REVIEW

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Matrix metalloproteinases expression in lentigo maligna/ lentigo maligna melanoma – a review of the literature and personal experience

ALICE BRÎNZEA[1,2)], ROXANA IOANA NEDELCU[1)], DANIELA ADRIANA ION[1)], GABRIELA TURCU[2,3)], MIHAELA ANTOHE[1)], ANASTASIA HODOROGEA[1,2)], ANDREEA CĂLINESCU[2)], DANIEL PIRICI[4)], RALUCA POPESCU[2)], CĂTĂLIN MIHAI POPESCU[2)], CRISTIANA GABRIELA POPP[5)], LUCIANA NICHITA[5)], MIRELA DANIELA CIOPLEA[5)], MARIANA CORDUN[6)], SABINA ANDRADA ZURAC[5)]

[1)] *Department of Pathophysiology, Laboratory of Experimental Medicine and Fundamental Research, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

[2)] *Department of Dermatology I, "Colentina" Clinical Hospital, Bucharest, Romania*

[3)] *Department of Dermatology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

[4)] *Department of Research Methodology, University of Medicine and Pharmacy of Craiova, Romania*

[5)] *Department of Pathology, "Colentina" Clinical Hospital, Bucharest, Romania*

[6)] *Faculty of Kinetotherapy, National Academy of Physical Education and Sports, Bucharest, Romania*

Abstract

Cutaneous melanoma is the most aggressive type of skin cancer, with high invasive potential. Lentigo maligna melanoma (LMM) is a relatively rare type, accounting for about 10% of all melanomas, while the most common subtype of melanoma on the face, typically on chronically sun-exposed skin of elderly people. Its *in situ* stage is lentigo maligna (LM). During the process of transformation from LM to LMM, tumor cells secrete or induce the release from neighboring cells of large amounts of matrix metalloproteinases (MMPs) that degrade the extracellular matrix. Some MMPs, as MMP3 and MMP9 expressed melanoma cells is associated with statistical significance in both *in vitro* and *in vivo* studies, with an invasive phenotype. Unfortunately, there is scarce data published about MMPs expression in LM/LMM, as majority of research on melanoma refer to superficial spreading and nodular melanoma. Our personal, unpublished yet fully data is an attempt to complete a specific panel of immunohistochemical markers that could explain the slow growing rate of LMM.

Keywords: lentigo maligna/lentigo maligna melanoma, MMP3, MMP9.

Introduction

The skin is the first interface of the human body with the ultraviolet (UV) radiation and can suffer complex changes, depending on genetics [1], the type and length of exposure [2, 3] from acute sunburn to premature photo-aging and even cancer [4, 5]. Cutaneous melanoma is the most aggressive form of skin cancer, with high invasive potential [6]. Lentigo maligna (LM) is a melanocytic proliferation that follows a two-dimensional horizontal growth pattern, dermal–epidermal junction limited. Left untreated, it invades the dermis and becomes lentigo maligna melanoma (LMM), at a rate with high variability (5–50%) [7]. LMM is a relatively rare type, accounting for about 10% of all melanomas, while the most common subtype of melanoma on the face, typically on chronically sun-exposed skin of elderly people, with increasing incidence in people older than 45 years, at a greater rate than for any other melanoma subtype [8]. Diagnosing LM is challenging clinically and histopathologically, as it shares many features with other pigmented macules on photo-exposed skin. The difficulty of differentiating between the tumor and photodamaged perilesional skin may lead to inadequate therapeutic decisions.

Matrix metalloproteinases

The integrity of cell and tissue activities is maintained by extracellular matrix (ECM) [9, 10]. ECM proteolysis is mediated by two classes of enzymes, plasmin/plasminogen activator system and matrix metalloproteinases (MMPs) family [11]. MMPs family, zinc-dependent endopeptidases, is involved also in complex physiological or pathological processes of tissue remodeling associated with morpho-genesis, wound repair, inflammation, angiogenesis and metastasis. Many studies assessed the MMPs roles in inflammatory, autoimmune and tumor skin conditions [12, 13]. At skin level, MMPs are mainly produced by epidermal keratinocytes and dermal fibroblast. Synthesis of ECM is finely tuned by a balance between MMPs and their endogenous inhibitors, called tissue inhibitors of matrix metalloproteinases (TIMPs), that reversibly inhibit the MMPs in a 1:1 stoichiometric ratio [14, 15]. Most migratory cells express membrane MMPs or these are secreted by advancing edge cells that spawn through digested ECM and thus support cell propulsion. MMPs are able to degrade all essential components of ECM and basal membrane and their high activity correlates with malignant tumor progression [16, 17]. After degradation, ECM releases and activates cytokine and matrix fragments that modulate

cellular growth, migration and angiogenesis [18]. Some MMPs are associated with growth and expansion of tumor mass, while others are responsible for *in situ* progression, microvascular invasion and metastasis [19]. Tumor cells use this capacity of ECM degradation for spreading. It seems that MMPs also promote cellular growth once they metastasis [20].

Also, the enzyme/inhibitor balance has been shown to favor the enzyme in some cancers tissue compared with adjacent, non-tumor tissue [14], and thus, consideration of the levels of natural MMPS inhibitors (such as TIMPs) should be made. Specifically, an increase in MMPs levels and low TIMPs level is traditionally believed to be involved in tumor expansion.

As such, during the process of transformation from LM to LMM, tumor cells secrete or induce the release from neighboring cells of large amounts of proteases that degrade the ECM. There is a non-homogenous temporal expression of MMPs that allows selective protein degradation in ECM and efficient migration of tumor cells. Expression of several proteinase invasiveness can be an important issue regarding the ability of metastasis.

So far, more than 20 types of MMPs have been described, either secreted or membrane bound, divided in five groups, according to their substrate: gelatinases (72 kDa gelatinase – MMP2 and 92 kDa gelatinase – MMP9), collagenases (MMP1, 8 and 13), stromelysins (MMP3, 10 and 11), matrilysins (MMP7) and membrane type (MMP14 and 17) though some new MMPs that cannot be classified in the aforementioned groups. Gelatinases are involved in the first steps of tumor invasion and these have the ability to degrade the collagen in the basal membrane [21].

UV rays and MMPs

Aged skin includes intrinsic changes (chronological ageing) and extrinsic changes (caused by chronic UV exposure) [8, 22]. Type B UV radiation (UVB) plays a key role in sunburn and skin cancer development by inducing deoxyribonucleic acid (DNA) damage [23]. While in the normal skin MMPs are in a perfect equilibrium with their TIMPs, while ageing, it was described an exaggerated MMPs activity which was associated with the excess degradation and damage of dermal ECM, and furthermore with the clinics of aged skin as wrinkles. High level of MMP induced by UV radiation disrupts the cutaneous physiological equilibrium. There is quite a difference between physiological aged skin, where it is described an increase in MMPs activity associated with downregulation of TIMPs, and the profile of photoaged skin where a strong increase in MMP activity was described, without affecting the amount or activity of TIMPs, creating a misbalance that favors MMPs activity and further, a lytic status of the ECM.

Type A UV radiation (UVA) penetrates the skin more deeply than UVB and is mainly involved in photoaging by also indirect generation of reactive oxygen species [24].

MMPs in melanoma

Of MMPs, expression of MMP1, 2, 3, 9, 14, 15 and 16 by melanoma cells is associated with statistical significance in both *in vitro* and *in vivo* studies, with an invasive phenotype [25, 26]. From this point of view, the majority of research on melanoma refers to superficial spreading and nodular melanoma. As far as we know, a study of this specific constellation of immunohistochemical (IHC) markers has not been reported yet in the literature in LM/LMM. The few reports in the literature describe the effect of the cell-culturing process on the expression pattern changes in MMPs. Cell lines derived from human cancers are the most widely utilized model for *in vitro* cancer research [27]. Although *in vitro* experiments can never reproduce the complexity of a whole organism, the simplicity of cell lines provides the ability to manipulate and analyze individual parameters specifically [28].

It is well known that lack of proper attachment to ECM leads to melanocyte apoptosis [29, 30]. Tumor invasion as well as metastasis are complex processes with several stages involving loss of growth control, cell attachment and increased migration capacity, ECM proteolysis and cell colonies formation in the target organs of metastasis. The induction of cell motility and survival in the ECM are crucial for a cell to become metastatic. When the melanocyte cell succeeds in leaving the epidermis and invading the dermis, it must survive in a different type of environment made mainly out of collagen. Receptors for growth factors activation protects the cell from apoptosis and induces cell migration in three-dimensional collagen environment [30, 31]. Metastatic tumor spreading is the most important cause of death in cancer [32].

MMP9 overexpression has been proved to greatly contribute to tumor invasion and spreading, being associated with tumor dissemination [28]. MMP9 digests, through proteolysis, gelatin (denatured collagen), type IV, V, XI and XVI collagen, elastin, decorin, fibrillin, laminin, and also activates growth factors transforming growth factor beta precursor (proTGFβ) and tumor necrosis factor alpha precursor (proTNFα), being involved in photoaging and horizontal (radial) growth phase of melanoma and tumor angiogenesis [32].

Because the data presented in different reports present a wide range of activity of MMPs in melanoma in general but nothing specific for LM/LMM, we have aimed for a focused approach to MMPs' expression in this type of melanoma. What is particular to LMM *versus* other melanomas is the fact that it evolves very slow, compared with superficial spreading melanoma, at same level of invasion. Some preliminary data are already available (unpublished data) about MMPs expression in a group of LM/LMM cases. We conducted a retrospective study on MMPs expression in a batch of 22 LM/LMM. A control group represented by fragments of photoaged skin from people with the same age and gender profile was studied. It consisted in 30 samples of skin taken from approximately the same anatomical regions, as the LM/LMM lesions were located.

We assessed the expression of MMP9 in LM/LMM group *versus* the controls. Only 3/22 cases studied showed positivity for MMP9, while all cases from the control batch were MMP9 negative (p=0.037, Fischer's Exact Test) (Figure 1). This evidence suggests its role as a prognostic factor. In order to clarify the role of MMP9 in the pathogenesis of LM/LMM it would be with of great value not only to assess its expression but also to

correlate MMP9 protein expression with the survival of patients. Furthermore, the relatively low expression of MMP9 in our study, especially in the studied batch of LM/LMM, may need epigenetic analysis in order to distinguish if MMP9 overexpression is directly associated with tumor progression and not with other co-morbidities or with inflammatory status given by other medical condition. MMP9, aberrantly expressed even in early melanoma stages could be of therapeutic value. This may contribute to an improvement in the quality of life and life span of these patients, as MMP9 expression is usually associated with melanoma progression and poor prognosis.

MMP3 (stromelysin-1) was among the first proteinases proved to be associated with cancer. It can hydrolyze fibronectin, type IV, V, IX and X collagens, elastin, laminins, gelatin and proteoglycan backbone protein. It can also activate other proMMPs, including the MMP1 and MMP13 collagenases. MMP3 has not been detected in 'normal' skin areas at distance from melanoma tumors, while a high expression has been reported in the deep edges of melanoma and in the ECM surrounding blood vessels, suggesting an important contribution of this enzyme to tissue remodeling phenomenon that associates with the high invasive growth of melanoma [19, 33]. Considering the epithelial cell-specific expression pattern of MMP3, we have investigated also (unpublished data) the MMP3 protein expression in cases of LM/LMM *versus* the control tissues, by immunohistochemistry. All participating patients expressed no MMP3, not even in chronically sun-aggressed skin (Figure 2).

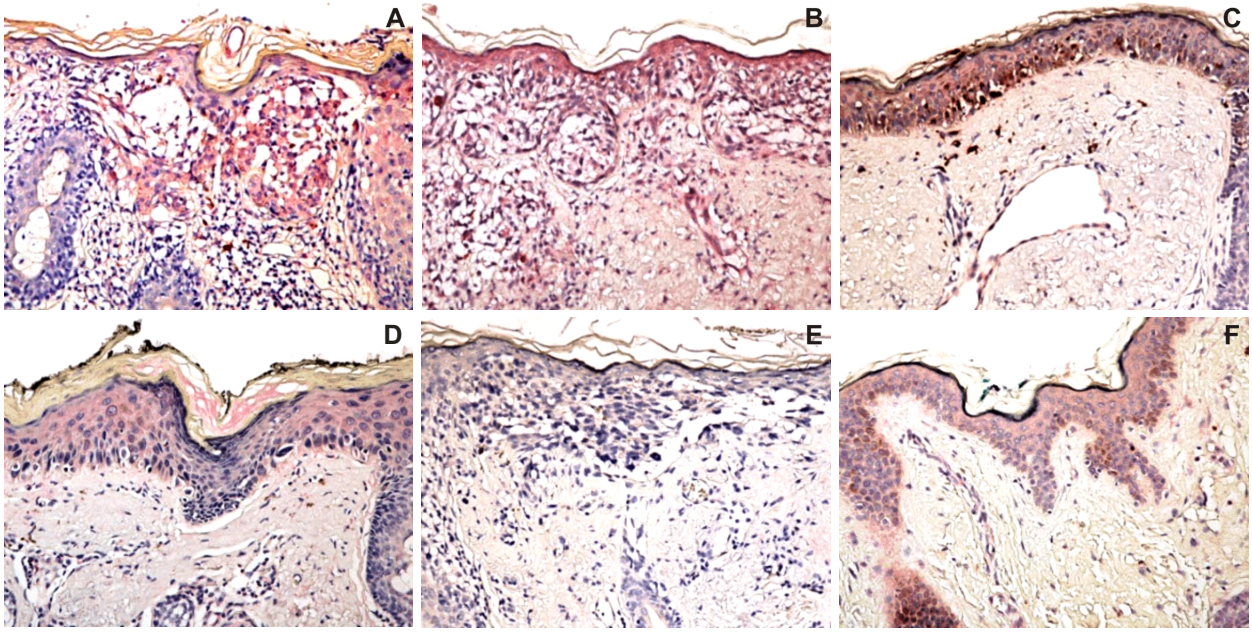

Figure 1 – ***MMP9 immunoexpression: (A) LMM – junctional tumor nests are positive for MMP9; (B) LMM – junctional tumor nests are light focal positive for MMP9; (C) LM – some of junctional tumor cells are positive for MMP9; (D) LM – tumor cells are negative for MMP9; (E) LMM – junctional tumor nests are negative for MMP9; (F) Control skin – basal hypertrophic melanocytes are negative for MMP9. Anti-MMP9 antibody immunomarking: (A–F) ×200. MMP9: Matrix metalloproteinase 9; LMM: Lentigo maligna melanoma; LM: Lentigo maligna.***

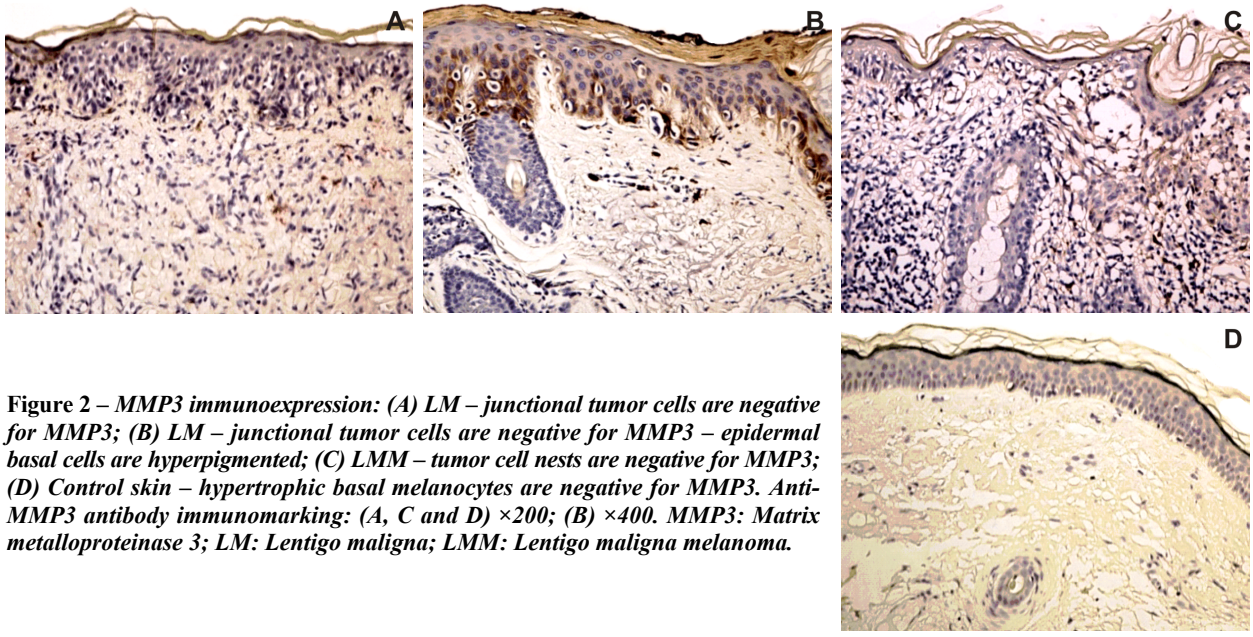

Figure 2 – ***MMP3 immunoexpression: (A) LM – junctional tumor cells are negative for MMP3; (B) LM – junctional tumor cells are negative for MMP3 – epidermal basal cells are hyperpigmented; (C) LMM – tumor cell nests are negative for MMP3; (D) Control skin – hypertrophic basal melanocytes are negative for MMP3. Anti-MMP3 antibody immunomarking: (A, C and D) ×200; (B) ×400. MMP3: Matrix metalloproteinase 3; LM: Lentigo maligna; LMM: Lentigo maligna melanoma.***

In the present study, MMP3 does not seem to be involved neither in benign nor in malignant transformation of LM, probably because this type of melanoma is not an aggressive one. Moreover, in the same line, functional polymorphism in the promoter regions of MMP2 and MMP3 has been recently showed not to be related to an increase risk for melanoma progression [16].

Conclusions

Understanding complex molecular mechanisms and identification their interactions may lead to important information about initiation and tumor progression as well as about patients' prognosis. For this purpose, these markers should be assessed in different stages of carcinogenesis in order to identify potential prognostic and therapeutic targets. The importance of MMPs in malignant melanocytes behavior was revealed, but there are scarce reported data in the literature describing IHC expression of MMP3 and MMP9 in this specific type of melanoma – LM/LMM. Results from our study, although not specific, being performed on human skin pathology cases, are becoming more valuable mainly because inhibition of expression and/or enzymes' activity of these markers may be effective targets for preventing tumor invasion and metastasis.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

This paper is supported by the Executive Agency for Higher Education, Research, Development and Innovation (UEFISCDI) under the identification code PN-III-P4-ID-PCE-2016-0641 (Contract No. 183).

References

[1] Goggins W, Tsao H. A population-based analysis of risk factors for a second primary cutaneous melanoma among melanoma survivors. Cancer, 2003, 97(3):639–643.
[2] Armstrong BK. Epidemiology of malignant melanoma: intermittent or total accumulated exposure to the sun? J Dermatol Surg Oncol, 1988, 14(8):835–849.
[3] Ting W, Schultz K, Cac NN, Peterson M, Walling HW. Tanning bed exposure increases the risk of malignant melanoma. Int J Dermatol, 2007, 46(12):1253–1257.
[4] Brenneisen P, Sies H, Scharffetter-Kochanek K. Ultraviolet-B irradiation and matrix metalloproteinases: from induction *via* signaling to initial events. Ann N Y Acad Sci, 2002, 973:31–43.
[5] Kim HH, Cho S, Lee S, Kim KH, Cho KH, Eun HC, Chung JH. Photoprotective and anti-skin-aging effects of eicosapentaenoic acid in human skin *in vivo*. J Lipid Res, 2006, 47(5):921–930.
[6] Gajda M, Kamińska-Winciorek G, Wydmański J, Tukiendorf A. "Better do not touch" and other superstitions concerning melanoma: the cross-sectional web-based survey. Postepy Dermatol Alergol, 2016, 33(5):329–335.
[7] McKenna JK, Florell SR, Goldman GD, Bowen GM. Lentigo maligna/lentigo maligna melanoma: current state of diagnosis and treatment. Dermatol Surg, 2006, 32(4):493–504.
[8] Swetter SM, Boldrick JC, Jung SY, Egbert BM, Harvell JD. Increasing incidence of lentigo maligna melanoma subtypes: northern California and national trends 1990–2000. J Invest Dermatol, 2005, 125(4):685–691.
[9] Fridman R. Metalloproteinases and cancer. Cancer Metastasis Rev, 2006, 25(1):7–8.
[10] Ortiz R, Díaz J, Díaz N, Lobos-Gonzalez L, Cárdenas A, Contreras P, Díaz MI, Otte E, Cooper-White J, Torres V, Leyton L, Quest AFG. Extracellular matrix-specific caveolin-1 phosphorylation on tyrosine 14 is linked to augmented melanoma metastasis but not tumorigenesis. Oncotarget, 2016, 7(26):40571–40593.
[11] Zhang Z, Zhu S, Yang Y, Ma X, Guo S. Matrix metalloproteinase-12 expression is increased in cutaneous melanoma and associated with tumor aggressiveness. Tumour Biol, 2015, 36(11):8593–8600.
[12] Terp G, Christensen IT, Jørgensen FS. Structural differences of matrix metalloproteinases. Homology modeling and energy minimization of enzyme–substrate complexes. J Biomol Struct Dyn, 2000, 17(6):933–946.
[13] Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA. Matrix metalloproteinases: a review. Crit Rev Oral Biol Med, 1993, 4(2): 197–250.
[14] Giricz O, Lauer JL, Fields GB. Variability in melanoma metalloproteinase expression profiling. J Biomol Tech, 2010, 21(4): 194–204.
[15] Edwards DR. The tissue inhibitors of metalloproteinases (TIMPs): biology and regulation. In: Clendeninn NJ, Appelt K (eds). Matrix metalloproteinase inhibitors in cancer therapy. Series "Cancer Drug Discovery and Development", Springer Science–Humana Press, 2001, 67–84.
[16] Cotignola J, Roy P, Patel A, Ishill N, Shah S, Houghton A, Coit D, Halpern A, Busam K, Berwick M, Orlow I. Functional polymorphisms in the promoter regions of MMP2 and MMP3 are not associated with melanoma progression. J Negat Results Biomed, 2007, 6:9.
[17] Stetler-Stevenson WG, Aznavoorian S, Liotta LA. Tumor cell interactions with extracellular matrix during invasion and metastasis. Annu Rev Cell Biol, 1993, 9:541–573.
[18] McCawley LJ, Matrisian LM. Matrix metalloproteinases: they're not just for matrix anymore! Curr Opin Cell Biol, 2001, 13(5): 534–540.
[19] Bodey B, Bodey B Jr, Siegel SE, Kaiser HE. Matrix metalloproteinase expression in malignant melanomas: tumor–extracellular matrix interactions in invasion and metastasis. *In Vivo*, 2001, 15(1):57–64.
[20] John A, Tuszynski G. The role of matrix metalloproteinases in tumor angiogenesis and tumor metastasis. Pathol Oncol Res, 2001, 7(1):14–23.
[21] Stetler-Stevenson W. Type IV collagenases in tumor invasion and metastasis. Cancer Metastasis Rev, 1990, 9(4):289–303.
[22] Kvaskoff M, Siskind V, Green AC. Risk factors for lentigo maligna melanoma compared with superficial spreading melanoma: a case-control study in Australia. Arch Dermatol, 2012, 148(2):164–170.
[23] Trautinger F. Mechanisms of photodamage of the skin and its functional consequences for skin ageing. Clin Exp Dermatol, 2001, 26(7):573–577.
[24] Lallas A, Tschandl P, Kyrgidis A, Stolz W, Rabinovitz H, Cameron A, Gourhant JY, Giacomel J, Kittler H, Muir J, Argenziano G, Hofmann-Wellenhof R, Zalaudek I. Dermoscopic clues to differentiate facial lentigo maligna from pigmented actinic keratosis. Br J Dermatol, 2016, 174(5): 1079–1085.
[25] Hendrix MJ, Seftor EA, Hess AR, Seftor RE. Molecular plasticity of human melanoma cells. Oncogene, 2003, 22(20): 3070–3075.
[26] Bauman P, Zingrino P, Mauch C, Breitkreutz D, Nischt R. Membrane-type 1 matrix metalloproteinase-mediated progelatinase A activation in non-tumorigenic and tumorigenic human keratinocytes. Br J Cancer, 2000, 83(10):1387–1393.
[27] Masters JRW, Palsson B (eds). Human cell culture. Cancer cell lines Part 1. Vol. 1, Book Series "Human Cell Culture" (HUUC), Kluwer Academic Publishers–Springer, Dordrecht, 1999.
[28] Tuschl G, Mueller SO. Effects of cell culture conditions on primary rat hepatocytes – cell morphology and differential gene expression. Toxicology, 2006, 218(2–3):205–215.
[29] Alanko T, Rosenberg M, Saksela O. FGF expression allows nevus cells to survive in three-dimensional collagen gel under conditions that induce apoptosis in normal human melanocytes. J Invest Dermatol, 1999, 113(1):111–116.

[30] Geissinger E, Weisser C, Fischer P, Schartl M, Wellbrock C. Autocrine stimulation by osteopontin contributes to antiapoptotic signalling of melanocytes in dermal collagen. Cancer Res, 2002, 62(16):4820–4828.
[31] Russo A, Ficili B, Candido S, Pezzino FM, Guarneri C, Biondi A, Travali S, McCubrey JA, Spandidos DA, Libra M. Emerging targeted therapies for melanoma treatment (review). Int J Oncol, 2014, 45(2):516–524.
[32] Ansieau S, Hinkal G, Thomas C, Bastid J, Puisieux A. Early origin of cancer metastases: dissemination and evolution of premalignant cells. Cell Cycle, 2008, 7(23):3659–3663.
[33] Walker RA, Woolley DE. Immunolocalisation studies of matrix metalloproteinases-1, -2 and -3 in human melanoma. Virchows Arch, 1999, 435(6):574–579.

Corresponding author
Roxana Ioana Nedelcu, Assistant Professor, MD, PhD, Department of Pathophysiology, Laboratory of Experimental Medicine and Fundamental Research, "Carol Davila" University of Medicine and Pharmacy, "Prof. Dr. Matei Balş" National Institute of Infectious Diseases, 1 Dr Calistrat Grozovici Street, Sector 2, 021105 Bucharest, Romania; Phone +40721–916 624, e-mail: roxanaioana.nedelcu@yahoo.com

Received: October 21, 2019

Accepted: February 25, 2020