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Rac1B, Cancer, and Rac1

The Rac1B GTPase is an alternative splice variant of Rac1, and both GTPases are members of the Rho sub-family of the Ras super-family of GTPases. Insertion of 19 additional amino acids (a.k.a. exon 3b) in Rac1B confers faster GEF-independent GDP/GTP nucleotide exchange due to reduced affinity for GDP and reduced intrinsic GTP hydrolysis compared to Rac1¹⁻³(Fig. 1). Biochemically, Rac1B behaves similarly to a constitutively-active Rac1 GTPase, but through a mechanism distinct from oncogenic Rho sub-family GTPase mutants⁴. Rac1B participates in many phases of oncogenesis, including regulation of cell cycle progression and increased resistance to apoptosis. Additionally, the GTPase has multiple roles in tumor progression, including malignant transformation, epithelial-mesenchymal transition (EMT), metastasis, and invasion³. This newsletter briefly discusses some of the more recent findings regarding the role of Rac1B in tumorigenesis and its relationship with Rac1.

Rac1B is widely regarded as a pro-tumorigenic GTPase as studies find that Rac1B promotes cellular transformation in NIH3T3 mouse fibroblasts and may enhance tumor progression in cancers that over-express Rac1B (e.g., colorectal cancer, human lung adenocarcinomas, thyroid carcinomas)^{3,5}. Rac1B's role in tumorigenesis involves activation of pathways that result in chronic inflammation, transcription of pro-proliferative genes, inhibition of signaling pathways which inhibit growth and stimulate cell cycle arrest, and modulation of signaling pathways that reduce cell adhesion and promote cell migration³(Fig 2). Rac1B is also an important player in matrix metalloproteinase-3 (MMP3)-induced EMT in breast, lung, and pancreas carcinomas^{3,6-8}. Conversely, Rac1B can also

exert anti-tumor effects in certain cancers (e.g., pancreatic ductal adenocarcinomas [PDAC]). In PDAC-derived cells, Rac1B inhibited EMT induced by TGF- β 1, as determined by measuring changes in cell morphology, gene expression of EMT markers, signaling cascades downstream of TGF- β 1 activation, cell migration, and growth inhibition following RNAi-induced exon 3b-targeted knockdown of Rac1B^{3,9,10}. In addition, Rac1B offers potential as a therapeutic target as different GTPase inhibitor compounds are selective for it over Rac1¹¹. In addition, Rac1B may serve as a prognostic marker for some cancers (e.g., breast, colorectal, hepatocellular carcinoma, non-small cell lung cancer, pancreatic cancer, chronic pancreatitis, and thyroid cancer)¹²⁻¹⁴.

Upstream activators, downstream targets, and binding partners

Rac1B is an integral regulatory player in several signaling cascades implicated in cancer.(Fig 2) For example, Rac1B controls the activity of several kinases, including members of the mitogen-activated protein kinases which Rac1B negatively regulates³. Conversely, Rac1B activates NF κ B (nuclear factor kappa-light-chain-enhancer of activated B cells), a family of transcription factors that can enhance or inhibit tumorigenesis¹⁵, and it appears to do so in a manner that avoids activation of the RelB-mediated negative feedback pathway in colorectal cancer cells¹⁶. In thyroid cancer cells,

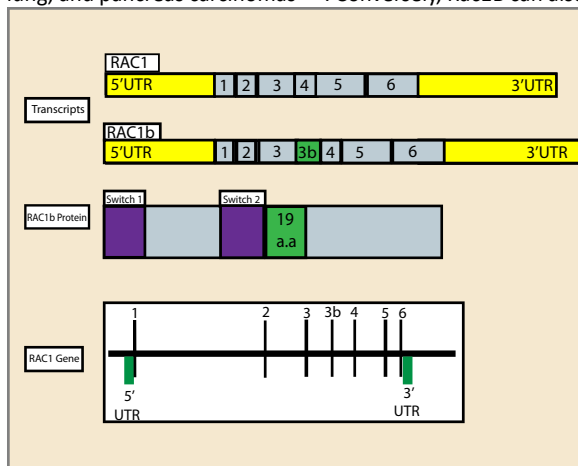


Figure 1: Diagram of RAC1 gene, alternative transcripts and RAC1b protein: 1,2,3,4,5,6 represent exons; a.a:amino acids.

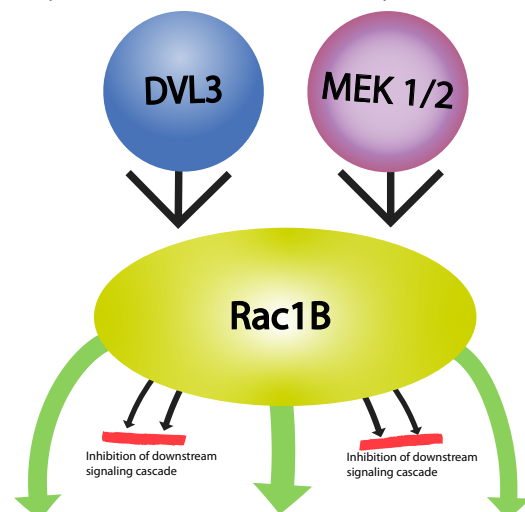


Figure 2: Rac1B-mediated signaling relevant in tumorigenesis. Green arrows oriented outwards from Rac1B depict activation of a downstream signaling cascade, while those oriented towards Rac1B depict either activation (DVL3) or increased protein expression (MEK1/2) of a pathway. Red lines depict inhibition of the downstream signaling cascade.



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SMALL G-PROTEIN PRODUCTS

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Rac1B over-expression stimulates NF κ B-mediated pro-proliferative and anti-apoptotic signaling pathways¹⁷. Additional signaling pathways impacted by Rac1B are Wnt/ β -catenin and TGF- β signaling³. Interestingly, Rac1B does not interact with Rho-GDP dissociation inhibitor (Rho-GDI), while Rac1 does^{5,15}. Rac1B can bind the GTPase binding domain of p21-activated kinase (PAK) in a GTP-dependent fashion, but not full length PAK¹⁵. In comparison with Rac1, Rac1B either does not bind or binds with a reduced affinity to Rho-family effectors such as Rho-GDI, GIT-1, and IQGAP. In contrast, it strongly binds those proteins [e.g., SmgGDS, RACK1, and p120(ctn)] involved in transcriptional regulation, cell-cell adhesion, and motility^{3,18}. The inability of Rac1B to interact with Rho-GDI means that most of the Rac1B is bound to the plasma membrane in the absence of Rho-GDI-mediated sequestration. Thus, although cells express low levels of Rac1B, there is a greater proportion that is available for activation compared to Rac1^{3,5,15}. Furthermore, Rac1B is immune from ubiquitination at the plasma membrane (Rac1B cannot activate Jun-N-terminal kinase which mediates Rac1 ubiquitination)^{3,19}.

Rac1B inhibits Rac1

Rac1B negatively regulates Rac1 activity^{20,21}. In HeLa cells, Rac1B expression prevents PDGF-induced Rac1 activation, reduces the proportion of membrane-bound Rac1, and elevates Rho activity. As might be expected, these changes manifest as altered cell morphology and motility due to the altered actin cytoskeleton dynamics which are regulated by Rho and Rac GTPases and which underlie these basic cellular functions²⁰. Similarly, siRNA-mediated knockdown of Rac1B in a pancreatic carcinoma cell line correlates with an increase in Rac1 protein levels¹⁰. Rac1 and Rac1B differentially modulate TGF- β 1-induced cell migration with the former promoting TGF- β 1-mediated increases in cell migration, while Rac1B inhibits it^{9,21}.

Summary

Despite obvious advances in understanding the unique role of Rac1B in tumorigenesis, much remains undiscovered, and the full potential of Rac1B as a prognostic indicator and therapeutic target very much remains in question. Further differentiation of Rac1B versus Rac1 in cancer-associated signaling cascades is also of paramount importance. Several studies focused on elucidating Rac1B's potential in these areas include development of a biosensor-based system that determines the ratio of Rac1/Rac1B in blood serum in real-time²² and studying the therapeutic feasibility of switching the ratio of Rac1/Rac1B in those cancers delineated by Rac1B over-expression³. To support this research, Cytoskeleton offers a wide range of GTPase reagents, including Rho-family GTPase activation assay kits, antibodies, GEF and GAP activity assay kits, live cell imaging probes for F-actin, and Signal Seeker kits for studying the post-translational modifications of target proteins up- and downstream of Rac1B.

G-LISA Activation Assay Kits

Product	Assays	Cat. #
RhoA G-LISA Activation Assay (Luminescence format)	96	BK121
RhoA G-LISA Activation Assay Kit (Colorimetric format)	96	BK124
Rac1,2,3 G-LISA Activation Assay (Colorimetric format)	96	BK125
Rac1 G-LISA Activation Assay (Luminescence format)	96	BK126
Cdc42 G-LISA Activation Assay (Colorimetric format)	96	BK127
Rac1 G-LISA Activation Assay Kit (Colorimetric Based)	96	BK128
Ras G-LISA Activation Assay Kit (Colorimetric Based)	96	BK131
RhoA / Rac1 / Cdc42 G-LISA Activation Assay Bundle 3 Kits	3 kits 24 per kit	BK135
Total RhoA ELISA	96	BK150

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Acti-Stain Phalloidins

Product	Amount	Cat #
Acti-stain 488 phalloidin	300 Slides	PHDG1-A
Acti-stain 555 phalloidin	300 Slides	PHDH1-A
Acti-stain 670 phalloidin	300 Slides	PHDN1-A
Rhodamine Phalloidin	1 x 500 ul	PHDR1

Rho Family Small G-Protein Tools

Product	Amount	Cat #
Rho Activator I	5 x 10 units 20 x 10 units	CN01-A CN01-B
Rac/Cdc42 Activator II	5 x 10 units 20 x 10 units	CN02-A CN02-B
Rho Activator II	3 x 20 ug 9 x 20 ug	CN03-A CN03-B
Rho/Rac/Cdc42 Activator I	3 x 20 ug 9 x 20 ug	CN04-A CN04-B
Rho Inhibitor I	1 x 20 ug 5 x 20 ug 20 x 20 ug	CT04-A CT04-B CT04-C