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Unshielding Exosomal RNA Unleashes Tumor Growth And Metastasis

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Reciprocal interactions between tumor cells and their microenvironment drive cancer progression and therapy resistance. In this issue, Nabet et al. demonstrate that dynamic feedback between tumor and stroma subverts normal inflammatory responses by triggering the release of exosomes containing unshielded RNAs that activate pattern recognition receptors, thereby promoting tumor growth and metastasis.

During cancer progression, the tumor and its microenvironment co-evolve, due to constant communication. As the tumor reshapes its local microenvironment, coaxing it to support cancer growth, it exerts systemic effects, conquering the immune system and distant organs, leading to metastasis. The local and far-reaching effects of tumors are mediated by tumor-secreted factors, such as soluble factors (cytokines and chemokines) and exosomes—nanovesicles that carry complex cargo, including proteins, DNA, and coding as well as non-coding RNAs (Becker et al., 2016).

A hallmark of cancer progression is the generation of local and systemic inflammation, driven by pro-inflammatory cytokines and a shift in the balance of both adaptive and innate immune subsets toward tumor-promoting phenotypes. Interestingly, in the absence of cancer, systemic inflammation is crucial for eliminating pathogens or noxious stimuli and repairing tissue damage (Huang et al., 2015). While an interplay between pathogen- and danger-associated molecular pattern (DAMP) recognition and cancer-induced inflammation has been previously suggested (Escamilla-Tilch et al., 2013), the specific mechanisms through which tumors hijack pro-inflammatory pathways to promote tumor growth remain unclear.

In an elegant study in this issue of *Cell*, Nabet et al. (2017) address this conundrum by building upon their previous work, which demonstrated that stromal cells induce interferon-stimulated genes (ISGs) in a subset of aggressive, triple-negative breast cancer (TNBC) cells via exosome secretion, rendering these breast cancer cells therapy resistant (Boelens et al., 2014). The authors show that inflammatory pathways normally only activated in response to exogenous, viral RNAs are instead ectopically induced in cancer by deregulated release of exposed endogenous 5′ triphosphorylated double-stranded RNA in tumor stroma exosomes (Nabet et al., 2017). The unshielded non-coding RNAs enriched in exosomes from tumor-activated stromal cells then act as DAMP signals, triggering a feedback loop that activates the pattern recognition receptor (PRR) retinoic acid-inducible gene I (RIG-I) and ISGs in breast cancer and innate immune cells.

Since endogenous RNAs can act as DAMPs in response to injury induced by radiation, chemotherapy, or during autoimmune diseases, Nabet et al. first characterized the RNAs packaged in exosomes released by fibroblasts co-cultured with ISG-responsive breast cancer cells. Metabolic labeling and single nucleotide polymorphism (SNP) analysis confirmed that stromal RNA was transferred to breast cancer cells. Interestingly, stromal exosomal RNA (exoRNA) was enriched in endogenous non-coding RNAs, including repeat and transposable elements (ALU and SINE) and Y-RNA and snRNA, as well as srpRNA. Importantly, the authors demonstrated that an exposed 5′ triphosphate (5′ ppp) was responsible for stromal exoRNA-mediated activation of the RIG-I receptor, and thus ISG induction, in breast cancer cells.

Next, using both chemical and genetic ablation of RNA polymerase III (POL3) function, Nabet et al. revealed that POL3 is responsible for 5′ ppp production in stromal cells co-cultured with ISG-responsive breast cancer. The authors then devised a novel 5′ ppp sequencing method to identify the specific POL3-driven exoRNA responsible for RIG-I activation, utilizing enzymatic modification of the 5′ RNA end before constructing their sequencing library. This ingenious approach allowed Nabet et al. to deplete RNAs lacking 5′ppp modifications and thus enrich for RNAs of interest, specifically RN7SL1, a 5′ ppp srpRNA with an extensive secondary structure, normally highly shielded in cells. Importantly, in contrast to normal cytoplasmic RN7SL1, the 5′ ends of RN7SL1 packaged in stromal exosomes derived from the breast cancer cell co-culture were not protected by RNA binding proteins (RBPs), such as SRP9 and SRP14, accounting for its unique capacity to stimulate RIG-I.

Thus, what is the mechanism responsible for the unshielding of stromal exosome RN7SL1? The authors reasoned

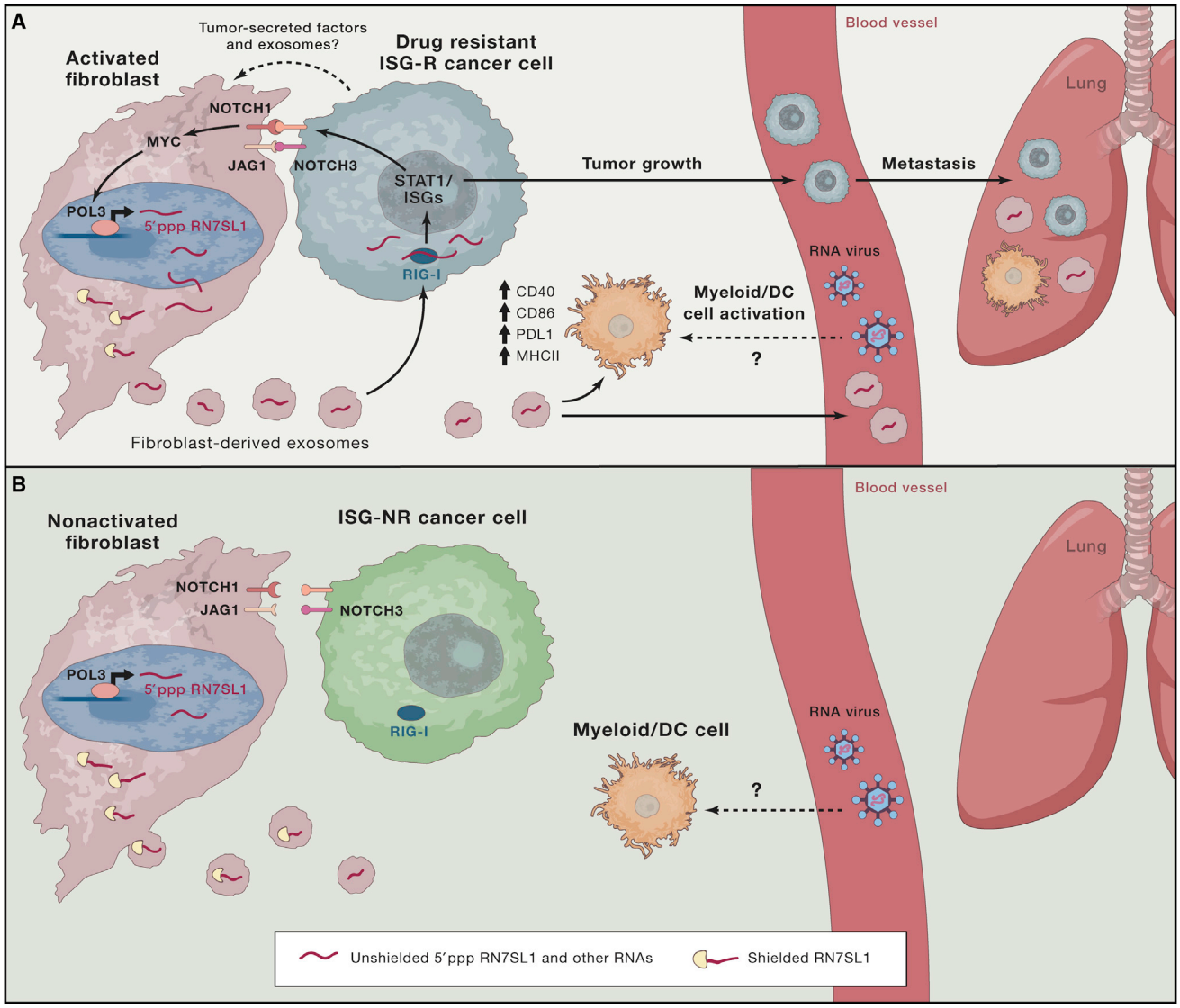

Figure 1. Reciprocal Tumor-Stromal Activation Increases Unshielded RN7SL1 in Stromal Exosomes to Enhance Inflammation, Tumor Growth, and Metastasis

(A) Heterotypic tumor-stroma interactions induce interferon-stimulated gene (ISGs) in a subset of breast cancer cells, called ISG responders (ISG-R). Cancer cell-stromal cell contact and engagement of breast cancer NOTCH3 and stromal JAG1 increases exosome production and triggers stromal NOTCH1-MYC activation. Whether tumors cells also release soluble factors and exosomes that further reinforce reciprocal communication remains to be determined. Stromal NOTCH1-MYC signaling increases RNA polymerase III (POL3) transcriptional activity and, consequently, RN7SL1. The increase in RN7SL1 levels, in the absence of a proportional increase in SRP9 and SRP14, which normally shield this transcript, markedly increases unshielded RN7SL1. Unshielded RN7SL1 is released in stromal exosomes and transferred to ISG-R breast cancer cells, where it activates the pattern recognition receptor (PRR) RIG-I, leading to STAT1 activation and ISG induction. Unshielded exosomal RN7SL1 also activates myeloid/dendritic cells, inducing CD40, CD86, PDL1, and MHCII expression. Unshielded exosomal RN7SL1 and RIG-I activation promotes primary tumor growth and metastasis. Interestingly, viral particles contain endogenous non-viral RNAs, including RN7SL1, but not SRPs (Telesnitsky and Wolin, 2016), which activate RIG-I in a manner similar to that of breast cancer exosomes.

(B) ISG non-responder (ISG-NR) breast cancer cells fail to enhance stromal exosome transfer and to induce ISGs upon tumor-stroma interactions. ISG-NRs do not increase stromal RN7SL1 transcription or stromal exosome production. Shielding of RN7SL1 by SRP9 and SRP14 inhibits RIG-I activation and ISG induction.

that a MYC-driven increase in POL3 transcriptional activity results in an excess of RN7SL1 (and other) 5′ ppp transcripts, without a proportional increase in the amount of SRP9/SRP14 RNA binding proteins, leading to accumulation of unshielded RN7SL1 in stromal exosomes. Indeed, co-culture of breast cancer cells, expressing the NOTCH ligand JAGGED1, with NOTCH1-expressing stromal fibroblasts activates NOTCH and its downstream transcriptional targets, such as *MYC,* in stromal cells (Figure 1). To complete the vicious cycle in cancer progression, transfer of unshielded stromal exoRN7SL1 to breast cancer and immune cells activates RIG-I signaling and induces upregulation of maturation/activation markers by dendritic cells and myeloid cells, events that are required for ISG-responsive tumor growth and

lung metastasis in murine models of breast cancer.

Lastly, confirming the clinical relevance of their mechanistic studies, the authors demonstrate that abundant POL3 and RN7SL1 transcripts are present in exosomes isolated from the serum of cancer patients. This finding suggests that these molecules could serve as biomarkers of inflammation and poor prognosis. Moreover, examination of exoRNA released by tumor-associated fibroblasts isolated from cancer patients revealed a significant increase in unshielded exoRN7SL1, as compared to healthy control fibroblasts. It would be interesting to determine whether this mechanism is commonly activated in highly metastatic cancers other than TNBC. Should a common mechanism of PRR recognition be identified in a subset of aggressive cancers, it would be crucial to further investigate the features that distinguish responder versus non-responder tumors and target them to hinder cancer progression, since directly blocking NOTCH activation in breast cancer has proven challenging due to ubiquitous expression of NOTCH receptors and their essential functions (Liu et al., 2016).

Nabet et al. demonstrate that aggressive cancers can activate stromal cells, inducing the release of endogenous exosomal RNAs that mimic viral components, to co-opt anti-viral immune responses into promoting tumor growth and inflammation. This comprehensive study opens the door to future studies into the complex interactions between DAMP signaling and cancer—specifically, do other DAMPs drive sterile inflammation during cancer progression? How is interferon (IFN) response activation, normally central to anti-tumor responses, beneficial for cancer cells? Furthermore, is there a right balance of IFN responses that restrains rather than promotes tumor growth and can it be harnessed to fight cancer?

Exosomes are ubiquitous messengers in the intricate intercellular communication system established during cancer progression. The central role of exosomes in linking stromal activation and PRR signaling in cancer, demonstrated by the authors, begs the question of whether exosomes carrying DAMPs are also acting on disseminated tumor cells and stromal cells at metastatic sites. Is uncapped RNA (or other DAMPs) preferentially shed in exosomes? And more broadly, what is the role of DAMPs in metastasis, and is this due to effects on tumor cells or the immune system (or both)?

Importantly, the quality and strength of the immune response to these exosomal RNA DAMPs requires further evaluation. How do exosomal DAMPs induce an immune response, what are the immune subsets orchestrating it, and what is the link to tumor progression and metastasis? Future studies addressing these questions will no doubt reinforce the idea that exosomal DAMPs, such as RN7SL1, are key factors regulating immune responses during tumor progression.

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