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Overexpression of Rac GTPase Activating Protein 1 Contributes to Proliferation of Cancer Cells by Reducing Hippo Signaling to Promote Cytokinesis

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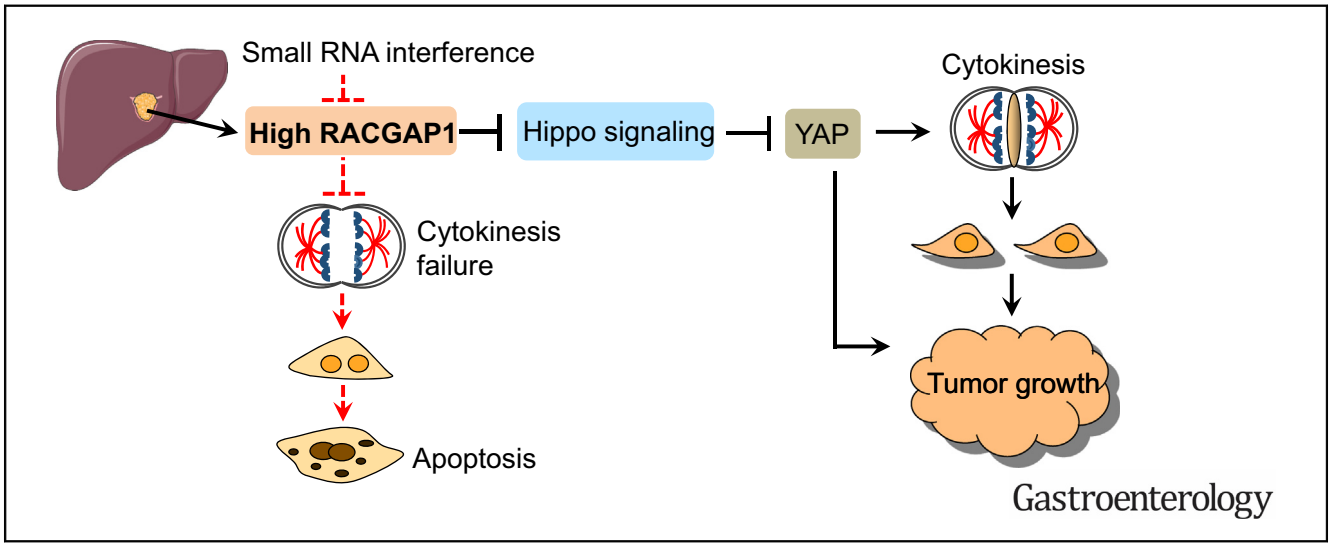

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BACKGROUND & AIMS: Agents designed to block or alter cytokinesis can kill or stop proliferation of cancer cells. We aimed to identify cytokinesis-related proteins that are overexpressed in hepatocellular carcinoma (HCC) cells and might be targeted to slow liver tumor growth. **METHODS:** Using the Oncomine database, we compared the gene expression patterns in 16 cancer microarray datasets and assessed gene enrichment sets using gene ontology. We performed immunohistochemical analysis of an HCC tissue microarray and identified changes in protein levels that are associated with patient survival times. Candidate genes were overexpressed or knocked down with small hairpin RNAs in SMMC7721, MHCC97H, or HCCLM3 cell lines; we analyzed their proliferation, viability, and clone-formation ability and their growth as subcutaneous or orthotopic xenograft tumors in mice. We performed microarray analyses to identify alterations in signaling pathways and immunoblot and immunofluorescence assays to detect and localize proteins in tissues. Yeast 2-hybrid screens and mass spectrometry combined with co-immunoprecipitation experiments were used to identify binding proteins. Protein interactions were validated with co-immunoprecipitation and proximity ligation assays. Chromatin immunoprecipitation, promoter luciferase activity, and quantitative real-time polymerase chain reaction analyses were used to identify factors that regulate transcription of specific genes. **RESULTS:** The genes that were most frequently overexpressed in different types of cancer cells were involved in cell division processes. We identified 3 cytokinesis-regulatory proteins among the 10 genes most frequently overexpressed by all cancer cell types. Rac GTPase activating protein 1 (RACGAP1) was the cytokinesis-regulatory protein that was most highly overexpressed in multiple cancers. Increased expression of RACGAP1 in tumor tissues was associated with shorter survival times of patients with cancer. Knockdown of RACGAP1 in HCC cells induced cytokinesis failure and cell apoptosis. In microarray

analyses, we found knockdown of RACGAP1 in SMMC7721 cells to reduce expression of genes regulated by yes-associated protein (YAP) and WW domain containing transcription regulator 1 (WWTR1 or TAZ). RACGAP1 reduced activation of the Hippo pathway in HCC cells by increasing activity of RhoA and polymerization of filamentous actin. Knockdown of YAP reduced phosphorylation of RACGAP1 and redistribution at the anaphase central spindle. We found transcription of the translocated promoter region, nuclear basket protein (TPR) to be regulated by YAP and coordinately expressed with RACGAP1 to promote proliferation of HCC cells. TPR redistributed upon nuclear envelope breakdown and formed complexes with RACGAP1 during mitosis. Knockdown of TPR in HCC cells reduced phosphorylation of RACGAP1 by aurora kinase B and impaired their redistribution at the central spindle during cytokinesis. STAT3 activated transcription of RACGAP in HCC cells. **CONCLUSIONS:** In an analysis of gene expression patterns of multiple tumor types, we found RACGAP1 to be frequently overexpressed, which is associated with shorter survival times of patients. RACGAP1 promotes proliferation of HCC cells by reducing activation of the Hippo and YAP pathways and promoting cytokinesis in coordination with TPR.

Keywords: Binucleated Cells; Large Tumor Suppressor Kinase; Pan-overexpressed; TEA Domain Transcription Factor.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Inhibiting cytokinesis is emerging as a tolerable and effective strategy for HCC treatment in preclinical studies. However, crucial regulators and underlying mechanisms of cytokinesis in carcinogenesis are not well defined.

NEW FINDINGS

RACGAP1 is a key pro-proliferation cytokinesis regulator that reduces Hippo signaling and activates YAP to promote cytokinesis of cancer cells along with its target gene nucleoporin TPR.

LIMITATIONS

This study did not use preclinical models such as the patient derived xenograft model to further confirm the effects of blocking RACGAP1 on HCC cancer development.

IMPACT

This study demonstrates a novel interplay between RACGAP1 and the Hippo pathway in promoting cytokinesis of HCC cells and suggests RACGAP1 as a potential tolerable and effective target for HCC therapy.

Cell cycle aberrations, a hallmark of cancer, have been the target of anticancer therapeutics for decades. Classical microtubule-targeting chemotherapy drugs are effective but lack cancer cell specificity and have severe adverse effects. Numerous inhibitors targeting specific mitotic regulators, such as Aurora kinases, Mps1, and Cenp-E, have been explored and tested in clinical trials.[1,2] However, few were clinically effective in solid tumors.

Recently, targeting post-metaphase events was found to be a promising strategy for cancer treatment in preclinical experiments.[3–6] Cytokinesis failure can increase chromosomal instability (CIN) above a threshold that affects cell viability and has been suggested as a promising anticancer therapeutic approach for human cancers.[3,4] In the liver, binucleation and polyploidy are differentiation markers and features of the aging process that can be a protective response to cellular loss or DNA damage.[7] Liver cancers, such as hepatoblastoma and hepatocellular carcinoma (HCC), are highly dependent on cytokinesis.[8,9] Inhibiting cytokinesis by blocking anillin actin binding protein (ANLN) can inhibit liver tumor growth in multiple cancer models without affecting normal liver function or regeneration.[6] Thus, inhibiting cytokinesis might be a tolerable and effective strategy for HCC treatment. Nevertheless, the significance and regulatory mechanisms of cytokinesis, the final step of mitosis, in carcinogenesis are poorly understood.

In this study, using the Oncomine database, we compared the global gene expression in various cancer microarray datasets and found that the top-ranked universally overexpressed genes were enriched in cell division processes. Notably, 3 cytokinesis regulatory proteins were among the top 10, and Rac guanine triphosphatase-activating protein 1 (RACGAP1) was ranked the most universally overexpressed gene.

RACGAP1 is a member of the guanine triphosphatase (GTPase) activation protein family that regulates the RhoGTPase transformation from guanine triphosphate–bound to guanine diphosphate–bound form.[10] RACGAP1 has been implicated in various cellular processes, including cytokinesis, differentiation, and migration,[11–15] and controls cytokinesis by forming the centralspindlin complex and mediating the Rho-dependent signaling required for actomyosin contractile ring assembly.[16,17] RACGAP1 phosphorylation events are critical in regulating its functions throughout mitosis.[18] The mitotic kinase Aurora B (AURKB) phosphorylates RACGAP1 at Ser387 in the GAP domain, which has been proposed to convert RACGAP1 to an active GAP toward RhoA and is critical in mediating cytokinesis.[19] Recently, an association between RACGAP1 expression and tumor malignancy was reported in some cancers, including HCC.[20–24] However, the molecular role and regulatory

***Authors share co-first authorship.**

Abbreviations used in this paper: **AURKB, aurora B kinase; ChIP, chromatin immunoprecipitation; CIN, chromosomal instability; CNL, noncancerous tissue; ECT2, epithelial cell transforming 2; GTPase, guanine triphosphatase; HCC, hepatocellular carcinoma; LATS, large tumor suppressor; mRNA, messenger RNA; PCR, polymerase chain reaction; PLA, proximity ligation assay; RACGAP1, Rac guanine triphosphatase-activating protein 1; sh, short hairpin; STAT3, signal transducer and activator of transcription 3; TAZ, WW domain containing transcription regulator 1; TCGA, The Cancer Genome Atlas; TMA, tissue microarray; TPR, translocated promoter region; YAP, Yes-associated protein.**

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