

Suppression of Antimicrobial Defense and Stabilization of STAT3 by IRAK-M Expression in Tumor Cells Promotes Colorectal Carcinogenesis

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Different environmental and genetic factors have been attributed to the etiology of colorectal cancer. Dysbiotic gut microbiota is associated with initiation and progression of colon carcinogenesis. Hyperactivation of STAT3 promotes carcinogenesis by upregulating cell proliferation, survival, tumor-induced immunosuppression and angiogenesis. IRAK-M is a negative regulator of toll-like receptor signaling and inhibits innate immune response. The cancer cell may exploit this property of IRAK-M and evade host immune surveillance. Recently, it has been found that IRAK-M provide controlled feed back to bacteria involved in colorectal cancer by reducing antibacterial response in mice. Furthermore, IRAK-M increased the stability of STAT3 in tumor cells that support tumor promotion by upregulating cell proliferation and survival. Thus, it is suggested that IRAK-M promotes colitis associated colon cancer by enhancing bacterial colonization and stabilization of STAT3.

Key Words: IRAK-M, Microbiota, STAT3, Colon cancer

In Cancer Cell on 9th May 2016, Kesselring *et al.* reported that IRAK-M expression in tumor cells supports colorectal cancer progression through reduction of antimicrobial defense and stabilization of STAT3 (1). Colorectal cancer (CRC) represents one of the most prevalent fatal malignancies worldwide (2). Mammalian gut is colonized by trillions of commensal bacteria termed as indigenous microflora (3). Abnormal response of the host immune system to the commensal microbiota leads to the initiation of chronic inflammatory disorder of the gut (4). Although gut microbiota have a critical role in human health by maintaining gut homeostasis, a dysregulated microbiota is involve in the

pathogenesis of several diseases including colon cancer (5). *Bacteroides vulgatus* and *Bacteroides fragilis* toxin (BFT) exacerbate inflammation and colon cancer in IL-10^{-/-} and APC^{Min/+} mice respectively (6). Previous studies reported relative abundance of *Bacteroidaceae*, *Fusobacteriaceae*, *Streptococcaceae*, *Veillonellaceae*, *Pasteurellaceae* and *Peptostreptococcaceae* in cancerous tissues as compared to normal intestinal tissues (7).

Signal transducer and activator of transcription (STAT) 3 is a pleiotropic transcription factor that play a critical role in regulation of transcriptional programs that are prerequisites for carcinogenesis, including cell proliferation, survival,

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tumor-induced immunosuppression and angiogenesis (8). IRAK-M is the member of interleukin-1 receptor-associated kinases (IRAKs) family. Unlike other member of the family IRAK-M lacks kinase activity and is the negative regulator of TLR signaling. IRAK-M suppresses excessive inflammatory responses by deactivating immune signaling and thus helps the cancer cell in evading the host immune surveillance. Previously, it has been reported that IRAK-M level increased in tumor tissue and facilitate cancer progression by down-regulating host immune defense (9). Here, we summarize the results of Kesselring *et al.* which demonstrate that IRAK-M expression in tumor cells supports colorectal cancer progression through reduction of antimicrobial defense and stabilization of STAT3 (1).

Previously it was shown that IRAK-M protects against acute colitis induced by dextran sulphate sodium (DSS) (9). Thus, Kesselring *et al.* investigated the impact of IRAK-M on development of chronic colitis and colorectal cancer. They found that IRAK-M^{-/-} mice showed severe symptoms of intestinal inflammation and more weight lost as compared to wild type (WT) mice in azoxymethane (AOM)/DSS model of colitis associated colon cancer. As compared to WT mice, IRAK-M^{-/-} mice showed more inflammation throughout the mucosa, loss of crypts, alterations of epithelial structure, and increased infiltration of lymphocytes. In spite of high level of inflammation, IRAK-M^{-/-} mice showed low tumor load as compared to WT mice. Although, activation of Wnt pathway was not different as evident by nuclear β -catenin staining and level of active β -catenin. Only 57% of IRAK-M^{-/-} mice developed macroscopic tumors, whereas 92% of WT mice developed tumors in their distal colon. Similarly average tumors number per mice were about 3 times less in IRAK-M^{-/-} mice as compared to WT mice. Regarding tumor size, WT mice developed 61% of large tumors (> 2 mm in diameter), in case of IRAK-M^{-/-} mice, only 5% of tumors were large size. These results demonstrate that in spite of increased colitis in IRAK-M^{-/-} mice, some protective mechanism exist that protects against inflammation associated colon carcinogenesis that is normally found in inflamed microenvironment of the tumor (1).

Antibiotic treatment causes reduction of tumor numbers

in APC^{Min/+} mice, showing that gut microbiota has cancer promoting role (6). In this study they analyzed the composition of intestinal microbiota of IRAK-M^{-/-} mice and WT mice. The total bacterial contents in the feces of WT type mice were approximately 8 times higher than IRAK-M^{-/-} as evident the number of 16S-rDNA gene copies. Furthermore, they estimated species richness and found that IRAK-M^{-/-} mice have less diverse composition of bacteria as compared to WT mice. The data obtained from deep 16S-rRNA sequencing was analyzed on taxonomical level and some remarkable differences were detected. Hence, some bacterial taxa including *Parasutterella*, *Roseburia* and *Blautia* were found to be significantly less abundant or almost absent in the feces of IRAK-M^{-/-} mice, while some others were more abundant like *Turicibacter* and some undefined members of *Erysipelotrichaceae* family. Similar differences in bacterial taxa and bacterial species richness were observed in tumor and colonic tissues of respective mice. By estimating the diversity of fecal microflora between WT and IRAK-M^{-/-} mice through principle coordinates analysis, they found that all samples obtained from IRAK-M^{-/-} mice clustered apart from those derived from WT control (1).

To investigate whether the enhanced antibacterial response or more intact epithelial barrier are responsible for reduced bacterial contents in IRAK-M^{-/-} mice, they examined intestinal permeability and antibacterial response separately from tumors site and non-tumorous epithelium of IRAK-M^{-/-} mice compared with WT mice. In case of non-tumorous epithelium, IRAK-M^{-/-} mice have more permeability as detected by fluorescein isothiocyanate dextran level measurements. Furthermore, they found decreased thickness of mucus layer in IRAK-M^{-/-} mice. At the tumor site they observed a remarkably increased deposition of Mucin2 and elevated expression of antimicrobial peptides including Cathelicidin and Galectin 8 in tumors derived from IRAK-M^{-/-} mice. These results demonstrate that luminal bacteria cannot translocate into tumors in IRAK-M^{-/-} mice due to highly increased antibacterial response (1).

Various studies have found that STAT3 gets activated in different adenomas and carcinomas. Activation of STAT3 leads to the elevated expression of different genes which

have critical role in cell proliferation (such as PCNA and cyclin D) and inhibition of apoptosis (Bcl-2, Bcl-Xl and Mcl-1) (2). To check whether increased antimicrobial defense is the only mechanism that explained reduced tumor growth in IRAK-M^{-/-} mice or some other pathways are involved. Therefore they check for activation of STAT3. They found that both total and activated STAT3 protein were decreased or absent in tumors from IRAK-M^{-/-} mice as compared to WT mice. Downstream target of STAT3 including regulators of cell proliferation and apoptosis (Cyclin D1, c-myc, Hsp70-Survivin, Bax2, Bcl-2, Bcl-x) were decreased in IRAK-M^{-/-} tumors. Next they evaluated for the level of PIAS3 (protein inhibitor of activated Stat3), which is a known inhibitor of STAT3 and found that the level of PIAS3 was strongly upregulated in IRAK-M^{-/-} tumors as compared to WT mice. They confirmed the interaction of IRAK-M with STAT3 in human CRC tissues through in situ proximity ligation assays. They analyzed the activation of caspase-3 in IRAK-M^{-/-} tumors and found increased level of cleaved caspase3 showing increased apoptosis in IRAK-M^{-/-} tumors. Further, they checked for correlation of IRAK-M and STAT3 expression in human CRC sample and found that STAT3 expression was positively correlated with IRAK-M. When they checked for correlation of IRAK-M with survival rate of CRC patients, they found that patient positive for IRAK-M had worse survival (1).

Kesselring *et al.* showed that IRAK-M promotes CRC by regulating microbial colonization and stabilization of STAT3 protein in tumor cell. So, there are two different functional role of IRAK-M expressed in tumor cells. It reduce antimicrobial defense against dysbiotic microbiota and secondly it increase the stability of STAT3 protein. Both of these mechanisms collectively support the progression of colorectal cancer. Furthermore, IRAK-M expression was found to be linked with poor prognosis. Thus, IRAK-M represents an important target for the development of new therapeutic approaches for treatment and prevention of colorectal cancer.

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