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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-PI3K 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, involving proteins of MAPK mfamily as end effector; and pathways of PI3K (Phosphatidylinositol 3-Kinase)/Akt/NF-KappaB (Nuclear Factor-Kappa B) pathway, p120-GAP/p190-B/Rac/NF-Kappa-B, and Raf/MEKK1/IKK (I-Kappa-B Kinase)/I-Kappa-B/NFKappa-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 cancers, and is also targeted by tumor suppressor genes (TSGs) when exerting their anti-oncogenic activities, through downregulation of cell cycle entry and angiogenesis, and potentiation of apoptosis. Mitogen-activated protein kinase (MAPK) is a serine/threonine kinase, which activates transcription factors and other cytoplasmic factors leading to mitogenesis [3]. The modulation of RAF-MEK-MAPK pathway in the context of oncogenes-TSGs interaction, and of intervention of anticancer drugs is to be discussed in the present paper.

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-kinase 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-RAFMEK-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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[1,12]. 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 regualtors 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 oncoproteins, 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 RASSF1, 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, hetromerization 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.

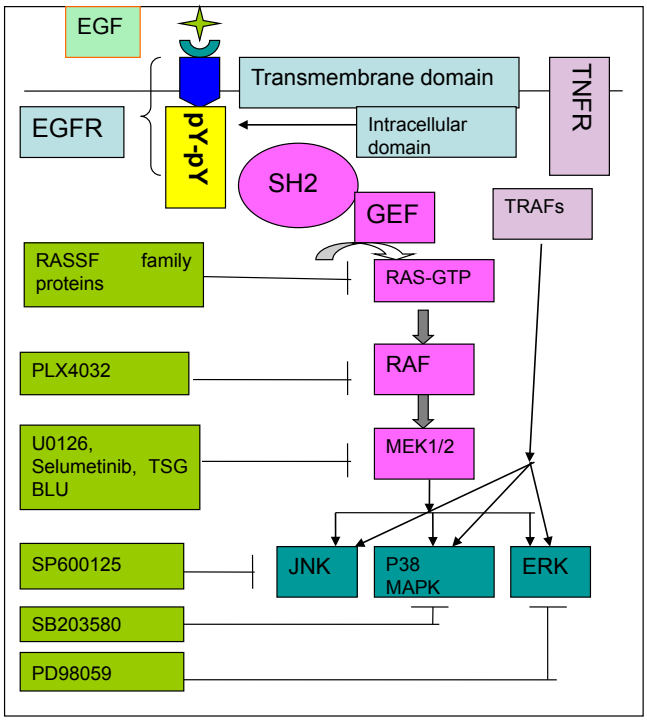
The tumor suppression of a family of proteins RASSF is exerted by downregulation of RAS-RAF-MEK1/2-ERK pathway. RASSF1A, the founding member of the RASSF family and RASSF5/NORE inhibits tumor growth and proliferation by targeting to signaling molecules of the MAPK family [23,35]. Phosphorylated ERK (pERK) 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 (Elk-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 occurence of hepatocellular carcinome (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



EGFR transmits growth signals when dimerizing triggered by EGF. Its intracellular domain of protein tyrosine kinase (PTK) catalyzes phosphorylation of tyrosine residues (p-Y), and the altered spatial configuration of the intracellular portion recruits adapter protein with SH2 motif, and then guanidine exchange factor to transfer GTP to RAS proteins. Through a cascade activation of RAS-RAF-MEK kinases, the effectors JNK, p38 MAPK and ERK are activated to induce transcription factor activity and then gene transcription is initiated. It is indicated that activity of components of this pathway are regulated by TSGs and small molecule inhibitors. MAPK family proteins are activated by trimerized TNFR triggered by its corresponding ligands, in a TRAF dependent manner with kinase activation [55].

Figure 1: Scheme of regulation of the signaling of RAS-RAF-MEK-MAPK pathway.

Targeting component	Small molecule inhibitor	References
EGFR Tyrosine Kinase	Genistein; AG 1478; gefitinib, erlotinib; afatinib, Icotinib	[62,63]
Mutant BRAF	PLX4032; dabrafenib, sorafenib	[64,65]
MEK1/2	PD 98059; Pimasertib U0126, Selumetinib (AZD6244, ARRY-142886) cobalt(II) complex 2	[55,56,66-69]
P38 MAPK	SB202190, SB203580	[70,71]
JNK	SP600125	[72]
ERK	PD98059	[73]

Table 1: Small molecules generated for targeting the components of the RAS-RAF-MEK-MAPK signaling pathway.

cell lung cancers (NSCLC) and many other epithelial cancers [43]. Besides the mutations in EGFR and HER2, KRAS genes are generally identifiable in over 40% of NSCLCs [44,45]. Tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib acting as reversible inhibitors for binding to the ATP pocket of the protein tyrosine kinase were clinically approved agents targeting EGFR. In NSCLC, gefitinib was shown to induce partial responses (PR) in approximately 10% of cases [46]. The downstream kinases were also tested as target for therapeutic intervention, resulting in the development of several potent MEK inhibitors [47,48]. Patients with lung cancer whose tumors harbor EGFR and HER2 mutations respond to EGFR TKIs but develop drug resistance can still benefit from the use of second generation tyrosine kinase inhibitors [49-51].

The downstream signaling that EGFR triggers is the RAS/RAS/MEK/ERK pathway. While RAF-MEK-MAPK pathway is targeted to be downregulated by TSGs, it has always been thought as an attractive pathway for anticancer therapy because it plays a central role in regulating various cellular processes from a broad spectrum of human tumors [52]. The activities of the pathway are often aberrant in tumors it therefore serves as a potential target for inhibitors of small molecule inhibition [53]. The blocking the post-translational modifications that promote Ras membrane association have been adopted as a strategy of anticancer therapy [54]. In recent years, various inhibitors have been developed for RAS downstream effector signaling, with efforts focused on the ERK/MAPK pathway (Figure 1).

In the recent years, small molecule intervention has been used targeting for the members of the RAS–RAF–MEK–ERK signaling pathway. The potential of AZD6244, a selective MEK1/2 inhibitor [55,56] which exerts anti-proliferative effects on NSCLC cell lines was characterized [57,58]. The dual inhibition of MEK and EGFR or MEK and signal transducer and activator of transcription 3 (STAT3) signaling pathways may constitute a potent therapeutic strategy for the treatment of KRAS mutant NSCLCs. But AZD6244 displays insufficient efficacy in non-small cell lung cancers (NSCLCs) due to deregulated expression and/or mutations of PIK3CA and PTEN [59-63]. A rational basis has been validated for choosing the inhibitor to best combine with the MEK inhibitor in cells on the basis of the expression and mutations of several oncogenes [64-74].

Emerging problems of using kinase inhibitors to treat cancer include acquired resistance in cancer cells [75]. A strategy to overcome to obstacle so as to achieve efficacy was to administer 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 efective; 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 glabridin, an isoflavone isolated from licorice as 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).

Conclusion

In conclusion, small molecule inhibitors to RAS-RAF-MEK-MAPK pathway have been validated as effective in therapy against a variety of cancers, and have gained wide application (Table 1). To circumvent the problems like acquired resistance, agents with multiple targeting are to be developed, and modality with combination of agents should be considered based on cancer related gene profile of the individual patients.

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