EDITORIAL

RalGAPα2 and NLRP3 Orchestrate Tumor Invasion in Colitis-Associated Cancers

Chronic inflammation facilitates cancer development, as illustrated by the increased incidence of colitis-associated cancers (CAC) in patients with inflammatory bowel diseases. CAC have a greater malignant potential than sporadic colorectal cancer, because they are frequently diagnosed at an advanced stage with locally advanced or metastatic disease. Molecular mechanisms involved in CAC development also differ from those in sporadic colorectal cancer, reflecting the prominent role of inflammation-induced carcinogenesis in the development of CAC.[1] How inflammation favors and what are the signaling nodes linking inflammatory and oncogenic pathways are still being uncovered, with the important clinical endpoint to identify therapeutic targets. However, the molecular mechanisms responsible for the acquisition of metastatic ability have not been fully identified.

This issue of *Cellular and Molecular Gastroenterology and Hepatology* features an original article by Iida et al[2] that sheds light on the molecular events underlying CAC development. They show that down-regulation of the RalGAPa2 subunit, the major inhibitory regulator of the small guanosine triphosphatase Ral in the colon, is a key factor driving the invasive tumorigenesis of CAC with up-regulation of matrix metalloproteinase (MMP)-9 and MMP-13 and reveal that it occurs via induction of the NLRP3-IL1β pathway.

The oncogenic effects of Ral, a member of the Ras subfamily, have been known for 2 decades, expanding investigations into the role of Ral in controlling multiple cellular functions.[3] Ral regulates tumorigenesis and cancer progression in different ways: through activation of Ral effector proteins, via activation of several signaling pathways, and by phosphorylation of Ral proteins. Ral activation was reported in several human cancers, including bladder, colon, and pancreas cancers, and involved in cell proliferation, migration, and metastasis. This protein is activated by Ral guanine nucleotide exchange factors and inactivated by Ral–guanosine triphosphatase activating proteins (RalGAPs), the latter of which consist of heterodimers containing a catalytic α1 or α2 subunit and a common β subunit. Tumors harboring the down-regulated α2 subunit of the RalGAPs appeared to be more prone to invasion or metastasis.

Iida et al reveal that invasive human CAC tumors at more advanced stage display decreased RalGAPα2 expression compared with matching normal tissue and that this might be the cause of aberrant Ral activation. Of note, the expression of RalGAPα2 in CAC was significantly lower than that in sporadic colorectal cancer, suggesting that the mechanisms of Ral activation might be different in the 2 clinical settings. What the host and environmental cues are that determine "low" RalGAPα2 expression profile in CAC remain to be investigated. Consistent with their findings in humans, Iida et al demonstrate that in the AOM/DSS model, RalGAP2 KO mice exhibited significantly larger CAC sizes

and more invasive phenotypes compared with their sibling wild-type mice, leading to the important perception that RalGAP2 expression level may be a useful predictive biomarker in the treatment of patients with CAC.

Next the authors sought to dissect the mechanism underlying the acquisition of the invasive phenotype in Ral-mediated CAC. By microarray analysis of colon epithelial cells isolated from both wild-type and RalGAPα2 KO mice, Iida et al point to the contribution of MMP-9 and MMP13, previously implicated in metastatic processes in many cancers including colon cancer. What is promoting the overexpression of MMPs and tissue remodeling? Normal inflammatory response typically aids our fight with infection and involves also activation of pathways responsible for tissue remodeling. Inflammation can therefore regulate proteases, whose activity is implicated in metastatic process. Various inflammatory cytokines that are involved in the pathophysiology of inflammatory bowel diseases and in the tumor initiation and promotion have been also implicated in the enhancement of metastatic behavior in colon cancers.

Here the authors unexpectedly find extremely high interleukin (IL) 1β expression in the colon tumors of RalGAPα2 KO mice in comparison with other cytokines such as Th1, Th2, and Th17 cytokines. IL1β plays a crucial role in carcinogenesis and invasiveness of the tumor by up-regulating also MMP gene expression. In agreement, increased levels of IL1β in cancer patients are correlated with bad prognosis.[4] Notably, the work of Iida et al shows that IL1β expression in cancer cells with down-regulated RalGAPα2 is sustained by the AP1-NLRP3 inflammasome axis activation, and treatment of tumor cells or RalGAPα2 KO mice with NLRP3 inhibitor significantly reduces tumor invasion, decreasing the expression of MMPs. To date, the role of the inflammasome in CAC progression remains controversial.[5] It might be argued that inflammasome activation in tumors depends on tissue context to whether inhibition or activation of tumorigenesis results. However, association among Ral, Ap1, and NLRP3 in CAC has not been reported previously. Whether AP1-NLRP3-MMPs axis plays a role in the gut under homeostatic or non-tumorous inflamed conditions remains to be determined.

Overall, Iida et al provide an original mechanism of CAC tumor progression, whereby Ral drives the concomitant expression of the component of the inflammatory machinery and activation of genes that are pivotal in tumor invasion and metastasis. The RalGAPs have been discovered only recently, and much more remains to be learned regarding their roles in regulation of Ral activity and signaling. With increasing evidence for key roles of Ral guanosine triphosphatases as drivers in cancer growth, it will be crucial to identify pharmacologic approaches for targeting aberrant

Ral function for cancer treatment.[6] From a clinical prospective, it is most important to learn how to target tumor progression and metastasis, because more than 90% of cancer-related deaths are because of metastasis and not because of the primary tumor growth. In this regard, identification of downstream effectors or key regulators of Ral guanosine triphosphatases can be exploited for anti-Ral drug development in cancer therapy.

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References

1. Terzic J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology 2010; 138:2101–2114 e5.
2. Iida T, Hirayama D, Minami N, Matsuura M, Wagatsuma K, Kawakami K, Nagaishi K, Nojima M, Ikeuchi H, Hirota S, Shirakawa R, Horiuchi H, Nakase H. Down-regulation of RalGTPase-activating protein promotes colitis-associated cancer via NLRP3 inflammasome activation. Cell Mol Gastroenterol Hepatol 2020;9:277–293.
3. Bodemann BO, White MA. Ral GTPases and cancer: linchpin support of the tumorigenic platform. Nat Rev Cancer 2008;8:133–140.
4. Bent R, Moll L, Grabbe S, Bros M. Interleukin-1 beta: a friend or foe in malignancies? International Journal of Molecular Sciences 2018;19.
5. Moossavi M, Parsamanesh N, Bahrami A, Atkin SL, Sahebkar A. Role of the NLRP3 inflammasome in cancer. Molecular Cancer 2018;17:158.
6. Yan C, Theodorescu D. RAL GTPases: biology and potential as therapeutic targets in cancer. Pharmacol Rev 2018;70:1–11.

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