The Ras/Raf/MAPK Pathway

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The Ras/Raf/MAPK pathway is probably the best characterized signal transduction pathway in cell biology. The function of this pathway is to transduce signals from the extracellular milieu to the cell nucleus where specific genes are activated for cell growth, division and differentiation. The Ras/Raf/MAPK pathway is also involved in cell cycle regulation, wound healing and tissue repair, integrin signaling and cell migration.[1–3] Finally, the Ras/Raf/MAPK pathway is able to stimulate angiogenesis through changes in expression of genes directly involved in the formation of new blood vessels.[4] Thus, signaling through the Ras/Raf/MAPK regulates a variety of cellular functions that are important for tumorigenesis.

Dysregulation of this pathway is a common event in cancer as Ras is the most frequently mutated oncogene in human cancer. Mutations in the K-ras oncogene have been localized in codons 12, 13, 59 and 61 with those at codons 12 and 61 occurring most frequently. K-ras mutations are present in 15-50% of lung cancers and in 72–90% of pancreatic cancers.[5,6] Even in the absence of activating mutations, K-ras still plays a role in oncogenesis via Ras gene amplification, overexpression or upstream activation of the pathway. Each of these potential cellular alterations will produce increased activation of Ras effectors, thereby promoting development of tumors. For example, 40% of esophageal cancers have amplification of the K-ras gene[7] and in approximately 50% of breast cancers, Ras is highly active in association with expression of HER-2/Neu receptors.[8] Mutations in other members of the pathway are also common in other forms of cancers. Somatic *B-raf* mutations have been found in 60-70% of malignant melanomas and are also seen in papillary thyroid cancer, colon and ovarian cancers.[9] Furthermore, activation of MAPK –not associated with either K-ras or BRAF activating mutations are seen in 41% of low-grade ovarian serous carcinomas.[10]

The ras superfamily of genes encodes small GTP-binding proteins that are responsible for regulation of many cellular processes, including differentiation, cytoskeletal organization, and protein trafficking.[11] Oncogenic ras genes in human cells include H-ras, N-ras and K-ras.[12] The 21-kd transforming proteins H- and K- ras genes were first identified as the counterparts of the oncogenes of the Harvey and Kirsten rat sarcoma viruses, whereas the N-ras oncogene was isolated from a neuroblastoma and has not been found in any retroviruses.[12] Since mutations in K-ras and not in H-ras or N-ras are common in lung cancer, this review will focus on K-ras. K-ras is initially synthesized as an inactive cytosolic pro-peptide. Then the protein undergoes a series of post-translational modifications at its carboxyl terminus that increase its hydrophobicity allowing its localization to the lipid-rich cell membrane.[13] An important post-translational modification is farnesylation in the hydrophobic tail of the carboxyl terminal group. This reaction is catalyzed by the enzyme farnesyltransferase which adds a 15-carbon hydrobobic farnesyl isoprenyl to the carboxyl terminus of Ras. Once in the cell membrane, K-ras cycles between inactive guanosine diphosphate–bound and active guanosine triphosphate (GTP) –bound states, thereby activating a series of effector kinases –such as Raf and MAPK- that phosphorylate a cascade of signaling proteins.[14] The principal consequence of the mutated proteins is a marked decrease in interactions between Ras and its GTPase activator protein.[15] Instead of reverting to its inactive guanosine diphosphate–bound state, the modified conformation of mutant Ras favors its active GTP-bound state, which has a higher propensity to activate downstream effectors even in the absence of growth factor stimulation, conferring a proliferative advantage to tumors.

Activation of the pathway begins when a signal binds to a protein tyrosine kinase receptor. The epidermic growth factor receptor (EGFR) and the platelet-derived growth factor receptor (PDGFR) are the best-known receptors in the pathway. However, multiple upstream receptors including other receptor tyrosine kinases, integrins, serpentine receptors, heterotrimeric G-proteins and cytokine receptors are able to activate K-ras.[16] Binding of a ligand to EGF receptor induces oligomerization of the receptor, a process that results in juxtaposition of the cytoplasmic, catalytic domains in a manner that allows activation of the kinase activity and transphosphorylation.[17] Adaptor proteins such as Grb2 are now able to recognize sequence homology 2 (SH2) domains such as Shc, which in turn, recruit guanine nucleotide exchange factors (GEFs) like SOS-1 or CDC25 to the cell membrane[17] (figure 1). The GEF becomes capable of interacting with Ras proteins at the cell membrane to promote a conformational change and the exchange of GDP for GTP. Following Ras activation, Raf is recruited to the cell membrane through

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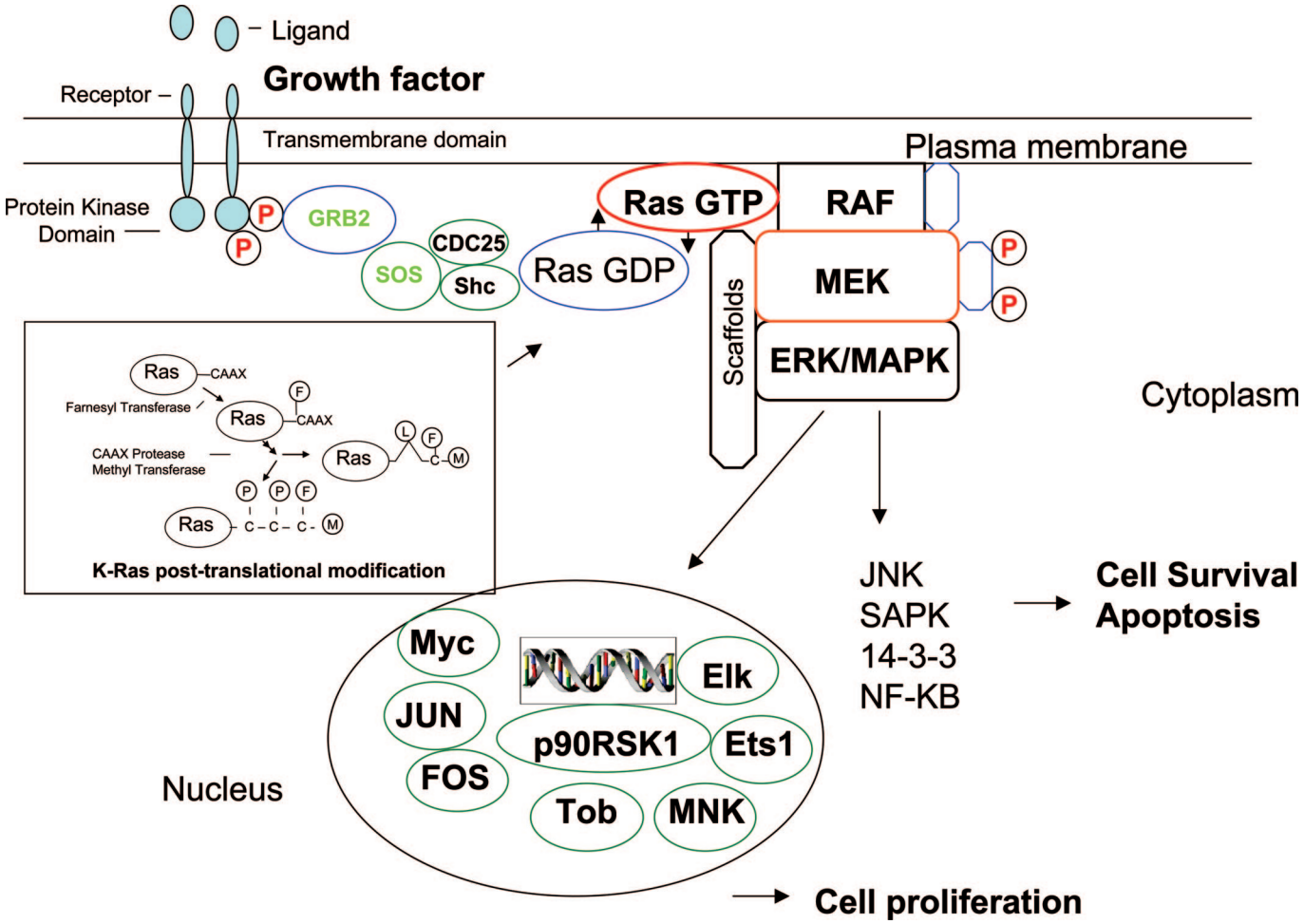

FIGURE 1. Ras/Raf/MAPK pathway

binding to the switch I domain of Ras and also by lipid binding.[18]

Raf is the best characterized Ras effector and is a member of a family of serine/threonine kinases, that includes Raf-1, A-Raf and B-Raf. Raf activation stimulates a signaling cascade by phosphorylation of MAPK which successively phosphorylate and activate downstream proteins such as ERK1 and ERK2 (figure 1). Activation of ERK is critical for a large number of Ras-induced cellular responses. ERK1 and ERK2 phosphorylate and activate a variety of nuclear transcription factors and kinases, including Elk-1, c-Ets1, c-Ets2, p90RSK1, MNK1, MNK2, as well as other proteins such as the anti-proliferative protein Tob (figure 1). Many of these MAPK/ERK targets have been implicated in Ras induced cell transformation.[14]

MAPK, which in mammalians is also called MEK, is a serine/threonine kinase activated in response to multiple signals including growth factors and cytokines to promote cell survival and apoptosis through a number of mediators such as JNK, SAPK, 14-3-3 and NF-KB[19] (figure 1). MAPK may also regulate both Raf and ERK, providing for cross talk between multiple signaling pathways. Indeed, MAPK appears to induce apoptosis by dysregulation of a number of pathways including ERK, JNK and p38.[20] MAPK has been shown to directly interact with K-ras in a GTP-dependent manner.[19,20]

Raf and MAPK are not the only downstream targets of K-ras, other downstream effectors of K-ras include the PI3K cell survival pathway, the small GTP-binding proteins Rac and Rho, and the stress-activated protein kinase pathway (also referred to as the c-jun N-terminal kinase (JNK) pathway).[19] In addition, in response to cellular stress and cytokine stimulation mediated through K-ras, the dual-specificity $p38^{MAPK}$ kinases (MKK3 and MKK6) and the JNK kinases (MKK4 and MKK7) phosphorylate $p38^{MAPK}$ and JNK, respectively.[20]

Numerous studies have been performed evaluating the prognostic importance of oncogenic ras mutations in human tumors. Results to date have been conflicting in non-small cell lung cancer, while some studies are negative or equivocal in non-small cell lung cancer.[21,22] However, the majority of data favors K-ras mutations as being a negative prognostic factor.[23,24] Possible explanations for the inconsistent results include the predominantly retrospective nature of these studies, the small numbers of patients studied, and the inability to correct for other confounding prognostic factors. Further-

more, activating K-ras mutations have been shown to diminish responsiveness to EGRF inhibitors.[25]

As Ras/Raf/MAPK signaling is complex, there are many steps at which to target therapies designed to interfere with signaling. Targeting the Ras/Raf/MAPK pathway could be achieved by:

1. Inhibiting Ras protein expression, [antisense Ras DNA, adenovirus expressing antisense K-ras and small interference RNA (siRNAs)].

2. Inhibiting membrane localization through post-translational modification or trafficking, [Farnesyltransferase inhibitors (FTIs and geranylgeranyltransferase inhibitors)].

3. Blocking Ras interaction with GEF and enhancing Ras/GAP interactions.

4. Targeting oncogenic K-ras, (immunological therapies against mutant K-ras and yeast expressing systems).

5. Inhibiting downstream targets of K-ras such as Raf and MEK, (Raf kinase inhibitor-BAY 43-9006 and MEK inhibitors such as CI-1040, PD0325901 and ARRY-142886)

Phase I and phase II clinical trials testing these approaches are currently ongoing.

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