New insights in melanoma biology: running fast towards precision medicine

Cutaneous melanoma is one of the most aggressive and the deadliest form of skin cancers characterized by a high degree of intra-tumor heterogeneity harboring mutations in genes involved in different signaling pathways. The majority of melanomas are diagnosed in the early stage and are treatable with surgical resection with a five-year survival rate ranging 82% to 98% for stages I-II. On the other hand, the prognosis of highly aggressive, late stage, melanoma is still very poor. For later stage and metastatic melanoma the alkylating agents, Dacarbazine or Temozolomide were the earliest treatment option; however, patients easily become resistant to these drugs and they also have serious side effects with low overall response and survival rates. 'Targeted therapies', such as Vemurafenib and Dabrafenib ($BRAF^{V600E}$ inhibitors) , were developed in order to specifically target mutated proteins, with a response rate around 50%; however, most patients relapse within few months [1]. The combination of BRAF inhibitors with MEK inhibitors, such as Cobimetinib and Trametinib, achieved response rates as high as 70%; however, most patients still face progressive disease even if treated with combination therapy, with less than 25% of patients being progression-free at 3 years. Along with the development of 'targeted therapies', the immune-checkpoint inhibitors, such as Ipilimumab (a CTLA-4 inhibitor), Nivolumab and Pembrolizumab (PD-1 inhibitors), were developed to improve the endogenous antitumor immune response. However, they are effective in only a subset of patients and may be associated with serious side effects and the development of acquired resistance in initially responders [2, 3]. Thus, many questions still remain to be answered: what is the best first- and second-line treatment or the best treatment sequence? New combinations of drugs, 'targeted therapies' combined with immunotherapies, immunotherapies combinations, and sequencing strategies are now underway in different clinical trials, highlighting the tendency towards a personalized medical approach [4-9].

Based on these data, we have foreseen this special issue to provide a critical survey of the current knowledge of the molecular mechanisms underlying melanoma development; we strongly believe that a deeper understanding of the complex biology network of melanoma will likely lead to the identification of novel molecular markers of disease progression/response to therapies and biotargets for innovative and complementary therapeutic approaches.

Melanoma is a tumor characterized by a high intra-tumor and inter-tumor molecular heterogeneity; these levels of tumor heterogeneity are responsible for the lack of accurate diagnosis and effective treatments. Therefore, in the last few years, a tremendous effort has been put in the development of 'omics' technologies (genomics, transcriptomics, proteomics and metabolomics) with the aim to dissect the immense heterogeneity in the biological mechanisms in tumors, including melanoma. In their article, Donnelly and coworkers [10] point out how the recently

available 'omics' platforms allowed the discovery of several novel biomarkers in melanoma; these include both tumor tissue-related and circulating biomarkers in liquid biopsies. Tumor tissues biomarkers, in addition to the well known BRAF (occurring in 40-60% of patients) and NRAS (occurring in 5-15% of patients) genetic alterations, include mutations in genes involved in the mechanisms of cell proliferation (KIT, GNA11/GNAQ, NF1, CDKN2A) as well as altered epigenetic mechanisms and immune destruction and inflammation mechanisms. On the other hand, circulating biomarkers in liquid biopsies of melanoma patients include ctDNA, miRNAs, lncRNAs, proteins/metabolites. The pivotal role of these biomarkers in the diagnosis, prognosis and treatment response prediction in melanoma is discussed in this article.

Angiogenesis, the formation of new blood vessels from pre-existing blood vessels, is a complex and highly regulated process that plays a key role in tumor growth and progression. This process provides tumor cells with oxygen and nutrients, critical for their continued proliferation and aggressiveness. Angiogenesis is regulated by a sequence of well-coordinated events mediated by tightly regulated interactions between proangiogenic factors and their receptors expressed on vascular cells components (endothelial cells and pericytes) and stromal components forming the extracellular matrix. Cho and coworkers [11] discuss the most relevant growth factors and cytokines (VEGF-A, bFGF, PIGF, Ang, PDGF, IL-8) endowed with pro-angiogenic activity (endothelial cells proliferation and migration, capillary sprouting) and the factors involved in the degradation of the extracellular matrix components and in tissue remodelling (uPA, integrins, matrix metalloproteinases). The molecular mechanisms of the synergistic activity of these factors in promoting melanoma angiogenesis is deeply discussed. Interestingly, high levels of these factors in liquid biopsies as well as in tissues biopsies from melanoma patients were reported to be correlated with a poor response and reduced overall survival, supporting their role as effective biomarkers of tumor development/drug resistance.

Tumor cells need high amounts of energy as well as of building blocks for the synthesis of new cellular components for their growth and survival. In particular, they display increased activity of the glycolysis pathways accompanied by high levels of glucose uptake (the 'Warburg effect') [12]. On the other hand, cancer cells can also possess functional mitochondria with active oxidative phosphorylation (OXPHOS) and oxygen consumption [13]. Another aspect of the metabolic reprogramming in cancer cells is the high rate of fatty acid synthesis and of glutaminolysis, leading to the conversion of glutamine into glutamate to fuel the TCA cycle (tricarboxylic acid cycle) and into intermediates necessary for aminoacids and nucleotides synthesis [14]. In their article, Ruocco et al. [15] discuss the metabolic alterations in melanoma cells, i.e. increased glycolysis and

mitochondrial glutamine uptake, as well as the intracellular pathways involved in these modifications. Mitochondrial dynamics (fission vs. fusion) are also dysregulated in melanoma cells. Energetic metabolism of melanoma cells can change in response to extracellular signals, such as hypoxia, microenvironment acidification and nutrient deprivation, or intracellular signals modulated by the BRAF/MAPK mutated pathways. This altered melanoma metabolism is associated with cellular growth, proliferation, invasion and metastasis, and has been shown to be involved in reistance to both BRAF/MEK inhibitors (BRAFi/MEKi) and immunotherapy. Melanoma cells-microenvironment interaction is bidirectional thus resulting in a biophysical reciprocity whereby cancer cells recruit host tissue cells that in turn participates in the progression, metastatic behavior and in the metabolic support and regulation of the tumor [16]. In their review, Ruocco et al. [15] also address the metabolic interplay between cancer cells and tumor microenvironment that modulate immune response and cancer progression. Specifically, tumor glycolysis pathways have a strong immunosuppressive role in melanoma; this is related to the increased rates of glycolysis and expression of glycolytic enzymes in melanoma cells that generate a glucose-deficient microenvironment, in which tumor cells and tumor infiltrating lymphocytes compete for glucose uptake, thus interfering with immune surveillance. The authors conclude that a better clarification of the metabolic alterations in melanoma cells and the metabolic interplay between cancer cells and tumor immune microenvironment may allow the development of new therapeutic strategies ameliorating the efficacy of currently used therapies.

Epigenetic mechanisms (such as DNA methylation, histone modifications and miRNAs) are essential for both a normal development and the maintainence of a tissue-specific gene expression pattern. On the other hand, alterations of epigenetic processes can promote altered gene functions and malignant cellular transformation [17, 18]. It is actually well accepted that changes in the epigenetic landscape represent a hallmark of tumors. Thyagarajan and coworkers [19] address the altered expression of miRNAs involved in several key cellular processes of melanoma cells. Specifically, miR-21 and miR-155, involved in proliferation and metastatic behavior of cancer cells, and miR-211, miR-193, miR-126, miR-145, miR-200 family, involved in apoptosis and tumor suppressor signalings, were reported to be up-regulated and down-regulated, respectively, in both melanoma tumor tissues (intracellular miRNAs) and serum/plasma samples (extracellular miRNAs). The key role of these miRNAs as diagnostic/prognostic biomarkers or molecular targets for future therapeutic intervention for malignant melanoma is also deeply discussed.

Cancer stem cells (CSCs) are a slow-cycling subpopulation of cells defined by their ability to: 1) self-renew indefinitely, 2) form single cell-derived clonal cell populations, and 3) differentiate into various cell types [20, 21]. The existence of CSCs in melanoma, as well as their role in tumor

relapse, is well documented [22]. From the molecular point of view, melanoma CSCs are identified and characterized by the expression of stemness markers, such as surface markers; however, the role of these markers has been a matter of debate [23]. In melanoma cells, a subpopulation of cells expressing the ATP-binding cassette transporter ABCG2, associated with autofluorescence and endowed with stemness traits, has been recently identified [24]. In their article, Marzagalli et al. [25] discuss the different functional features of melanoma stem cells. In particular, it is reported that melanoma CSCs play pivotal roles in the processes of epithelial-to-mesenchymal transition (EMT), angiogenesis and dissemination, mediated by specific intracellular signaling pathways; moreover, they are characterized by a unique metabolic reprogramming. Melanoma CSCs are also characterized by a low immunogenic profile and by the ability to escape the immune system, through the expression of a negative modulation of T cell functions and the secretion of immunosuppressive factors. Based on these biological features melanoma CSCs are able to escape standard treatments, thus being deeply involved in tumor relapse. Targeting the CSCs subpopulation is now considered an attractive treatment strategy; combination treatments, based on both CSCs-targeting and standard treatments, will likely increase the therapeutic options for melanoma patients. The characterization of CSCs in liquid biopsies from single patients will hopefully pave the way towards personalized treatments [25].

Melanomas are among the most immunogenic tumors being endowed with a great potential to elicit specific adaptive antitumor immune responses and to respond favorably to immunotherapy. Unfortunately, the majority of melanomas rapidly acquire different suppressive mechanisms, which generally act in concert, to escape innate and adaptive immune detection and destruction; a 40 to 65% treatment failure for patients treated with anti-PD-1 and a 70% treatment failure in patients treated with anti-CTLA-4 were reported. Intense research into the cellular and molecular events associated with melanomagenesis, which ultimately lead to immune suppression, has resulted in the discovery of new immune system-therapeutic targets and synergistic combinations of immunotherapy, 'targeted therapy' and chemotherapy. In their article, Marzagalli and coworkers [26] review the evolution of the immune system during melanomagenesis, the mechanisms exploited by melanoma to suppress anti-tumor immunity and the methods that have been developed to restore immunity. After the initial participation of the immune landscape in the control of transformation of pre-malignant cells (innate immunity, adaptive immunity), melanoma cells that most fit to evade immunity can proliferate leading to tumor progression (analogous to Darwinian selection). Specifically, different mechanisms can lead to immune escape: 1) escape of melanoma cells from T cell recognition through the modulayion of the expression of highly immunogenic proteins (i.e., tumor-associated antigens, TAAs); 2) a dysfunction of T cells which lose their

effector activities (i.e., cytotoxicity) due to chronic antigen presentation, eventually by non-professional presenting cells (such as melanoma cells); 3) secretion of cytokines and chemokines by melanoma cells leading to the recruitment of pro-tumor, and the suppression or exclusion of anti-tumor, immune subsets (T cells, macrophages, neutrophils); 4) a rapid reprogramming of melanoma cell metabolism, responsible for an inhibitory activity of immune cells activity and proliferation; 5) altered expression of miRNAs involved in the modulation of the immune microenvironment in malignant melanoma; 6) secretion from tumor cells of exosomes that, through their cargos, can exert a pro-apototic activity in immune cells. In summary, this review integrates available knowledge on melanoma-specific immunity, molecular signaling pathways and molecular targeting strategies that could be utilized for the development of therapeutics with broader application and greater efficacy for melanoma patients, increasing the feasibility of personalized treatment to overcome tumor heterogeneity and to achieve greater clinical benefits.

Melanoma cells produce a variety of extracellular vesicle (EV) types including shedding vesicles and exosomes (EXOs). These EVs are defined by their mechanism of cellular production. To date, the majority of EV investigations has focused on melanoma EXOs or small EVs (sEVs). Hood's article aims to examine the contribution of natural unmodified melanoma EVs to melanoma pathogenesis, and briefly explore their translational potential [27]. In particular, the different roles of natural melanoma sEVs in pro-tumor processes, such as angiogenesis, immune regulation and modification of tissue microenvironments, are deeply discussed. A thorough examination of these processes reveals that they are interdependent, working in concert to support tumor growth and survival. Pro-tumor functions attributed to melanoma cells themselves are recapitulated through melanoma sEVs, and this ensures a certain degree of redundancy within the melanoma pathogenic process, allowing for rapid adaptation of melanoma cells to changing microenvironments, anti-tumor immune responses, and therapeutic challenges. These specific roles of sEVs in melanoma growth and progression are strictly associated with their cargos, such as lipids, proteins, noncoding RNA types, particularly miRNAs. Further, as a result of their composition and inherent ability to engage the immune system, natural melanoma EVs possess excellent biomarker potential and might be used therapeutically as tumor vaccines.

Treatment strategies for melanoma patients are known to be associated with development of drug resistance and severe side effects. In recent years, natural compounds have been extensively investigated for their anti-melanoma effects: tumor growth inhibition, apoptosis induction, angiogenesis and metastasis suppression, cancer stem cells eradication. Moreover, a considerable number of studies reported the synergistic activity of phytochemicals and standard therapeutics, as well as the effectiveness of their synthetic derivatives and novel formulations. The molecular

mechanisms of the antitumor activity of different natural compounds such as quercetin, fisetin, apigenin, luteolin, genistein, epigallocatechin-3-gallate, curcumin, resveratrol, sulforaphane, ursolic acid, ginsenosides, tocotrienols and berberine in melanoma cells are highlighted by Fontana and coworkers [28]. These authors also underline that, unfortunately, clinical data confirming these promising effects in patients and, therefore, the potential application of the most studied natural products for melanoma prevention and treatment are still scanty. Given their anticancer activities, widely reported in in vitro and preclinical studies, together with their bioavailability and lack of side effects, clinical trials addressing the efficacy of these compounds as novel anti-tumor treatment strategies are urgently needed.

Over the past 20 years, the adjuvant treatment of high-risk melanoma relied on interferon alpha, with a consistent effect on relapse-free survival and a less consistent and less impressive effect on overall survival, at the cost of severe toxicities, especially when used at high doses. The past 5 years have witnessed the results of many practice-changing studies that have dramatically improved the landscape of adjuvant therapy in patients with resected, high-risk melanoma, with a reduction of the risk of recurrence of about 50% for anti-PD-1 drugs and BRAF+MEK inhibitors compared with placebo. In their review, Spagnolo et al. [29] discuss the results of such studies in light of other revolutionary changes in the surgical management of high-risk melanoma. In fact, while the prognostic value of sentinel lymph node biopsy-based staging was confirmed, completion lymph-node dissection in patients with positive sentinel node was demonstrated to have no impact on overall survival in two randomized trials and is no longer recommended by the most recent clinical guidelines. The authors suggest that this conservative approach may lead to reduced risk of surgical morbidity and is supported by the outstanding results obtained by adjuvant anti-PD-1 drugs and BRAF+MEK inhibitors, limiting the need for lymphadenectomy in patients who relapse in regional lymph nodes only despite adjuvant therapy. The authors propose an algorithm for the management of resected melanoma at high risk of recurrence in everyday clinical practice, with a look on the future directions of the field, giving some insights on neoadjuvant treatments.

Despite melanoma has always been described as an immunogenic tumor and a model of cancer immunotherapy, “old generation” immunotherapies such as vaccines and cytokines provided an impact on survival in a very limited subset of patients. The revolution in the field of melanoma immunotherapy was brought by the introduction of 'new generation' immune-checkpoint inhibitors, such as anti-CTLA-4 and anti-PD-1 drugs. This strategy proved to be effective not only in melanoma, but also in a number of other solid tumors and hematologic malignancies, and was considered one of the most important breakthrough in modern oncology. In their article, Queirolo and colleagues [30] explain the mechanism of action of such treatments and report the results of the

main clinical trials that led to their approval. To improve the efficacy of immunotherapy with a limited impact on toxicity, the investigation of new combinations and sequences of treatments is underway and many clinical trials are ongoing. Despite the high number of treatments and combinations of treatments, no validated predictive biomarker exist to select patients, and the authors suggest that more efforts should be addressed into the pursuit of predictive biomarkers that may guide the clinicians towards personalized medicine.

The Guest Editors and the authors of these articles sincerely hope that the recently discovered biological features of melanoma addressed in this special issue of the journal Seminars in Cancer Biology will be of interest not only for biologists but also for pharmacologists and oncologists providing the molecular basis for the identification of novel diagnostic/prognostic markers as well as innovative therapeutic strategies, paving the way towards precision medicine.

Acknowledgements

We, Patrizia and Paola, are deeply grateful to the Editor-in-Chief, Professor Theresa Vincent, for the opportunity to serve as Guest Editors of this special issue dedicated to the complex biology network of melanoma cells and its key role in the develoment of novel therapeutic approaches in terms of precision medicine. We also like to sincerely thank Carly Middendorp and Rohini Nair for their prompt, kind and very useful assistance in the processes of both articles handling and issue preparation.

We also thank Comitato Emme Rouge Onlus (Milano, Italy) for supporting young postdoctoral fellows giving them the opportunity to carry out the research in melanoma biology summarized and discussed in our articles of this special issue.

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