Biomarkers in Cutaneous Melanoma

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Contributor: Simona Roxana Georgescu , Cristina Iulia Mitran , Madalina Irina Mitran , Clara Matei , Carolina Constantin , Monica Neagu , Mircea Tampa

Melanoma is the most frequent of the aggressive cutaneous malignancies, being the first cause of death for patients with skin cancer. Melanoma is considered the tumor with the highest mutation rate. Mutations in the *BRAF* gene are present in 50–60% of melanomas and the *CDKN2A* gene is altered in 16–41% of melanomas.

melanoma biomarker immune cells microRNA

1. Introduction

Melanoma is the most frequent of the aggressive cutaneous malignancies, being the first cause of death for patients with skin cancer [1]. Cutaneous melanoma develops from melanocytes and can be classified into four major types, superficial spreading melanoma (70%), the most prevalent form, nodular melanoma (15–30%), lentigo maligna melanoma (4–10%) and the less common form, acral lentiginous melanoma (<5%) [2]. However, it should be kept in mind that melanoma can develop wherever there are melanocytes appending to tissues; thus, it can develop in the skin, eye, nasopharynx or gastrointestinal tract [2]. The main risk factors associated with melanoma are phototypes I-II, an increased number of nevi, history of sun exposure, advanced age and family history of skin cancer [3][4][5]. Melanoma is considered the tumor with the highest mutation rate. Mutations in the *BRAF* gene are present in 50–60% of melanomas and the *CDKN2A* gene is altered in 16–41% of melanomas [6]. Other mutated genes associated with melanoma development are *NRAS* and *C-KIT* [7]. The intimate mechanisms of melanoma development are complex and still incompletely elucidated [8][9][10]. The main intracellular signaling pathways described as being involved in its pathogenesis are the MAPK, PI3K-AKT, MITF, NF-kB and Wnt pathways [11]. Although several markers have been described and algorithms for the rapid diagnosis of melanomas have been established, the tumors are often diagnosed in their late stages and the prognosis is poor in that phase of evolution [12].

2. Novel Genetic Aspects in Melanoma

Melanoma exhibits significant heterogeneity, with diverse genomic alterations being one of the highest mutated tumors. Therefore, tumor genetic heterogeneity is an important player in the process of immune evasion, invasion and metastasis of new tissues. This genetic plasticity interferes with the proper diagnosis and treatment of melanoma [13]. Currently, melanoma staging is based on clinical and histopathological characteristics, but to identify new prognostic markers, it is important to investigate gene expression in the case of each melanoma

patient [14], as new technologies have opened an entire new research area in genomic biomarkers in melanoma [15] [16].

Data from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases provide new genetic profiles aimed to predict patient outcomes. It was identified that downregulation of the genes *CDCA8* and *DPF* and upregulation of the genes *ABCC3*, *CAPS2*, *CCR6*, *CLU*, *PTK2B*, *SATB1*, and *SYNE* represent indicators for a positive prognosis of cutaneous metastatic melanoma [14]. Another study, using the same databases, showed that the over-expression of the following six hub genes, *CDC20*, *GNB2*, *PPP2R1A*, *AURKB*, *POLR2E*, and *AGTR1*, is associated with low overall survival [17]. Gao et al. proposed six genes, including *IQCE*, *RFX6*, *GPAA1*, *BAHCC1*, *CLEC2B*, and *AGAP2*, as candidate genes for melanoma prognosis [18]. Liu et al. showed that melanoma cells are characterized by low expression of fibulin 1 (FBLN1) and its low expression is associated with a poor outcome. By acknowledging that FBLN1 is involved in maintaining the junctions between cells and extracellular skeleton construction [19], its low expression can contribute to an accelerated metastatic process.

Gene expressions influence the tumor microenvironment that plays an important role in the progression and prognosis of melanoma. Yingjuan et al. analyzed genes related to stromal and immune cells in melanoma patients using TCGA database. They identified 10 genes (*IL15*, *CCL8*, *CLIC2*, *SAMD9L*, *TLR2*, *HLA.DQB1*, *IGHV1–18*, *RARRES3*, *GBP4*, *APOBEC3G*) involved in processes such as inflammation and immune response, which differentiate melanoma patients according to survival rate into two classes, with low and high risks [12]. Yan et al. identified four genes (*NOTCH3*, *DBN1*, *KDELC2*, and *STAB1*) that support the infiltration of pro-tumoral M2 macrophages in melanoma and even validated a prognostic risk score to evaluate mortality in patients with melanoma, based on the expression of these genes [20]. Li et al. identified *RTP4* (genes involved in the expression of interferon-responsive proteins) as a novel gene associated with cutaneous melanoma and revealed that there is a link between *RTP4* and tumor inflammatory infiltrate [21]. Another recent study analyzed 84 genes involved in cancer-related inflammation and immune response and highlighted a greater magnitude of the fold change in the CC chemokine ligand 18 (CCL18) gene (C-C motif) in plasma circulating cell free messenger ribonucleic acid from patients with melanoma with high disease burden, compared to those with low disease burden. The same results were obtained for the genes that encode the chemokine receptor (CCR) 1 and CCR4. Plasma transcriptomic levels of cluster of differentiation (CD) 274were also identified as increased in patients with progressive disease. The researchers concluded that the plasma transcriptomic profile could represent a potential biomarker for melanoma [22].

The role of the hedgehog pathway in basal cell carcinoma is well known. In fact, the hedgehog (HH) pathway has been described as being involved in several malignant tumors, including melanoma [23][24]. Recently, Dunjic et al. analyzed whether there is a link between genetic polymorphisms in the sonic hedgehog (SHH) pathway and melanoma risk and identified a significant correlation between mutations in *GLI1 rs2228224 G* and *GLI1 rs2228226 G* alleles and susceptibility to melanoma. Moreover, the variant mutant genotype GG of *GLI1 rs2228226* polymorphism was associated with a lower survival rate [25]. In line with this, Peng et al. showed that blocking the HH pathway in melanoma cells was correlated with inhibition of cell growth and aggressiveness and

upregulation of apoptosis. The study was performed on melanoma A375 cells using cyclopamine as an inhibitor of the HH signaling pathway [26]. Moreover, a crosstalk between the HH-GLI and ERK5 pathways has been described. GLI upregulates the expression of ERK5 in melanoma cells. GLI1 binds to the *MAPK7* promoter, the gene encoding the ERK5 protein. Inhibition of the HH-GLI and MEK5-ERK5 pathways can display an important antitumor effect [27]. Therefore, genes that can be markers for melanoma prognosis can also become future therapy targets.

3. miRNA Signature in Melanoma

In recent years, epitranscriptomics has gained its place in the biomarker domain, along with the important progress made in the field of sequencing methodologies [28]. Noncoding RNAs are among the most promising biomarkers in cutaneous melanoma in terms of tumor progression and recurrence rate; one of these, miRNAs, represents the class on which most studies have focused in the last decade [29]. miRNAs modulate the activity of both tumor and immune cells [30]. Ghafouri-Fard et al. elaborated an extensive list that includes numerous miRNAs that are upregulated or downregulated in melanoma [31]. miR-214, miR-148a, miR-221, miR-16, miR-29c, miR-146a-5p, miR-205, miR-203, miR-148, miR-155, miR-182, miR-200c, miR-211, miRNA-222 and miR-106b are the main miRNAs identified in the tumor samples or in the blood of patients with cutaneous melanoma [32]. Several miRNAs have been shown to distinguish healthy subjects from melanoma patients and melanoma patients without metastases from patients with metastatic melanoma. Thus, the role of miRNAs in improving the current approach for predicting the prognosis and guiding certain therapies was suggested [31]. However, Korfiati et al. conducted a review on the role of miRNAs in cutaneous melanoma and concluded that the results available so far are inconsistent, which may be the consequence of the various methodologies used and suggested that studies on a larger number of patients, along with improved bioinformatic tools, are required [29].

The investigation of miRNAs in melanoma is currently ongoing. In recent years, several new miRNAs related to the diagnosis and prognosis of melanoma have been reported. Downregulation or upregulation of certain miRNAs can predict patient outcomes. Sun et al. described miR-431 as a potential prognostic biomarker in melanoma. Low miR-431 expression in melanoma cells indicates tumor ulceration and is associated with a low survival rate [33]. Low expression of miR-107 was also correlated with a poor prognosis, with the lowest level being identified in metastatic melanoma [34]. miR-135b is a novel miRNA that was found to be upregulated in melanoma cells and it can promote cell proliferation and invasion [35]. Additionally, statistically significant correlations were identified between miR-424 expression and tumor characteristics (thickness, stage, ulceration, and metastasis). Increased expression of miR-424 is associated with decreased overall survival and disease-free survival [36].

Several studies have focused on identifying panels of miRNAs, not just one type of miRNA. Lu et al. identified a group of five miRNAs (miR-25, miR-204, miR-211, miR-510, miR-513c) that can be used to predict prognosis in melanoma patients. They identified a positive correlation between miR-204 and the prognosis of the disease, while the other four miRNAs correlated negatively with the prognosis [37]. These results are consistent with the research of Galasso et al., who pointed out the role of miR-204 in melanoma progression [38]. Another recent study reported four miRNAs (miR-142-5p, miR-550a, miR-1826, and miR-1201) that could be potential biomarkers for the

development of primary melanoma [39]. Sánchez Sendra et al. analyzed circulating free miRNAs and identified that low circulating levels of miR-182-5p, and high circulating levels of miR-199a-5p, miR-877-3p, miR-1228-3p and miR-3613-5p indicate advanced melanoma and this panel may be used to detect micrometastatic regional lymph node disease [40].

It should be taken into account that miRNAs are degraded by RNA enzymes, but if they are packed in exosomes, degradation becomes more difficult. Thus, exosomes can be used in disease detection because they represent a simple method with good sensitivity and, moreover, are a stable family of molecules that can be identified in body fluids [41][42]. Tengda et al. identified significant differences between exo-miRNA-532-5p and exomiR-106b levels when they compared melanoma patients and healthy subjects. Moreover, they demonstrated that the two miRNAs have discriminatory power between patients with and without metastases [42]. Guo et al. also studied exosomal miRNA in melanomas and highlighted a negative correlation between the miR-1180-3p level and pro-metastatic processes, such as migration and cell invasion, concluding that the detection of exosomal miR-1180-3p in plasma from patients diagnosed with melanoma can be a promising tool for the diagnosis of melanoma [41]. The domain of miRNAs in melanoma biomarkers is still an intriguing and open-to-research subject and **Table 1** summarizes the novel miRNAs or miRNA panels that can be good candidates for future development as biomarkers.

Table 1. Summary of the novel miRNAs or miRNA panels in melanoma.

miRNA/miRNA Panels	Function/Significance
miR-431	Downregulates cell proliferation, migration and invasion via NOTCH2 [33]
miR-107	Inhibits cell growth, migration and invasion via POU3F2 [34]
miR-135b	Promotes cell proliferation and migration via LATS2 [35]
miR-424	Associated with decreased overall survival [36]
miR-25, miR-204, miR-211, miR-510, miR-513c	Regulate genes related to PI3K-Akt pathways, ubiquitin-mediated proteolysis and focal adhesion [37]
miR-142-5p, miR-550a, miR-1826, and miR-1201	Involved in the development of primary melanoma [39]
miR-182-5p, miR-199a-5p, miR-877-3p, miR-1228-3p and miR-3613-5p	Associated with micrometastatic regional lymph node disease [40]
exo-miRNA-532-5p and exo-miR-106b	May be used to identify patients with early-stage melanoma [42]
exo-miR-1180-3p	Negatively corelates with melanoma cell proliferation [41]

NOTCH2—neurogenic locus notch homolog protein 2, POU3F2—POU class 3 homeobox 2, LATS2—large tumor suppressor kinase 2.

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