REVIEW

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TIMPs expression in lentigo maligna/lentigo maligna melanoma *versus* aged skin – a review of the literature and personal experience

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Abstract

Mechanisms involved in melanoma invasiveness and metastasis are essential to understanding the behavior of this aggressive melanocytic skin cancer. The epithelial–mesenchymal transition (EMT) is considered fundamental for overcoming the *in situ* stage of melanoma and its proliferation beyond the basal membrane. Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are key molecules involved in EMT about whose expression in lentigo maligna (LM) and lentigo maligna melanoma (LMM) little has been studied so far. In this article, our main aim was to review the role of TIMPs in invasiveness and aggressiveness of LM/LMM in order to detect EMT modifications in this type of melanoma. We also presented some partial personal unpublished results. It is well established by now that progression of melanoma depends on ECM remodeling, TIMPs family being one of the most important regulators of this process. Considering the multitude of molecules involved in cancer invasiveness and their complex interaction, it is too early to analyze and to conclude upon the significance of different expression of TIMPs in LM/LMM. We consider some correlations are needed to be done also with other consecrated histological (as Clark level, Breslow indexes, presence of ulceration, mitotic index, intratumor inflammatory infiltrate, etc.) and immunohistochemical markers [cadherins, vascular endothelial growth factor (VEGF), bcl-2, etc.] of prognosis and metastasis. In this light, we consider that our study could further clarify the significance of TIMPs expression in this specific type of melanoma.

Keywords: lentigo maligna, lentigo maligna melanoma, TIMP-1, TIMP-2, TIMP-3.

Introduction

Melanoma *in situ* is reported as a disproportionately high percentage of the total melanoma, all melanoma types having an *in situ* stage. Lentigo maligna (LM) is the *in situ* stage of lentigo maligna melanoma (LMM) and it is specific in elderly, appearing on photo-aged skin [1]. The melanocytic proliferation is above the basal membrane. Of all *in situ* forms, LM has a quite long evolution before becoming invasive, years, even decades. For becoming invasive, meaning that the melanocytes proliferate beyond the basal membrane, a specific key stage should be reached and that is epithelial–mesenchymal transition (EMT) [2]. EMT represents a key phenomenon involved in the biology of tumor genesis, in general. While suffering EMT, a tumor is acquiring invasiveness and the ability to metastasize. Deepening the knowledge on EMT could lead to advances in understanding the pathophysiology of melanoma and to optimize the therapeutic approach. Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are key molecules involved in EMT [3, 4] but about their expression in LM/LMM is little published. Clinical and histopathological diagnosis is difficult, immunohistochemical (IHC) examination being currently helpful.

The main objective of this review is to establish the role of TIMPs, key molecules of EMT, in invasiveness and aggressiveness of LM/LMM in order to detect EMT modifications in this type of melanoma. Unfortunately, the data on this subject is scarce, and, as far as we have searched, a specific panel of IHC markers has not been reported yet in scientific papers. The data presented in this review is more about TIMPs involvement in melanoma in generally, as most of the IHC studies included only specific types of melanoma, as nodular melanoma and superficial spreading melanoma. What is particular to LM is the very slow evolving, in decades, of melanocytic proliferation above the basal membrane but once the melanocytes invades the dermis and becomes LMM has the same prognostic with any other type of melanoma, prognostic that depends mainly on tumor thickness in the moment of diagnosis [5].

TIMPs, MMPs and metastasis

It is well known in dermato-oncology and dermatopathology that melanoma is highly metastatic, being one of the most aggressive tumors at all and, most of times, resistant to current chemotherapeutic treatments [6]. This may explain why this type of cancer is associated with high mortality rates [7]. The production of melanoma metastases depends on fulfilling some essential steps. First of all, melanoma cells develop specific characteristics that permit them to detach from the tumor matrix. Essential next step is the degradation of basement membrane. Detaching and remodeling of the tumor extracellular matrix

(ECM) is made possible by proteolytic enzymes such as MMPs, which facilities the motility of the tumor cells through ECM [8, 9]. Next, they have to migrate and invade the stromal tissue. Further, melanoma cells must penetrate into blood vessels where they have to survive in order to extravase and to invade secondary sites. Once extravased, metastatic melanoma cells must attach and proliferate in order to form a secondary tumor [10]. There is not described yet a fully histopathological, molecular, or IHC markers panel to clearly define and predict melanoma behavior [11].

In vitro studies showed that fibronectin and laminin, that are non-collagenous ECM glycoproteins important for cell adhesion, enhances migration of melanoma cells. Both have domains with unique functions that promote binding to specific collagens and proteoglycans, as well as to cell surfaces [12]. The major proteinases involved in ECM catabolism are the MMPs, which include collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs (MT-MMPs) and others as ADAMTS (a disintegrin and metalloproteinase with thrombospondin domains) metalloproteinases [13].

The activities of these MMPs are precisely regulated under physiological conditions at the levels of transcription, zymogen activation and inhibition by endogenous inhibitors. Disruption of the balance between the production of active enzymes and their inhibition may result in diseases associated with uncontrolled ECM turnover, inflammation, cell growth and migration, such as cancer.

MMPs activity is modulated by a family of tissular inhibitor of matrix metalloproteinase (TIMP), a family with four members (TIMP-1 to TIMP-4). They actively inhibit MMPs by binding to their catalytic domain. More than that, TIMP-1 and TIMP-2 regulate activation of some proMMPs by binding to the COOH-terminal hemopexin-like domain. They are consequently important regulators of ECM turnover, tissue remodeling and cellular behavior. In addition to their MMP inhibitory activity, TIMPs have other biological activities such as promoting cell proliferation, anti-angiogenic, pro- and anti-apoptotic and synaptic plasticity activities, many of which are independent of MMPs inhibition [13, 14].

All four TIMPs inhibit the 23 MMPs identified in humans, but with different affinities that vary. Among the four TIMPs, TIMP-3 has the broadest inhibition spectrum inhibiting also members of the ADAM and ADAMTS families [15, 16]. It also differs from the other TIMPs by being firm bound to the ECM [13]. *In vivo* overexpression of TIMP-3 is associated with apoptosis of melanoma cells, indicating that TIMP-3 may inhibit melanoma growth on different levels. Thus, overexpression of TIMPs has been shown to reduce tumor growth and metastasis formation [17].

Quite opposite to TIMP-3, TIMP-1 has the most restrictive area of inhibition. It has a relatively low affinity for the MT-MMPs, MMP-14, MMP-16, MMP-24 and MMP-19 [18]. It is quite well studied the preference inhibition of TIMP-1 against MMP-9, MMP-3 and MMP-1. TIMP-1 is expressed mostly at the front of cell invasion and, as at that level MMP-1 and MMP-13 are not so often detected, its action is directed to the inhibition of MMP-9 [19].

When talking about differences between the affinities of different TIMPs for different MMPs, it is well known that TIMPs-2 and -3 are weaker inhibitors than TIMP-1 for MMP-3 and MMP-7 and that TIMP-2 acts against MMP-2 rather than MMP-9 [13].

Overexpression of TIMP-2 reduces invasive properties of the cells and angiogenesis of melanoma cells *in vivo* and protects them from apoptosis [20]. It is well expressed in melanomas, regardless of Clark stages. MMP-14 specifically binds to TIMP-2 generating a complex that functions as a receptor for the proenzyme MMP-2 [21].

It is very interesting the relation between TIMP-2 and MMP-2 as it depends on TIMP-2 concentration the relation it establish with MMP-2. It was demonstrated that, at low concentrations, TIMP-2 promotes the formation of a complex with proMMP-2 and MT1-MMP on the cell surface, complex that lead to activation of MMP-2. Therefore, at low concentrations, TIMP-2 promotes processing of MMP-2 to its proteolytically active form. On the other hand, high concentrations of TIMP-2 inhibit MMP-2 activation. The fine balance between levels of activated MMP and free inhibitors seems to be critical for MMP activity [21]. TIMP-2 is detectable in melanoma cells, fibroblasts or endothelial cells in most tumors and associated with low invasiveness.

Premalignant lesions, such as tumors of grade I are negative for collagenases-1, -3 and TIMP-1 and -3. TIMP-1 and -3 are abundantly expressed in the stromal cells or melanoma cancer cells grade III and IV, while TIMP-2 was detected in melanomas with decreased invasive potential.

Despite the complex and sometimes-controversial effect of TIMPs, it is accepted by quite all scientists that their overexpression decreases melanoma experimental metastasis [22] because they can directly suppress MMPs activity [23]. TIMP expression may reflect the host response to tumor invasion in an effort to control MMP activity and preserve the integrity of the ECM.

TIMPs in photo-aged skin

Despite their role in cancer metastasis, MMPs play an important role in physiological turnover and maintenance dermal ECM as the MMPs degrade basically every component of the dermal ECM, including collagen. In the aged skin, there was reported an exaggerated MMPs activity, which was associated with the excess degradation and damage of dermal ECM, and furthermore with the clinics of aged skin. The balance between MMPs and TIMPs is critical in maintaining skin's matrix stability and homeostasis in dermal connective tissue. Any misbalance between MMPs and TIMPs affects the skin, even if is about overexpression of MMP and down-regulation of TIMP, which will lead to excessive degradation and damage of the ECM, specifically the collagen, causing wrinkles and skin laxity or is about a deficiency in MMP level associated with an excess of TIMPs, which also affects the turnover and recycling of the ECM.

Physiological aged skin is characterized by a strongly increased MMPs activity associated with down-regulation of TIMPs that leads on one hand to important loss and damage of collagen and matrix and on the other hand to impaired cell growth and survival. Photo-aging, on the other hand, leads to a strong increase in MMP activity without affecting the amount or activity of TIMPs, creating a misbalance that favors MMPs activity [17, 24, 25].

Because the data in scientific papers present a wide range of activity of TIMPs, but nothing specific for LM/LMM, we have already started a focused approach to TIMPs expression in this particular lesion. Some preliminary data are already available (unpublished data) about TIMPs expression in a batch of LM/LMM compared to its expression in a control batch consisted of 30 samples of photo-aged skin. The expression of TIMP-1 was negative in 50% of control batch and in all cases of LM/LMM at cytoplasmic level (p=0.000 Fisher's exact test) (Figure 1).

The cytoplasmic expression of TIMP-2 was positive in 4/22 cases in the LM/LMM batch. In all cases, the control group cytoplasmic expression of TIMP-2 was negative (p=0.015 Fischer's exact test) (Figure 2). Nuclear and cytoplasmic TIMP-3 expression were appreciated both separately and concomitant. 18/22 cases from LM/LMM batch and 12/30 from control batch were presenting cytoplasmic +/- nuclear positivity (p=0.002 Fischer's exact test). We found out that TIMP-3 cytoplasmic expression was present in 7/30 cases in control batch and in 18/22 cases from LM/LMM batch (p=0.000 Fischer's exact test). TIMP-3 immunoexpression was positive at nuclear level in 4/22 cases in LM/LMM batch and seven of cases in the control batch, but the association is not having any statistical significance (Figure 3).

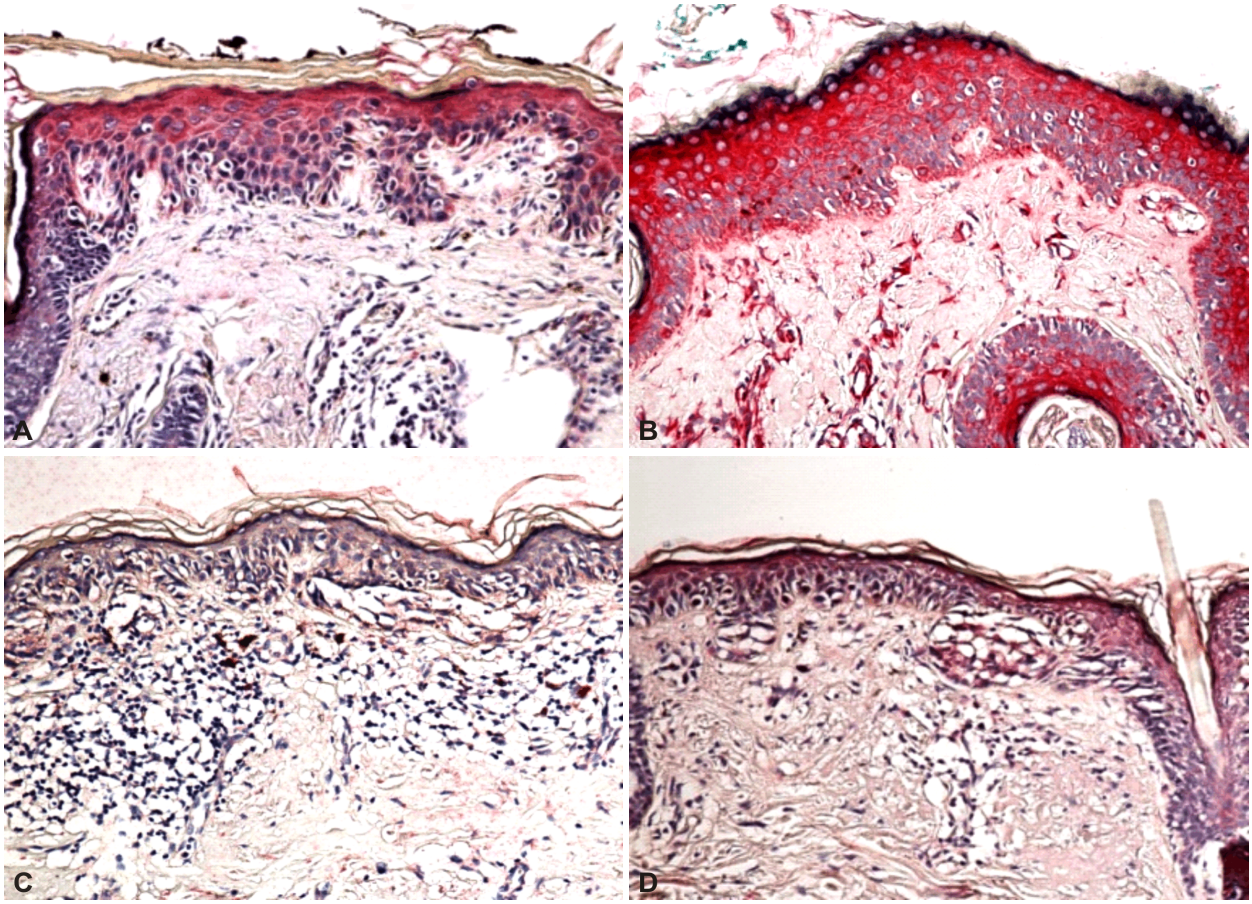

Figure 1 – ***TIMP-1: (A) Control skin – basal hypertrophic melanocytes are positive at cytoplasmic level for TIMP-1 (×200); (B) Control skin – basal melanocytes express variable nuclear positivity for TIMP-1 (×400); (C) LMM – tumor nests are negative for TIMP-1 (×200); (D) LM – tumor junctional cells are negative for TIMP-1 (×200).***

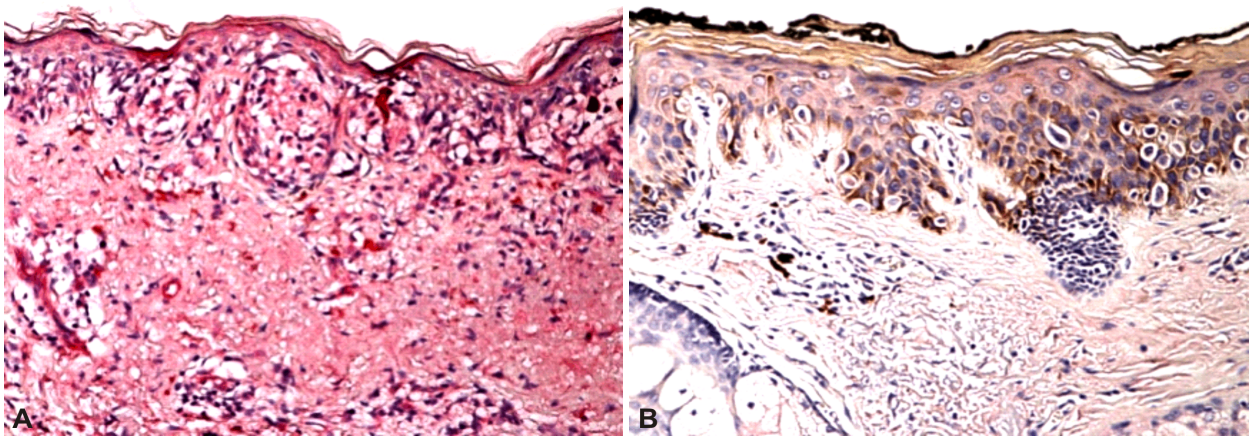

Figure 2 – ***TIMP-2: (A) LMM – junctional tumor nests are positive for TIMP-2 (×200); (B) LM – junctional tumor nests are negative for TIMP-2 (×200).***

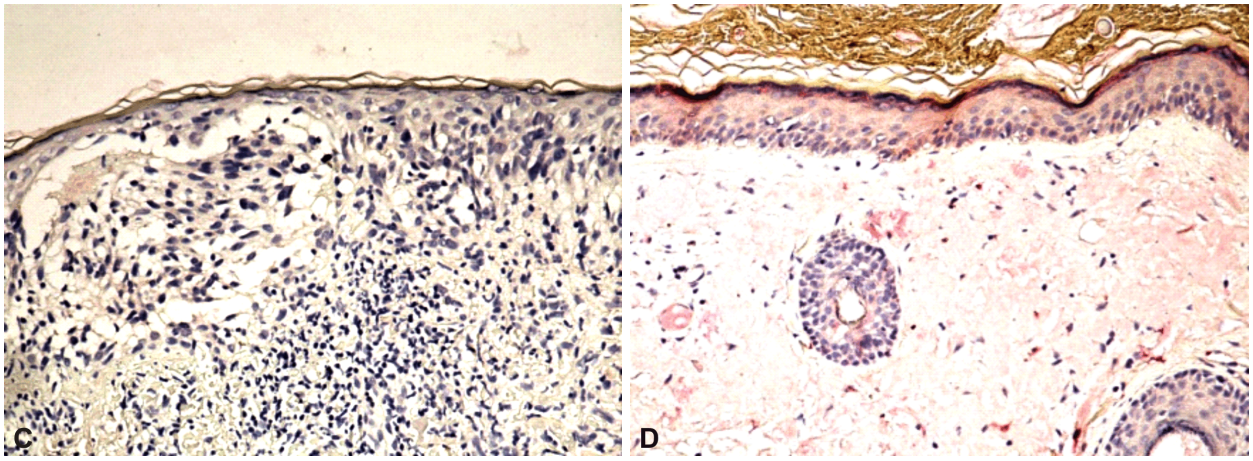

Figure 2 (continued) – ***TIMP-2: (C) LMM – junctional tumor nests are negative for TIMP-2 (×200); (D) Control skin – basal hypertrophic melanocytes are negative for TIMP-2 (×200).***

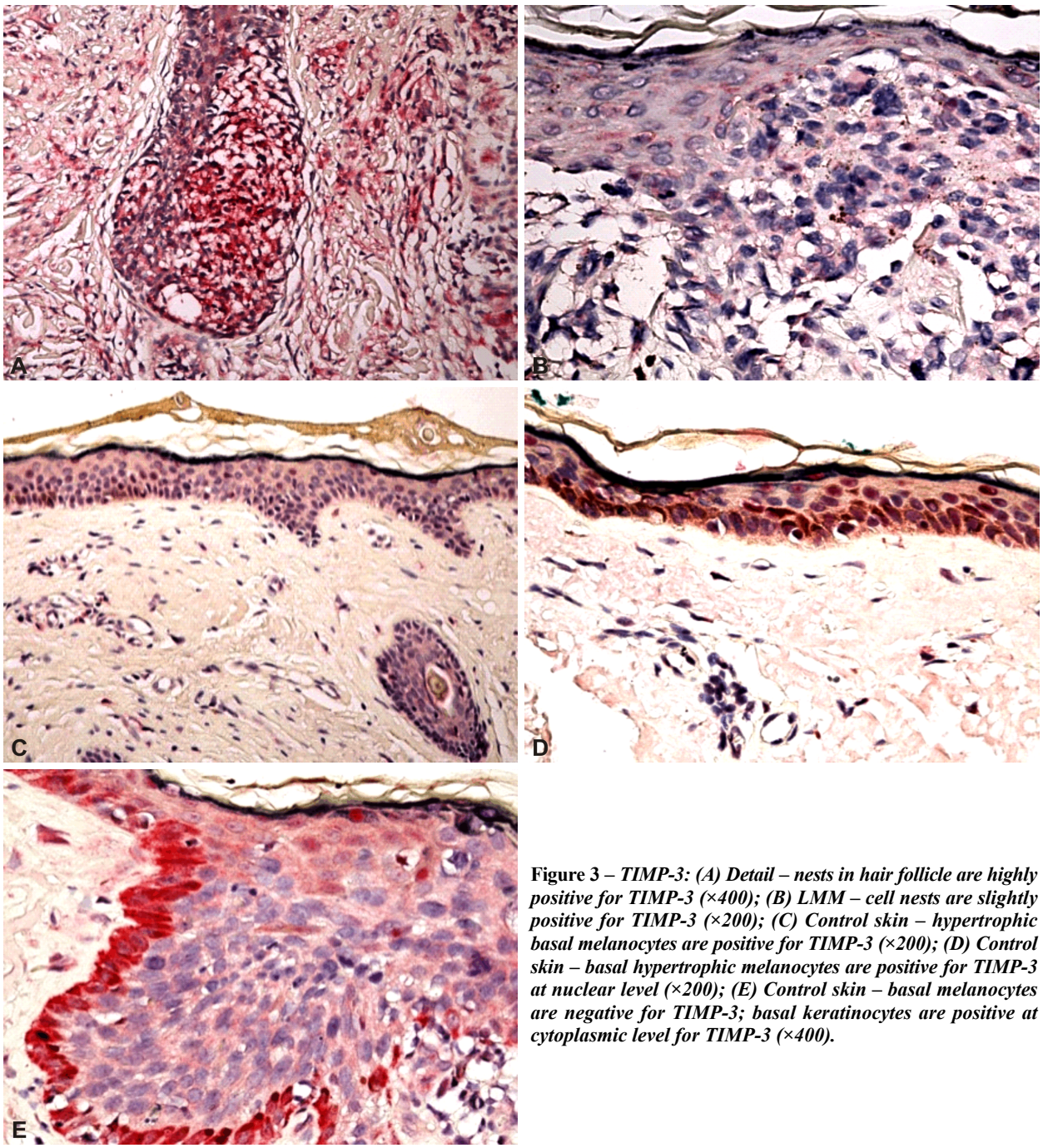

Figure 3 – ***TIMP-3: (A) Detail – nests in hair follicle are highly positive for TIMP-3 (×400); (B) LMM – cell nests are slightly positive for TIMP-3 (×200); (C) Control skin – hypertrophic basal melanocytes are positive for TIMP-3 (×200); (D) Control skin – basal hypertrophic melanocytes are positive for TIMP-3 at nuclear level (×200); (E) Control skin – basal melanocytes are negative for TIMP-3; basal keratinocytes are positive at cytoplasmic level for TIMP-3 (×400).***

Conclusions

It is well established by now that progression of melanoma depends on ECM remodeling, that depends on many molecules, TIMPs family being one of the most important regulators of this process. Considering the multitude of molecules involved in cancer invasiveness and their complex interaction, it is too early to analyze and to conclude upon the significance of different expression of TIMPs in LM/LMM especially because not only the qualitative but also the quantitative and semi quantitative expression of TIMPs could be relevant. Some correlations are needed to be done also with other consecrated histological (as Clark level, Breslow index, presence of ulceration, mitotic index, intratumor inflammatory infiltrate, etc.) and immunohistochemical markers [cadherin, vascular endothelial growth factor (VEGF), bcl-2, etc.] of prognosis and metastasis. In this light, we consider that our study could further clarify the meaning of TIMPs overexpression and loss of expression in this specific type of melanoma.

Conflict of interests

The authors declare that they have no conflict of interests.

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