

A personalized Approach for Targeting the Melanoma: Inhibition of Oncogenic Signaling in Combination with Small Molecules

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Rec date: February 22, 2016; Acc date: April 28, 2016; Pub date: April 30, 2016

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Abstract

Melanoma is responsible for the most of skin cancer deaths each year. Standard therapies haven't shown much efficacy against malignant melanoma. Therefore, new "individualized medicine" approaches are expected to overcome this deadly disease. Recent studies show that specific targeting of oncogenic signalling pathways using small molecule drugs in combinations or immuno-therapies have shown an improved advantage both in non-clinical and clinical research. This article will provide an overview of the progress in pathway targeting therapies in melanoma.

Keywords: Melanoma; MAPK pathway; Cell cycle; Cancer stem cells; Targeted therapy

Abbreviations:

MAPK: Mitogen-Activated Protein Kinase;
PI3K: Phosphatidylinositol-3-Kinases;
PIP2: Phospholipid Phosphatidylinositol-4,5-Bisphosphate;
PIP3: Phosphatidylinositol-3,4,5-Trisphosphate;
CTLA-4: Cytotoxic T-Lymphocyte-Associated Protein 4;
PD-1: Programmed Cell Death Protein 1;
CDK4: Cyclin-Dependent Kinase 4;
CSCs: Cancer Stem Cells

Introduction

Melanoma, also known as malignant melanoma is the most fatal type of skin cancer. Based on the American Cancer Society's estimates for melanoma in the United States alone for 2015, about 73,870 new melanomas will be diagnosed and approximately 9,940 people are expected to die of melanoma [1]. Melanoma begins in cells called melanocytes, which are found between the epidermis and the dermis. When melanocyte begins to proliferate out of control, it becomes the early stage of melanoma. Melanomas are usually caused by DNA damage due to unprotected exposure of ultraviolet (UV) light from the source or sun. Some studies suggested that exposure to ultraviolet radiation is one of the major contributing factors of the melanoma [2,3]. Another reason that greatly increases melanoma susceptibility is the gene mutations, which often happens in families with the history of melanoma disease [4,5]. For the past decades, significant progress has been made towards understanding the molecular and genetic mechanisms of malignant melanoma, thereby, those therapies, which

target either key effectors of the deregulated pathways or immune regulatory molecules that involved in antitumor immune responses are very promising strategies for personalized medicine [6]. This review will focus on important molecular signaling pathways that are associated with the targeted therapies of melanoma.

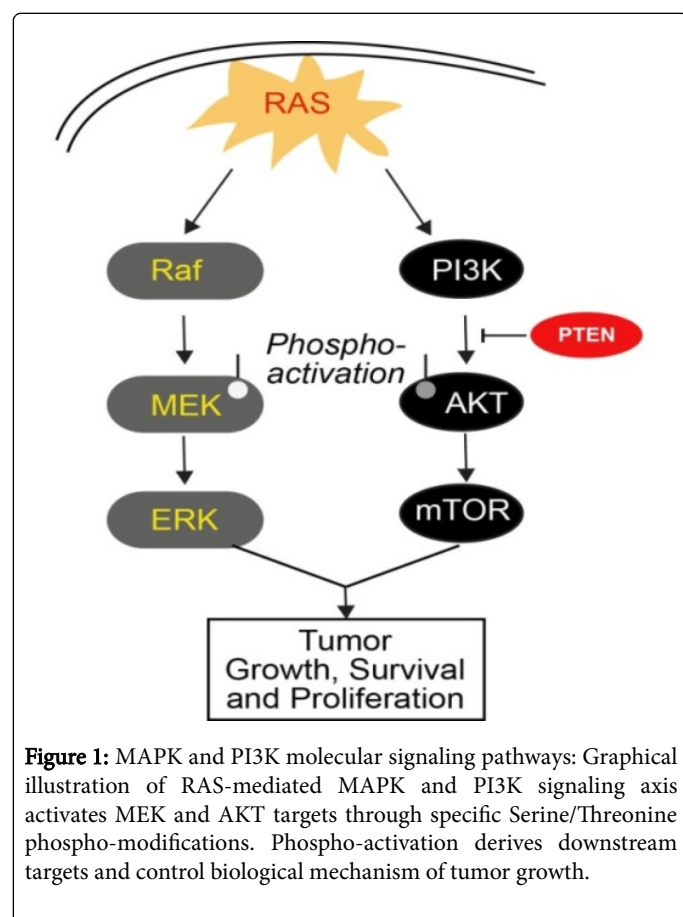
MAPK pathway

MAPK pathway also known as the Ras-Raf-MEK-ERK pathway is one of the most frequently deregulated signaling pathways that plays a critical role in regulating cell growth, survival and proliferation. Among three specific isoforms of Ras mutants i.e., H-Ras, N-Ras and K-Ras, the N-Ras mutation is the most prevalent in melanoma [7-10]. There are two important downstream target proteins of N-Ras mutation, RAF and PI3K (phosphatidylinositol 3 kinase) (Figure 1) [9].

In melanocytes, BRAF induces the activation of MEK kinase, which in turn activates ERK, the final effector of MAPK cascade. BRAF gene is mutated in 40–60% of cases of melanoma and 90% of mutated cases are represented by BRAFV600E variant - replacement of glutamic acid with valine at codon 600 [11]. Vemurafenib is the first BRAF kinase pharmacological inhibitor that was approved by the Food and Drug Administration (FDA) in 2011 to treat patients with advanced melanoma, especially to treat patients harbouring BRAFV600E tumors [12]. Recent research studies also approved that Vemurafenib has significant effect on melanoma survival rate in the cases where patients were positive for BRAF mutant [13,14].

Another inhibitor had been approved by FDA in 2013 is Dabrafenib that shows dramatic effect on melanoma patients with brain metastasis. A phase II clinic trial among six countries was performed on 172 melanoma patients whom were histologically confirmed with BRAF-mutant (Val600Glu or Val600Lys) and asymptomatic brain metastases. In this study, Dabrafenib shows both strong activity and an acceptable safety profile in Val600Glu BRAF-mutant melanoma patients with brain metastases [15]. Although the pharmacological

BRAF inhibitors showed a promising effect, a small subset of melanomas that harboring mutated BRAF amplification showed a resistant effect to BRAF targeting inhibitors [16]. Moreover, these resistant cells expressed a truncated form of mutated BRAF, which lost the RAS-binding domain and keep BRAF to stay in a constitutively active status. To overcome the drug-resistance issue, scientists adopted the combination therapy strategies. As BRAF inhibitor and MEK inhibitor are both the potent blockers of the MAPK signaling pathway, an on-going phase I study using BRAF and MEK combined inhibitors received a very promising result [17].



PI3K/Akt pathway

The PI3K/AKT pathway is an intracellular oncogenic signaling pathway important in regulating cell survival, growth and proliferation dynamics, and is hyper activated in most of the malignancies [18]. In melanoma cells, N-Ras mutation activation is a major reason to cause PI3K activation. Once mutation is activated as phospho-cascade initiates, PI3K phosphorylates PIP2 and PIP3, and then AKT. AKT is a serine-threonine kinase that normally exists in the cytoplasm, which will be recruited to the inner surface of plasma membrane after phosphorylation/activation [19]. Thr308 and Ser473 are two major AKT phosphorylation sites, which will lead to AKT activation (Figure 1). Active phospho-AKT targets and phosphorylates a wide variety of substrates, most of them are important regulators of many key cellular processes [20].

Another reason that causes AKT activation in melanoma is the loss of function of PTEN, which happened in 10% to 30% of patients

suffering with melanomas [21]. Usually, PTEN mutation is mutually exclusive with N-Ras mutation in melanoma patients. Because AKT can also be phosphorylated by mTORC2 complex in response to the growth factor or DNA damage stimulation. Single target inhibitors like PI3K inhibitor, showed limited effect in advanced melanomas [22]. Recently, a study using the dual mTORC1/2 inhibitor showed promising inhibitory effects in melanoma cells. At the meantime, studies by Maria et al., and Marone et al., have also shown an impressive anti-melanoma activity after dual PI3K-mTOR inhibitors treatment [23-25]. However, there are still challenges that require further in depth investigates. Such as feedback down-regulation of receptor tyrosine kinase signaling, a frequent event in tumor cells with constitutive mTOR activation.

mTOR inhibition induces insulin receptor substrate-1 expression and abrogates feedback inhibition of the pathway, resulting in Akt activation both in cancer cell lines and in patient tumors treated with the mTOR inhibitor [26]. Therefore, combined therapy with other pathway inhibitors would be the better option. For example, BRAFV600E mutant melanoma cells harboring MEK or N-RAS mutations also demonstrated resistance to BRAF and MEK inhibitors. More important, studies have shown that combinatorial treatment of a BRAF and the PI3K inhibitor significantly suppressed transformed cell growth [27].

CTLA4 and PD-1 pathway

Cytotoxic T cell lymphocyte antigen 4 (CTLA-4) is a protein receptor that functions as an immunological checkpoint and plays an important role of the anti-tumor immune response. As a member of the immunoglobulin superfamily, CTLA-4 is mainly expressed on the surface of the helper T cells and transmits an inhibitory signal to T cells [28]. Programmed cell death protein 1 (PD-1) is a cell surface receptor that also belongs to the immunoglobulin superfamily and is expressed on the surface of T-cells. PD-1 can bind two ligands, PD-L1 and PD-L2, which are members of the B7 family. PD-L1 is expressed on most tumor cells and mostly escapes from host's immune signals, while PD-L2 is mainly expressed on dendritic cells and a few tumor cells as well [29-32]. Therefore, blockade the binding between PD-1 and PD-L1 by using pharmacological PD-1 inhibitors may provide a promising strategy for tumor immunotherapies.

The CTLA-4 and PD-1 pathways function at different stages of the immune response. Generally, CTLA-4 modulates the early immune events. T cells are activated by antigen presenting cells (APCs). After the activation of T-cells, CTLA-4 localization from Golgi to plasma membrane takes place, where it binds to B7 ligands on APCs to inhibit further T-cell activation. In tumor cells, blocking CTLA-4 may lead to consistent activation of T cells, thus leading to more effective antitumor responses [33,34]. Importantly, Ipilimumab is a monoclonal antibody that overcomes CTLA-4 mediated T-cell inactivation to enhance the anti-tumor immune response (Figure 2) [35].

The patients with stage III melanoma who received Ipilimumab had a longer overall survival comparing to those treated with placebo [36]. Comparing with the negative regulatory effect of CTLA-4 on early T-cell activation, the PD-1 pathway appears to function as a late event. In response to chronic infections or tumor cells, PD-1 is up-regulated on T-cells. When PD-1 binds to its ligand (PD-L1), the T-cells receive an inhibitory signal leading to the blockage of a productive antitumor immune response [37,38]. Expression of PD-L1 is a mechanism for tumors to evade antitumor immune responses (Figure 2) [39]. In many tumor types including melanoma, the presence of tumor infiltrating

lymphocytes (TILs) has been associated with improved patient's outcomes [40,41]. Blockade of the PD-1/PD-L1 signaling axis may restore the activity of TILs. Monoclonal antibodies like nivolumab,

pembrolizumab and oidilizumb are designed to prevent PD-1 from binding to its ligands.

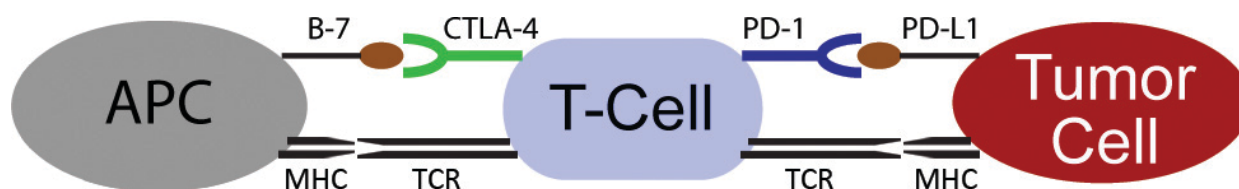


Figure 2: Cytotoxic T cell lymphocyte-mediated signaling axis: T- cells are primed by APCs (antigen presenting cells), shortly after activation, T cells upregulate CTLA-4 expression to limit further activation. Activated T cells traffic to the tumor sites and bind to the PD-L1 through PD-1 that involves participation of B7, T cell receptors (TCR) and Major histocompatible factors (MHC).

Cyclin-dependent kinase pathway

Cyclin-dependent kinases (CDKs), which are involved in regulating transcription, mRNA processing, and the differentiation of nerve cells, are a family of protein kinases first discovered for their role in regulating the cell cycle. In melanoma patients, the CDK4 pathway dysregulation is associated with genomic alterations for more than 90% of cases [42], and, in melanoma mouse models, mutant BRAF or NRAS potently cooperates with the activation of the CDK4 pathway [43]. Recent clinical developments to treat melanoma using cyclin-dependent kinase inhibitors showed very promising outcomes. LEE011 is a highly selective small molecule inhibitor of CDK4/6, which interrupts the CDK4/cyclin-D1 and CDK6 complexes. LEE011 demonstrated anti-tumour activity in melanoma, but requires the functional retinoblastoma protein [44]. Palbociclib is another highly selective CDK4/6 inhibitor that directly targets the ATP-binding site of the CDK4-cyclin-D1 complex. Current Studies investigating palbociclib in treating advanced melanoma are still underway [45].

Conclusions and Future Directions

Modern molecular clinical biology techniques for genetic analyses provide us a much better understanding of the mechanisms involved in the pathogenesis of melanoma. Mutation analyses of the main oncogenic pathway components, for example B-RAF of the MAPK signaling cascade, is being utilized for treatment prediction and personalized medicine [46,47]. However, the drug resistance problem is still a worrying problem that has not been resolved. Combination therapies are one promising choice to overcome the shortcomings of single agents, leading to more durable responses, and, longer patient survivals [48]. The combination of dabrafenib and trametinib is the current treatment of clinic choice for advanced melanomas carrying BRAF [49]. Up to now, specific targeted therapies for most patients with malignant melanomas bearing genetic alterations are not available, new therapies are still wanted.

Micro-RNAs (miRNA) are a set of small, single-stranded, non-protein-coding RNA molecules, which mediate gene expression at the posttranscriptional and translational levels. One of the first connections between miRNA and melanoma is about 86% of primary melanoma cell lines had DNA copy number alterations in genomic loci containing miRNA genes [50]. miR-137 was identified and proved as a trigger for microphthalmia-associated transcription factor (MITF), which is a master regulator of melanocyte development [51]. MiR-34a turns to act as a tumor suppressor in melanoma cell proliferation and migration by down-regulation of c-Met [52]. Regulation of miRNA

represents a promising means for targeted therapy against melanoma. It is known that miRNAs can interact with many of the most important regulatory pathways during melanoma development and progression (like MAPK/ERK and PI3K/Akt). Understanding miRNA roles in many complex signaling pathway networks will lead to the development of better personalized therapies.

The first evidence of cancer stem cells (CSCs) in human melanoma was provided in 2005, which demonstrated that the metastatic melanoma lesions from human patients contained a small population of cancer stem cell-like (CSC) properties [53]. CSCs have a higher ability to survive under hypoxic conditions, as well as overcome targeting therapies, comparing to "normal" cancer cells [54,55]. Thus, CSCs-targeted therapies might play a catalytic role for advanced melanomas. One successful temptation to target CSCs in melanomas is through targeting the signaling pathways that regulate the stem-like fate of CSCs. By depleting the expression of Sox10, the melanoma formation was completely abolished both in mice and in human melanoma cells [56,57]. Research related to melanoma CSC-mediated therapy is getting attention, however, there are no effective drugs approved for clinical tests to treat specifically CSCs in melanoma, but targeting the signaling pathways that used by resistant CSCs represents a promising new field for melanoma treatment.

Acknowledgements

We acknowledge Dr. Mangi (MD) and Dr. Bukhari (MD) for their input in order to improve the manuscript. The authors declare that there is no conflict of interest regarding the publication of this article.

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