Friend and foe, HNRNPC takes on immunostimulatory RNAs in breast cancer cells

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The RNA-binding protein HNRNPC is highly expressed in breast cancer, but its contribution to tumorigenesis was unclear. In this issue, Wu *et al* (2018) demonstrate that elevated HNRNPC is essential for the proliferative ability of two breast cancer cell lines. Reducing HNRNPC results in the accumulation of short Alu-derived dsRNAs that bind RNA receptor RIG-I and stimulate the production of IFN, a cytokine with known antiproliferative activity.

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See also: Y Wu *et al* (December 2018)

Innate immunity, commonly defined as a first line of defense against invading pathogens in the host cell, also plays a key role in regulating tumor progression. In this issue of *The EMBO Journal*, Wu *et al* (2018) explore the complex relationship between breast cancer and the innate immune system.

The innate immune response is mediated by a set of proteins termed pattern recognition receptors (PRRs) which patrol each cell for the presence of foreign molecules. For example, RNA viruses can generate double-stranded RNA (dsRNA) structures, normally absent from the cytosol of uninfected cells. Upon binding dsRNAs, PRRs activate a signal transduction pathway which results in the production and release of cytokines called interferons (IFNs) by the infected cell. IFNs are the central orchestrators of innate immunity: They signal to IFN-producing and neighboring cells to initiate a massive

transcriptional program including over 400 IFN-stimulated genes (ISGs), with a broad variety of antiviral and antiproliferative functions (Schneider *et al*, 2014).

Given their antiproliferative potential, IFNs were eagerly seized upon in the 1970s as potential blockers of cancer cell proliferation. IFN injections have indeed proven successful against some cancers and are now part of the immunotherapy tool set—see (Parker *et al*, 2016) for review. Other therapeutic strategies coax cancer cells into producing IFNs themselves. DNA methyltransferase inhibitors, for example, relieve transcriptional repression on endogenous retroviruses, resulting in the accumulation of dsRNAs and activation of the IFN response by PRRs (Chiappinelli *et al*, 2015). IFNs can also be naturally secreted in the tumor microenvironment. IFN induction by endogenous retroviral dsRNAs has been observed in hypomethylated testicular germ cell tumors (Haffner *et al*, 2018). At the same time, and perhaps explaining the variable effectiveness of IFN therapies, many tumors display resistance to IFN through defects in IFN induction or responsiveness. IFN resistance likely protects cancer cells from the antiproliferative effects of naturally secreted IFNs (Parker *et al*, 2016). The intricacies between cancer and innate immunity are still poorly understood: Which endogenous nucleic acids spur IFN induction by cancer cells? What is the impact of IFNs on cancer progression? The answers to these questions will no doubt vary between cancer types and across stages of tumor development.

In this issue, Wu *et al* (2018) focus on HNRNPC as a critical RNA-binding protein (RBP) at the intersection between cancer and innate immunity. The authors were initially intrigued by a strikingly elevated level of HNRNPC in breast cancers relative to healthy tissue. However, it was unclear whether high levels of HNRNPC were required for tumorigenicity or an insignificant bystander to global changes accompanying transformation. Knock-down (KD) of HNRNPC in two specific human breast cancer cell lines, MCF7 and T47D, was sufficient to restrict cell proliferation. This was corroborated *in vivo* by decreased tumor growth in mice transplanted with these cell lines and treated with siRNAs targeting HNRNPC. These findings paralleled a previous study of glioblastomas, where elevated HNRNPC promoted cancer proliferation by directly stabilizing an anti-apoptotic microRNA (Park *et al*, 2012). Here however, Wu *et al* (2018) chose an unbiased approach to understand how HNRNPC promotes proliferation in breast cancer. RNA-Seq of HNRNPC KD cells revealed a massive upregulation of ISGs indicative of IFN signaling. To determine whether IFN was directly mediating the observed antiproliferative effect, the authors transferred supernatant from HNRNPC KD cells to unaltered breast cancer cells and observed a growth-inhibitory effect, confirming that HNRNPC depletion leads to the release of an antiproliferative agent into the media. Second, blocking IFN β signaling in HNRNPC KD cells, either through antibody inhibition of IFN β and its receptor IFNAR2 or drugs blocking the ISG transcription factor

STAT1, prevented both ISG induction and antiproliferative effects. Elevated HNRNPC in breast cancer cells thus promotes tumor growth by preventing IFN β production—but by what mechanism?

In mammalian cells, IFN β can be induced by the PRRs RIG-I and MDA5, two cytosolic receptors for foreign RNAs. Knock-down of RIG-I, but not MDA5, abrogated the induction of the IFN response and inhibition of cell proliferation in HNRNPC KD cells. RIG-I can be activated by short dsRNAs with di- or tri-phosphorylated 5' ends (Schneider *et al*, 2014). The authors therefore isolated putative RIG-I targets: Total RNA was immunoprecipitated with an anti-dsRNA antibody, size-selected for species below 500 nucleotides, and sequenced. RNA sequences

indicated that HNRNPC KD cells accumulate short (< 100 bp) dsRNAs that map to intronic Alu elements. The authors therefore hypothesized that Alu-derived dsRNAs might be responsible for activating RIG-I.

Alus are repetitive elements scattered throughout the genome including in the intronic and untranslated regions of protein-coding genes. When transcribed as part of pre-mRNA transcripts, Alus can alter splicing and lead to the retention of intronic RNA in a process termed “Alu exonization”. Interestingly, HNRNPC has been shown to bind and obscure Alu sequences on pre-mRNAs and protect against Alu exonization (Zarnack *et al*, 2013). The authors thus hypothesized that in the absence of HNRNPC, the cytoplasm becomes overwhelmed by

Alu-containing mRNAs, which can carry premature stop codons (PTCs). To ensure proper translation, it is well known that transcripts with PTCs are eliminated by the nonsense-mediated decay (NMD) pathway through a series of endo- and exonucleases. However, Alu RNAs, being highly structured, may be partially resistant to NMD nucleases, leading to the accumulation of short Alu-derived dsRNA degradation intermediates—possible substrates for RIG-I. In support of this hypothesis, knocking-down HNRNPC alongside components of the NMD pathway, UPF1 or the endonuclease SMG6, lowered both dsRNA levels and IFN β induction. In summary, Wu *et al* (2018) suggest that HNRNPC promotes the proliferation of breast cancer cells by preventing the export

Figure 1. HNRNPC prevents the accumulation of immunostimulatory RNAs in breast cancer cells.

Wu *et al* (2018) demonstrate that high levels of HNRNPC are necessary for the proliferation of certain breast cancer cell lines. High levels of HNRNPC shield intronic Alu sequences from splicing machinery, resulting in proper transcript production. When levels of HNRNPC are decreased, cryptic splice sites in intronic Alu sequences are exposed resulting in their inappropriate “exonization” into transcripts. These aberrant mRNAs are then exported to the cytoplasm where, due to premature stop codons, they undergo nonsense-mediated decay (NMD) by a series of endo- and exonucleases. The resulting fragments (dsRNA structures derived from Alu sequences) serve as substrates for RIG-I, triggering the induction of IFN β and its downstream effectors, ISGs, a gene set with known antiproliferative activity.

of Alu sequences to the cytosol, where they may be partially degraded by the NMD to generate immunostimulatory short Alu-derived dsRNAs (Fig 1).

NMD processed Alu fragments join a rapidly expanding list of endogenous RNA species comprising the “immunostimulatory self”. Alu sequences are known to generate long dsRNAs that must be edited by ADAR1 to prevent sensing by MDA5 (Ahmad *et al*, 2018). In contrast, the shorter Alu-derived RNAs identified by Wu *et al* (2018) are sensed by RIG-I. Yet, it remains unclear how NMD-derived RNAs would acquire the 5' di/triphosphate normally required for RIG-I recognition. One recently reported endogenous ligand of RIG-I is the 7SL RNA (evolutionarily related to Alu), but this is an RNA polymerase III product with a known 5' triphosphate end (Nabet *et al*, 2017). Another study however, reports that cells lacking exosome component SKIVL2 similarly generate small transcript-derived RNA fragments capable of activating RIG-I through endonuclease IRE-I (Eckard *et al*, 2014). Our current understanding of RIG-I substrates may therefore be incomplete.

An important direction for future studies will be to understand the unique RNA processing demands of breast cancer cell lines MCF7 and T47D that necessitate elevated HNRNPC to avoid IFN production and maintain proliferation. By contrast, healthy cells and even other breast cancer cell lines tested by the authors display no dsRNA accumulation or IFN production even when expressing comparatively low levels of HNRNPC. Perhaps altered levels or activity of proteins mediating other steps in RNA processing and clearance could have combinatorial effects leading to

accumulation of Alu-derived fragments specific to these cells.

With their study of HNRNPC, Wu *et al* (2018) explore another fascinating instance of immune evasion by cancer cells and, in the process, unveil new aspects of RNA biology. Their work enters the ongoing debate weighing IFN's collective effect on tumorigenesis, including counterintuitive reports of a proliferative role of chronic IFNs in tumor microenvironments (Nabet *et al*, 2017). IFN not only can recruit but also can exhaust adaptive immune players such as lymphocytes within the tumor microenvironment, and the effects of elevated HNRNPC in this context remain to be explored.

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