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CYTOSKELETON NEWS

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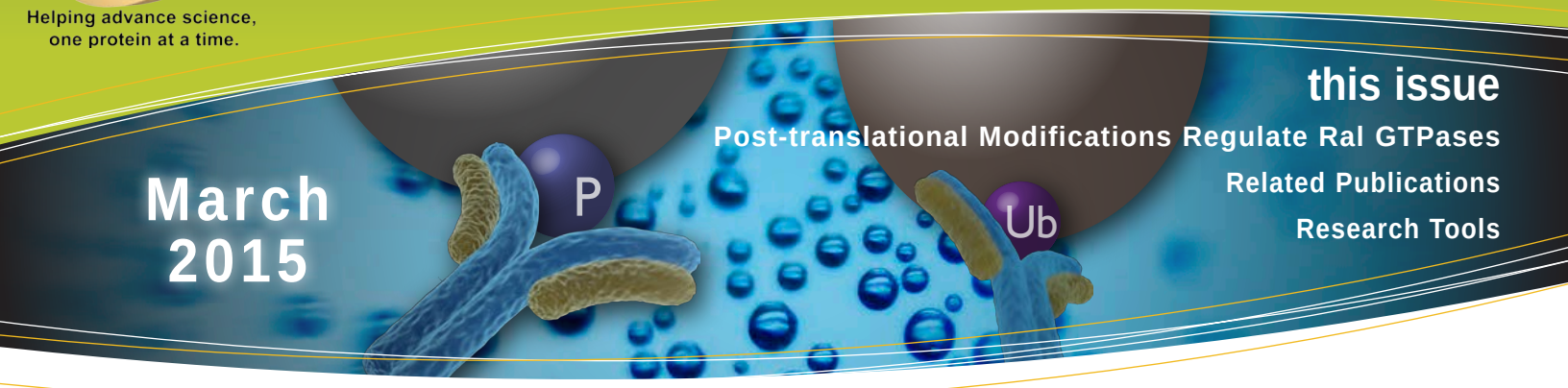

this issue

Sponsorships

Emerging Concepts of the Neuronal Cytoskeleton
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Puerto Varas, Chile

3rd International Meeting of the German Society for Cell Biology on Actin Dynamics
May 2-5, 2015
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Fibronectin, Integrins, & Related Molecules (GRS)
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Pint of Science
May 18-20, 2015
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Cytoskeleton Products

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Post-translational Modifications Regulate Ral GTPases

RalA and RalB GTPases regulate cell motility, morphology, signaling, vesicular trafficking, and endo/exocytosis. The regulation of these functions is critical for the development and spread of cancer[1-4], implicating Ral in oncogenesis and metastasis. Both isoforms are integral for Ras-mediated tumorigenesis, metastasis, and invasion[2-6]. Despite sharing 82% amino acid sequence identity, effectors, and structural/biochemical properties[4], RalA and RalB have their own unique functions in oncogenesis due to distinct subcellular localization and differential effector interactions[2,4,7,8]. Ral localization, binding partners, and function are regulated by post-translational modifications (PTMs)[2,4,9-11]. Ral PTMs are found within the Switch 1 and C-terminal hypervariable regions and include geranylgeranylation, carboxymethylation, palmitoylation, phosphorylation, and ubiquitination[3,4] (Tables 1 and 2).

Table 1. Ral Isoforms, PTMs, and Enzymes

Ral Isoform	PTM	Enzymes	Modified Ral aa	Ref
RalA	Phosphorylation / Dephosphorylation	AKA / PP2A Aβ	Ser194	21
RalA	Phosphorylation / Dephosphorylation	Unknown / PP2A Aβ	Ser183	20
RalB	Phosphorylation / Dephosphorylation	PKC / Unknown	Ser198	24
RalB	Phosphorylation / Dephosphorylation	PKC alpha / Unknown	Ser192	9
RalB	Ubiquitination / Deubiquitination	Unknown / USP33	Lys47	26

aa, amino acid; Ser, Serine; Lys, Lysine

Table 2. Ral Switch 1 and Hypervariable Regions

RalA	EDYEPTKADSY ARKMEDSKEKNGKKKRKSLAKRIRERCCIL
RalB	EDYEPTKADSY TKKMSENKDKNGKKSSKN-KKSFKERCCLL

Bold residues are Switch 1. Others are Hypevariable Region. Red indicates sequence overlap. Phosphorylation sites are green. Ubiquitination site is purple.

Geranylgeranylation

Geranylgeranylation (GG) is necessary for proper membrane localization and functionality of Ral[12,13]. Geranylgeranylated RalA and RalB are both similarly localized to the plasma membrane[13]; however, others have reported differential localization[11]. Both isoforms terminate in a CAAX motif (C=cysteine, A=aliphatic amino acid, X=terminal amino acid), the signal motif for GG[4]. GG is followed by carboxymethylation of the modified now terminal cysteine residue following enzymatic cleavage of the AAX residues[4,14]. A subset of Ral proteins express the alternative CCAX motif, which signals modification by palmitoylation instead of carboxymethylation on the second cysteine[4,15]. Formation of two differentially modified Ral proteins raises therapeutic dilemmas because CAAX processing enzymes are druggable targets[16,17]. Proteins with the CCAX motif are likely immune to such treatments.

Inhibition of GG by either mutation of the cysteine residue or pharmacological inhibition of geranylgeranyltransferase (a.k.a. GGTase I) disrupts Ral association with the membrane and functionality[13]. GGTase I inhibitors target both isoforms; however, differential effects follow the inhibition[13]. GGTase I inhibitors prevent plasma membrane localization of either Ral isoform; instead the Ral GTPases are found throughout the cytoplasm and perinuclear region[13]. Additionally, a GGTase I inhibitor significantly decreases proliferation of various cancer cell lines *in vitro*[18,19], an effect replicated by genetic ablation of RalA[18]. Similar GGTase I inhibitor-mediated anti-tumorigenic effects are observed *in vivo*[19].

Phosphorylation

The Ral isoforms undergo phosphorylation on different serine residues mediated by different kinases *in vitro*. The C-terminal RalA S183[20] and S194[21] residues are phosphorylated, which activates RalA[10,21]. Both are specifically dephosphorylated by PP2A Aβ, leading to decreased RalA activity[20]. Loss of PP2A Aβ expression increases phosphorylation of S194 and S183, increasing RalA activation and inducing RalA-mediated cell transformation[20]. The kinase responsible for phosphorylating S183 is unknown[22], while S194 is phosphorylated by Aurora A kinase (AAK)[21]. Besides increasing activity, AAK-mediated phosphorylation of S194 causes trafficking of active RalA and the effector RalBP1 to internal membranes, followed by increases in RalA/RalBP1-mediated downstream signaling[10]. For example, phosphorylated RalA and RalBP1 re-localize to the mitochondria itself or mitochondrial vesicles, initiating a signaling cascade that results in mitotic mitochondria fission[23]. Upon phosphorylation, RalA preferentially binds RalBP1 rather than exocyst subunits Sec5 or Exo84[10]. In addition, phosphorylation of S194 regulates the *in vivo* and *in vitro* oncogenic functions of RalA[10,21]. RalA is also phosphorylated

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by PKA[22] on yet to be described residues. AKA inhibitors significantly decrease proliferation of cancer cells *in vitro*[18,19], reduce *in vivo* tumorigenesis[19], and prevent RalA activation in cancer cells[19].

RalB is phosphorylated by PKC on S198[24] and PKC alpha on S192[9]. Phosphorylation of S198 stimulates activation of RalB[9], causes relocalization from plasma membrane to the perinuclear region[24], including endocytic vesicles[9], and regulates RalB oncogenic functions such as anchorage-independent growth, cell motility, and actin cytoskeleton dynamics *in vitro*[24]. *In vivo*, RalB S198 phosphorylation is necessary for tumor growth and metastasis[24]. Additionally, S198 phosphorylation alters RalB's effector interactions. Phospho-mimetic RalB is bound to RalBP1 preferentially at the endomembranes while phospho-deficient RalB is bound to Sec5 at the plasma membrane. The phosphorylation state of RalB regulates vesicle trafficking to and fusion with the plasma membrane and the subsequent release of vesicle cargo proteins[9].

Ubiquitination

Ubiquitination identifies proteins destined for proteasome-mediated degradation as well as regulating a protein's activity, localization, or binding partner(s). The Ral isoforms are mainly monoubiquitinated in a nondegradative manner independent of activation state[25]. However, RalA, but not RalB, is modified by nondegradative ubiquitination under anchorage-independent conditions. While both Ral isoforms can be found at the plasma membrane and endomembranes, constitutively ubiquitinated Ral constructs induce robust plasma membrane enrichment of RalA, but not RalB, which is localized as distinct puncta intracellularly. RalA ubiquitination triggers lipid raft enrichment at the plasma membrane. RalA is deubiquitinated in the raft microdomains which is necessary for endocytosis of the rafts[25]. In a cyclical manner, endocytosis of lipid rafts caused an increase in RalA ubiquitination while blockade of endocytosis triggered a decrease. Several lysine residues are viable sites for Ral ubiquitination[25]. Ubiquitination of the Lys47 RalB residue determines effector binding and subsequent functionality. When ubiquitinated, RalB binds to Sec5 rather than Exo84; however, upon deubiquitination, RalB binds to Exo84 preferentially[26]. Binding to the former effector mediates innate immune response signaling while binding to the latter mediates autophagocytosis[26].

Conclusion

Ral GTPases undergo a multitude of PTMs that regulate their activity, subcellular localization, effector binding, and ultimately, function. Given the involvement of Ral proteins in Ras-mediated oncogenesis, there is a clear impetus to develop therapeutics that target Ral activation[27]. Besides directly targeting Ral binding to downstream effectors[27], another therapeutic avenue is modulation of Ral activity by increasing or decreasing certain PTMs. To study this untapped therapeutic potential, Cytoskeleton offers Ral activation assays and monoclonal antibodies that specifically target such PTMs as acetylation, phosphorylation, and ubiquitination.

Select Small G-protein Products

Product	Cat. #	Amount
G-LISA RalA Activation Assay Biochem Kit (colorimetric format)	BK129	96 assays
RalA Activation Assay Biochem Kit (bead pull-down format)	BK040	50 assays

New PTMtrue™ Antibodies

Product	Cat. #	Amount
Anti-Acetyl Lysine Mouse Monoclonal Antibody	AAC01 AAC01-S	2x100µl 1x25µl
Anti-Ubiquitin Mouse Monoclonal Antibody	AUB01 AUB01-S	2x100µl 1x25µl
Anti-Phosphotyrosine Mouse Monoclonal Antibody	APY03 APY03-S	2x100µl 1x25µl

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