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Review

The mitogen-activated protein kinase pathway in melanoma part I – Activation and primary resistance mechanisms to BRAF inhibition

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Abstract Mitogen-activated protein kinase (MAPK) pathway has an important role in normal cells and can be activated under physiological conditions. MAPK pathway activation is a fundamental step in several intracellular processes requiring a sequential phosphorylation of the different pathway components. In normal cells, when MAPK pathway activation occurs, it leads to cell growth and differentiation. In order to prevent persistent MAPK pathway activation, physiological upstream negative feedback also takes place. In cells harbouring *BRAFV600* mutations, the process leading to MAPK pathway activation is different, and the negative physiological feedback does not exist thus leading to permanent MAPK pathway activation, which ultimately can lead to uncontrolled proliferation.

Targeted therapy with rapidly accelerated fibrosarcoma – B (BRAF) and/or mitogen-activated extracellular signal-regulated kinase kinase (MEK) inhibitors is indicated in patients with metastatic melanoma harboring BRAFV600 mutations. However, several different resistance mechanisms to this therapy were identified. In this review, we focus on primary or intrinsic resistance mechanisms to BRAF and MEK inhibition. In this setting, although a

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BRAF mutation is identified, there is no response to treatment with either BRAF or MEK inhibitor.

1. Introduction

The mitogen-activated protein kinase (MAPK) pathway is an important intracellular signalling pathway. Activation of the MAPK pathway occurs under physiological conditions through extracellular binding of growth factors to receptor tyrosine kinases (RTKs) [1].

MAPKs are responsible for signal transduction that controls intracellular processes, namely acute hormone responses, embryogenesis, cellular differentiation, proliferation and apoptosis [1].

Interaction between an RTK and its ligand is necessary to activate the MAPK pathway and its rapidly accelerated fibrosarcoma (RAF) components (ARAF, BRAF and CRAF). When such interactions occur, a cascade of intracellular events is activated, leading to cellular growth, increased survival and inhibition of apoptosis. Without RTK–ligand interaction, RAF kinases are in their inactive (non-phosphorylated) form, and no signal transduction occurs. After an RTK–ligand interaction, RAF phosphorylation takes place, and BRAF and CRAF serine/threonine kinases can act as downstream mediators. Activated RAF homo- or heterodimers interact with and phosphorylate mitogen-activated extracellular signal-regulated kinase kinase (MEK) that further phosphorylates and activates mitogen-activated extracellular-signal regulated kinase (ERK) [2,3]. ERK activation has an important role in oncogenesis, promoting cellular growth and differentiation. Activated ERK is also responsible for the upstream negative feedback, which occurs at different levels of the MAPK pathway (Fig. 1a). Other intracellular pathways, namely the phosphatidylinositol-3-kinase (PI3K) pathway can also be activated by RTK–ligand interactions.

MAPK pathway activation is a central step in melanoma pathogenesis [4]. In melanoma cells harbouring a *BRAFV600* mutation, MAPK pathway activation occurs differently (Fig. 1b). In these cells, *BRAFV600* activation does not require RAS activation through RTK–ligand interaction. *BRAFV600* monomers are constitutively activated, which continuously activate MEK followed by ERK, leading to cell growth and proliferation. As a consequence of permanent ERK activation, the resulting negative feedback is also more intense, which translates into low RAS expression, making RAS-dependent RAF dimerisation and PI3K activation through the RTK–ligand interaction extremely unlikely. When this negative feedback is released or attenuated by BRAF inhibitors (BRAFis), activation dependent on RTK–ligand signalling becomes again an important mechanism of MAPK pathway activation, leading to resistance [5].

Resistance to BRAFi is mainly associated with MAPK reactivation through MEK [6,7]. In order to overcome or delay resistance, combination therapy of BRAFi and MEK inhibitors (MEKis) was evaluated [8–12].

Menzies *et al.* reported data on overall survival (OS) rate in patients treated with BRAFi and BRAF/MEKi, after a median follow-up of 15.7 months [13]. Results showed that 2-years (2y)OS rate was 43% and the 3y and 4y OS rate was 24%. Other results from combination therapy trials were recently updated. Dabrafenib in combination with trametinib versus dabrafenib alone achieved a median OS of 25.1 months versus 18.7 months, median progression-free survival (PFS) of 11.0 versus 8.8 months, overall response rate of 69% versus 53% and a 3y OS rate of 44% versus 32% [14]. Combination therapy with vemurafenib and cobimetinib also improved OS [9].

Although combination therapy improves OS in these patients, resistance development appears to affect long-term survival. Moreover, some patients do not respond to therapy, even if they carry a *BRAFV600* mutation, which could be due to intrinsic or early induced resistance.

2. MAPK pathway in benign lesions

BRAFV600 mutations are also present in benign lesions, i.e. in melanocytic nevi [4,15].

Fig. 2 represents the currently known spectrum of mutations in melanoma and a possible model of their temporal evolution. The exact mechanisms involved in this transition/evolution are incompletely understood. Shain *et al.* proposed a model for progression of melanomas in chronically sun-damaged skin [4]. In this model, point mutations and copy number alterations increase in number as a time function. Ultraviolet (UV) radiation seems to play an important role in melanomagenesis although this is not universally agreed. It is clear that driver mutations in *BRAF, NRAS* and *TERT* are present in benign and intermediate lesions, as well as in melanoma *in situ*. Other mutations or alterations in driver genes such as *CDKN2A* and genes encoding for *SWI/SNF* subunits and *TP53* and *PTEN* are found in invasive melanomas only. As described further,