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Review

B-Raf mutation: A key player in molecular biology of cancer

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ABSTRACT

B-Raf is one of the more commonly mutated proto-oncogenes implicated in the development of cancers. In this review, we consider the mechanisms and clinical impacts of B-Raf mutations in cancer and discuss the implications for the patient in melanoma, thyroid cancer and colorectal cancer, where B-Raf mutations are particularly common.

Contents

Introduction

B-RAF is known as v-raf murine sarcoma viral homolog B1 which is a proto-oncogene. It is a member of Raf (Rapidly accelerated fibrosarcoma) kinase family proteins. Raf kinase family proteins are serine/threonine kinases, originally identified as retroviral oncogenes in 1983 (Moelling et al., 1984; Rapp et al., 1983; Zebisch and Troppmair, 2006). So far, the Raf kinase proteins have had three members identified and they are: A-Raf, B-Raf and C-Raf. C-Raf, also known as Raf-1, was first discovered in 1985 whereas A-Raf was discovered in 1986 and B-Raf in 1988 (Bonner et al., 1985; Huebner et al., 1986; Ikawa et al., 1988; Roskoski, 2010). A-Raf is the smallest isoform, at 68 kDa, C-Raf ranges from 72 to 74 kDa and due to some significant alternative splicing, B-Raf ranges from 75 to 100 kDa (Barnier et al., 1995; Storm et al., 1990; Wellbrock and Karasarides, 2004).

All Raf proteins share a common structure of three conserved region: CR1, CR2 and CR3 (Fig. 1). CR1 and CR2 are regulatory domains which exist in the N-terminus whereas CR3 is a kinase domain which is encoded in the C-terminus (Morrison and Cutler, 1997; Wellbrock and Karasarides, 2004). CR1 contains the Ras binding domain (RBD) and cysteine rich domain (CRD). It is responsible for binding with Ras and membrane phospholipids (Roskoski, 2010). Although Ras binds to RBD only in its active form (Ras-GTP), binding to CRD does not depend on GTP status of Ras (Wellbrock and Karasarides, 2004; Wittinghofer and Nassar, 1996). CR2 is a serine/threonine rich region. Protein–protein interactions and phosphorylation through CR2 interfere with Raf activation and localization (Guan et al., 2000; Morrison et al., 1993; Yao et al., 1995; Zhang and Guan, 2000; Zimmermann and Moelling, 1999). In addition, CR2 holds a phosphorylation site which is responsible for binding to the regulatory protein 14-3-3. Besides the

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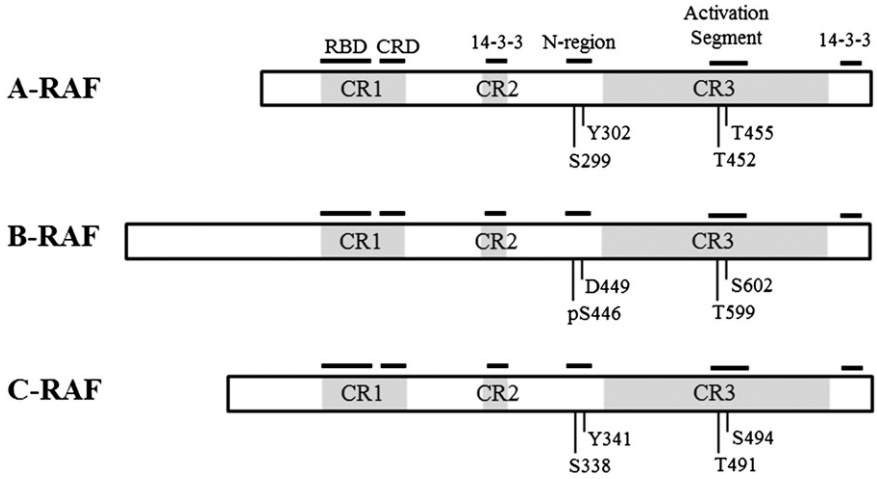

Fig. 1. Linear structure of Raf proteins. Three conserved regions of A-Raf, B-Raf and C-Raf proteins are: CR1, CR2 and CR3. Other important corresponding regions in Raf proteins are also indicated. The most important corresponding residues in each Raf proteins are numbered. In B-Raf, S446 is a constitutively phosphorylated residue and aspartic acid replaces the tyrosine residue at D449. Regulatory protein 14-3-3 binding sites in each Raf proteins are shown. RBD: Ras binding domain, CRD: cysteine rich domain, N-region: negatively charged region.

site in the CR2, there is another binding site for the 14-3-3 regulatory protein after the CR3 region. The CR3 region itself is a catalytic kinase domain which is regulated through phosphorylation (Roskoski, 2010).

B-Raf is the most potent activator of downstream effector MEK kinases in the Ras–Raf–MEK–ERK pathway when compared with A-Raf and C-Raf (Cantwell-Dorris et al., 2011; Moelling et al., 1984; Rapp et al., 1983). The reason for this is that residue S446 in B-Raf is constitutively phosphorylated and becomes negatively charged. In addition, residue D449 in B-Raf contains aspartic acid (instead of tyrosine) which is a negatively charged amino acid. Both of the residues are in N-region of B-Raf. As a result, B-Raf always has a negatively charged N-region, which facilitates its activation through Ras (Cantwell-Dorris et al., 2011; Garnett and Marais, 2004; Mason et al., 1999). On the other hand, C-Raf and A-Raf require their N-region (residues S338 and Y341 in C-Raf, residues S229 and Y302 in A-Raf) to be phosphorylated by Src in addition with phosphorylation by Ras for their full activation (Fabian et al., 1993; Garnett and Marais, 2004; Marais et al., 1995; Mason et al., 1999). B-Raf also possesses the highest basal kinase activity among all Raf isoforms. All these factors give B-Raf predominance over other Raf isoforms for MEK activation (Cantwell-Dorris et al., 2011).

The maintenance of homeostasis in the Ras–Raf–MEK–ERK pathway requires strict balance. If this pathway is hyper-activated, it leads to cell-cycle arrest. On the other hand, carcinogenesis is also the result of aberrant activation of the Ras–Raf–MEK–ERK pathway (Cantwell-Dorris et al., 2011; Davies et al., 2002). Mutations that are responsible for aberrant ERK signalling can present at different levels within the Ras–Raf–MEK–ERK pathway. B-Raf mutation is one of common types of mutations that lead to aberrant ERK signalling (Garnett and Marais, 2004). To avoid over activation of the Ras–Raf–MEK–ERK pathway and cell cycle arrest, ERK signalling should be maintained carefully (Kerkhoff and Rapp, 1998; Marshall, 1995; Sewing et al., 1997; Woods et al., 1997). To do that some multiple mutations are seen in the Ras–Raf–MEK–ERK pathway in cancer so that the cancer cells can still modulate ERK signalling despite aberrant pathway activity. Therefore, some B-Raf mutations are seen along with Ras mutations in the same type of cancer, cooperating with each other to modulate ERK signalling (Davies et al., 2002; Garnett and Marais, 2004; Yuen et al., 2002b). However, B-Raf V600E mutation is not seen in the same type of cancer where Ras is mutated. This may be because their combined stimulation will hyper-activate the ERK signalling pathway and will arrest the cell cycle (Davies et al., 2002; Garnett and Marais, 2004).

Clinical impacts of B-Raf mutation

Cancers arising through mutations in the Ras–Raf–MEK–ERK pathway are noted in approximately 30% of all cancers, where around 8% is due to B-Raf mutation (Davies et al., 2002; Garnett and Marais, 2004). The highest frequency of B-Raf mutations happens in melanoma (40–70%). This mutation also commonly found in thyroid cancer (36–53%), colorectal cancer (5–22%) and ovarian serous cancer (~30%). B-Raf mutation is also detected in other cancers such as gliomas, non-small cell lung carcinoma, hepatobiliary carcinoma and hairy cell leukaemia (Cantwell-Dorris et al., 2011; Davies et al., 2002; Dhomen and Marais, 2009; Garnett and Marais, 2004; Pakneshan et al., 2013; Zebisch and Troppmair, 2006). In addition, B-Raf mutation has been related clinically to the histological subtypes and prognosis of patients in several cancers (Pakneshan et al., 2013; Smith et al., 2011; Xing et al., 2013).

Most of the B-Raf mutations are found in exon 11 or 15 of its catalytic kinase domain (Ikenoue et al., 2004; Zebisch and Troppmair, 2006). So far, more than 65 B-Raf mutations have been discovered (Cantwell-Dorris et al., 2011; Davies et al., 2002; Roskoski, 2010). Among all the B-Raf mutations, V600E mutation is the most frequent type, making up more than 90% of observed B-Raf mutations. This mutation is a missense mutation in exon 15, occurring due to the substitution of the amino acid valine to glutamic acid at residue position 600 (Davies et al., 2002).

B-Raf wild-type and activation of MEK–ERK pathway

Ras–Raf–MEK–ERK pathway is an important cellular functional pathway that works in response to extracellular stimuli like hormones, cytokines and various growth factors (Cantwell-Dorris et al., 2011; Garnett and Marais, 2004; Robinson and Cobb, 1997). Extracellular stimuli activate Ras (small G-protein) kinases through their respective receptor on the cell membrane (Fig. 2). Activated Ras then recruits B-Raf protein from cytoplasm to the cell membrane. With the help of adaptor proteins, Ras phosphorylates two important residues in B-Raf (residues T599 and S602) that are located in the activation segment of B-Raf kinase domain (Garnett and Marais, 2004; Zhang and Guan, 2000). After being phosphorylated, B-Raf activates its downstream effectors MEK1 and MEK2 which afterwards phosphorylate ERK1 and ERK2. Activated ERK1/2 then forwards the signals to its downstream effectors (both cytosolic and nuclear effectors) for cellular proliferation, differentiation, growth and survival (Cantwell-Dorris et al., 2011). Experiments have shown that among the MEK kinase family, B-Raf only interacts with MEK1 and MEK2. The reason behind this is that