REVIEW

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

The role of tumor microenvironment in development and progression of malignant melanomas – a systematic review

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Abstract

To reveal the particular aspects of the tumor microenvironment of malignant melanomas, a systematic review including 34 representative papers was performed. The review took into account the aspects related the Wnt/β-catenin pathway-related epithelial–mesenchymal transition (EMT) *versus* mesenchymal–epithelial transition (MET) of keratinocytes, fibroblasts and melanoma cells, as possible tools for understanding genesis and evolution of malignant melanoma. The possible reversible features of EMT and the role of tumor microenvironment in the metastatic process were also analyzed. A particular issue was related on the cancer stem cells that include melanocyte stem cells (McSCs) and multipotent mesenchymal stem/stromal cells (MSCs). As the McSCs embryological development in mouse is not similar to human development, the role of stem cells in genesis and development of human melanoma should be proved in human melanoma cells only. For further development of targeted therapy, a better understanding of melanomagenesis pathways and its microenvironment particularities is necessary.

Keywords: epithelial–mesenchymal transition, mesenchymal–epithelial transition, β-catenin, cadherin, stem cells, Wnt/β-catenin pathway.

Introduction

Although melanomas represent less than 5% of all skin cancers, they are responsible for 70–80% of skin cancer deaths, with a five-year overall survival rate less than 15–20%, for metastatic tumors [1, 2]. For this reason, exploring the tumor–stroma interaction and its molecular particularities is necessary, taking into account all of the stroma components that includes fibroblasts, endothelial cells, immune cells, soluble molecules, and the extracellular matrix [2, 3].

To understand the multistep melanomagenesis, it is mandatory understanding the embryonic development of melanocytes. It is well known that, during embryogenesis, melanocytes derive from the N-cadherin-positive neural crest [4]. Delamination and migration of neural crest-derived progenitors of melanocytes (non-pigmented melanoblasts), from spinal ganglia to the epidermis, is controlled by specific molecular pathways [1, 4–7]. The Wnt family members (E-cadherin, N-cadherin, β-catenin, P-cadherin and H-cadherin) cooperate with other agents such bone morphogenetic proteins (BMPs) and their antagonists, transcription factors (SNAIL, SLUG, TWIST, PAX3, SOX10), the fibroblastic growth factor (FGF) and NOTCH pathway families [1, 4–7]. Disengage from neural crests and migration of neural progenitors requires epithelial–mesenchymal transition (EMT), defined by loss of the adhesion molecules E-cadherin, neuronal cell adhesion molecule (NCAM), cadherin 6B and N-cadherin and gain of other substances, such as cadherin 7, cadherin 11, etc. [4, 7–9].

After migration in the epidermis and differentiation into pigment-producing melanocytes, a reverse mesenchymal–epithelial transition (MET) is required, to facilitate the melanocytes–keratinocytes connection [9]. This connection is produced *via* E-cadherin, P-cadherin, H-cadherin, desmoglein and connexins [7]. E-cadherin and H-cadherin are highly expressed by basal layer melanocytes, whereas P-cadherin is rather expressed by melanocytes of the hair follicles [7, 9]. Although Wnt/β-catenin signaling is not essential for normal epidermal homeostasis [10], the cadherin-mediated adhesion of the epidermal melanocytes is controlled by β-catenin [1, 11]. N-cadherin is not expressed by the normal epidermis [12].

In this review, we aimed to present data about the possible role of Wnt signaling pathway and its regulatory proteins, together with cancer stem cells and other stromal components, in development and progression of malignant melanoma.

Methodology

Systematic search of literature on the PubMed database using the keywords "melanoma and epithelial mesenchymal transition", "melanoma and mesenchymal epithelial transition" and "melanoma and stem cells" was performed until March 1, 2018. Both experimental studies and publications referring to human tissues were taken into account. The selection of articles was firstly based on the titles, then based on the abstracts and finally, full text was readed to select the eligible papers.

Based on these criteria, from 366 publications identified

on the PubMed database, 34 articles were found eligible for this review (Figure 1). Other papers were added based on manual research or from personal publications in the field. The review was mainly focused on the impact of tumor microenvironment in development and progression of malignant melanoma.

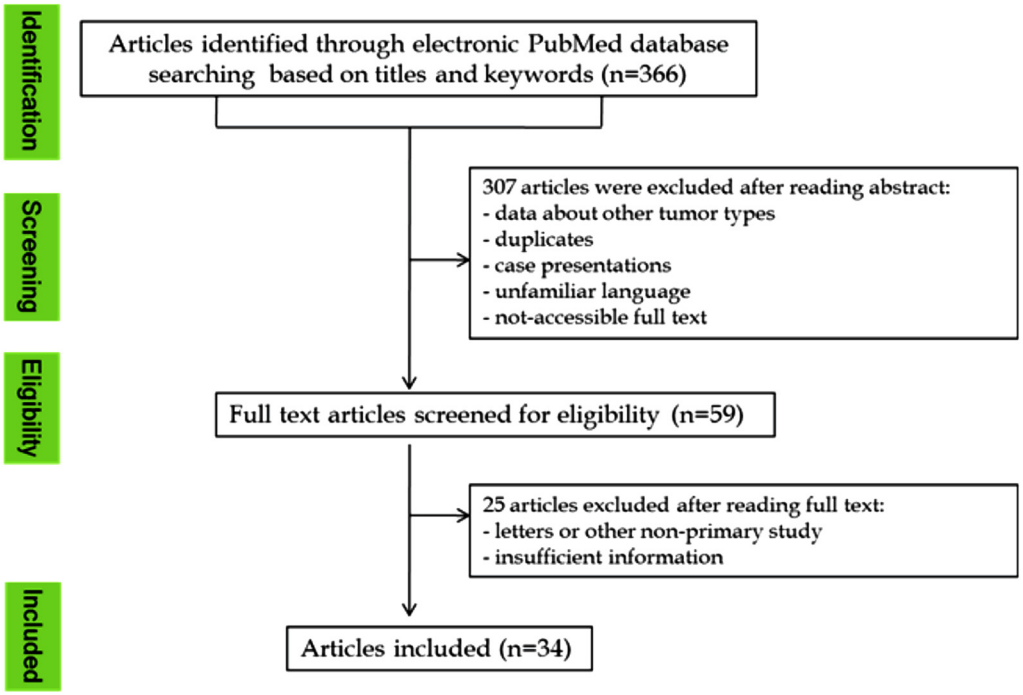

Figure 1 – ***Preferred reported items for systematic reviews and meta-analyses (PRISMA) flow diagram adapted for the data regarding melanoma and the tumor microenvironment published on the PubMed database between 1984 and March 1, 2018***

Development of malignant melanoma

The melanocytes–keratinocytes interaction

In the normal epidermis, localization and proliferation of melanocytes is controlled by keratinocytes through paracrine growth factors and adhesion molecules [3, 13]. Melanocytes are located in the basal layer of the epidermis, each melanocyte being surrounded by about 36 keratinocytes [3, 7].

When the nevus formation is started up, the melanocytes homeostasis is disturbed [3]. In melanomagenesis, due do downregulation of adhesion molecules, such as cadherins, the melanoma cells take over the control of the epidermal tumor microenvironment [3, 13]. Loss of E-cadherin induces N-cadherin upregulation, loss of keratinocytes control over melanocytes and architectural changes and increased motility to the cells with malignant potential [2, 7, 11, 13].

The melanoma cells show a highly heterogenic behavior and they cannot generate connections with the keratinocytes [3]. Each melanoma cell can present different cell-to-cell or cell-to-stroma interaction through endocrine or paracrine mechanisms mediated by hormones, growth factors, cytokines, connexins, etc. [3].

Wnt/β-catenin pathway

Although this molecular pathway was mainly described to induce EMT of carcinoma cells, the most recent studies showed that Wnt-signaling can also induce melanomagenesis and stimulate melanoma cells invasion [4, 14]. The Wnt family members and BMPs seem to be reactivated during melanomagenesis, being responsible by migration of melanocytes from epidermis to dermis [4]. Loss of Wnt/β-catenin signaling might induce malignant transformation of neural crest-derived melanocytes [1, 4, 5, 8].

In carcinomas, the E-cadherin and β-catenin membrane expression is considered an indicator of normal cell adhesion and the EMT is defined as E-cadherin loss, E-cadherin to N-cadherin switch, β-catenin membrane-to-nuclear translocation and gain of vimentin expression [2, 15, 16]. In melanomas, the EMT is defined as loss of cellular adhesion (between keratinocytes–melanocytes and melanoma cells-to-melanoma cells), with changing from an epithelial to a functional mesenchymal phenotype of tumor cells [12, 17–19]. Loss of E-cadherin together with gain of N-cadherin, *via* bcl-3 upregulation, is supposed to reflect the bidirectional interaction between tumor cells and stroma, which includes fibroblasts, inflammatory cells, immune cells, vascular endothelial cells and the extracellular matrix [3, 7, 12, 13, 17, 19, 20]. Fibroblasts and endothelial cells express N-cadherin and can easily interact with N-cadherin positive melanoma cells [13].

The dermal fibroblasts can be transformed in cancer-associated fibroblasts and exert pro-angiogenic/pro-inflammatory role, facilitating tumor cells proliferation and invasion of the remodeled extracellular matrix [13, 19, 20]. The melanoma cells stimulate activation of stromal fibroblasts [21] and their transformation in melanoma-associated myofibroblasts, *via* upregulation of transforming growth factor-β1 (TGF-β1), interleukins (IL6, IL8), or matrix metalloproteinase 1, which are released by melanoma cells in a paracrine manner [13, 22]. These myofibroblasts can secrete cytokines, pro-angiogenic substances and growth factors [19, 20–22].

Wnt/β-catenin signaling interacts with TGF-β1 that induces EMT *via* Wnt/β-catenin, phosphatidyl inositol 3-kinase/protein kinase B (PI3K/Akt), or suppressors of mothers against decapentaplegic (SMADs)-dependent pathways [2]. In human melanoma cell lines, TGF-β1 proved to induce angiogenesis, E-cadherin downregulation, stroma formation and enhancing expression levels of vimentin, Snail and β-catenin [2, 13]. This pathogenetic crosstalk strongly supports tumor–stroma interaction [12, 13].

Melanoma-associated fibroblasts express the immunohistochemical (IHC) markers smooth muscle actin (SMA), fibroblast-specific protein 1 (FSP1), fibroblast activation protein-alpha (FAP-α), platelet-derived growth factor receptor (PDGFR), N-cadherin and even keratin 7 [13, 19, 22]. The N-cadherin-positive transformed melanocytes can interact with the melanoma-associated fibroblasts and migrates in the underlying dermis [19]. Besides transformation in myofibroblasts, the dermal fibroblast can also be transformed in pericytes and promote angiogenesis [13].

SNAIL is considered to be the main repressor of E-cadherin, during both embryonal period (migration of melanoblasts) and melanomagenesis, by binding to E boxes in the E-cadherin promoter [2, 7]. SNAIL is not expressed by normal melanocytes but is diffusely positive in melanoma cells with downregulated E-cadherin [7]. E-cadherin can also be downregulated by other transcriptional factors, such SLUG, TWIST, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and ZEB1 [2, 7, 13, 18]. E-cadherin positivity was not described in the benign nevi [18]. N-cadherin is not expressed by the nevi (with or without dysplasia) confined to the epidermis but its positivity was reported for 24% of compound and 35% of intradermal nevi; this expression was confined to the nests of nevus cells from the dermis [12, 18].

The subcellular localization (membrane, cytoplasmic or nuclear) of β-catenin in melanoma cells seems to not influence the tumor behavior, most of the studies revealing positive or negative reaction, without importance of the expression localization [5, 23]. Cytoplasmic and nuclear β-catenin can be seen in both nevi and early-staged melanomas with low proliferative index, proving that it is rather a marker of melanocyte proliferation and differentiation in the epidermis and not an indicator of malignant transformation [5, 11]. In case of melanoma development, a close cooperation between β-catenin and NRAS was described [11, 24].

Progression of malignant melanoma

Wnt/β-catenin pathway

Discordant results have been published in literature regarding the IHC expression of E-caherin, N-cadherin, β-catenin or Wnt3a in human melanoma cells and few aspects about H-cadherin or cadherin 7 were analyzed.

E-cadherin expression was reported in 20% [17] until 90% [18] of primary melanomas and up to 79% of paired lymph node metastases [18]. It was recently proved that the more aggressive variants, such as desmoplastic melanomas showed a lower expression of E-cadherin compared to the non-desmoplastic melanomas (14% *versus* 61%) [25]. The cytoplasmic N-cadherin positivity can be seen in 28–40% of primary melanomas, especially ulcerated tumors, and 29–40% of lymph node metastases [12, 18, 25–27]. In contrast, up to 60% of desmoplastic melanomas may express N-cadherin [25, 26].

Although E-cadherin/N-cadherin switch was considered to be stage-dependent and reflect the EMT of melanoma cells, the IHC studies did not confirm this absolute switch [7, 12, 18]. The E-cadherin positive/N-cadherin negative expression was reported in 19–36% of malignant melanomas and the reverse expression in 5–23% of the cases [18]. Up to 17–30% of melanomas express both E-cadherin and N-cadherin and 11% are negative for both markers [18]. Other papers revealed that cadherin expression mostly reflect the tumor environment particularities than the tumor cell progression and the EMT is a reversible process that can be changed in every moment [7, 8]. Based on these aspect, the IHC expression of E-cadherin should not be used to estimate the active role of E-cadherin [7]. However, E-cadherin overexpression in melanoma cells might show restoration of keratinocytes control over melanoma cells [13]. On the other hand, N-cadherin can also be activated *via* Notch1 signaling pathway and may induce chemotherapeutics resistance [12]. The prognostic role of E-cadherin and N-cadherin is controversial, although a diffuse positivity of E-cadherin seems to reflect a lower risk for lymph node metastases [7], whereas strong N-cadherin expression indicates repression of the proapoptotic factors [13], high metastatic potential [25, 26] and decreased overall survival [27].

About one third of metastatic melanomas proved to express nuclear β-catenin [6, 23]. β-catenin intensity seems to be enhanced in the invasion front, in cells with spindle-like aspect, and reduced in the cells with epithelial-like morphology [4]. Independently from its subcellular localization, β-catenin was described to block invasion of malignant melanoma cells [5, 13, 23]. In most of the studies, β-catenin positivity was correlated with a low proliferation index [1] and proved to be an independent indicator of good survival [5], whereas loss of β-catenin was considered as a negative prognostic factor [1, 4]. In cell lines, loss of E-cadherin induced β-catenin cytoplasmic upregulation and N-caherin positivity [5].

If β-catenin indeed reduces melanoma cells migration [5, 23], it can be considered that, loss of Wnt/β-catenin homeostasis induces melanoma development [1] but the invasive properties of melanoma cells could be down-regulated *via* Wnt/β-catenin signaling pathway [8]. In cell cultures, loss of β-catenin signaling induces deactivation of cancer-associated stromal fibroblasts and decreases melanoma growth and progression [20]. The β-catenin deficient stromal fibroblasts were earlier recruited and were larger than fibroblasts developed in cultures with active β-catenin [20].

In other studies it was showed that β-catenin inhibits apoptosis and increases melanoma cells proliferation, migration and invasion [11]. Based on these aspects, it can be showed that β-catenin represses or promotes invasion of melanoma cells, depending on the protocol and cell lines [23]. These contradictory results prove that, in melanomas, β-catenin exerts different function compared to other cancers, this function being still unknown [5].

The melanoma-specific nuclear transcription factor MITF was shown to be amplified in 10% of primary and 21% of metastatic melanomas, exerting a central role in melanoma cell proliferation, migration and invasion [1, 6, 8]. MITF positivity is higher in desmoplastic melanomas (29% of cases) [21]. MITF seems to exert antiapoptotic effect against melanoma cells *via* bcl-2, livin or BPTF [8]. Other authors revealed a direct correlation of MITF with IHC expression of E-cadherin and reversely with N-cadherin and concluded that it has an anti-invasive role in melanomas [8]. MITF is a target of *β*-catenin–T-cell factor/lymphoid enhancer factor [22]. The prognostic impact of MITF and the molecular pathways of Wnt/MITF alterations are still unclear [6].

Wnt3a is also considered an independent prognostic marker, inversely correlated with the survival rate [4]. In experimental studies, Wnt3a positivity correlated with increased neural crest migration and primary melanoma cells migration from epidermis through the dermis [4]. In melanomas, Wnt3a positivity is correlated with decreased melanoma size and decreased metastatic rate [1] and also with low phosphatase and tensin homolog (PTEN) expression [4].

The activation of Wnt/*β*-catenin signaling in primary melanomas also leads to upregulation of specific genes that induce normal melanocyte differentiation, such as *met* or *kit* [1]. Although most of the authors admit the suppressive role of *β*-catenin against melanoma cells proliferation [1, 4, 5], other studies revealed opposite results and showed the necessity of molecular classification of melanomas. For example, Damsky *et al.* showed that, in BRAF-activated PTEN-deficient pigmented melanomas, *β*-catenin is the main mediator of occurrence of lymph node and distant metastases (in lung, bowel and spleen) and melanomagenesis is suppressed by inactivation of *β*-catenin [6]. This is probably the reason why the *β*-catenin-positive melanomas are usually resistant to the anti-BRAF and anti-programmed death-ligand 1/anti-cytotoxic T-lymphocyte-associated protein-4 (anti-PD-L1/anti-CTLA-4) therapy [4].

Tumor microenvironment and systemic metastases

A particular aspect of malignant melanoma cells is related on their extremely high motility and invasive capacity. These cells invade by modulating the extracellular matrix *via* matrix metalloproteinases (MMPs) or by modification of the cells architecture and of the tumor environment [4, 14]. The MMPs are highly expressed in melanomas (*e.g.*, MMP-2 in 69% of the cases) [17] and are controlled by the tissular inhibitor of MMP [14].

Because loss of E-cadherin and gain of N-cadherin is an indicator of highly invasive capacity, it was hypothesized that systemic metastases can occur *via* the gap junctions formed between tumor cells and vascular endothelium [3, 7]. N-cadherin promotes cell detachment and induces them invasive properties [7, 12]. N-cadherin is also a pro-angiogenic factor that facilitates transmigration of tumor cells through the vascular epithelium [12]. E-cadherin negativity and the E-cadherin/N-cadherin switch are predictors of poor distant metastasis-free survival [12, 18, 28], especially in patients with desmoplastic melanomas [26, 28].

On the other hand, the E-cadherin/N-cadherin switch-mediated melanoma cells–stroma interaction induces development of the vasculogenic mimicry network [3, 7, 17]. It is defined as formation of the extracellular matrix-rich vasculogenic-like channels with red blood cells, in three-dimensional culture [17]. These Periodic Acid–Schiff (PAS)-positive channels are not covered by true endothelium (CD34 negative structures); they are lined by tumor cells with EMT-like phenotype [17]. As in other tumors, vasculogenic mimicry, which is identified in more than 25% of malignant melanomas, indicates an increased risk of recurrence and a highly systemic metastatic risk [17]. Most of melanomas showing vasculogenic mimicry are MMP-2 positive/E-cadherin negative [17]. It was considered that MMP-2 might the main promoter of vasculogenic mimicry formation [17].

Although *β*-catenin inhibits melanoma cells migration, it promotes lung metastasis [23], through a poorly defined mechanism, and is an essential survival factor for aggressive metastatic melanoma cells [11]. It is supposed that *β*-catenin can induce melanoma cells bypassing senescence, especially in NRAS-driven melanomas, but also stimulates the cell–matrix adhesion, especially for cells located close to the lymphatic or systemic vessels [23]. Increased aggressivity was experimentally proved for melanoma cells that were directly injected in the mouse-tail vein [23].

In brain, the metastatic melanoma cells are surrounded by astrocytes. In normal conditions, the astrocytes prevent neuron apoptosis. It was then hypothesized that the peritumoral proliferation of activated astrocytes, but not fibroblasts, might prevent melanoma cells apoptosis and exerts a chemopreventive role [29].

Malignant melanoma and cancer stem cells

In adult mouse, the melanocyte stem cells (McSCs) were identified to be located in the dorsal skin, within hair follicles, and are absent in the epidermis [30–33]. These cells can be activated by several factors that alter the microenvironment, including depilation, ultraviolet type B irradiation, metabolic imbalance, chronic inflammation, wounding, or *via* BRAF, K-ras or PTEN gene mutations [30, 31]. Activation of the PAX3-positive McSCs may be followed by their migration from the follicular stem cell niche to the upper follicular epithelium and then to the basal layer of the epidermis [30, 31]. In the epidermis, an initial cell division of the follicle-derived melanocytes occurs and the follicular McSCs are transformed in melanocytes [30, 31]. They can act as a pigmented protective barrier against external factors or can be involved in the genesis of pigmented melanoma [30, 31].

In human skin, the PAX3-positive McSCs and differentiated melanocytes are present in both follicles and epidermis, during fetal and adult period of life [31]. In wounding denuded human skin, migration of the pigmented follicular melanocytes to the epidermis, during skin re-epithelization, was experimentally proved [31]. Some of the McSCs remain in the follicular niche, being responsible by the hair cycles, but the follicle-derived epidermal melanocytes can also be re-transformed in hair melanocytes [31]. On the other hand, the McSCs remain at the periphery of the wound, repopulate the denunded

epithelium and the intact follicles but are not able to re-pigmented the neogenic follicles from the wound center [31, 33].

In melanoma cell lines, multipotent mesenchymal stem/stromal cells (MSCs) proved to also play roles in both development and progression of malignant melanomas [34–36]. The MSCs show a strong tropism to the melanoma cells and induce them pro-proliferative [*via* galectin-1 or tumor necrosis factor-alpha (TNF-α)], pro-angiogenic [*via* secretion of vascular endothelial growth factor (VEGF)], anti-apoptotic, immunosuppressive (*via* IL2), and EMT-like properties (*via* paracrine secretion of TGF-β and SNAIL upregulation) [34–36]. About 70% of human MSCs present mutations in the BRAF oncogene at exons 11 and 15 but no BRAF mutations were identified in murine MSCs [35, 36]. It was also proved the active role of β-catenin in maintaining the stemness properties of CD34-positive cutaneous cancer cells [10, 11].

Other stem cell factors, such as SOX2, proved to be reversely correlated with the EMT markers E-cadherin and MITF and play central role in self-renewal and aggressivity of human melanoma cells [8]. Hepatocyte growth factor (HGF) and osteonectin also downregulate expression of the adhesion molecules E-cadherin and desmoglein in melanoma cells [13]. The stem-like glycoprotein CD44 is strongly expressed by cancer-associated fibroblasts and might facilitate their interaction with melanoma cells [18]. Expression of the EMT-related markers MITF and nuclear β-catenin are downregulated in the McSCs [31].

Outlook

Further studies are necessary to be performed for a proper understanding of the melanoma cells to stroma interaction. This review showed that the heterogenic aspect of melanoma cells should be examined with a large panel of markers, as the EMT occurs as result of interaction of several pathways and cannot be defined based on a single marker profile. The individualized therapy of malignant melanoma should take into account the reversible mechanism of EMT, should target the possibility of E-cadherin upregulation and the EMT interaction with cancer stem cells-like McSCs and MSCs. As the McSC embryological development in mouse is not similar to human development, the role of stem cells in genesis and development of human melanoma should be proved in human melanoma cells only.

Conflict of interests

None of the authors has any competing interests in the manuscript.

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Received: March 20, 2018

Accepted: April 17, 2018