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Review

Recent advances in melanoma research via "omics" platforms

Carmen Rodríguez-Cerdeira[a,b,*], Alberto Molares-Vila[a,c], Miguel Carnero-Gregorio[a,d], Alberte Corbalán-Rivas[e]

[a] Efficiency, Quality and Costs in Health Services Research Group (EFISALUD), Galicia Sur Health Research Institute (IIS Galicia Sur), SERGAS-UVIGO, Spain
[b] Dermatology Department, Complexo Hospitalario Universitario de Vigo (CHUVI), SERGAS, Vigo, Spain
[c] Department of Analytical & Food Chemistry, Universidade de Vigo (UVIGO), Spain
[d] Department of Biochemistry, Genetics & Immunology, Universidade de Vigo (UVIGO), Spain
[e] Nursery Department, Complexo Hospitalario Universitario de A Coruña (CHUAC), SERGAS, A Coruña, Spain

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ABSTRACT

Melanoma has a high mortality rate and metastatic melanoma is highly resistant to conventional therapies. "Omics" fields such as proteomics and microRNA and exosome studies have provided new knowledge to complement the information generated by genomic studies. This work aimed to review the current status of biomarker discovery for melanoma through multi-"omics" platforms. A few sets of novel microRNAs and proteins are described, some of them with important implications in suppressing melanoma at different stages. Upregulation of genes involved in angiogenesis, immunosuppressive factors, modification of stroma, capture of melanoma cells in lymph nodes and factors responsible for tumour cell recruitment have been identified in exosomes, among molecules with other functions. A remarkable series of proteins involved in epithelial-mesenchymal/mesenchymal-epithelial transitions, inflammation, motility, proliferation and progression processes, centrosome amplification, aneuploidy, inhibition of CD8 + effector T-cells, and metastasis in general were identified. Genomic and protein-protein interactions or metabolome levels were not analysed.

Proteomics tools such as Orbitrap shotgun mass spectrometry or deep mining proteomic analysis utilizing high-resolution reversed phase nanoseparation in combination with mass spectrometry are also discussed. The application of these tools together with bioinformatics approaches applied to the clinical setting will enable the implementation of personalized medicine in the near future.

1. Introduction

Melanoma has an overall mortality rate of 20% and metastatic melanoma is highly resistant to conventional therapies. Several factors are involved in the development of melanoma at the environmental, genetic, and individual level [1].

Within the "omics", genomics is one of the most advanced approaches, allowing the determination of genome amplifications and deletions, as well as gene fusions. Next-generation sequencing and array-based comparative genomic hybridization (Array-CGH) are two of the most-used techniques, and the latter is an accessible, reliable, and economical tool. One of the first uses of Array-CGH was reported in mouse melanoma to study the chromosomal aberrations in melanomas with the same phenotype [2].

One of the important genes for melanoma formation is the v-Raf murine sarcoma viral oncogene (*BRAF*), which is mutated in 50–60% of melanomas and whose product acts at the level of several mitogen-activated protein kinases (MAPK) pathways, causing cell proliferation and triggering mechanisms of apoptosis evasion [3]. This protein is not tumorigenic on its own and is used as a prognostic marker [4,5]. Another gene involved in melanoma is the tumour suppressor gene Cyclin-dependent kinase inhibitor 2 A (*CDKN2A*), which is mutated in 16–41% of melanomas [6,7]. One of the products of this gene is the $p16^{INK4A}$ protein that activates the retinoblastoma (RB) protein [8]. Amplification of the microphthalmia-associated transcription factor (MITF) locus is found in 20–30% of tumours and is usually accompanied with mutations in the BRAF gene and by the loss of $p16^{INK4A}$ [9]. Another gene associated with melanomas is Cyclin-dependent kinase 4 (*CDK4*), which together with *CDKN2A*, is involved in cell cycle control, affecting the G_1/S phase checkpoint [10,11].

Melanoma metastasis is linked to the activation of certain pathways responsible for embryogenesis [12]. Among these routes are the MAPKs that activate extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2), which in turn regulate several transcription factors, leading to

* Corresponding author at: Dermatology Service, Meixoeiro Hospital, Meixoeiro S/N, 36200 Vigo, Spain.
E-mail address: carmen.rodriguez.cerdeira@sergas.es (C. Rodríguez-Cerdeira).

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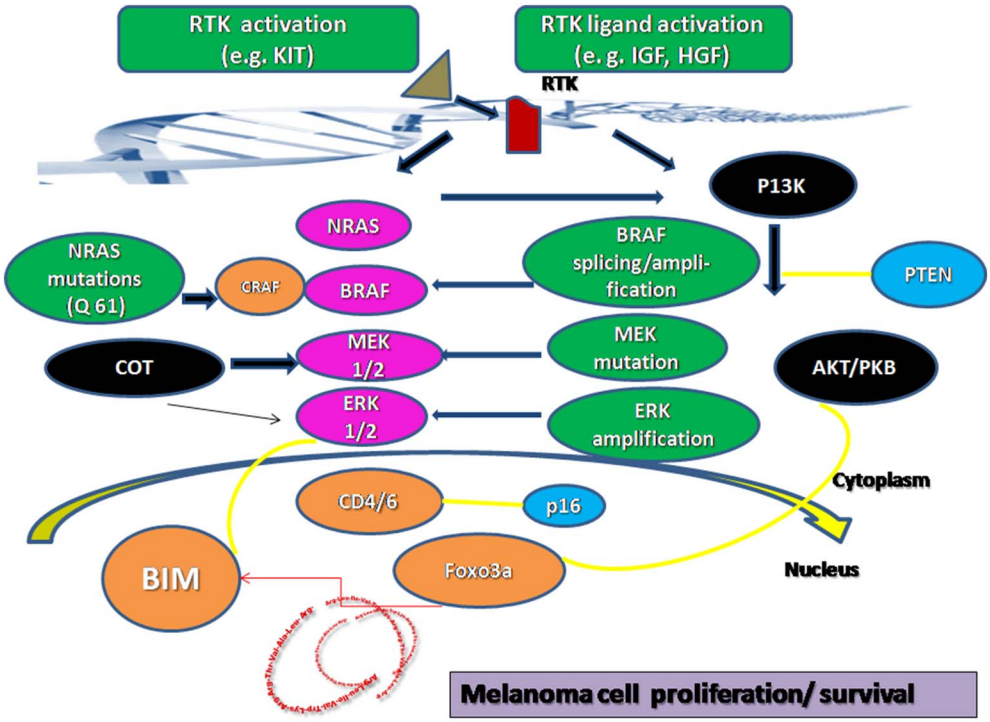

Fig. 1. Overview of receptor tyrosine kinase (RTK) networks and Bcl-2 interacting mediator of cell death (Bim) checkpoint blockade. RTK signalling cascades are responsible for coordinating intracellular signalling in response to various stimuli such as growth factors, chemokines, and other extracellular stimuli in order to control fundamental biological processes such as cell proliferation, metabolism, and survival. Bim appears to have an important role in cancer; its upregulation has a negative effect on the survival of patients with melanoma. Melanoma cells have developed mechanisms that suppress Bim expression to promote tumour progression and metastasis. Therefore, this process has become a new target that could help many patients who were previously considered untreatable. Abbreviations: c-Kit, a receptor tyrosine kinase; *HGF*, hepatocyte growth factor; *IGF*, insulin-like growth factor.

cell proliferation [13].

Another pathway commonly altered in melanoma is WNT signalling, which may be canonical or non-canonical depending on whether β-catenin is involved [14]. The canonical pathway contributes to melanoma formation, and the non-canonical pathway is involved in the metastatic process [15]. The canonical WNT pathway acts synergistically with the MAPK cascade, favouring melanoma formation and development [16]. In addition, the WNT5A protein involved in the non-canonical pathway induces the release of exosomes from tumour cells, which contain proangiogenic and immunosuppressive factors [17]. There is also evidence that the family of G-protein-coupled receptors (GPCRs) plays an important role in tumorigenesis and melanoma progression [18]. The RAS oncogene is known to stimulate the phosphatidylinositol-3-kinase (PI3K) pathway [19]; PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP_2) to phosphatidylinositol-3,4,5-trisphosphate (PIP_3), which entails phosphorylation of the serine-threonine-specific kinase (AKT) [20]. p-AKT acts as an oncogenic signal that promotes tumour progression [21] (Fig. 1).

In melanoma, cellular metabolism is altered; tumour cells bypass mitochondrial oxidative phosphorylation and depend on cytoplasmic aerobic glycolysis (Warburg effect) [3]. One of the key regulators of the switch to aerobic glycolysis is hypoxia inducible factor 1α (HIF1α), whose effects can be offset by peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α). Activation of the MAPK pathway via $BRAF^{V600E}$, negatively regulates MITF, thus suppressing the effect of PGC1-α, and increasing the expression of HIF1α through mTOR stimulation [3].

Melanoma cells can reverse their original phenotype through a process called the mesenchymal-epithelial transition (MET). E-cadherin downregulation is accompanied by an increase in N-cadherin, which alters cell adhesion and favours cell migration [22]. Decreased E-cadherin during tumour development correlates with a poor disease prognosis [23].

There are many other molecules that can be related to melanoma pathogenesis. One such molecule is p53 (associated with exposure to UV radiation), whose inactivation or low expression is found in 90% of tumours [24]. p53 reactivation together with $BRAF^{V600E}$ inhibition causes apoptosis and suppresses melanoma growth [24]. Another molecule that is involved is c-KIT, which is a receptor tyrosine kinase for stem cell factor (SCF) [25]. Mutations in c-KIT are involved in melanocyte development, migration, and survival processes; its expression is lost when the disease reaches the metastatic stage [26]. Another molecule linked to melanoma development is the tumour antigen melanotransferrin (MFI2) [27]. If MF-12 expression in tumour cells is silenced, cell proliferation, migration, and DNA synthesis, among other processes, are greatly reduced in mice [28]. Mutations in NRAS are present in 15–30% of melanomas [29,30], and lead to abnormal proliferation and melanocyte survival [31]. Other genes whose mutation can lead to melanoma are heterotrimeric G protein α-subunit (GNAQ) and G protein subunit alpha 11 (GNA11) [32,33], Rac family small GTPase 1 (RAC1, associated with chronic exposure to sunlight) [34], phosphatidylinositol-3,4,5-trisphosphate dependent Rac exchange factor 2 (PREX2) [35], telomerase reverse transcriptase (TERT, mutated in 85% of metastatic melanomas) [36], and phosphatase and tensin homolog (PTEN, which is lost in 25–30% of melanomas and favours cell survival and apoptosis inhibition) [37]. Fig. 2 depicts an action scheme for some of these biomarkers in melanoma progression.

Melanoma stage is evaluated using criteria such as lactate dehydrogenase (LDH) levels, sentinel node biopsy, or imaging tests. Surgical resection is the choice treatment for early-stage melanomas, but those in advanced stages require treatment with chemotherapeutic agents (dacarbazine alone or combined with vinblastine or cisplatin, high doses of interferon α-2b, etc.) [38,39].

Regarding treatments for melanoma, neither administration of cytokines like IL-2, nor dacarbazine improved the survival rate of patients prior to 2011 [40]. Since then, the FDA has approved several anti-tumour agents, but only vemurafenib is effective against cells harbouring the $BRAF^{V600E}$ mutation [41].

Dabrafenib is another BRAF inhibitor that has improved progression-free survival (PFS) compared to dacarbazine [42], as does trametinib (a MEK1/2 inhibitor) [43]. Imatinib is a c-KIT inhibitor that is currently in phase II and III clinical trials for patients with metastatic melanoma [44]. However, resistance to these treatments has appeared over time, and it has been suggested that a combination with several of these treatments may be of benefit to patients [45].

Other therapeutic targets are the immune checkpoint inhibitors