diffuse positivity for TIMP-1 in non-regressed component in tumor cells, few fibroblasts, few endothelial and few plasma cells. TIMP-1 × 400. (c) Melanoma with segmental regression, diffuse positivity for TIMP-1 in area of regression in fibroblasts, endothelial and inflammatory cells. TIMP-1 × 400. (d) Melanoma without regression, diffuse positivity for TIMP-2; TIMP-2 × 400. (e) Melanoma with partial regression, diffuse positivity for TIMP-2 in non-regressed component in tumor cells. TIMP-2 × 400. (f) Melanoma with partial regression, faint positivity for TIMP-2 in non-regressed component in tumor cells. TIMP-2 × 400. (g) Melanoma with partial regression, diffuse variable positivity for TIMP-3 in non-regressed component; TIMP-3 × 400. (h) Melanoma with partial regression, faint diffuse positivity for TIMP-3 in non-regressed component in tumor cells. TIMP-3 × 400. (i) Melanoma with partial regression, variable positivity for TIMP-3 in non-regressed component in tumor cells. TIMP-3 × 400.

noted for other MMPs (MMP2, MMP3, MMP11, and MMP13). Expression of TIMP-1 and/or TIMP-2 in NRC of tumors with PR was statistically significant increased comparing with either NRC of SR cases (TIMP-1 P = 0.011; TIMP-2 P = 0.009) or with NRC of SR-PR cases and AR (P = 0.002 and 0.037, respectively). There was no difference in TIMP-3 expression in NRC and/or AR cases according to the type of regression.

3.2. Differences in MMPs and TIMPs expression between RC and NRC in melanoma with regression

The differences in MMPs and TIMPs expression were evaluated in the same tumor, both in tumoral and stromal cells. In case of tumors with SR, the expression was evaluated in stromal cells (fibroblasts) present in regressed area. In the other cases (PR tumors, SR-PR tumors, and AR tumors), most of the cases showed similar expression of each marker in tumor cells versus stromal fibroblast for each tumor compartment.

In all the cases, there was either similar expression of MMPs in tumor cells in both areas (NRC and RC) or slightly overexpression in NRC comparing with RC. MMP-3 was the biomolecule with the most numerous cases of overexpression (76.93% had MMP-3 overexpression in NRC than in RC), followed by MMP-2 and MMP-11 (58.97% each), MMP-13 (48.71%), and MMP-1 (30.76%). Tumor stromal fibroblasts were also slightly more intense positive in NRC than in RC or showed similar expression in both components.

Considering the specific type of regression, MMP2 was over-expressed tumor fibroblasts in NRC than in RC in PR cases comparing with SR ones ($P = 0.023$). Also, differences occurred in case of MMP-13 expression—all SR-PR cases had MMP-13 overexpression in NRC versus RC comparing with PR and SR cases ($P = 0.003$, respectively, $P = 0.0003$). No significant differences occurred in case of MMP-1, MMP-3, and MMP-11 expression.

TIMPs expression had more variability in NRC versus RC component both in tumoral and stromal cells; there were cases with overexpression, similar expression or diminished expression for each type of TIMP investigated. However, most of the cases (66.66% for TIMP-1, 61.53% for TIMP-2, and 64.10% for TIMP-3) had TIMP overexpression in NRC versus RC.

The type of regression did not influence TIMP-1 and TIMP-2 expression in NRC and RC. In case of TIMP-3, all SR melanomas had TIMP-3 overexpression in stromal tumor fibroblasts in NRC when compared with those in RC component ($P = 0.007$).

4. Conclusions

Cancer biology is a complex phenomenon, several mechanisms concurring to tumor progression and metastasis. The role of the tumor microenvironment and its regulation by both tumor neoplastic cells and host response was lately established, the identification of several stromal-related biomarkers offering some explanations for different biology behavior of tumors with otherwise similar origin and classical histopathologic appearance.

MMPs and TIMPs are potent molecules involved in tumor development, progression, and metastasis with either pro- and anti-tumor activity; their correlation with regression in melanoma shows: (a) regressed and nonregressed components are in fact different tumor subclones and (b) in some cases of melanoma with regression (with a specific morphology), the biologic aggressiveness of the tumor and implicitly the overall prognosis may be more favorable than that of melanoma without regression, thus offering the possibility of a supplemental stratification of these patients beyond AJCC staging. More studies are needed to establish comprehensive pathways as a gate for identification of new biomarkers for either diagnostic or therapeutic purposes.

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Conflict of interest

The authors declare no conflict of interests.

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