

The Biological Function and Tumorigenesis Of ERK/MAPK Signaling in Melanoma

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Abstract. Melanoma, as a potentially lethal malignant tumor, has been increasing worldwide. As an aggressive and lethal skin cancer, discover the malignant melanoma at an early stage can reduce mortality. The deeper the tumor, the more difficult the prognosis of the melanoma, Timely detection and rapid treatment of melanoma is very important for the diagnosis of melanoma. Because the treatment of melanoma is resistant to conventional chemotherapy, the direction of treatment has shifted to molecular targeted therapy. The MAPK pathway is a central signal transduction element that regulates basic physiological activities such as cell proliferation and apoptosis, the occurrence and development of tumors. The over-activation of ERK Assume a pivotal role. RAS/Raf/MAPK (MEK) /ERK pathway is a part of the MAPK signaling pathway, which is the most pivotal signaling cascade. RAS/Raf/ MEK/ERK (MAPK) pathway inhibitors have shown significant results in the clinical treatment of tumors, and can slow the development of the disease. In this review, we discussed the principles, methods and recent progress in the targeted therapy of melanoma.of the ERK/MAPK pathway.

Keywords: Melanoma, ERK, MAPK, tumorigenesis, cancer.

1. Introduction

The cause of melanoma is the deterioration and transformation of melanocytes, which is often highly malignant and non-infectious. Most of the diseases occur in the skin, but also in different parts or tissues such as mucosa and uvea of the eye. Malignant melanoma is divided into in situ malignant melanoma and invasive malignant melanoma. And its tumor stage is 0-IV, the earlier the stage, the easier to cure. In addition to prompt surgical resection, there exists no specific remedy for malignant melanoma, rendering the prognosis bleak. Henceforth, timely detection and intervention of malignant melanoma assume paramount significance. Among the various influencing factors, ultraviolet radiation may be the most important environmental risk factor. In addition, race, genetics and trauma are also common causes [1]. Melanocytic nevi may arise from congenital or acquired growth of melanocytes or nevi cells, and in about 25% of melanoma cases, it occurs in the original nevi [2].

The MAPK pathway is a central signal transduction element that regulates basic physiological activities such as cell proliferation and apoptosis. Extracellular signal-regulated kinases (kinases), a member of the MAPK family, function to regulate the cascade of signal transduction and act as a transmission channel to transmit extracellular signals to intracellular targets. The basic core units of extracellular signal-regulated kinase are its first three layers, while the last two layers are present in some cascades and may vary with cellular activity and external stimuli [3]. MAPKKK, the upstream kinase, in response to a myriad of intracellular and extracellular signals, triggers the activation of the intermediate kinase MAPKK through direct phosphorylation.

The MAPK pathway is an important therapeutic target for melanoma and plays a very important role in the occurrence and development of tumors. In normal cells, the transfer of extracellular signals from the cell membrane to the nucleus is in a tightly regulated manner through a cascade of phosphorylation events. In melanoma, the activation of B-RAF and RAS by mutations or epigenetic modifications leads to dysregulation of the MAPK pathway, resulting in increased signaling activity, which ultimately promotes cell growth, apoptosis, metastasis, and angiogenesis.

2. Biological function of ERK/MAPK signaling

The MAPK signaling pathway is highly conserved in cell evolution. Different extracellular stimuli can use different MAPKs signaling pathways to mediate different cell biological responses. There are four major subfamilies of MAPK, corresponding to four MAPK pathways: ERK, p38, JNK, BMK1 (ERK5). Among them, the ERK pathway includes proteins RAS, Raf, MEK and ERK, and the abnormality of any of these proteins may induce the occurrence and proliferation of tumors, so they have become popular targets for various innovative drugs.

In the MAPK/ERK signaling pathway, tyrosine kinase receptors (RTKS) are stimulated to activate MAPK in a series of steps. Activated Ras activates the protein kinase activity of RAF kinase. RAF kinases activate MEK (MEK1 and MEK2) by phosphorylating them. MEK can phosphorylate ERK, thereby activating it. Studies have shown that the MAPK/ERK pathway plays a key role in the process of transducing extracellular signals to cellular responses.

The ERK cascade is a cascade that can highly regulate cell physiological activities and is involved in basic physiological processes such as cell proliferation or apoptosis. The activation of the ERK cascade is observed in the majority of cancer types, and dysregulation of this pathway plays a pivotal role in the initiation and progression of most malignancies.

2.1. Activation of ERK/MAPK signaling

Upstream growth factor receptors (EGFR, TGF- α) activate and mediate the reaction process of the MAPK/ERK pathway. The guanine nucleotide exchange factor (GEF) catalyzes the binding of RAS proteins to guanosine triphosphate (GTP), which keeps RAS in an activated state. Activated RAS recruits the downstream RAF protein in the cytoplasm and binds to its N-terminal CR1 domain to transport RAF protein to the cell membrane for its activation. RAF in the activated state further interacts with the downstream MEK via its C-terminal CR3 domain, which in turn activates MEK. The activated MEK then interacts with ERK to activate tyrosine (Tyr) and threonine (Thr) residues in ERK, thereby activating downstream ERK.

2.2. Upstream of the EPK/MAPK signaling

Under the normal pathway mechanism, the activation of ERK has the function of negatively regulating the MAPK/ERK pathway to maintain the homeostasis of the body. This negative feedback mechanism is achieved by two pathways: direct phosphorylation of ERK upstream components (EGFR, RAS, RAF, MEK) at specific sites, and induction of de novo synthesis of specific inhibitors within the pathway (DUSP, Sprouty). Under the normal physiological mechanism, the activation of RAS protein mediated by upstream growth factors is usually transient, and There are many ways for RAS to participate in the reaction and be activated, thereby ensuring the normal cycle of cell proliferation, apoptosis and and other cellular activities. RAS proteins and exchange factors require the help of ERK1/2 in positive cycling. The activation of RAS proteins is regulated by the upstream RAS. In the activated state, RAS recruits the downstream RAF proteins located in the cytoplasm, which is bound by RAS and transported to the cell membrane for activation. Proteins, such as epidermal growth factor (EGF) and tumor necrosis factor (TNF), can alternately regulate signal transduction between the two conformations. When extracellular signals bind to receptors, receptor-Grb2-SOS complexes are synthesized. Taking the exchange factor SOS1 as an example, it was found that ERK2 activation may regulate molecular association by phosphorylating 1132 (Ser), 1167 (Ser), 1178 (Ser), 1193 (Ser) and 1197 (Ser) sites of SOS1, which is a 1333 codon protein. The interaction between SOS1 and Grb2 was interfered, and the ERK negative feedback mechanism was regulated by SOS1.

3. The role of ERK/MAPK in melanoma

3.1. Tumorigenic role of EPK/MAPK in melanoma

The formation of cutaneous melanoma goes from superficial to deep, starting with obvious precursor lesions such as benign melanocytic nevus or dysplastic nevus, and then the deterioration becomes more and more serious after a series of lesions. The fundamental cause of malignant melanoma is damage to the DNA in melanocytes. In addition to genetic diseases, long-term UV exposure or external damage can easily cause DNA fragmentation, abnormal methylation or mutation in melanocytes. Eventually, the disease deteriorates and malignant tumor cells appear.

In melanoma, when growth factors bind to RTKS, the MAPK pathway is stimulated and guanosine triphosphatase (gtpase) activity that stimulates RAS is activated. Signals propagate through RAF, MAP2K1, and ERK cascades, which enter the nucleus and activate transcription factors, and at the same time the cell cycle is promoted. In the MAPK-ERK pathway, GPCR stimulation leads to PLC activation. This promotes DAG, which in turn activates PKC. RTKS are activated by binding to extracellular growth factor ligands, which activate RTKS activity and initiate signaling cascades. RAF subtypes include RAF1, BRAF and other protein kinases whose activity is activated by activated RAS.

3.2. ERK/MAPK signaling in the proliferation, metastasis and apoptosis of melanoma

In the MAPK signaling pathway, a series of reactions and changes in specific genes ultimately affect melanoma proliferation, apoptosis, and metastasis. Cellular activation of the MAPK/ERK signaling pathway is the primary step in the development of melanoma, and protein kinases such as BRAF rely mainly on activated RAS to activate their activities. Each RAF subtype has a unique ability to activate MEK. BRAF is the most potent activator of MAPK and plays a key role in the phosphorylation of MEK and the activation of downstream proteins, such as ERK1 and ERK2.

ERK can phosphorylate a variety of transcription factors into the nucleus and regulate cell growth and development cycle. MITF is a target of ERK by regulating the occurrence and development of melanocytes in the human body, while also controlling the cell cycle and survival. The KIT-bound ligand (SCF) activates MAPK and PI3K pathways. In the PI3K-AKT pathway, activated RTKS recruit PI3K to the plasma membrane. GPCR, IGF-1R, and RAS activate PI3K, and PI3K activates AKT. The activation pathway of mTOR signaling pathway is through ERK and AKT, which mediate the normal physiological activities of cells, such as proliferation and apoptosis. In the TNFR pathway, TAK1 binds and is activated under the influence of TNF- α and TNFR1 cytokines and their receptors. Aggregation of the downstream kinase complex IKK after TAK1 activation I κ B phosphorylation by the IKK complex leads to NF κ B release. NF κ B enters the nucleus and is involved in cell growth after activation of cell survival and anti-apoptotic genes.

In melanoma, the activity of ERK is increased through the activation of RAS on the inner surface of the cell membrane. In the state of continuous activation of ERK, the MAPK signaling pathway will be simultaneously dysregulated, which eventually leads to subsequent cell proliferation, apoptosis, and angiogenesis. As a result, cell proliferation is increased, cell survival is improved, and anti-apoptotic ability is enhanced. ERK activation can also promote integration of tumor invasion, induced by expression of the metastatic potential of melanoma.

Several mutations in the BRAF gene occur more frequently in cancers, and BRAFV600 mutations are common in skin lesions. BRAF kinase activity increases after BRAFV600 mutation, whereas downstream targets continue to be activated [4]. Among KRAS mutations in melanoma, KRASQ61 is the most common mutation, and its internal hydrolytic activity and the final active state of KRAS are of reduced importance. Overstimulation of RAS can also result from other mutations, such as loss-of-function mutations in neurofibromin 1 (NF1). In most melanomas with nf1 alterations, the basic function of the cells is altered, with the possibility of mutation or loss. Among them, neurofibrins lose their ability to inactivate RAS, leading to enhanced stimulation of RAF and its downstream targets, and stimulation of the MAPK pathway, leading to enhanced cell proliferation.

Other mutations occur frequently in melanoma, such as mutations in the promoter of the telomerase reverse transcriptase (TERT). According to the Cancer Genome Atlas (TCGA), it occurs mainly in mutant subtypes BRAF, RAS, and NF1, suggesting a causal relationship between MAPK activation and TERT expression [5].

4. ERK/MAPK in melanoma treatment, application of inhibitors

4.1. ERK/MAPK inhibitors

In the MAPK-ERK pathway, each RAF subtype can deactivate MEK in a specific way, and BRAF is the strongest activator in different categories [6]. BRAF and NRAS mutations, one of their roles, are oncogenic and are targets for new therapeutic strategies.

In BRAF mutation-related inhibitor therapy, the principle of treatment is to inhibit the growth of melanoma cells and to control apoptosis through molecular targeted regulation. The inhibitors of BRAF and NRAS mutations in melanoma are selective MEK inhibitors [7]. Experiments have confirmed that single inhibitors are often less effective than combination therapy. For example, in three randomized phase 3 studies involving patients with BRAF-mutated metastatic melanoma, treatment with a single inhibitor was substantially worse than that with combined BRAF and MEK inhibition [8].

Vemurafenib is an inhibitor that is superior to dacarbazine chemotherapy in the treatment of metastatic BRAF-mutated melanoma, according to a phase 3 trial. Similar results were seen for another BRAF mutation inhibitor, amidefene, which was approved in 2013.

Another group of new drugs, MEK inhibitors, have been shown in preclinical studies to be more effective than vemurafenib in inhibiting mutant BRAF and NRAS. Since trametinib was first approved as a MEK inhibitor in 2014, it has been used for the inhibition and clinical treatment of advanced BRAF-mutated melanoma by monotherapy or in combination with Vemurafenib. Inhibitors based on other pathways are also in development. Through multiple genetic mechanisms, we could overcome the simultaneous inhibition of MEK and BRAF and thus show a more durable tumor response than BRAF monotherapy.

4.2. Application of combination therapy

Combination therapy prevents the development of acquired resistance and reduces malignant skin problems. Such skin problems are often triggered by aberrant activation of the MAPK pathway. BRAF inhibitor induction was the main factor causing MAPK pathway abnormalities. The combination of BRAF and MEK inhibitors has a good therapeutic prospect and is effective in clinical application. In addition, novel mechanisms of triple therapy are being investigated [9]. BRAF and MEK inhibitors are multipotent, and an important one is their ability to induce high levels of metabolism while simultaneously inhibiting aerobic glycolysis, effectively causing apoptosis or cell death in BRAF-mutated cancer cells. The experimental results showed that the combined application of inhibitors is far from the same signaling pathway, such as the cooperation with mitochondrial inhibition, and the application of MAPK/ERK pathway inhibitors at the same time, so as to improve the application effect of inhibitors. [10]. By exploring these new targeted therapies and aiming to use and test the effect of inhibitors in clinical trials, we can achieve better anti-tumor effect. The introduction of inhibitors of kinases associated with the ERK/MAPK pathway in clinical trials offers new hope for overcoming the challenge of MAPK pathway resistance and significantly enhanced effects in controlling melanoma growth and progression [11].

5. Conclusion

This article reviews the effects of ERK/MAPK signaling pathway on human health. Further data and clinical trials will show more evidence of the role of MAPK signaling in melanoma. Since cellular signaling pathways are a complex network. The activation of upstream and downstream signaling

molecules can promote or inhibit tumor cell growth. The application of MAPK signaling pathway related inhibitors in the treatment of metastatic melanoma has shown great results, which can improve the utilization rate of drugs and reduce the risk of malignant melanoma deterioration. At the same time, with the deepening of research and understanding, more inhibitors are in the experimental. Targeted therapies combined with BRAF/MEK inhibition have yielded high response rates. Because of the good response to treatment and the high incidence of resistance, combination therapy has been and is continuously being investigated. MAPK/ERK inhibitors in related fields have also been significantly developed and achieved good clinical results, which provides broad ideas and methods for better treatment of melanoma in the future.

References

- [1] Sera F, Gandini S, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. *Eur J Cancer* 2015, 41: 45-60.
- [2] Lejeune FJ. Epidemiology and etiology of malignant melanoma. *Biomed Pharmacother.* 1986;40(3):91-9.
- [3] Guo YJ, Pan WW, Liu SB, Shen ZF, Xu Y, Hu LL. ERK/MAPK signalling pathway and tumorigenesis. *Exp Ther Med.* 2020 Mar;19(3):1997-2007.
- [4] Davies H., Bignell G.R., et al. Mutations of the BRAF gene in human cancer. *Nature.* 2002, 417:949–954.
- [5] Nissan M.H., Pratilas C.A., et al. Loss of NF1 in cutaneous melanoma is associated with RAS activation and MEK dependence. *Cancer Res.* 2014, 74:2340–2350.
- [6] Teixido C, Castillo P, Martinez-Vila C, Arance A, Alos L. Molecular Markers and Targets in Melanoma. *Cells.* 2021 Sep 5;10(9):2320.
- [7] Grimaldi AM, Simeone E, Ascierto PA. The role of MEK inhibitors in the treatment of metastatic melanoma. *Curr Opin Oncol.* 2014 Mar;26(2):196-203.
- [8] Queirolo P, Picasso V, Spagnolo F. Combined *BRAF* and MEK inhibition for the treatment of *BRAF*-mutated metastatic melanoma. *Cancer Treat Rev.* 2015 Jun;41(6):519-26.
- [9] Grimaldi AM, et al. Novel mechanisms and therapeutic approaches in melanoma: targeting the MAPK pathway. *Discov Med.* 2015 Jun;19(107):455-61.
- [10] Marchetti P, Trinh A, Khamari R, Kluza J. Melanoma metabolism contributes to the cellular responses to *MAPK/ERK* pathway inhibitors. *Biochim Biophys Acta Gen Subj.* 2018 Apr;1862(4):999-1005.
- [11] Savoia, P.; Fava, P.; Casoni, F.; Cremona, O. Targeting the ERK Signaling Pathway in Melanoma. *Int. J. Mol. Sci.* 2019, 20, 1483.