The role of tumor microenvironment in melanoma therapy resistance

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Practice points

- Melanoma is highly invasive and incurable in advance stages.
- Low survival rate and therapy resistance.
- Tumors are heterogeneous and genetic analyses have revealed high mutation rates in melanoma.
- The role of tumor microenvironment in melanoma heterogeneity.
- Enrichment or induction of tumor initiating cells during therapy.
- Designing personalized therapy to target tumor initiating cells and/or the tumor microenvironment to obtain long lasting remission.

Melanoma patients develop resistance to both chemotherapy and targeted-therapy drugs. Promising preclinical and clinical results with immune checkpoint inhibitors using antibodies directed against cytotoxic T-lymphocyte-associated protein 4 and programmed cell death protein 1 have re-energized the field of immune-based therapies in melanoma. However, similar to chemotherapy or targeted therapies, immune checkpoint blockade responds in only subsets of melanoma patients. A number of factors, including gene mutations, altered cell-signaling pathways and tumor heterogeneity can contribute to therapy resistance. Recent studies have highlighted the role of inflammatory tumor microenvironment on therapy resistance of cancer cells. Cancer cells either alone or in conjunction with the tumor stroma can contribute to an inflammatory microenvironment. Multimodal approaches of targeting the tumor microenvironment, in addition to malignant cells, may be necessary for better therapy responses.

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Melanoma is an aggressive form of cutaneous neoplasia accounting for approximately 80% deaths in skin cancer patients [1,2]. Although patients with early stage of melanoma can be cured with surgical excision of the primary tumor, treatment of advanced metastatic melanoma patients continues to be a major challenge [2]. This is due to the rapid development of resistance to most available therapies including chemotherapy and targeted-therapy drugs [2–6]. Until very recently, melanoma was considered as a single disease entity and was treated with dacarbazine, an alkylating agent, or temozolomide, a second-generation drug derivative of dacarbazine. The objective response rate with these drugs was low (<15%) and it had limited overall survival benefits [5].

KEYWORDS

- cancer associated
- fibroblasts • hypoxia
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Immunological agents such as IL-2 or IFN- α (see reviews [2,7,8]) were tried as an alternative and the response rates were not any appreciably better than the conventional therapy. Long-term remissions were observed in a small subgroup of patients with high-dose IL-2 at the cost of severe toxicities [9,10]. Besides cytokines, other immunological agents such as use of BCG, a nonspecific vaccine, and whole cell allogeneic melanoma vaccines (Canvaxin and Melacine), or melanoma antigen-specific vaccines (gangliosides, peptides [gp100, MART, MAGE-1/3 and tyrosinase]) in presence and absence of dendritic cells (DCs) were all tried but failed to go beyond clinical trials [11]. Adoptive cell therapy using *in vitro* expanded tumor-infiltrating lymphocytes (TILs) has shown mixed responses in melanoma patients [12]. Melanoma patients in the younger age group fared better in the trials as they could withstand high toxicity issues [13]. As in the case of any other therapy, only a subset of melanoma patients responded to adoptive cell therapy treatment [14].

Advances in molecular medicine have reclassified melanoma as a highly complex heterogeneous disease comprising of several subpopulations of tumor cells [4,5]. These tumor cells harbor a number of distinct gene mutations and aberrant cell-signaling pathways [2,4,5,15]. Discovery of new generation of targeted-therapy drugs such as vemurafenib and dabrafenib targeting BRAFV600E mutation of the MAPK pathway or MEK inhibitors such as cobimetinib or trametinib targeting MEK have led to a substantial increase in overall response rate and has extended the survival of melanoma patients [6,15–17]. Still, most patients experience emergence of drug resistance and disease progression within a year.

Recent successful introduction of immune checkpoint reagents such as anti-CTLA-4 (ipilimumab) and anti-PD-1 (pembrolizumab, nivolumab) antibodies in the clinics have led to renewed interest in the use of immune-based therapies to treat melanoma patients [13,18–21]. Clinical studies have shown improved and impressive increase in durable regression and curative outcome in patients treated with combined checkpoint inhibition [19,22,23] and raised enormous interest in further development of these immunotherapies by combination with drugs providing a synergetic acting principal.

Currently, many studies address the nature of immune nonresponsiveness at the tumor site.

Poor lymphocytic infiltration of anti-melanoma-specific T cells at the tumor site may be partly responsible for the lack of therapy responses to anti-CTLA-4 or anti-PD-1 treatment [24]. Efforts are underway to improve mobilization of T cells to the tumor site. Some studies have suggested that tumor microenvironment plays a key role in the recruitment of immune cells to the tumor site (see reviews [25,26]). Many chemotherapy and targeted-therapy drugs also play a role in recruiting immune cells to the tumor site [27–29]. However, the precise mechanism of immune cell infiltration after therapy is not very well understood. Once the immune cells are in the tumor stroma, cancer cells can modulate their function to support their own growth and survival. Modulated immune cells can induce tumor heterogeneity resulting in therapy resistance. In the following paragraphs, we discuss the role of the tumor microenvironment in therapy-related resistance.

The tumor microenvironment & therapy resistance

Here, we will focus on the acquired resistance mechanisms mediated by the tumor microenvironment and the readers are referred to many excellent reviews that provide an overview of other drug resistance mechanisms [26,30–32].

Cells of the tumor stroma generally comprise of noncancer and neoplastic cells. Noncancer cell types comprise of adipocytes, epithelial and endothelial cells, fibroblasts, smooth muscle cells, and the cells of the immune system such as dendritic cells, macrophages, T or B cells. Cells of the tumor stroma collectively play an important role in cancer cell survival, in particular their growth and acquisition of metastatic phenotype. A number of events contribute to this process which include cell to cell interactions of the cancer cells with tumor stroma. These well-orchestrated interactions between different cell types are mediated either directly or indirectly via soluble growth factors such as cytokines or chemokines present in the tumor milieu. Transcription and the translation of cytokine and chemokine genes are generally triggered by the hypoxic environment present in the core of growing tumor cells. Increased presence of the cytokine/chemokine factors results in the recruitment of fibroblasts, lymphocytes and monocytes/macrophages into the tumor stroma. Presence of cytokines/chemokines and infiltrating cells creates an inflammatory environment

that favors tumor growth. Neoplastic cells in the tumor stroma modulate the biological functions of the infiltrating cells to further support the growth of cancer cells, their invasion and resistance to therapy (see review [33]). Thus, hypoxia can trigger a cascade of events that can lead to tumor progression.

Hypoxia & its role in therapy resistance

Studies in many cancers including melanoma have shown differing O_2 levels in the tumor tissues. These regions are often referred as hypoxia, anoxia or normoxia based on their O_2 levels. In **Box 1**, we have briefly summarized the biological effects of hypoxia.

Early studies have shown that many therapies including radiation and chemotherapy are less effective under hypoxic conditions [34]. Altered cell metabolism due to hypoxia is known to be one of the mechanisms contributing to therapy resistance [34,35].

In preclinical studies of melanoma, induction of tumor heterogeneity and therapy resistance has been attributed to hypoxia. In our study, we have shown the induction of slow cycling JARID1B+ subpopulation when melanoma cells were cultured *in vitro* under hypoxic conditions [36,37]. Furthermore, JARID1B+ subpopulation of melanoma cells are required for continuous tumor growth in mouse xenograft model [36]. *In vitro* treatment of melanoma cells with chemotherapy or targeted-therapy drugs also resulted in enrichment of JARID1B+ tumor cell subpopulation, indicating their role in therapy resistance [37]. In the same study, using mouse melanoma xenografts, the authors have shown beneficial effects of targeting of JARID1B+ subpopulation with a metabolic inhibitor and targeted-therapy drug [37]. However, the direct role of HIF-1 α , a hypoxia-induced transcription factor involved in metabolic switches, in the induction

of JARID1B+ melanoma cells remains unclear. In clinical studies of prostate and renal cancer, prolonged disease-free survival was seen with the use of HIF-1 α inhibitors [38].

Hypoxia often results in the alteration of transcription factor levels that can lead to a cascade of events including changes in cell-signaling and modified metabolic pathways. Recent studies have shown that hypoxia can induce down-modulation of microphthalmia-associated transcription factor, which plays a key role in the regulation of many melanocytic differentiation antigens. Immunohistochemistry study of primary cutaneous melanoma tissues has shown down-modulation of melanoma-associated antigen recognized by T cells in hypoxic areas [39]. Melanoma-associated antigen recognized by T cells is a T-cell-defined melanoma antigen and it has been suggested that loss of such antigens will result in immunological resistance. In the same study, the authors have also shown higher expression of glucose transporter 1, which is often associated with altered glucose metabolism and therapy resistance. Combination of glucose transporter 1 inhibitors and therapy drugs has shown improved response in experimental models (see review [40]). Chronic hypoxic conditions caused by growing tumor results in release of cytokines/chemokines such as IL-1 β , IL-6, IL-8, IL-12, IFN- γ , TNF- α and VEGF causing influx of leukocytes and endothelial cells creating inflammatory conditions. Influx of cells into the hypoxic environment of the tumor microenvironment (TME) can trigger induction of cytokines/growth factors such as IL-6 and VEGF by the endothelial cells that can further support neoangiogenesis and tumor growth [41]. Endothelial cells in the TME are known to express multi-drug resistance genes that contribute to therapy resistance [42]. Recent

Box 1. Hypoxia and its biological effects.

- Hypoxia-mediated activation of HIF-1 α results in transcription of growth factors including cytokines and chemokines
- Release of cytokine/chemokine factors by tumor cells attracts a number of leukocytes such as dendritic cells, macrophages, neutrophils and lymphocytes into the tumor stroma
- Interaction of tumor cells with the stromal-derived cells promotes cancer cell survival, angiogenesis, invasion and metastasis
- Epithelial–mesenchymal transition, induction of slow-cycling cells, induction of side population and tumor heterogeneity
- Altered glucose metabolism, increased lactate formation, changes in glucose transporter, GLUT-1
- Down-modulation of microphthalmia-associated transcription factor, modulation of melanoma-associated antigens, loss of tumorigenicity and possible induction of immune-related resistance

studies have shown that VEGF inhibitor therapy using anti-VEGF antibody can improve therapy responses [43]. Explorative trials are underway to determine if anti-VEGF therapy can enhance antitumor circulating T cells to improve immune-based therapy responses. In this regard, role of galectins and their modulation in hypoxic environment is gaining significant attention [44]. Galectin expressions are associated with increase in angiogenesis and they play a role in immune down-modulation by increasing antitumor T-cell apoptosis [44]. Galectin-3 can bind to CD45 molecules on lymphocytes and can trigger apoptosis whereas galectin-1 can prevent T-cell receptor dimerization with CD4/CD8 molecules on CD4⁺ or CD8⁺ T cells that can result in immune down-modulation [44]. Hypoxia also alters cellular metabolism that results in stress response, reactive oxygen species (ROS) production that are known to impact therapy responses.

Inflammatory cytokines, reactive oxygen species & their role in therapy resistance

Infiltrating leukocytes in the TME produce a number of proinflammatory cytokines such as TNF- α and IL-1 β . These cytokines are shown to increase HIF-1 α transcriptional activity and trigger a cascade of events resulting in stabilization of HIF-1 α , increase in NF- κ B activity, transcription and enhanced production of proangiogenic as well as inflammatory cytokines. Many of the inflammatory cytokines are distinctly associated with infiltrating leukocytes and their role in therapy resistance is described with each leukocyte cell type.

Growing tumor results in hypoxic environment and stress response. Many studies suggest that under stress environment there is an increase in ROS production within the cells (see reviews [45,46]). Increase in ROS production causes genetic instability, DNA damage, changes in amino acid residues and protein composition, inactivation of DNA repair enzymes that can affect DNA repair mechanisms [45]. Increase in ROS can modulate MAPK pathway, ERK and AKT signaling that can result in inhibition of apoptosis and enhanced tumor survival. Additionally, most of the above events support tumor heterogeneity and poor response to therapy drugs [45,47]. It is known that besides the tumor-generated ROS activity, stromal-derived cells such as cancer-associated fibroblast (CAF) are also involved in ROS production.

Cancer-associated fibroblasts & their role in therapy resistance

Solid tumor cells grow in a complex environment comprising of many different stromal-derived cells including fibroblasts. Stromal-derived fibroblasts are frequently referred to as CAF and their presence is associated with tumor progression and therapy resistance. Origins of CAFs may vary in many tumor cell types and they differ significantly from normal fibroblasts resembling myofibroblasts (see Box 2). CAFs are activated by growth factors secreted by the tumor cells and they express markers such as alpha smooth muscle actin and fibroblast activation protein. Activated CAFs secrete growth factors such as HGF, IGF-1, TGF- β and other cytokine/chemokine factors (see Box 2) to promote tumor growth and therapy resistance.

Many *in vitro* and *in vivo* studies using mouse models in solid tumors have confirmed the role of CAFs in therapy resistance mechanisms [48–53]. In one *in vitro* study, elevated levels of matrix metalloproteinase-1 in co-culture of CAFs and squamous-cell carcinoma resulted in resistance to anti-EGFR (cetuximab) treatment [50]. In a mouse breast cancer model, activated fibroblasts are implicated in the induction of tamoxifen resistance [51], and in pancreatic-ductal carcinoma, increased IL-1 β secretion by CAFs in resistance to etoposide-induced apoptosis [52], as does CAF-secreted HGF in resistance to EGFR tyrosine kinase inhibitors in a lung cancer xenograft model [53]. Furthermore, adhesion of neoplastic cells to fibronectin confers therapy resistance in lung cancer and multiple myeloma.

Unlike that of other solid tumors, the role of CAFs in melanoma is not well understood. In an *in vitro* study, it has been shown that activated fibroblasts can confer resistance to melanoma cells treated with chemotherapy drugs [49]. In the same study, the mechanism of therapy resistance has been attributed to the secretion of extracellular matrix proteins by activated fibroblasts. Another factor may be HGF secreted by stromal-derived fibroblasts as shown by its contribution to resistance against targeted drugs in melanoma [54,55]. Recruitment of macrophages by chemokines secreted by activated CAFs in prostate cancer [56,57] and the reported expression of c-Met, a receptor for HGF, on tumor-associated macrophages in murine glioma tissues (see review [58]) argue for a paracrine activation of tumor-associated macrophages to result in the recruitment of additional monocytes into the tumor tissue.

Box 2. Cancer-associated fibroblasts and their role in tumor modulation.

- Originate from pre-existing normal fibroblasts resident in the TME or from normal epithelial or endothelial cells through epithelial–mesenchymal transition or endothelial–mesenchymal transition or recruitment of bone marrow-derived mesenchymal stem cells
- Cancer-associated fibroblasts (CAFs) are generally present in an activated state and express markers such as α -SMA, fibroblast specific protein and fibroblast activation protein
- TGF β , PDGF and bFGF secreted by tumor cells can activate resident fibroblasts, fibrocytes or pericytes to become CAFs
- Activated CAFs secrete growth factors such as PDGF α/β , VEGF, SDF-1, HGF, MMPs, IL-6, CCL-7 and CXCL-14
- Factors produced by CAFs support angiogenesis, promote tumor growth and progression

Tumor-associated macrophages & their role in therapy resistance

Infiltration of macrophages has been widely reported in many solid tumors such as cancers of the breast, colon, kidney, liver, lung, prostate, ovaries, skin and uterus (see review [59]). Many cytokines and chemokines such as CCL-2, CCL-3, CCL-4, CCL-5, CXCL-8, CXCL-12, HGF, PDGF and VEGF are involved in the recruitment of monocytes/macrophages [59]. There are mixed views on the role of tumor-associated macrophages (TAMs) in tumor progression. The majority of the reports suggest that high infiltrations of macrophages are associated with poor prognosis, while a few suggest that they are correlated with better prognosis [59]. In breast and prostate cancer patients, the presence of TAMs is associated with therapy resistance. Patients receiving cytotoxic or targeted-therapy drugs are known to attract TAMs into the cancer tissue. This is primarily due to the induction of cytokine/chemokine attractants such as colony stimulating factor-1, CCL-2, CXCL1 and CXCL2 in tumor cells after therapy [48]. In some instances, chemotherapy stimulates stromal endothelial cells to release TNF- α which causes CXCL1 or CXCL2 secretion by tumor cells. Induction of cytokines/chemokines results in the recruitment of myeloid lineage cells that can differentiate into TAM, resulting in cancer cell survival [60]. Unlike CAFs, phenotypic markers used for identification of TAM are not very well defined. CD68 and CD163 are the two markers of TAM that are frequently used for its identification (see [Box 3](#)). Macrophages are classified as M1 or M2 subtypes based on their cytokine stimuli profile that is based on T-helper (Th1 or Th2) functional phenotype subpopulations. Unlike the distinct T-helper cell phenotypes, TAMs derived from tumor tissues are rather heterogeneous and express both M1

and M2 phenotypes. TME stress can influence macrophage subtypes to change their cytokine profile from M1 to M2 to support tumor survival [61]. Many groups are actively working on the role of TAM in therapy resistance and tumor progression. Recently, using a mouse model of squamous cell carcinoma, it was shown that activated macrophages (M2 phenotype) can induce therapy resistance that is mediated by proangiogenic chemokine such as CXCL-10 [62]. Fc receptor activation in macrophages is triggered by binding of immune complex formed by B-cell-secreted immunoglobulin molecules. Activation of Fc-receptor of macrophages results in secretion of cytokines that promotes therapy resistance. In clinical study, targeting TAM in addition to the cancer cells has shown improved therapy response and survival in patients with diffuse-type giant cell tumor [63].

In both early and late stages of melanomas, a high number of infiltrating macrophages are associated with poor prognosis [64] (see review [65,66]). In a number of studies, secretion of chemokine CCL-2 by melanoma cells is associated with a high infiltration of TAM [64,65,67]. Furthermore, increased density of TAM is frequently observed in highly invasive and metastatic melanoma phenotypes (see review [59]). Studies have shown that secretions of proteolytic enzymes such as matrix metalloprotease-1/-2 and cytokine factors such as IL-1, EGF, PDGF and VEGF by macrophages contribute to neo-vascularization and invasiveness of the tumor (see review [59]). In our laboratory, a microarray-based analysis on *in vitro* induced TAM using melanoma-conditioned media confirms the presence of invasion and prometastatic genes in these cells [61].

In mouse melanoma models, macrophage infiltration after BRAF and MEK inhibitors treatment is associated with increased secretion

of TNF- α that is known to be associated with therapy resistance [68]. Inhibition of TNF- α signaling with I κ B kinase inhibitors enhanced the effectiveness of BRAF and MEK inhibitors treatment. Using an *in vitro* co-culture system, we have shown that BRAF inhibitors can paradoxically activate the MAPK pathway in macrophages to produce VEGF that in turn can enhance melanoma growth, resulting in therapy resistance [64]. There are indications that lymphoid cells may play a role in the induction of TAM. However, very few studies are available to confirm the role of lymphoid cells in the induction and activation of TAM.

Role of dendritic cells in the TME

Monocytes, macrophages and DCs share similar functional role of antigen-presenting cells [69–72]. DCs can be broadly categorized as myeloid- or plasmacytoid-derived cells based on their cellular origin. They are further categorized as classical type 1 (DC1) and type 2 (DC2) DC, and plasmacytoid DCs based on their phenotype markers (CD8a⁺ and CD103⁺ for DC1; CD11b⁺ and CD172a⁺ for DC2) and distinct set of transcription factors (basic leucine zipper BATF3 for DC1, interferon regulatory factor 4 for DC2 and E2–2 for pDC) that are needed for DC maturation [72]. Chemokines attract DCs into the TME where they are known to be modulated by a number of cytokines and growth factors such as GM-CSF, IL-6, IL-8, IL-10, TGF- β and VEGF [71]. In many solid tumors including melanoma, infiltration of DCs in early primary lesion is associated with good prognosis [71]. There are also reports of DCs undergoing apoptosis or inhibition of DC maturation including DC polarization or induction of regulatory DCs in growing tumor environment [69,70]. All of these events can contribute to lack of antigen presentation and immunosuppression biasing the lymphocyte immune responses [69,70].

Role of lymphoid cells in therapy resistance

TILs are well documented in many cancers. In most studies, their presence is attributed to good prognosis, while in others to poor prognosis and tumor progression [73]. T cells are the most preferred lymphoid cell population studied in a majority of cancers and very little information is available on B-cell subpopulations. T-cell subtypes can be broadly categorized into CD4⁺ Th cells, CD8⁺ cytotoxic T lymphocyte or CD4⁺, CD25⁺ (FOXP3⁺) T-regulatory cells based on their functional and cytokine profiles in an immune response (Box 4). Unfortunately, there are no well defined phenotype markers available to sub-categorize human B-cell subpopulations. Readers are referred to reviews on various subpopulations of T and B cells, and their prognostic significance in solid tumors (see reviews [73–76]). Recent studies in solid tumors have shown increased infiltration of TILs in patients treated with targeted therapy drugs [68]. In some patients, the type of lymphocyte infiltration is linked to therapy response [27,60]. However, the role of TILs in induction of therapy resistance is poorly understood.

Studies in melanoma have indicated that drugs targeting the MAPK pathway can cause nonspecific activation of lymphocytes and increase their infiltration into the tumor tissues [68,77]. Deep sequencing analysis of T-cell receptor of TILs obtained from patients before and after therapy indicates that a good therapy response is dependent on a preferential infiltration of melanoma reactive subpopulations of T cells into tumor tissues. The cause of preferential accumulation and infiltration of anti-melanoma-specific T cells in tumor tissues remain unclear. Also, the possible role of nontumor-specific T cells in therapy resistance remains to be explored.

In mouse tumor models, chemotherapeutic agents such as 5-fluorouracil and gemcitabine

Box 3. Characteristics of tumor-associated macrophages in solid tumors.

- Originate from bone marrow-derived precursor monocytes or resident tissue macrophages
- Attracted by tumor cells by chemokines such as CCL-2
- Tissue resident macrophages are identified by surface expression of CD68 and CD163 markers
- Based on the cytokine stimuli, macrophages are delineated as M1 (IFN- γ , TNF- α) or M2 (IL-4, IL-10) macrophages
- M2 macrophages are proinflammatory and protumorigenic
- Tumor-associated macrophages secrete cytokines such as IL-1, TNF- α , PDGF and VEGF
- Tumor-associated macrophages support angiogenesis, promote tumor growth, progression and therapy resistance

Box 4. Characteristics of tumor-infiltrating lymphocytes in solid tumors.

- They are mainly circulating lymphoid cell population attracted by chemokines secreted by the tumor due to hypoxia
- Mainly comprising of T and B cells and sub-categorized into CD3⁺, CD4⁺ or CD8⁺ T cells:
- Minor sub-population of CD4⁺, CD25⁺ T cells (T-regs; FOXP3⁺) are frequently observed in infiltrates
- There are no good phenotypic markers available to sub-categorize CD19⁺ or CD20⁺ B cells
- In most cases, presence of tumor-infiltrating lymphocytes (TILs) are associated with good prognosis and in some patients associated with tumor progression
- TILs generally secrete a variety of cytokines/chemokines based on the TME:
- Tumor programs TILs to support proinflammatory environment that supports cancer cell growth and progression
- Presence of inflammatory cytokines promotes tumor heterogeneity, epithelial-to-mesenchymal transition, loss of cancer-associated antigens and therapy resistance

can indirectly activate CD4⁺ T cells to release IL-17 that can result in resistance to therapy drugs [78]. Induction of IL-17 is dependent on IL-1 α released by chemotherapy-induced stimulation of myeloid-derived suppressor cells. Secreted IL-17 has a paracrine effect on tumor cells nullifying therapy effect. Recently, the release of proinflammatory cytokine such as TNF- α by TILs has been shown to cause dedifferentiation of mouse and human melanoma cells resulting in immune resistance by down-modulation of melanocytic antigens [79]. Furthermore, release of IFN- γ by anti-melanoma-specific cytotoxic T lymphocytes has resulted in an increased expression of immune checkpoint molecules PD-1 and PD-1L that can down-modulate T-cell function [79,80]. In many solid tumors, T and B cells co-exist in infiltrating tissues and the cross-talk between these two cell types cannot be ruled out in TME.

Reports on tumor-associated B cells are few and their biological significance is not well defined. This is despite the prevalence of infiltrating B cells in many solid tumors including cancers of the breast, colon, ovaries, prostate and skin [74,76]. Using mouse tumor models of prostate and squamous-cell carcinoma, it has been shown that B-cell-derived proinflammatory factors are actively involved in therapy resistance of cancer cells [62,81]. In a murine squamous-cell carcinoma model, Fc γ receptor mediated activation of myeloid-derived cells by infiltrating B-cell-derived immunocomplexes, resulting in tumor progression and resistance to chemotherapy [62]. In a murine prostate carcinogenesis model, tumor-infiltrating B-cell-derived lymphotoxin promotes inflammation and transformation to castration-resistant carcinomas [81].

Conclusion & future perspective

In the recent years, major advances have been made in the treatment of melanoma with targeted-therapy drugs or immune-based therapies. Despite the introduction of new therapy drugs, treatment of melanoma continues to be a major challenge as still many patients develop resistance or are nonresponsive to the therapy. Causes of therapy resistances are many including tumor heterogeneity, and the presence of hostile TME favoring tumor growth and progression. This includes hypoxia and the presence of inflammation through tumor-infiltrating leukocytes that have been re-programmed by the cancer cells to support tumor growth and spread. So far targeted and immunotherapies in patients with advanced disease were developed to directly or indirectly target the tumor cell itself. The more we understand the role of the TME in therapy resistance, additional modulation of the hostile inflammatory environment is becoming a more and more compelling strategy for future combinatorial therapies. First encouraging results are seen in patients in which targeting the tumor stroma in addition to the cancer cells have resulted in improved therapy responses.

Most recently, an additional immune classification via immunoscore of the TME has been proposed for implementation of a new prognostic component of the AJCC/UICC TNM classification of primary tumors [74]. A deepened understanding of the functional role of the cellular components of the TME and its re-programming through cancer cells are prerequisites for a further development of this score toward prediction of therapy response and, besides tumor genotype analyses, of paramount importance for the management of patients with metastatic disease.

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References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest.

- 1 Siegel R, Naishadham D, Jemal A. Cancer statistics. *CA Cancer J. Clin.* 62(1), 10–29 (2012).
- 2 Schadendorf D, Fisher DE, Garbe C *et al.* Melanoma. *Nat. Rev. Dis. Prim.* 3(1), 1–20 (2015).
- An excellent review on melanoma, a primer on signaling pathways to disease progression.
- 3 Miller AJ, Mihm MC Jr. Melanoma. *N. Engl. J. Med.* 355(1), 51–65 (2006).
- 4 Shtivelman E, Davies MQ, Hwu P *et al.* Pathways and therapeutic targets in melanoma. *Oncotarget* 5(7), 1701–1752 (2014).
- 5 De Souza CF, Morais AS, Jasiulionis MG. Biomarkers as key contributors in treating malignant melanoma metastases. *Dermatol Res. Pract.* 2012, 156068 (2012).
- 6 Robert C, Karaszewska B, Schachter J *et al.* Improved overall survival in melanoma with combined dabrafenib and trametinib. *N. Engl. J. Med.* 372(1), 30–39 (2014).
- 7 Srivastava N, McDermott D. Update on benefit of immunotherapy and targeted therapy in melanoma: the changing landscape. *Cancer Manag. Res.* 6, 279–289 (2014).
- 8 McDermott D, Lebbe C, Hodi FS *et al.* Durable benefit and the potential for long-term survival with immunotherapy in advanced melanoma. *Cancer Treat. Rev.* 40(9), 1056–1064 (2014).
- 9 Atkins MB, Lotze MT, Dutcher JP *et al.* High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. *J. Clin. Oncol.* 17(7), 2105–2116 (1999).
- 10 Melero I, Berman DM, Aznar MA, Korman AJ, Gracia JL, Haanen J. Evolving synergistic combinations of targeted immunotherapies to combat cancer. *Nat. Rev. Cancer* 15(8), 457–472 (2015).
- 11 Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* 39(1), 1–10 (2013).
- An excellent review on current trends in tumor immunology.
- 12 Dudley ME, Yang JC, Sherry R *et al.* Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. *J. Clin. Oncol.* 26(32), 5233–5239 (2008).
- 13 Ribas A, Flaherty KT. Gauging the long-term benefits of ipilimumab in melanoma. *J. Clin. Oncol.* 33(17), 1865–1866 (2015).
- 14 Rosenberg SA, Yang JC, Sherry RM *et al.* Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin. Cancer Res.* 17(13), 4550–4557 (2011).
- 15 Miller DM, Flaherty KT, Tsao H. Commentary: molecular testing in melanoma. *J. Am. Acad. Dermatol.* 70(5), 863–870 (2014).
- 16 Larkin J, Ascierto PA, Dreno B *et al.* Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. *N. Engl. J. Med.* 371(20), 1867–1876 (2014).
- 17 Long GV, Stroyakovskiy D, Gogas H *et al.* Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N. Engl. J. Med.* 371(20), 1877–1888 (2014).
- 18 Miller DM, Flaherty KT, Tsao H. Current status and future directions of molecularly targeted therapies and immunotherapies for melanoma. *Semin. Cutan. Med. Surg.* 33(2), 60–67 (2014).
- An excellent review on current status of targeted- and immune-based therapies.
- 19 Berman D, Korman A, Peck R, Feltquate D, Lonberg N, Canetta R. The development of immunomodulatory monoclonal antibodies as a new therapeutic modality for cancer: the Bristol-Myers Squibb experience. *Pharmacol. Ther.* 148, 132–153 (2015).
- 20 Postow MA, Chesney J, Pavlick AC *et al.* Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N. Engl. J. Med.* 372(21), 2006–2017 (2015).
- 21 Robert C, Schachter J, Long GV *et al.* Pembrolizumab versus ipilimumab in advanced melanoma. *N. Engl. J. Med.* 372(26), 2521–2532 (2015).
- 22 Brahmer JR, Hammers H, Lipson EJ. Nivolumab: targeting PD-1 to bolster antitumor immunity. *Future Oncol.* 11(9), 1307–1326 (2015).
- 23 Wolchok JD, Kluger H, Callahan MK *et al.* Nivolumab plus ipilimumab in advanced melanoma. *N. Engl. J. Med.* 369(2), 122–133 (2013).
- 24 Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. *Nature* (523(7559), 231–235 (2015).
- 25 Somasundaram R, Herlyn D. Chemokines and the microenvironment in neuroectodermal tumor-host interaction. *Semin. Cancer Biol.* 19(2), 92–96 (2009).
- 26 Somasundaram R, Villanueva J, Herlyn M. Intratumoral heterogeneity as a therapy resistance mechanism: role of melanoma subpopulations. *Adv. Pharmacol.* 65 335–359 (2012).
- 27 Zitvogel L, Kepp O, Kroemer G. Immune parameters affecting the efficacy of chemotherapeutic regimens. *Nat. Rev. Clin. Oncol.* 8(3), 151–160 (2011).
- Immune parameters and role in therapy resistance.
- 28 Cooper ZA, Reuben A, Amaria RN, Wargo JA. Evidence of synergy with combined BRAF-targeted therapy and immune checkpoint blockade for metastatic melanoma. *Oncoimmunology* 3(9), e954956 (2015).
- 29 Niezgoda A, Niezgoda P, Czajkowski R. Novel approaches to treatment of advanced melanoma: a review on targeted therapy and immunotherapy. *Biomed. Res. Int.* 2015, 851387 (2015).
- 30 Zhao Y, Butler EB, Tan M. Targeting cellular metabolism to improve cancer therapeutics. *Cell Death Dis.* 4, e532 (2013).
- 31 Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. *Nat. Rev. Cancer* 13(10), 714–726 (2013).
- 32 Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat. Rev. Cancer* 5(4), 275–284 (2005).
- 33 McMillin DW, Negri JM, Mitsiades CS. The role of tumour-stromal interactions in

- modifying drug response: challenges and opportunities. *Nat. Rev. Drug Discov.* 12(3), 217–228 (2013).
- 34 Tredan O, Galmarini CM, Patel K, Tannock IF. Drug resistance and the solid tumor microenvironment. *J. Natl. Cancer Inst.* 99(19), 1441–1454 (2007).
- 35 Cuvillier O, Ader I, Bouquerel P, Brizuela L, Gstalder C, Malavaud B. Hypoxia, therapeutic resistance, and sphingosine 1-phosphate. *Adv. Cancer Res.* 117, 117–141 (2013).
- 36 Roesch A, Fukunaga-Kalabis M, Schmidt Ec *et al.* A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. *Cell* 141(4), 583–594 (2010).
- 37 Roesch A, Vultur A, Bogeski I *et al.* Overcoming intrinsic multidrug resistance in melanoma by blocking the mitochondrial respiratory chain of slow-cycling JARID1B(high) cells. *Cancer Cell* 23(6), 811–825 (2013).
- 38 Ranasinghe WK, Sengupta S, Williams S *et al.* The effects of nonspecific HIF1alpha inhibitors on development of castrate resistance and metastases in prostate cancer. *Cancer Med.* 3(2), 245–251 (2014).
- 39 Widmer DS, Hoek KS, Cheng PF *et al.* Hypoxia contributes to melanoma heterogeneity by triggering HIF1alpha-dependent phenotype switching. *J. Invest. Dermatol.* 133(10), 2436–2443 (2013).
- 40 Zhao Y, Butler EB, Tan M. Targeting cellular metabolism to improve cancer therapeutics. *Cell Death Dis.* 4, e532 (2013).
- 41 Cuvillier O, Ader I, Bouquerel P, Brizuela L, Gstalder C, Malavaud B. Hypoxia, therapeutic resistance, and sphingosine 1-phosphate. *Adv. Cancer Res.* 117, 117–141 (2013).
- 42 Huang L, Perrault C, Coelho-Martins J *et al.* Induction of acquired drug resistance in endothelial cells and its involvement in anticancer therapy. *J. Hematol. Oncol.* 6, 49 (2013).
- 43 Hodi FS, Lawrence D, Lezcano C *et al.* Bevacizumab plus ipilimumab in patients with metastatic melanoma. *Cancer Immunol. Res.* 2(7), 632–642 (2014).
- 44 Braeuer RR, Shoshan E, Kamiya T, Bar-Eli M. The sweet and bitter sides of galectins in melanoma progression. *Pigment Cell Melanoma Res.* 25(5), 592–601 (2012).
- 45 Schumacker PT. Reactive oxygen species in cancer: a dance with the devil. *Cancer Cell* 27(2), 156–157 (2015).
- 46 Sabharwal SS, Schumacker PT. Mitochondrial ROS in cancer: initiators, amplifiers or an Achilles' heel? *Nat. Rev. Cancer* 14(11), 709–721 (2014).
- Discusses reactive oxygen species and its role in therapy resistance.
- 47 Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 140(6), 883–899 (2010).
- Discusses reactive oxygen species and its role in therapy resistance.
- 48 Junttila MR, De Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. *Nature* 501(7467), 346–354 (2013).
- 49 Flach EH, Rebecca VW, Herlyn M, Smalley KS, Anderson AR. Fibroblasts contribute to melanoma tumor growth and drug resistance. *Mol. Pharm.* 8(6), 2039–2049 (2011).
- 50 Johansson AC, Ansell A, Jerhammar F *et al.* Cancer-associated fibroblasts induce matrix metalloproteinase-mediated cetuximab resistance in head and neck squamous cell carcinoma cells. *Mol. Cancer Res.* 10(9), 1158–1168 (2012).
- 51 Shekhar MP, Santner S, Carolin Ka, Tait L. Direct involvement of breast tumor fibroblasts in the modulation of tamoxifen sensitivity. *Am. J. Pathol.* 170(5), 1546–1560 (2007).
- 52 Muerkoster S, Wegehenkel K, Arlt A *et al.* Tumor stroma interactions induce chemoresistance in pancreatic ductal carcinoma cells involving increased secretion and paracrine effects of nitric oxide and interleukin-1beta. *Cancer Res.* 64(4), 1331–1337 (2004).
- 53 Wang W, Li Q, Yamada T *et al.* Crosstalk to stromal fibroblasts induces resistance of lung cancer to epidermal growth factor receptor tyrosine kinase inhibitors. *Clin. Cancer Res.* 15(21), 6630–6638 (2009).
- 54 Straussman R, Morikawa T, Shee K *et al.* Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. *Nature* 487(7408), 500–504 (2012).
- Discusses innate immunity and therapy resistance.
- 55 Wilson TR, Fridlyand J, Yan Y *et al.* Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. *Nature* 487(7408), 505–509 (2012).
- 56 Cirri P, Chiarugi P. Cancer associated fibroblasts: the dark side of the coin. *Am. J. Cancer Res.* 1(4), 482–497 (2011).
- 57 Mizuno S, Nakamura T. HGF-MET cascade, a key target for inhibiting cancer metastasis: the impact of NK4 discovery on cancer biology and therapeutics. *Int. J. Mol. Sci.* 14(1), 888–919 (2013).
- 58 Kennedy BC, Showers CR, Anderson DE *et al.* Tumor-associated macrophages in glioma: friend or foe? *J. Oncol.* 2013, 486912 (2013).
- 59 Chanmee T, Ontong P, Konno K, Itano N. Tumor-associated macrophages as major players in the tumor microenvironment. *Cancers (Basel)* 6(3), 1670–1690 (2014).
- 60 Denardo DG, Brennan DJ, Rexhepaj E *et al.* Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. *Cancer Discov.* 1(1), 54–67 (2011).
- 61 Wang T, Ge Y, Xiao M *et al.* Melanoma-derived conditioned media efficiently induce the differentiation of monocytes to macrophages that display a highly invasive gene signature. *Pigment Cell Melanoma Res.* 25(4), 493–505 (2012).
- 62 Affara NI, Ruffell B, Medler TR *et al.* B cells regulate macrophage phenotype and response to chemotherapy in squamous carcinomas. *Cancer Cell* 25(6), 809–821 (2014).
- 63 Ries CH, Cannarile MA, Hoves S *et al.* Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. *Cancer Cell* 25(6), 846–859 (2014).
- 64 Wang T, Xiao M, Ge Y *et al.* BRAF inhibition stimulates melanoma-associated macrophages to drive tumor growth. *Clin. Cancer Res.* 21(7), 1652–1664 (2015).
- 65 Heusinkveld M, Van Der Burg SH. Identification and manipulation of tumor associated macrophages in human cancers. *J. Transl. Med.* 9, 216 (2011).
- 66 Jensen TO, Schmidt H, Moller HJ *et al.* Macrophage markers in serum and tumor have prognostic impact in American Joint Committee on Cancer stage I/II melanoma. *J. Clin. Oncol.* 27(20), 3330–3337 (2009).
- 67 Varney ML, Johansson SL, Singh RK. Tumour-associated macrophage infiltration, neovascularization and aggressiveness in malignant melanoma: role of monocyte chemotactic protein-1 and vascular endothelial growth factor-A. *Melanoma Res.* 15(5), 417–425 (2005).
- 68 Smith MP, Sanchez-Laorden B, O'Brien K *et al.* The immune microenvironment confers resistance to MAPK pathway inhibitors through macrophage-derived TNFalpha. *Cancer Discov.* 4(10), 1214–1229 (2014).
- 69 Fricke I, Gabrilovich DI. Dendritic cells and tumor microenvironment: a dangerous liaison. *Immunol. Invest.* 35(3–4), 459–483 (2006).

- **Dendritic cells, myeloid-derived cells and their role in immune modulation.**
- 70 Shurin GV, Ma Y, Shurin MR. Immunosuppressive mechanisms of regulatory dendritic cells in cancer. *Cancer Microenviron.* 6(2), 159–167 (2013).
- 71 Ma Y, Shurin GV, Peiyuan Z, Shurin MR. Dendritic cells in the cancer microenvironment. *J. Cancer* 4(1), 36–44 (2013).
- 72 Williams M, Ginhoux F, Jakubzick C *et al.* Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat. Rev. Immunol.* 14(8), 571–578 (2014).
- 73 Jochems C, Schlom J. Tumor-infiltrating immune cells and prognosis: the potential link between conventional cancer therapy and immunity. *Exp. Biol. Med. (Maywood)* 236(5), 567–579 (2011).
- 74 Galon J, Pages F, Marincola FM *et al.* Cancer classification using the Immunoscore: a worldwide task force. *J. Transl. Med.* 10, 205 (2012).
- 75 Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. *Oncogene* 27(45), 5904–5912 (2008).
- 76 Erdag G, Schaefer JT, Smolkin ME *et al.* Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma. *Cancer Res.* 72(5), 1070–1080 (2012).
- 77 Cooper ZA, Frederick DT, Ahmed Z, Wargo JA. Combining checkpoint inhibitors and BRAF-targeted agents against metastatic melanoma. *Oncoimmunology* 2(5), e24320 (2013).
- 78 Bruchard M, Mignot G, Derangere V *et al.* Chemotherapy-triggered cathepsin B release in myeloid-derived suppressor cells activates the NLRP3 inflammasome and promotes tumor growth. *Nat. Med.* 19(1), 57–64 (2013).
- 79 Landsberg J, Kohlmeyer J, Renn M *et al.* Melanomas resist T-cell therapy through inflammation-induced reversible dedifferentiation. *Nature* 490(7420), 412–416 (2012).
- 80 Taube JM, Anders RA, Young GD *et al.* Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. *Sci. Transl. Med.* 4(127), 127ra137 (2012).
- 81 Ammirante M, Luo JI, Grivennikov S, Nedospasov S, Karin M. B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature* 464(7286), 302–305 (2010).