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Mini-review

A thirty-year quest for a role of R-Ras in cancer: from an oncogene to a multitasking GTPase

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ABSTRACT

Since the identification of R-Ras, which is the first Ras-related GTPase isolated based on sequence similarity to the classical *RAS* oncogene, more than 160 members of the Ras superfamily of GTPases have been identified and classified into the Ras, Rho, Rap, Rab, Ran, Arf, Rheb, RGK, Rad, Rit, and Miro subfamilies. R-Ras belongs to the Ras subfamily of small G-proteins, which are frequently implicated in cell growth and differentiation. Although the roles of R-Ras in cellular transformation and integrin-mediated cell adhesion have been extensively studied, the physiological function of this enigmatic G-protein was only revealed when a mouse strain deficient in R-Ras was generated. In parallel, a plethora of research findings also linked R-Ras with processes including tumor angiogenesis, axon guidance, and immune cell trafficking. Several upstream factors that modulate R-Ras GTP-binding were identified including Notch, semaphorin, and chemokine C-C motif ligand 21. A review of our evolving understanding of the role of R-Ras in oncogenesis is timely, as this year marks the 30th anniversary of the publication describing the cloning of R-Ras.

Introduction

Small GTPases are molecular switches that cycle between GTP-bound active and GDP-bound inactive forms [1–4]. The localization of these proteins to the inner leaflets of the plasma membrane suggests potential roles in transmitting extracellular cues [5]. The approximately 160 identified members of the Ras superfamily share functional motifs involved in phosphate and purine ring binding, as well as a carboxyl (C) terminal CAAX sequence that facilitates membrane attachment [2,6](Fig. 1A). The activation states of G-proteins are controlled by three key regulators: guanosine nucleotide dissociation inhibitors (GDIs), which promote GDP-binding; guanine nucleotide exchange factors (GEFs), which stabilize the nucleotide-free state; and GTPase activating proteins (GAPs), which enhance intrinsic GTPase activity [7–9]. Small GTPases can also be regulated via post-translational modifications such as phosphorylation and ubiquitination, which can modulate protein stability and subcellular localization [10].

HRAS, *NRAS*, and *KRAS* were the earliest identified human oncogenes. These oncogenes were found to be mutated in more than 30% of human cancers [1,11,12]. A vast number of Ras-related genes that did not exhibit readily detectable transforming activities in conventional NIH3T3 focus-forming assays were subsequently isolated [13–15]. These Ras-related GTPases were grouped based on sequence similarities into 11 major subtypes in which members share similar biological properties. For instance, Ras subfamily members play a predominant role in cell proliferation [14], whereas Rab subfamily members participate in intracellular vesicular trafficking [16].

Among the 32 Ras subfamily members, *RRAS* was the first Ras-related gene isolated by Lowe et al. via low-stringency

Abbreviations: BM, Bone marrow; C, Carboxyl; CCL21, C-C motif ligand 21; CXCL12, Cysteine X cysteine ligand 12; DC, Dendritic cells; DRG, Dorsal root ganglion; EAE, Experimental autoimmune encephalomyelitis; EC, Endothelial cells; ECMs, Extracellular matrices; EHD, Eps15-homology domain; FLNa, Filamin A; GAPs, GTPase activating proteins; G-CSF, Granulocyte-colony stimulating factor; GDIs, Guanosine nucleotide dissociation inhibitors; GEFs, Guanine nucleotide exchange factors; HPC, Hematopoietic progenitor cells; HSC, Hematopoietic stem cells; ICAM1, Intercellular adhesion molecule 1; IL3, Interleukin 3; ILK, Integrin-linked kinase; LFA, Lymphocyte function-associated antigen 1; LPS, Lipopolysaccharide; MAPK, Mitogens-Activated Protein Kinase; N, Amino; NRP, Neuropilin; PI3-K, Phosphatidylinositol 3-kinase; RBD, Rho GTPase binding domain; SCF, Stem cell factor; Sema, Semaphorin; Treg, Regulatory T-cells; VE-Cadherin, Vascular Endothelial-Cadherin; VEGFR2, Vascular endothelial growth factor receptor 2; VSMC, Vascular smooth muscle cells.

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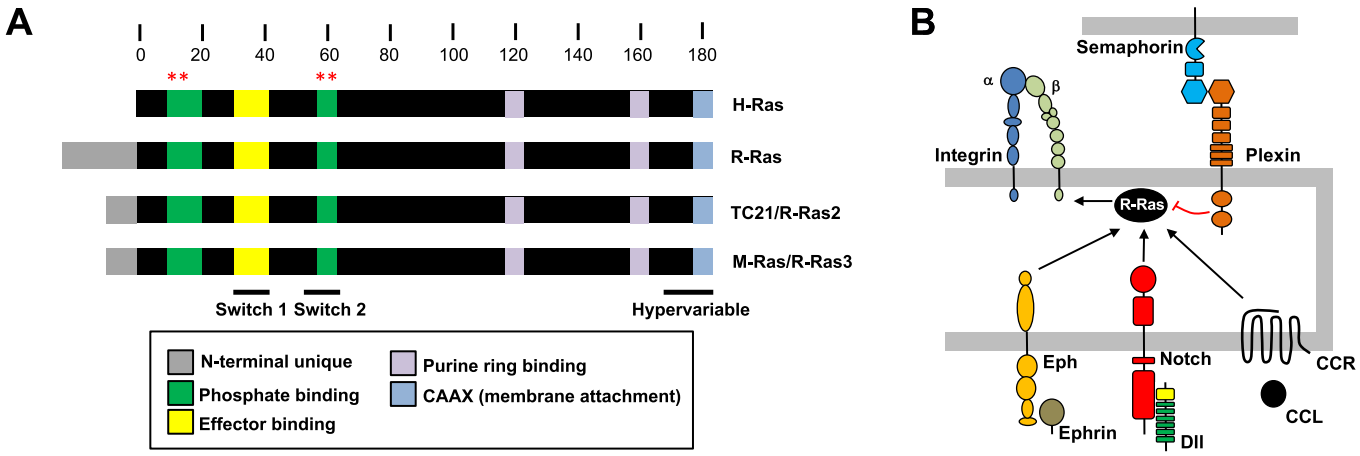

Fig. 1. **(A)** Ras subfamily GTPases. Schematic representation of four members of the Ras subfamily showing the key functional motifs (colored bars) shared among small GTPases. The switch 1 and 2 domains, and the carboxyl terminal hypervariable region are shown. Codons at positions 12, 13, 59, and 61 of H-Ras that are frequently mutated in human cancers are indicated with an asterisk. Amino acid positions are indicated (numbers). **(B)** R-Ras regulated signaling events. Schematic representation of key positive (Eph, Notch, CCR) and negative (Plexin) receptors that regulates the GTP-binding states of R-Ras. The cognate ligands that bind to these receptors are shown. R-Ras positively regulates the activation state of integrin receptors.

hybridization with a v-H-Ras cDNA probe [17,18]. R-Ras and two closely related members, TC21/R-Ras2 and M-Ras/R-Ras3, are the only small Ras GTPases that possess transforming activities, albeit weakly [19–23]. R-Ras is known to promote inside-out signaling leading to integrin activation, although the underlying mechanisms have not been fully delineated [24]. Additionally, considerable efforts have been devoted to deciphering the pro-proliferative and pro-migratory role of R-Ras in various epithelial tumor types. However, evidence supporting a definitive role for R-Ras in human cancer remains elusive.

New findings have implicated a role for R-Ras in some unexpected cellular systems [25]. In the vascular system, R-Ras has been implicated in endothelial cell junctional stability [26,27]. In the neural system, R-Ras activation is controlled by semaphorin-plexin signaling events in migrating axons [28](Fig. 1B). In the immune system, R-Ras is required for the trafficking of hematopoietic progenitor cells and T cells, and for immunological synapse formation with dendritic cells [29–31]. Therefore, a review of the key R-Ras research milestones from the past three decades and redefinition of the role of this GTPase in carcinogenesis is timely.

Identification of R-Ras as a Ras-related GTPase

The first two known human oncogenes, *HRAS* and *KRAS*, were isolated by transfection-transformation assay with high-molecular-weight DNA from the T24/EJ bladder and LX-1 lung carcinoma cell lines [32–35]. *NRAS* was subsequently cloned from the SK-N-SH neuroblastoma and SW-1271 lung carcinoma cell lines [36,37]. Invariably, mutations in these oncogenes are confined to the GTP-binding domain and switch II region in codons 12/13 and 59/61, respectively (Fig. 1A). However, certain human tumor types, such as breast, brain and prostate, rarely harbor *RAS* mutations [38–42].

These initial observations prompted a search for additional Ras-related oncogenes using two general approaches. First, novel Ras-related oncogenes were isolated through NIH3T3 transfection-transformation assays, using either high-molecular-weight tumor DNA or expression cDNA libraries. For example, *TC21/RRAS2* was cloned from an expression cDNA library derived from the human ovarian carcinoma cell line, A2780 [43]. The *TC21* Q72L mutation identified resembled that of the Q61L oncogenic lesions found in classical *RAS* oncogenes. To this day, only 21 tumor specimens have been reported to harbor *TC21* mutations at the hotspot codon23/24 and codon70/72 [43–45]. The second approach utilized low stringency hybridization technique to isolate Ras-related oncogenes. *RRAS* (*R*-stands for "related") was the first gene isolated by this strategy with a viral H-Ras DNA probe [17,18]. A SMARTBLAST analysis revealed 95% sequence identity between the human and mouse R-Ras proteins. Although the 218-amino acid R-Ras shares 48% sequence identity with H-Ras, it is evolutionarily more similar to another human Ras subfamily member, TC21/R-Ras2, with which it shares 61% sequence identity (Fig. 1A). Similar orthologues have been identified in *Drosophila melanogaster* (fruit fly; 56% identity), *Caenorhabditis elegans* (round worm; 50%), and *Danio rerio* (zebrafish; 62%).

Structurally, R-Ras possesses the five domains characteristic of small GTPases: the GTP-binding domain, Raf binding domain, Switche I and II regions, and CAAX box [17,18]. However, R-Ras uniquely possesses a glycine-rich, 30-amino-acid amino (N)-terminal region (MSSGAASGTGRGRPRGGGPGPGDPPPSETH) that is present in most mammals, but absent from the zebrafish, fly and worm (Fig. 1A). The effector-binding domain, which encompasses amino acids 58–66 (YDPTIEDSY), is identical in all Ras subfamily members, including TC21/R-Ras2, M-Ras/R-Ras3, and H-Ras [46]. Numerous studies have shown that R-Ras can interact with three key downstream effectors: c-Raf, phosphatidylinositol 3-kinase (PI3-K), and Ral-GDS [46–50]. The C-terminus of R-Ras possesses a prenylation motif (CVLL) that has been shown to undergo geranylgeranylation instead of farnesylation [51]. A palmitoylation site at cysteine 213 is thought to participate in membrane attachment as well [52]. The C-terminal hypervariable region also contains unique sequence motifs, including a proline-rich domain (PPSPPSAP), a short basic stretch (RKK), and a three-glycine run. Wang et al. reported a SH3-binding motif (PXXP) that could interact with the signaling adaptor Nck and mutation of this motif reduced the ability of R-Ras to promote cell adhesion in 32D cells [53]. These unique C-terminal features of R-Ras are expected to regulate its subcellular localization. In fact, unlike classical Ras, R-Ras is enriched in early and recycling endosomes and less localized to the plasma membrane [54].

GEFs and GAPs regulate the guanine nucleotide binding status of GTPases. Five GEFs—RasGRF, C3G, CalDAG-GEFI, CalDAG-GEFII (RasGRP), and CalDAG-GEFIII—stimulate GTP binding of R-Ras [55].