

Cutaneous melanoma dissemination is dependent on the malignant cell properties and factors of intercellular crosstalk in the cancer microenvironment (Review)

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Abstract. The incidence of cutaneous malignant melanoma has been steadily increasing worldwide for several decades. This phenomenon seems to follow the trend observed in many types of malignancies caused by multiple significant factors, including ageing. Despite the progress in cutaneous malignant melanoma therapeutic options, the curability of advanced disease after metastasis represents a serious challenge for further research. In this review, we summarise data on the microenvironment of cutaneous malignant melanoma with emphasis on intercellular signalling during the disease progression. Malignant melanocytes with features of neural crest stem cells interact with non-malignant populations within this microenvironment. We focus on representative bioactive factors regulating this intercellular crosstalk. We describe the possible key factors and signalling cascades responsible for the high complexity of the melanoma microenvironment and its premetastatic niches. Furthermore, we present the concept of melanoma early becoming a systemic disease. This systemic effect is presented as a background for the new horizons in the therapy of cutaneous melanoma.

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1. Introduction

Similarly to other malignant diseases, the incidence of cutaneous malignant melanoma (CMM) is increasing worldwide (1). This increased incidence seems to be influenced by many factors, including ageing of the population, behavioural habits, and climatic and environmental changes. Formation of CMM is associated with the main genetic drivers such as BRAF, NF1 and NRAS mutations, also usually associated with chronic skin sun damage (2). Aberrant activation of the RAS/BRAF/MEK/ERK signalling pathway causes uncontrolled proliferation of malignant cells in the majority of CMM (3). Melanoma causes most of the skin cancer-related deaths. The patient overall survival at five years depends on the thickness of the primary melanoma. CMM is also known for its remarkable ability to metastasise. Despite the new therapeutic options, the curability of advanced-stage melanoma is still limited. These recent therapeutic approaches modulate the immune response of the organism (e.g., via application of anti-CTLA-4 and anti-PD-1 antibodies) or target proliferation in specifically mutated melanomas (e.g., by application of BRAF or MEK inhibitors). In order to establish novel targeted melanoma therapies, it is

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Abbreviations: CAFs, cancer-associated fibroblasts; CMM, cutaneous malignant melanoma; EMT, epithelial to mesenchymal transition; NC, neural crest; SCs, stem cells; SMA, smooth muscle actin

Key words: melanoma, cancer microenvironment, cancer-associated fibroblast, cytokine, chemokine, growth factor

of fundamental importance to understand the mechanisms activated in the permissive tumour microenvironment. In particular, interactions between melanoma cells and the tissue microenvironment play key roles in the disease progression. This article summarises data on the multifaceted roles of CMM microenvironment in tumour spreading. This concept may be extended to the intravasation of bioactive molecules participating in the melanoma cell crosstalk with non-malignant cells forming the CMM microenvironment. These molecules also participate in premetastatic niche formation. Finally, their role in patient wasting is also widely discussed. The concept of CMM microenvironment as a complex system suitable for therapeutic targeting is introduced in this article.

2. Cutaneous malignant melanoma (CMM) disseminates extensively in the organism of the patient

The critical feature associated with melanoma is its enormous capability to spread and form lymph node or distant visceral metastases (Fig. 1). Almost any tissue in the patient's body can host metastatic cells, and even a small and thin primary tumour can metastasise to the entire body, leading to the death of the patient (1). Metastatic spread is a complex multistep process, as was noted almost 200 years ago by surgeon Stephen Paget, who coined the 'seed and soil' hypothesis (4). Surprisingly, cutaneous melanoma can spread to different organs without any particular predilection, and thus differs from, e.g., uveal melanoma of similar histogenesis. However, the first predictive site of metastatic disease is a lymph node. The presence of tumour cells in this lymph node is generally investigated in melanoma patients with tumours thicker than 1 mm. This procedure is routinely called sentinel lymph node biopsy. The presence of melanoma cells in the lymph node is a powerful predictor of melanoma recurrence, but not of survival, in the melanoma patients (5). VEGF-C, which is involved in lymphangiogenesis and promotes increased lymphatic vessel density, can also play a role in lymph node metastasis (6).

CMM metastasis to the lungs and brain and other visceral organs. In the case of visceral melanoma metastasis, the most predictive localisations are the lungs and pleura (7). Lung metastases are also the most frequent metastases in mouse models of metastatic melanoma (8). In these mouse models using the B16 model of melanoma, chloride channel accessory 2 (CLCA2), an extracellular protein expressed predominantly in the lung, was identified as a factor mediating interactions with $\alpha 6 \beta 4$ -integrin, which is expressed by tumour cells (9). Brain metastases are associated with poor prognosis. Historically, melanoma patients with brain metastases have had dismal outcomes and very limited treatment options. Systemic treatment with BRAF inhibitors and immunotherapy offers therapeutic responses in up to 55-58% of patients (10). The actual mechanism of brain metastases is not clear, but mouse models point to some factors that play a role in this process. The original model suggested a role of transferrin receptors and their interaction with their ligand, transferrin, mediating metastases of melanoma cells to the brain. Another study highlighted the importance of neurotrophins and neurotrophin receptors in the process of brain-specific melanoma metastases (11).

Other common sites for melanoma metastases are the liver (up to 20% of patients), bones (11-17%), or skin and subcutaneous tissue (12).

CMM metastasis to the skin. Skin metastasis represents haematogenous dissemination of melanoma cells. Specific interactions between chemokines C-C motif chemokine receptor 10 (CCR10) and C-C motif chemokine ligand 27 (CCL27) have been determined as crucial factors in melanoma metastasis to the skin (13). CCL27 is a chemokine expressed in the epidermis by normal keratinocytes. In addition, high expression in supratumoral epidermis is associated with more prolonged melanoma-specific survival (14). Presumably, CCL27 interacts with the chemokine receptor CCR10, which is expressed in melanoma cells. Experiments with blocking antibodies to CCL27 showed inhibition of development of skin metastasis in a mouse model (15).

CMM metastasis and somatic mutations. Despite that, no specific biomarker with predictive potential to determine the metastatic site exists to date. In melanoma, there is also an observed lack of association between the site of visceral or lymph node metastasis and either the clinicopathological variant or location of the primary tumour (16). The dependence on the presence of somatic mutations has been reported. One study suggested that BRAF mutation is associated with lymph node metastasis as the first metastasis and sentinel lymph node positivity. BRAF and NRAS mutations were associated with different metastatic patterns, with metastases more frequently affecting the central nervous system and the liver. NRAS-mutated tumours formed lung metastases (17). This highlights an earlier-unexpected internal heterogeneity of the group of tumours nowadays collectively called melanoma. Although intense visceral organ-specific surveillance may be initiated in patients with tumours harbouring these somatic mutations, this does not necessarily lead to a decrease in mortality. It is not easy to understand this metastatic potency of CMM, which represents the main, frequently fatal, complication in the treatment of patients. The complexity of these mechanisms is also shown by the concept of pre-malignant melanocyte dissemination, suggesting that benign melanocytes may exist at disseminated sites in the body and may be capable of undergoing malignant progression. It is not uncommon to find benign melanocytic nevi in the lymph node during sentinel lymph node biopsy or in non-melanoma patients (up to 7% of patients) (18). These findings support the hypothesis mentioned above. It is also critically important to identify the mechanisms driving the metastatic behaviour.

CMM cells are similar to neural crest-originated stem cells. CMM cells arise after malignant transformation from pigment-producing cells called melanocytes. Melanocytes originate from the embryonic neuroectoderm structure called the neural crest (NC). NC cells are multipotent stem cells derived from the neuroectoderm that delaminate from the neural tube in early vertebrate development (in the 4th week) and migrate throughout the developing embryo. Consequently, NC cells differentiate into various cell lineages, including melanocytes (19).

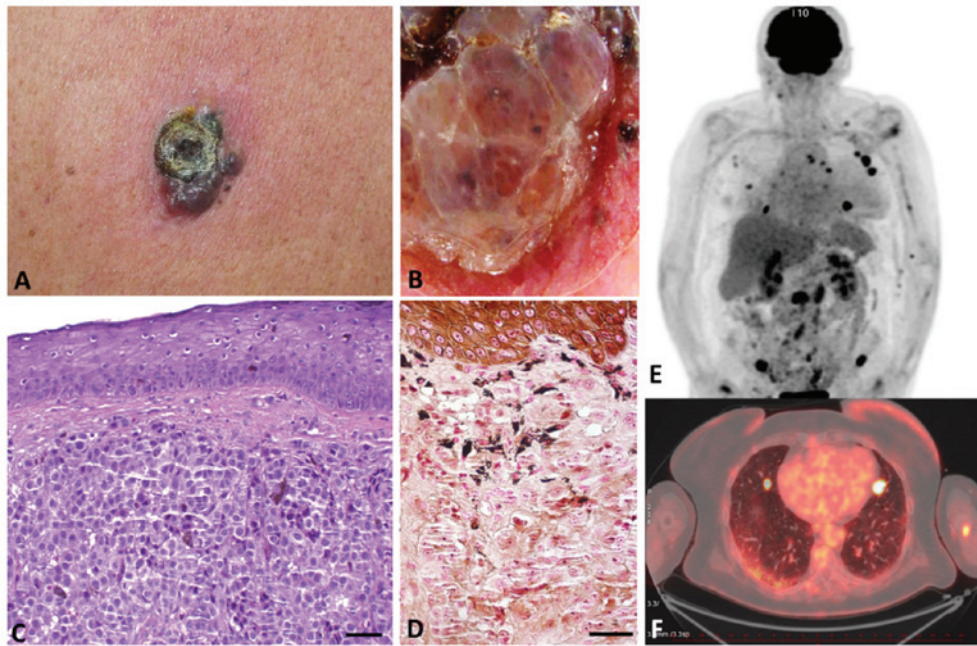


Figure 1. This figure presents the extensive capability of melanoma to form lymphatic/distant metastases in all tissues in the patient's body documented at a single patient level. (A) Primary cutaneous melanoma (Breslow thickness 6 mm) with clinical apparent ulceration in a 69-year-old female, and (B) dermoscopy of this primary cutaneous melanoma with atypical black dots, dotted vessels and erythema in regression areas. (C) The same primary tumour stained with H&E; magnification, x200. (D) After staining with Fontana-Masson (melanin), dark black granules of melanin in melanoma are visualised; magnification, x400. (E) PET-CT scan shows generalisation of the tumour to the lung, bones, lymph nodes, and soft tissue. (F) Horizontal section of PET-CT scan demonstrates metastasis in the lung and left humerus. The images were kindly provided by the Department of Dermatovenereology, First Faculty of Medicine, Charles University with explicit informed consent.

NC cells are unique because of their remarkably broad differentiation potential (Table I) (20-22). Once they have reached the final tissue niche in the skin, NC cells differentiate to melanocytes by a cascade of events controlled by transcription factors such as microphthalmia-associated transcription factor (MITF) and sex-determining region Y-box 10 (Sox10). This process occurs during the prenatal period of human development (23,24). The signalling molecules and transcription factors that are required for NC cell specification, migration and differentiation form a highly orchestrated gene regulatory network. Every individual signalling molecule has either individual or combinatorial roles in transcriptional regulation (25). The precise understanding of this mechanism seems to be critically important because similar pathways are activated in malignancy, and they could control the biological properties of malignant CMM cells (26). The signalling pathways regulating epithelial to mesenchymal transition (EMT) can be triggered by transcription factors that are active in both NC development and cancer progression (27).

Interestingly, both melanoblasts and NC cells also reside in the bulge region of the hair follicle in the outer root sheath. In this highly specialised niche, NC cells retain their multipotency during adult life. NC cells can be isolated and expanded *in vitro* with the remarkable features of highly multipotent stem cells (SCs). It is possible to differentiate NC cells to various specialised cell types such as melanocytes, adipocytes, osteoblasts, chondroblasts, smooth muscle cells, neurons, and Schwann cells (28). The NC cell phenotype is defined by expression of multiple markers, and NC cell identification cannot be based on a single molecule. Of note, there is a significant overlap with the marker profile of CMM [Table II based on (29-37)].

This highlights the low differentiation frequently observed in melanoma, where many cells typically have properties of stem cells (37). These cancer-initiating cells of CMM have an indispensable role in CMM resistance to therapy, progression and generalisation (38).

The life-long postnatal presence of NC cells in hair follicles raises important questions regarding the maintenance of their multipotency and regulation of their normal behaviour within this niche. There is strong evidence that the microenvironment is a critical condition of this steady-state. The signalling cues within the proper microenvironment, via both extrinsic and intrinsic factors, orchestrate the interplay necessary for healthy tissue dynamics. The importance of the normal tissue microenvironment was highlighted in several studies using transplantation of malignant cells to animal embryos. In experiments performed in the early chicken embryo, labelled CMM cells were injected into the region of the neural tube. It was demonstrated that melanoma cells migrate to the same regions as the autologous embryonic NC cells (39). Similar experiments performed later in zebrafish embryos supported these findings. Both the embryonic NC cells and the cells of CMM in zebrafish express specific protein crestin, which is absent in normal melanocytes (40).

Taking into account the low differentiation status of NC cells and their natural migratory activity, the similarity of CMM and NC cells can also explain the highly metastatic behaviour observed in melanoma in the clinic.

Circulating CMM cells in disease dissemination. Similarly to other types of malignant tumours, cells of CMM can also be detected in the circulation. These circulating melanoma

Table I. Examples of cells originated from neural crest cells.

Cell type	Specification
Peripheral neurons	Sensory, sympathetic + parasympathetic ganglia
Glial cells	Schwann cells
Merkel cells	Mechanoreceptor function
Parafollicular cells	Production of calcitonin
Adrenal medullar cells	Chromaffin cells
Osteoblasts/odontoblasts	Facial skeleton
Chondroblasts	Facial skeleton
Myoblasts	Striated/smooth-facial region
Dental pulp cells	Multipotent stem cell potential
Fibroblast/mesenchymal cells	Facial region
Cornea	Stromal cells
Melanocytes	All parts of the body

cells harbour the functional properties of cells of the primary tumour, including their SC-like properties (41,42). These cells leave the primary tumour and penetrate the vessels and use them as a highway for dissemination through the patient's body to target the organ/tissue where they form metastases. Using an identical vascular path, the normal adult tissue SCs can migrate in order to facilitate body repair processes during wound healing (43,44). From this point of view and based on histological/molecular similarity, cancer again resembles wound healing. With a certain hyperbole, cancer can be seen as a distorted cascade of wound repair events (45). These data can also predict the great invasive metastatic potential of CMM.

3. The microenvironment of CMM participates in the control of its invasive potential

Melanoma is a complex ecosystem. Malignant cells define the type of tumour. However, there are other non-cancerous populations forming the tumour stroma. It is the interaction of both components of this microenvironment that finally defines the biological behaviour of the tumour. It is truly applicable to solid tumours in general, and CMM is no exception (46-48). Concerning CMM, the cancer microenvironment is formed by cancer-associated fibroblasts (CAFs) and several types of leukocytes, as comprehensively reviewed by Lacina *et al.* (49) (Fig. 2A).

The origin of CAFs is not fully understood. Local normal dermal fibroblasts, attracted mesenchymal SCs and pericytes are frequently mentioned as source cell populations from which CAFs are recruited (50). However, the transition of cancer cells to CAFs cannot be entirely excluded, although its likeliness is not very high. This fact is difficult to prove in the experimental model (51).

Treg lymphocytes, tumour-associated macrophages and myeloid-derived immunosuppressive cells stimulating the CMM progression, as well as NK cells, macrophages and

Table II. Comparison of markers of hair follicle NC SCs and CMM cells.

Factor	NC SCs	CMM cells
BMP4 ^a	+	+
SNAIL ^a	+	+
SLUG ^a	+	+
SOX9 ^a	+	+
TWIST ^a	+	+
MITF ^b	+	+
Desmin ^c	+	+/-
Calponin ^c	+	+/-
β -III tubulin ^d	+	+

^aNC marker, ^bmelanocyte progenitor marker, ^csmooth muscle differentiation marker, ^dneuronal marker. NC, neural crest; SCs, stem cells; CMM, cutaneous malignant melanoma; BMP3, bone morphogenetic protein 3; SOX9, SRY-box transcription factor 9; MITF, microphthalmia-associated transcription factor. Based on Person *et al.* (29), Stasiak *et al.* (30), Yang *et al.* (31), Lee *et al.* (32), Tudrej *et al.* (33), Iwakami *et al.* (34), Goding and Arnheiter (35), Campbell *et al.* (36), Krejčí and Grim (37).

CD8-positive T lymphocytes, are attracted to the CMM site. Interestingly, CAFs stimulate the activity of immune cells supporting melanoma cells and inhibit the cancer-suppressing cells (52,53).

Unlike in other epidermal tumours, keratinocytes are also an important component of the CMM microenvironment. Melanoma cells can stimulate surrounding keratinocytes (54). On the other hand, keratinocytes control growth and differentiation of melanocytes and potentiate the invasiveness of melanoma cells during early progression as observed in a reconstructed skin model (55).

Role of intercellular contact. Cell-cell adhesion molecules (cadherins) and cell-extracellular matrix adhesion proteins (integrins) play a critical role in the regulation of cancer invasion and metastasis. Many members of the cadherin superfamily play an important role in cancer biology. However, the most significant explanation is seen in the E-/N-cadherin switch, and its role in epithelial to mesenchymal transition (EMT), in cancer progression. N-cadherin expression in CMM cells helps cancer cells to interact with fibroblasts and extracellular matrix and stimulates the invasive potential of melanoma cells and their proliferation, but also activation of PI3/AKT, mTOR, and ERK kinase. Inhibition of N-cadherin represents an interesting possibility, with potential clinical use (56).

Intercellular contacts of normal melanocytes, or malignant melanoma cells, respectively, and their non-cancerous neighbours within the tissue environment influence their properties mutually (62). Keratinocytes reduce expression of N-cadherin not only via cell-cell contacts, but also via cell-derived extracellular matrix and conditioned medium with calcium regulators (57). These findings support the importance of the balance in communications between melanoma cells and non-cancerous cells in the melanoma microenvironment.

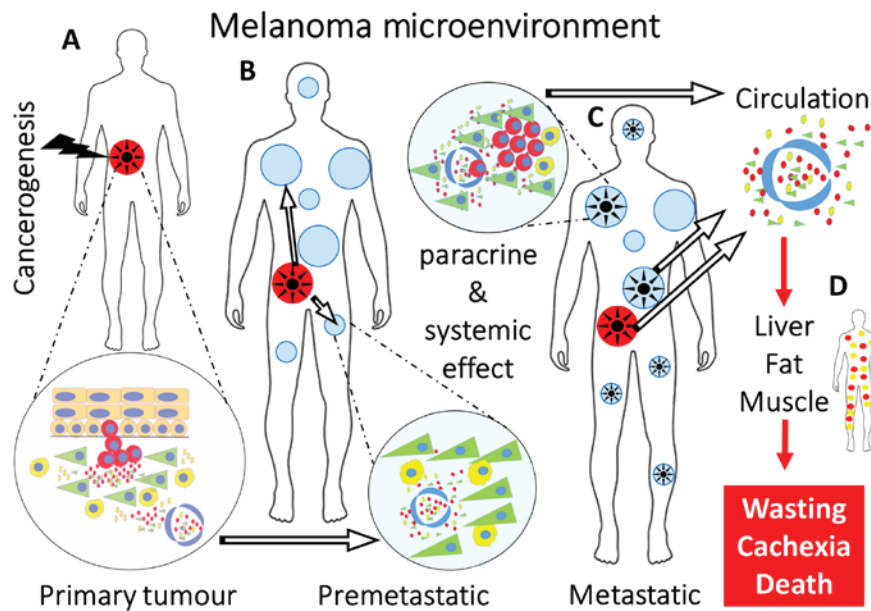


Figure 2. The figure demonstrates the paracrine and systemic effect of CMM during progression. (A) Once initiated, the tumour grows and via paracrine signalling influences surrounding non-cancerous tissues (detail in the pink-filled circle). This paracrine interaction strengthens the malignant potential of CMM. However, signalling molecules diffuse and also leak to the circulation via capillaries. (B) Exposed to the released cytokines, chemokines and growth factors, the normal distant tissues become activated and form premetastatic niches (detail in the blue-filled circle). This is an early systemic effect of the primary tumour. (C) In some of these niches, the metastasising malignant cells will harbour and initiate a metastatic tumour (detail in the blue-filled circle). The concentration of circulating bioactive molecules is further enhanced by their production in metastases. This represents a later systemic effect typical of generalised malignancies. (D) Circulating bioactive molecules induce physical and functional organ changes. This consequently results in wasting, cachexia and death. CMM, cutaneous malignant melanoma.

Integrins $\beta 1$ and $\beta 3$, as adhesion cell-extracellular matrix proteins, are differentially expressed during the transformation of melanoma radial growth to the vertical invasion (58,59). The differentiation status of melanoma cells and the ability to invade the surrounding tissue also highlights this impact. For example, the expression of connexin-43 in CMM cells indicates the ability of CMM cells to metastasise (52,60,61). Expression of desmoglein-2, which participates in the contacts with keratinocytes, has an inhibitory effect on CMM cell migration.

On the other hand, the expression of desmoglein-2 promotes the vasculogenic mimicry of CMM cells, which is associated with a poor outcome of patients (62,63). Furthermore, in melanoma cells that do not express $\beta 3$ integrins, $\beta 1$ integrins instead play a role in promoting their transendothelial migration by binding to vascular cell adhesion molecule 1 (VCAM-1) (64). Integrins also play an important role in connecting the extracellular matrix with the melanocyte and melanoma cell cytoskeleton. Cytoskeletal rearrangements, such as the increase of the overall contractility, impact cell mechanical properties and cell deformability. These changes may then potentiate prometastatic phenotypes of melanoma cells. Expression of $\alpha v\beta 3$ integrin increases elasticity in human melanoma cells in adherent and non-adherent conditions (65). Intercellular contacts and molecules play an important role in the mechanisms of targeted therapy. Targeting of the CMM cell surface receptor Notch-dependent pathway improves the activity of Erk inhibitors in BRAF-V600E mutated tumours. Further, it can be combined with inhibition of ERBB3 to suppress melanoma cell growth (63). On the other hand, Notch expression in CAFs reduces the growth and migration potential of melanoma cells (66).

Role of paracrine signalling in communication across the CMM microenvironment. The paracrine mode of signalling between cancerous and non-cancerous cells in CMM has been extensively studied (Fig. 2B and C). For example, CAFs, keratinocytes and infiltrating immune cells produce a variety of growth factors/cytokines/chemokines that significantly influence the biological properties of malignant CMM cells (67,68) (Table III). Interestingly, many of these factors are also associated with skin ageing. Collectively, these bioactive molecules are called the senescence-associated secretome (69). As expected, chemokines attract inflammatory cells to the tumour site. However, they also have multiple other functions (70). CMM cells express receptors for CXCL1 and interleukin (IL)8, and these factors enhance their invasiveness (71,72). Antagonists of CXCR1/2 receptors have a well-documented inhibitory effect on migration of CMM cells (73). The IL8 chemokine also stimulates vascularisation of CMM (74,75), and in general, its high expression indicates poor prognosis of CMM patients (76).

Expression of chemokine CXCL16 seems to participate in the malignant transformation of a melanocytic nevus to CMM (77). Activation of CXCR6 recognising this chemokine induces SC-like properties in CMM cells and initiates their migration (78).

IL1 β is predominantly produced by macrophages. It participates in the progression of CMM in collaboration with IL8 and caspase recruitment domain family member 8 (CARD8) (79).

IL6 is a crucial factor initiating the immune response. IL6 has a multifaceted role in cancer progression (80). While the initial stage of CMM growth can be inhibited by IL6 (81), the more advanced stages are associated with production of

Table III. Examples of factors produced by CAFs, Kerat and melanoma cells in CMM.

Symbol	Gene name	CAFs	Kerat	CMM cells
<i>IL8</i>	Interleukin 8	+	+	+
<i>CXCL1</i>	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity α)	+	-	-
<i>CXCL16</i>	Chemokine (C-X-C motif) ligand 16	+	-	+
<i>IL1B</i>	Interleukin 1 β	+	-	-
<i>IL6</i>	Interleukin 6	+	+	+
<i>IL17D</i>	Interleukin 17D	+	-	+
<i>ACAN</i>	Aggrecan	+	+	+
<i>HBEGF</i>	Heparin-binding EGF-like growth factor	+	+	-
<i>BDNF</i>	Brain-derived neurotrophic factor	-	-	+
<i>TGFB2</i>	Transforming growth factor, β 2	-	-	+
<i>IGFBP7</i>	Insulin-like growth factor binding protein-7	+	+	-
<i>GAP43</i>	Growth associated protein 43	+	+	-
<i>BMP2</i>	Bone morphogenetic protein 2	+	-	+
<i>BMP6</i>	Bone morphogenetic protein 6	+	-	-
<i>VEGFA</i>	Vascular endothelial factor A	+	+	+
<i>VEGFC</i>	Vascular endothelial factor C	+	+	+
<i>CTGF</i>	Connective tissue growth factor	+	-	+
<i>PDGFRL</i>	Platelet-derived growth factor receptor-like	+	-	+
<i>LEPRE1</i>	Leucine proline-enriched proteoglycan (Leprecan)	+	-	-
<i>LEPREL1</i>	Leprecan-like 1	+	-	+
<i>KAZALD1</i>	Kazal-type serine peptidase inhibitor domain 1	+	-	-

CAFs, cancer-associated fibroblasts; CMM, cutaneous malignant melanoma; Kerat, keratinocytes. Based on Kodet *et al* (54), Jobe *et al* (67).

this interleukin (82,83). IL6, frequently in cooperation with IL8, exhibits an additive effect on WNT5A in the stimulation of CMM cell invasiveness (67,84). IL6 induces Twist and N-cadherin expression in CMM angiogenesis in a mechanism dependent on the p50 subunit of nuclear factor κ B (85).

In general, IL17D (IL27) has an anti-tumoral effect in CMM (86), where it participates in generating tumour-specific cytotoxic T cells (87). The effect of IL17D on CMM cells seems to be TRAIL-dependent (88). On the other hand, it is also known as a potent inducer of the production of IL6 and IL8 in endothelial cells. It is highly expressed in the initial stages of CMM (89), stimulating the CMM growth and tumour vascularisation.

Glycoprotein aggrecan is produced by cells of the CMM, CAFs and keratinocytes. It is usually secreted during the process of chondroblast differentiation, and it has an inhibitory effect on CMM progression (90). A similar anti-CMM effect is produced by insulin-like growth factor-binding protein 7 (IGFBP-7), namely in BRAF-mutated V600E-positive dysplastic nevi (91). On the other hand, heparin-binding EGF-like growth factor has a stimulatory impact on CMM growth (92). The same result was described in the case of another growth factor, neurotrophin (93,94). miRNA-328 controls production of TGF- β 2, and attenuation of its expression has a strong inhibitory effect on CMM cell proliferation (95). VEGFA and VEGFC are generally responsible for the activation of CMM neovascularisation by blood/lymphatic vessels that support the CMM growth and progression. Connective

tissue growth factor has a synergistic effect on VEGFA and stimulates the tumour site neovascularisation (96).

As mentioned earlier, CAFs do not support tumour growth and metastases exclusively. CAFs are also implicated in the acquisition of resistance to the targeted therapy in BRAF-mutated melanomas. Under the influence of BRAF inhibitors, CAFs secrete factors that contribute to CMM cell survival and melanoma resistance. CAFs release factors such as hepatocyte growth factor (HGF) and neuregulin 1 (NRG1), which can trigger alternative cascades in MAP kinase signalling (97,98).

The short overview in Table III demonstrates the complexity of signalling between CMM cells and non-cancerous cells, where intercellular contacts, cytokines, chemokines, and growth factors with both the stimulatory and inhibitory effect influence the tumour growth and generalisation.

Concluding this paragraph, paracrine signalling represents a critical aspect in the control of biological properties of CMM. In addition to the crosstalk between melanoma cells, this process includes an exchange of information between CMM cells and non-malignant cells of the microenvironment.

Role of exosomes. In addition to paracrine signalling via soluble products, exosomes represent another tool of CMM cell communication with non-cancerous partners within the microenvironment. All affected cell populations of the cancer microenvironment produce these bodies. Exosomes thus influence CMM cell biological properties (99).

Exosomes stimulate CMM cell metastasis via support of the epithelial-mesenchymal transition. Exosomes influence vascularisation of the lymph node in order to prepare the vascular bed for the metastasising (100,101). Exosomes significantly participate in the regulation of local invasiveness and also in the entrance of melanoma cells to the target organs (102). This effect is frequently associated with the presence of miRNAs in CMM-derived exosomes. It was confirmed both *in vitro* and *in vivo* in clinical material (103). Exosomes exert a robust immunosuppressive effect on the cancer microenvironment, where they inhibit IL2-dependent proliferation of CD8-positive T lymphocytes (104). Moreover, CMM exosomal miRNA-125b-5p induces a tumour-promoting phenotype in macrophages (105). These changes can induce a mixed M1, and M2 tumour-promoting macrophage activation included production of CCL22, IL-12B, IL-1 β , IL-6, i-NOS, and TNF- α (106). These data highlight exosomes as a critically important component of the CMM microenvironment significantly participating in its biological properties, with the ability to stimulate the immune response of the melanoma microenvironment.

4. Differences in serum proteins between CMM patients and healthy individuals

Serological biomarkers represent a diverse group of biomolecules with importance in diagnosis, staging, and monitoring the therapeutic response. Serum lactate dehydrogenase (LDH) is the only serum biomarker that has been accepted as a prognostic biomarker for routine clinical use in melanoma patients with a predictive therapeutic outcome and has been implemented in the American Joint Committee on Cancer (8th edition) staging system (107). Routinely used S100B (S100 calcium binding protein B) protein is highly specific for melanoma patients. Its increased levels can be detected in patients with advanced melanoma during melanoma prognosis (108). Another serological protein is MIA (melanoma inhibitory activity), which interacts with extracellular matrix proteins. Its expression can also be detected in normal tissue such as cartilage. In pathological processes, its overexpression is observed in breast cancer or colorectal cancer, in addition to melanoma (109).

Introduction of a new treatment strategy for advanced melanoma leads to the search for new biomarkers to improve both prognostic and predictive outcome. Likely, the high intensity of molecular exchange between cancer cells and other members of the microenvironment via cytokines, chemokines and growth factors can lead to leakage out from the tumour microenvironment, and mediators can be consequently detected in systemic circulations in the serum (110,111) (Fig. 2A-D). Therapy by monoclonal antibodies targeting immune checkpoint inhibitors is one of the most potent treatments of CMM patients. Measurement of current concentrations and dynamics of these mediators in the serum can have the potential of liquid biopsy. Indeed, the serum protein signature even reflects the efficiency of anti-PD-1 therapy of CMM patients and can be substantial for therapeutic indications (112).

Similarly to other types of tumours, an elevated serum level of IL6 in CMM has been observed (111), which has reached some prognostic validity (113). Serum elevation of

IL6 sensibly reflected the tumour burden and indicated a relapse of the disease or insensitivity to tumour therapy in several studies (114-116).

A similar finding was observed in the case of serum levels of IL8 (71,117,118). Interestingly, the levels of IL6, IL8, and VEGFA correlated with the level of Breslow index at the time of diagnosis (117,119). Moreover, the amount of VEGFA also depended on the stage of the disease (120). As IL8 also supports CMM neovascularisation, it is not surprising that the elevation of the serum level of both IL8 and VEGFA correlates with the progression of the disease and poor survival of CMM patients (121). Proteins of the TGF- β family are also elevated in the sera of CMM patients, and the prognostic relevance of these factors has been proposed (122). Elevated concentration of factors with immunomodulatory activity such as IL6 and IL10 influence the presence of self-renewal tumour-initiating (stem) cells in CMM (123).

Serum protein imbalance influences premetastatic niche formation. Based on the selected examples, it is possible to demonstrate the systemic effect of CMM. The serum/plasma of CMM patients contains numerous bioactive proteins and exosomes that are transported to the distant parts of the patient's body through the vessels (Fig. 2B-D). These factors participate in the preparation of a premetastatic niche suitable for cancer cell homing and metastasis formation (124). Under the influence of CMM-derived exosomes, the dermal fibroblasts reprogram their metabolism significantly (125). The distant dermal fibroblasts from CMM patients at the stage of metastatic tumour dissemination differ from the normal dermal fibroblasts from healthy donors. The phenotype of distant dermal fibroblasts, as well as the expression profile and methylation profile of gene promoters, is shifted closer to CAFs (126). Due to this activation, it is possible to hypothesise that the tissue microenvironment in the distant body parts is influenced by the released bioactive factors from the melanoma microenvironment. It is, therefore, likely that melanoma becomes a systemic disease very early. If so, it is the signalling in the primary tumour that already prepares the rest of the organism to host CMM cells and facilitate metastases (114) (Fig. 2B).

5. Intravasation and extravasation of CMM cells and their inhibition via migrastatics

In recent years, the term 'migrastatics' has been introduced for drugs interfering with all modes of cancer cell invasion (115). Migrastatics inhibit local invasion and consequent metastasis. This group of drugs was recently established to define and distinguish them from conventional cytostatic drugs that traditionally target cell proliferation. Malignant melanoma, therefore, seems to be a tempting disease for validation of this concept.

Endothelial cells of capillaries are also an important structure of the cancer microenvironment. Migrating CMM cells adhere to the capillary endothelium, intercalate between endothelial cells, and migrate throughout the vessels in both directions. From the endothelial cell perspective, this process is not passive. Endothelial cells actively participate in extravasation, where the role of N-cadherin has been broadly

investigated (117). It seems to be also controlled by CD146, which cooperates with VEGFA. CD146 is also elevated in the patients' serum/plasma (127).

On the other hand, VE-cadherin expressed on the surface of endothelial cells prevents the migration of cancer cells through the endothelium of capillaries. VE-cadherin must, therefore, be eliminated from the site of malignant cell migration (128). P-selectin has an essential role in the recruitment of inflammatory cells to the site of inflammation, so-called homing.

P-selectin expression on endothelial cells is under the control of the local microenvironment. Expression of this molecule on endothelial cells and blood platelets seems to be a prerequisite for successful metastasising of CMM cells to the target tissue (129,130). P-selectin expression on the surface of endothelial cells is induced by STAT3 activation (129). IL6 is available in the serum of CMM patients, and it is known as a potent activator of STAT3. The observation that capsular polysaccharides from *E. coli* attenuate adhesion of CMM cells to the endothelium via P-selectin demonstrates the specificity of this interaction (130). Endogenous lectin galectin-3 locally accumulates in inflamed tissues, including endothelium. This lectin also enhances invasion of CMM cells, e.g., to the lungs (131). These examples show that an imbalance in serum proteins can participate in the process of extravasation of CMM cells to the target tissue and metastasising.

Therefore, the combination of migrastatics with other groups of traditional oncologic drugs may be possible. Beyond that, we suggest that directed therapy (biologics, small-molecule receptor-associated kinase inhibitors) against the most prominent inflammatory cytokines, namely IL6, could bring highly desirable synergism. However, it seems evident that inhibition of the IL6 signalling axis is not sufficient and must, therefore, be accompanied by simultaneous blockade of other proteins/receptors such as IL8, VEGFA and MFG8 (48,132). The therapeutic blockade of IL6, in combination with checkpoint inhibitor anti-PD1, represents an interesting possibility of overcoming some immunological mechanisms of resistance. IL6 blockade upregulated expression of PD-L1 on melanoma cells in a mouse model and may sensitise melanoma to this treatment (133). These findings underscore the importance of the IL6-PD1/PD-L1 crosstalk in the tumour microenvironment of melanoma.

The local microenvironment and its control by bioactive factors can be a highly relevant target in the prevention of the deadly complication of malignant disease (Fig. 2D).

6. Cancer-associated wasting and cachexia as a terminal complication of CMM; clinically relevant complications are also associated with factors of intercellular crosstalk

Advanced stages of cancer, including CMM, are associated with metastasising, which in the case of CMM has a character of extensive generalisation. The increasing burden of tumour cells generates an imbalance in growth factors, cytokines and chemokines, among which IL6 seems to have the leading position (80). This stage of the disease is usually terminated by cancer-associated cachexia (CAC), which affects approximately 16 patients per 100,000 individuals (134)

(Fig. 2D). CAC is a highly complex and multifaceted catabolic process (135). IL6, in cooperation with TNF α and IL1 β influences the metabolism of striated muscle fibres, adipocytes and hepatocytes (136,137). The level of the mentioned factors in the serum can even predict the onset of CAC and survival of cancer patients (138). The terminal stage of cancer is also associated with decreased food intake in cancer patients, which is called anorexia (139). IL6 seems to be linked to the control of food intake, where it inhibits the appetite and participates in the development of anorexia (140). TNF α and IL6 can cross the blood-brain barrier (141). TNF α , IL1 and IL6 can interact with hypothalamic neurons and affect the serotonergic metabolism, which can be reflected by decreased food intake (142). Patients with advanced cancer frequently suffer from depression that seems to be associated with elevation of IL6, IL10 and TNF α (143). On the other hand, the serum levels of IL6 (and also IL8) are significantly elevated in tumour-free patients with bipolar disease, but not with major depressive disorder (144). This section demonstrates that factors produced by the cancer ecosystem have a strong systemic effect by which they influence the metabolic functions of cancer patients, resulting in wasting and death.

7. Conclusion

CMM, similarly to the majority of cancers, can be characterised as a genetic abnormality and a regulation failure in which cancer cells employ predestined pathophysiological pathways that are normally activated in the course of organism growth, tissue regeneration and repair. This deregulation is typically associated with accumulation of mutations acquired during the ageing of the individual.

The progression of CMM from tumour initiation to the systemic effect on the patient's metabolism is organised according to a quite uniform scenario (Fig. 2A-D). The intercellular crosstalk within this ecosystem is mediated either directly by intercellular contacts, or indirectly by paracrine secretion of numerous active molecules. This interconnection strengthens the malignant potential of cancer cells, and it can inhibit the anticancer immune response or protect malignant cells from the harmful effect of oncological therapy. A plethora of bioactive factors are transported via vessels and significantly influence even the distant tissue. Collectively, these factors prepare a suitable microenvironment for the malignant cell extravasation and metastasising, the premetastatic niche. The increasing mass of CMM cells in the body of patients generates a cancer-induced profile of inflammatory mediators in the patients' serum. The systemic availability of these bioactive molecules triggers mental disorders, depression, and mental anorexia-associated problems with food intake. Wasting leads to cancer cachexia and death. Based on this scenario, it is evident that besides the conventional anticancer therapy, it would be necessary to influence the migration of CMM cells and their metastasising - the concept of migrastatics (115). Because the CMM microenvironment stimulates malignant cell invasiveness (145), targeting both cancerous and non-cancerous cells of the tumour ecosystem and their products seems to be a promising approach. IL6 and its signalling pathway influence CMM cell growth and migration, but it can also

positively affect the entire patient metabolism and mental status (82). Therefore, targeting the IL6/IL6R/STAT3 axis as a new therapeutic modality was enthusiastically expected, but, unfortunately, the reality did not meet this expectation (146). The progress in the detection of clinically relevant markers using a robust omics approach that includes stromal factors can be translated into personalised therapy of CMM (147). For example, a combination of blocking the anti-IL6 axis with drugs blocking other signalling pathways seems to be promising for future trials (48). It can be hypothesised that the progress in diagnostics and therapy covering the complex ecosystem of melanoma can bring some benefit to CMM patients.

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Availability of data and materials

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Authors' contributions

Conceptualisation, data collection and manuscript preparation were carried out by KSm, OK and LL. Manuscript preparation was conducted by JK, JŠ, KSt and BD.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

The authors declare no competing interests.

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