RAF-MEK-MAPK Pathway Targeted by Tumor Suppression and Anticancer Therapeutic Agents

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Abstract

RAS-RAF-MEK-MAPK pathway comprises a group of kinases which regulates the activities of effector proteins in growth, proliferation and apoptosis. The extracellular signals from growth factors, cytokines and other stimuli transmitted by surface receptors and upstream signaling molecules are integrated by this cascade of kinases whose activity is regulated by the interaction of oncoproteins and tumor suppressors. The anomaly of the signaling pathway would lead to occurrence of malignancies. The RAS-RAF-MEK-MAPK pathway is therefore targeted by anticancer therapeutic agents. The present paper discussed the interaction of individual component with tumor suppressors and the impact of their inhibitor on the efficacy of anticancer therapy, and improvement on small molecule inhibitor of RAS-RAF-MEK-MAPK pathway with modified targeting has been proposed.

Keywords: Oncogene; RAS; RAF-MEK-MAPK pathway; Tumor suppression; Tyrosine Kinase Inhibitor (TKI); Anti-cancer therapy

Introduction

Kinases, notably those regulated the action of RAF-MEK-MAPK and AKT-mTOR-P13K pathways have attracted increasing attention to identify anticancer therapeutic targets, in view of their activity in integration of signals of growth, proliferation, angiogenesis and apoptosis. Oncoprotein RAS activates the RAF/MEK/ERK (Extracellular Signal-Regulated Kinases) pathways, involved proteins of MAPK family as effector; and pathways of PI3K (Phosphatidylinositol 3-Kinase)/Akt/NIK-KappaB pathway, p120-GAP/p190-B/Rac/NIK-Kappa-B, and Raf/MEKK1/IKK (1-Kappa-B Kinase)/I-Kappa-B/NIKappa-B pathway activate transcription factor NF kappa-B through signaling molecules PI3K and Akt [1]. RAS proteins are encoded by members of oncogene RAS family with H-, Ki- and N-RAS, whose mutational activation has been seen more than 50% of human cancer cases [2], leading to cancerous cell growth. Inhibition of the kinases has demonstrated efficacy in therapy against some of them are in clinical trials [8-11].

The Implications of RAF-MEK-MAPK Pathway in Promotion of Cell Growth and Proliferation

The family of RAS gene comprises of a group of oncogenes that are frequently mutated in human tumors like pancreas, lung, and colorectal cancers and neuroblastoma. The prominent members of the family include N-Ras (neuroblastoma cell line), H-Ras (Harvey murine sarcoma virus), and the alternatively spliced K-Ras (Kirsten murine sarcoma virus). Among these, K-Ras is most frequently constitutively activated in human cancers [2]. The genes of this family code for RAS proteins, which reversely binds guanidine nucleotides of GDP and GTP. The metabolic forms of guanidine phosphate correspond with functional statuses of RAS, a small molecule G protein. RAS is activated when recruiting adapter proteins such as Grb2 that in turn engages guanine nucleotide exchange factors (GEFs) like SOS to the cell membrane, GDP bound to RAS is replaced by GTP transferred by factors SOS [4,5].

RAS is normally activated in response to the binding of extracellular signals, such as growth factors, RTks (Receptor Tyrosine Kinases), TCR (T-Cell Receptors) and PMA (Phorbol-12 Myristate-13 Acetate). The GTP associated Ras triggers the activation of a sequential three-kinate phosphorylation cascade through RAF, MEK, and ERK. RAF-MEK-ERK is essential for the regulation of cellular proliferation and survival [6]; the pathway integrates a wide range of signals into major cellular programs such as proliferation, differentiation, or apoptosis. And half of all human malignancies display aberrations in the RAS-RAF-MEK-ERK pathway.

RAF is a downstream effector kinase of RAS, and is found in three isoforms: A, B and C-RAF (also called RAF-1 or C-RAF-1). Many studies showed the role of RAF kinase as a potential cellular oncogene for cancer therapy [7]. RAF-1 is a 74 kDa mitochondrial protein, ubiquitously expressed in adult tissues, with highest expression in muscle, cerebellum, and fetal brain. It was the first RAF isoform identified. The agents targeting the RAF family as a whole or C-RAF extensively examined in many pre-clinical studies and more recently some of them are in clinical trials [8-11].

B-RAF is a 94 kDa mitochondrial protein identified as second RAF isoform which acts as mutational target in various human cancers.

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It is the strongest RAF kinase in terms of induction of MEK activity. A-RAF isoform is the weakest activator of MEK, and can only activate MEK1 but not MEK2. At present, no mutations in A-RAF have been found in human cancers [13,14].

MEK activates MAPK, the members of the family include extracellular signal regulated kinase (ERK) or MAPK, p38 MAPK, and JNK [15]. While different MAPK family members involve the same pathway with similar components with similar activities, the downstream effects notably regulators of cell cycle are not completely overlapped between different molecules.

In the field of anticancer drug, success in screening drugs targeting to the upstream factor RAS have been limited [16]. RAF and MEK, however, are important intermediates in the MAPK pathway [17]. researchers invested efforts to screen inhibitors of RAF and MEK as agents in anticancer therapy.

The Regulation of RAF-MEK-MAPK Involving Interactions between Oncogenes and Tumor Suppressor Genes

The pathway is activated by oncproteins, and targeted by tumor suppressors while exerting their anticancer potential, and in some context, the activity involves interactions between TSGs and oncogenes. TSGs are implicated in the genesis of malignancies in case of inactivation, through loss of heterozygosity (LOH) mutation, or epigenetic inactivation, in a manner of loss-of-function. As a result, the transformed cells are no longer harnessed in growth and proliferation by the regulation of cell cycle progression [18,19]. In fact, cell cycle progression, and hence cell proliferation is regulated by cyclin dependent kinases (CDKs) in complexed with cyclins transcriptionally activated mostly by RAS-RAF-MEK-MAPK pathway [20]. Tumor suppressors have been shown to downregulate the signaling axis of MAPK-cyclin, for example, JNK-cyclin D1, and some through interactions with RAS proteins [21-23].

FHIT is tumor suppressor mapped on 3p14, a frequently lost chromosomal region in human cancers; its inactivation is an early event in development of the cancer [24-26]. Data obtained from lung cancer lines have not just indicate the importance of FHIT in carcinogenesis but also its potential to serve as an early biomarker for lung cancer [27]. A new role of FHIT in down-regulating the Ras/Rho GTPase-associated oncogenic signaling pathway has been suggested [28].

We have reported that BLU, a TSG mapped on the same chromosomal region as RASSF1a, i.e. 3p21 which is frequently lost in nasopharyngeal carcinoma (NPC) and a variety of human tumors, mainly of epithelial origin, suppressed the signaling of JNK pathway, and reduced the level of cyclin D1 to arrest cell cycle at G1 phase when it is re-expressed in negative NPC cells [29]. Structurally, BLU protein contains a zinc finger MYND domain on its amino-terminus. The molecular mechanisms underlying its downregulation of JNK-cyclin D1 axis remain to be elucidated.

Transcription factor AP1 is formed by, heteromerization of c-FOS and c-JUN, whose phosphorylation is catalyzed by JNK and another protein c-FOS. AP1 binds to the genomic DNA sequences upstream to the coding portion of a number of genes coding for cell cycle regulator, notably CCND1 coding for cyclin D1. Known as product of proto-oncogene, cyclin D1 promotes proliferation in malignancy through interaction with oncogenic molecules [30]. It has been reported that over-expressed cyclin D1 facilitates the infection of nasopharyngeal epithelial cells by a lymphotropic human herpesvirus, Epstein-Barr virus (EBV), a ubiquitous human virus that is tightly associated with the occurrence of NPC and Burkitt lymphoma (BL) [31]. It has been proposed that MAPK pathway and cyclin D1 forms a signaling axis to regulate cell proliferation, and amplification of the chromosomal region that harbors CCND1 is a frequent anomaly at cytogenetic level during the occurrence of human tumors [32,33].

We reported that re-expression of BLU downregulated JNK signaling through reducing phosphorylation on JNK and inhibiting formation of AP1. It is reasoned that the effect was due to the inhibition of upstream kinase(s) by reducing their levels via epigenetic mechanism. In fact, we have shown that BLU inhibited the expression of IKK alpha, reduced the level of NFkappaB and hence NFkappaB dependent anti-apoptotic factors, so as to promote death receptor induced apoptosis [34]. It is speculated that BLU binds HDACs or SIRT, to repress transcription of genes coding for kinases in the pathway of RAF-MEK1/2-MAPK to downregulate the pathway and exert tumor suppression.

Previous study has shown that lymphoid-specific helicase (LSH), a SNF2-SWI chromatin remodeler, plays an essential role in cancer progression via regulation of fumarate hydratase (FH) [35]. Mechanistically, together with histone methyltransferase G9a, LSH is critical for the normal development of mammals and is involved in the establishment and maintenance of DNA methylation [36]. Since apart from depositing of H3K9me2 [37], G9a and its partner modifier GLP also interact with DNA methyltransferases (DNMTs) and protect proper DNA methylation at certain loci [38], LSH might also directly interact with DNMTs and affect the patterns of DNA methylation in the cells. Therefore, in human NPC cells, investigation of chromatin loading of LSH and the patterns of DNA methylation at the BLU locus might shed light on the mechanisms that required for the regulation of BLU and the progression of these malignant carcinomas.

The tumor suppression of a family of proteins RASSF is exerted by downregulation of RAS-RAF-MEK1/2-ERK pathway. RASSF1A, the founding member of the RASSF family and RASSF5/NORE inhibits tumor growth and proliferation by targeting to signaling molecules of the MAPK family [23,35]. Phosphorylated ERK (pERK) is a key downstream component of the Ras/Raf/MEK/ERK signaling pathway. After phosphorylation, it translocates to the nucleus, and regulates various transcription factors such as Ets family transcription factors (Etk-1) [39].

The Implications of RAF-MEK-MAPK on Malignant Transformation of Cells

The aberrant activation of the RAF/MEK/MAPK signaling pathway is correlated with the occurrence of hepatocellular carcinoma (HCC) and a variety of human cancers. The activity of the RAF/MAPK signaling pathway was significantly higher, and the activity of ERK1/2 and MEK1 were upregulated threefold to fourfold in neoplastic liver specimens when compared to normal liver tissue adjacent to the HCC lesions [40,41]. Furthermore, it has been reported that the over-expression of RAF-1 could be regarded as an indicator of HCC prognosis [42]. These data suggest that the RAF/MEK/MAPK pathway may serve as an attractive target in the therapy of HCC.

The Applications of Components of RAF-MEK-MAPK Pathway in Anticancer Therapy

Epidermal growth factor receptor (EGFR) signaling is triggered by the binding of its ligand, resulting in the dimerization of EGFR molecules or heterodimerization with other closely related receptors, such as HER2/neu. EGFR is overexpressed in 40-80% of non–small
Table 1: Small molecules generated for targeting the components of the RAS-RAF-MEK-MAPK signaling pathway.

<table>
<thead>
<tr>
<th>Targeting component</th>
<th>Small molecule inhibitor</th>
<th>References</th>
</tr>
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<tr>
<td>EGFR Tyrosine Kinase</td>
<td>Genestein; AG 1478; gefitinib, erlotinib; afatinib, icotinib</td>
<td>[62,63]</td>
</tr>
<tr>
<td>Mutant BRAF</td>
<td>PLX4032; dabrafenib, sorafenib</td>
<td>[64,65]</td>
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<tr>
<td>MEK1/2</td>
<td>PD 98059; Pimasertib; U0126, Selumetinib (AZD6244, ARRY-142886) cobalt(II) complex 2</td>
<td>[55,56,66-69]</td>
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<tr>
<td>P38 MAPK</td>
<td>SB203180, SB203580</td>
<td>[70,71]</td>
</tr>
<tr>
<td>JNK</td>
<td>SP600125</td>
<td>[72]</td>
</tr>
<tr>
<td>ERK</td>
<td>PD98059</td>
<td>[73]</td>
</tr>
</tbody>
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Emerging problems of using kinase inhibitors to treat cancer include acquired resistance in cancer cells [75]. A strategy to overcome obstacles is to achieve efficacy by administering the drugs according to the gene profile of the individual patient, notably the status of EGFR, RAS and PTEN. Alternatively, combined administration of the inhibitor has been proved to be effective; for example, the use of BRAF inhibitor dabrafenib and MEK inhibitor trametinib in treating melanoma patients with BRAF V600E mutation has significantly improved progression-free survival of the patients [76]. We have identified abliradib, an isoflavone isolated from licorice as a BRAF and MEK double targeting, based on computational modeling and shown that the drug suppressed the proliferation of HCC cells by inhibiting the phosphorylation of MEK1/2 and the downstream molecules including ERK1/2 and transcription factors ATF1 and CREB [77] (Table 1).
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