EDITORIAL

JAM-ming miR-21

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In this issue of *Cell Death & Differentiation*, Lampis et al. report a novel molecular pathway involving miR-21 upregulation causing JAM-A downregulation that activates multiple oncogenic processes leading to increased metastasis and invasiveness in colorectal cancer (CRC), the third deadliest cancer in the world. This is new and interesting, as no data was available on JAM-A expression in early and metastatic CRC. The authors tested the prognostic and functional effects of JAM-A dysregulation and elucidated the molecular underpinnings of JAM-A loss in CRC.

JAM-A is a protein from the family of junctional adhesion molecules that is found to be deregulated in many cancers, more often associated with tissue and organ specificity. Loss of JAM-A is known to be a negative prognostic factor associated with poor survival in pancreatic [1] and gastric cancer [2] patients. JAM-A downregulation is known to be induced by loss of *Apc* (APC regulator of WNT signaling pathway) in the intestine of genetically engineered mouse models, thereby leading to loss of polarity and barrier function and increased inflammation. However, not much was known about JAM-A loss in CRC. In this interesting paper, Lampis et al. have performed a well-designed, systematic study by first identifying the deregulated JAM-A expression and localization in three different cohorts of CRC patients. Secondly, JAM-A expression was found to be reduced and scattered across the cytoplasm or completely lost in more than 50% of cases, both in primary and metastatic conditions of CRC that was validated by immune-histochemistry staining and histo-scores. Lastly, they also confirmed these findings in three independent cohorts of stage-II colon cancer by utilizing the public data repositories such as gene expression omnibus and The Cancer Genome Atlas that suggested that downregulation or loss of JAM-A imposes a negative prognostic in early CRC.

Lampis et al. also showed that downregulation of JAM-A promotes migration, proliferation, and metastatic ability in CRC cells such as CACO-2 and DLD-1. To confirm the *in vitro* findings, they recapitulated the events in 3D spheroids and *in vivo*. Knockdown of JAM-A significantly increased the tumors *in vivo* that was achieved by implanting luciferase positive CACO-2 cells expressing JAM-A or control shRNA constructs. The next question would be whether aberrations in JAM-A expression impacted the metastatic potential of CRC cells—to address this the authors performed tail vein injections of DLD-1 cells expressing the JAM-A versus control shRNA constructs and checked their potential of colonization to lungs. Formation of metastatic loci was observed that confirmed the association of JAM-A knockdown with increase in absolute number of lung metastatic foci, total volume of lung metastatic burden, and proliferation in the metastatic DLD-1 deposits. Further, lack of JAM-A in CRC preclinical models was found to activate the pro-survival and pro-migratory pathways by increasing AKT, ERK, and ROCK1 expression respectively

and downregulate the TGFβ1 antagonist Bone Morphogenetic Protein 7.

Coming to the most interesting finding in the paper, the authors show elevated levels of miR-21 as the root cause of JAM-A downregulation in CRC. Isn't it surprising to see that a microRNA (miRNA) of 22 nucleotides can have such a powerful effect on one of the key proteins? miRNAs are important as they play many key regulatory roles in diseases such as cancer. The first ever report on involvement of miRNAs in cancer opened the huge arena of opportunities to the research community and currently there are around 60,000 articles reporting the key roles of different miRNAs in cancer [3]. MiR-21 is known to be significantly upregulated in CRC [4]. A putative binding site for miR-21 was identified by the authors in the 3'UTR of JAM-A. The hypothesis was miR-21 is a negative regulator of JAM-A and the results supported the hypothesis as they found endogenous JAM-A protein levels to be higher in DLD-1 miR-21^{KO} compared to the DLD-1 miR-21^{WT} suggesting translational inhibition imposed by miR-21. Further carefully planned confirmatory experiments showed increase in JAM-A protein levels upon silencing of miR-21 in DLD-1 miR-21^{WT} cells while re-expression of miR-21 in DLD-1 miR-21^{KO} decreased JAM-A protein levels. This validated the previous findings of miR-21 regulating the translational process of JAM-A mRNA transcripts. Absence of miR-21 binding site on JAM-A 3'UTR rendered higher JAM-A protein expression and luciferase reporter assays confirmed the direct regulation of JAM-A mRNA translation by miR-21 through JAM-A-3'UTR binding. They also studied the impact of miR-21 overexpression in migration ability of DLD-1 cell line by performing 3D migration assays that showed overexpression of miR-21 induced increased migration in 3D spheroid conditions.

This remarkable paper shows for the first time evidence of a functional link between overexpression of oncogenic miR-21 and lower levels of JAM-A. Adding to the feather on the cap, they also demonstrated how loss of JAM-A is associated with activation of several oncogenic pathways in CRC by increased proliferative and migratory ability and thus links to poor survival outcome and disease progression. It is exciting to see how the clinical observations and preclinical data go hand in hand depicting a clear pro-oncogenic effect of JAM-A downregulation in CRC progression and tumorigenesis (Fig. 1). This finding has a potential clinical relevance unlike JAM-A as a target in breast cancer [5] and multiple myeloma [6], where JAM-A inhibitors may represent potential therapeutic implications while suppression of JAM-A expression in CRC imposes poor survival rate in CRC patients. Loss of JAM-A aggravates CRC tumorigenesis and maintains huge potential in promoting CRC phenotype in the absence of inflammatory or micro-environment related stimuli and responses. Previous reports [7] depicting JAM-A downregulation in normal fibroblasts accelerating cell proliferation and migration through activation of MAPK pathway and the current findings are aligned. MiR-21 is known to be a powerful regulator of inflammatory cytokines such as IL-23 and IL-17A [8, 9] in the colon mucosa that indicates a potential positive feedback loop involving miR-21 overexpression leading to

Fig. 1 Schematic illustration of miR-21 in regulation of JAM-A. (Adapted from Lampis et al.). Downstream molecular events upon deregulated JAM-A expression in CRC.

synthesis of pro-inflammatory factors thereby activating oncogenic pathways and further contributing to JAM-A inactivation and downstream effects.

The authors have also laid a proposal as the future direction of the findings. Even though they did not explore the causal relationship between β -catenin activation, miR-21 overexpression and JAM-A downregulation, previous reports [10, 11] have shown that analogous to effects of lack of JAM-A, miR-21 could promote pSer552 phosphorylation of β -catenin and its translocation to nucleus in the mutated APC CRC cells. Further, β -catenin/TCF activated pathway enhances STAT3 expression [12] whose binding site is present upstream of pri-miR-21 transcription start site. Considering all the important findings reported in this paper and previous reports, there could be a potential feedforward loop where loss of APC and activation of β -catenin triggering STAT3-mediated overexpression of miR-21 and successive miR-21 dependent downregulation of JAM-A. This concept is supported by activating mutations present in β -catenin in both the high miR-21 expressing CRC cell lines (HCT116 and CACO-2). Consequently, loss of JAM-A would further enhance pSer552 phosphorylation and localization of β -catenin to nucleus by enduring miR-21-mediated downregulation of JAM-A.

It is inevitable to expect more exciting studies elucidating the multi-faceted role of miR-21 and JAM-A that will help in decrypting hidden molecular microRNAs count, and we can't lose them!

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AUTHOR CONTRIBUTIONS

SM wrote the paper. GAC supervised and recommended the final editings.

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CONFLICT OF INTEREST

GAC is one of the scientific founders of Ithax Pharmaceuticals. The other author does not declare any competing interests.

ADDITIONAL INFORMATION

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