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Exosome-transferred lncRNAs at the core of cancer bone lesions

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ABSTRACT

Exosome-mediated transfer of regulatory RNAs is a key feature that enables cancer cells to shape a tumor-promoting environment. Cancers growing in the bone can use this communication modality to disrupt the homeostatic balance between bone forming and bone resorbing cells, which results in the release of bone-embedded factors supporting cancer growth and progression. Long noncoding RNAs (lncRNAs) are potent regulators of cell fate determination with exceptional cell- and tissue-specificity that are secreted by cancer cells via exosomes. In multiple myeloma (MM), the exosomal transfer of the lncRNA RUNX2-AS1 specifically inhibits the osteogenic differentiation capacity of mesenchymal stem cells (MSC) by repressing the master regulator of bone formation RUNX2. Detailed studies into the role of exosomal lncRNA transfer in the bone microenvironment *in vivo* might constitute the basis for the development of novel therapeutic strategies for tumor-associated bone lesions.

1. Main text

The intercellular crosstalk within the tumor microenvironment is a key force supporting tumor progression. In cancers growing in the bone, including multiple myeloma (MM), a complex network of signals released by or in response to tumor cells disrupts the delicate balance between bone formation and resorption, leading to pathological alterations of the bone architecture and, ultimately, bone lesions. In this process, numerous bone-embedded growth factors are released in the microenvironment, stimulating tumor growth in a self-perpetuating "vicious cycle". Apart from the well-established role of osteoclast activation, unbalanced bone remodeling in MM is associated with a severe impairment of bone formation, reflecting the complexity of the osteoclast-osteoblast functional uncoupling in cancer (Giuliani et al., 2006).

As osteoblast progenitor cells and critical immunomodulatory components of the tumor microenvironment, mesenchymal stem cells (MSC) emerged as key targets of MM paracrine activity. Indeed, MM-MSCs display impaired osteogenic differentiation capacity as wells a perturbed cytokine expression profile (Xu et al., 2018), which might explain the reduced number of osteoblasts observed in MM patients. In a recent issue of Oncogene, Li and colleagues (Li et al., 2018a) show that MM cells can subvert the MSC differentiation program by directly suppressing the master osteogenic transcription factor RUNX2 through the exosome-mediated transfer of the long non-coding RNA lncRUNX2-AS1.

Exosomes are endosome-derived extracellular vesicles (EVs) released by all cell types that selectively incorporate biologically active molecules of the cell of origin and are particularly enriched in regulatory non-coding RNAs (ncRNA). In cancer the horizontal transfer of exosome-associated signals between malignant and stromal cells modulates many aspects of tumor development and progression, including metastasis formation, immune escape and development of drug resistance (Zhou et al., 2014; Liu et al., 2016; Zhang et al., 2015; Haderk et al., 2017; Nabet et al., 2017; Boelens et al., 2014). While most studies have attributed the tumor-promoting effects of exosomal RNA to small ncRNAs, such as miRNAs and pol III-transcripts, recent evidence supports a role for EV-associated lncRNAs in cancer progression and in the propagation of drug resistance (Qu et al., 2016).

In their recent article (Li et al., 2018a) Li and colleagues show that MSCs exposed to MM-derived exosomes have a reduced capacity to form bone nodules, and narrow down the exosome-dependent phenotype to the large-sized RNA molecules contained in the vesicles. Further analyses revealed that human myeloma cells (HMCL) efficiently sort into exosomes the lncRNA lncRUNX2-AS1, which upon internalization by MSCs interferes with the splicing of RUNX2. Consistent with the central role of RUNX2 in osteogenic differentiation, disruption of its splicing and the consequent attenuation of its expression, reduces the differentiation capacity of MSCs. Importantly, RNA interference (RNAi)

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directed towards lncRUNX2-AS1 in exosome-producing HMCLs rescues the osteogenic differentiation capacity of exosome-treated MSCs. Finally, the authors show that blocking exosome secretion with the neutral sphingomyelinase inhibitor GW4869 prevents bone loss in a MM mouse model.

Initially believed to be transcriptional noise or simply non-functional by-products, lncRNAs are now recognized as potent regulators of key cellular processes, including proliferation and differentiation (Kretz et al., 2012). These RNA molecules are longer than 200 nucleotides and have little or no coding potential. Mechanistically, lncRNAs can exert their function in the nucleus or in the cytoplasm and act with distinct modalities. For instance, nuclear lncRNAs can form ribonucleoprotein complexes with chromatin modifiers (e.g. epigenetic erasers or writers) and directly regulate transcription or splicing at specific loci (Huarte et al., 2010; Gonzalez et al., 2015). Alternatively, they can alter RNA splicing by interacting with specific splicing factors or by forming complexes with pre-mRNA molecules (Bardou et al., 2014). Finally, enhancer RNAs (eRNAs) can modulate transcription by facilitating structural interactions between enhancers and nearby or distant promoters. In the cytoplasm, lncRNAs influence RNA stability or translation by forming RNA:RNA hybrids or by sponging microRNAs (miRNAs) away from their target transcripts (Kretz et al., 2012).

While increasing evidence shows that lncRNAs can be transferred between cells in association with EVs, many questions remain unanswered. For instance, it is not clear whether nuclear and cytoplasmic lncRNAs have the same propensity to be loaded into EVs, and how their sequence, structure or protein-binding partners influence their sorting. However, as lncRNAs are in general expressed at lower levels than other transcripts, the most compelling question remains whether their presence in purified vesicles is relevant from a physiological perspective. How many copies of a specific lncRNA are present in EVs, and how many are necessary to phenotypically impact target cells? This aspect requires careful evaluation as physiological exosome communication might drastically differ in time and intensity from *in vitro* treatment with purified vesicle preparations. Finally, while specific lncRNAs are overrepresented in tumor compared with normal tissues and can be detected in the circulation of cancer patients in association with EVs (Qu et al., 2016; Tan et al., 2018; Li et al., 2018b), it is not clear whether specific sorting mechanisms in cancer cells determine their incorporation into exosomes (Bebelman et al., 2018).

The study from Li and colleagues (Li et al., 2018a) opens new perspectives on the potential mechanisms underlying impaired bone formation in MM that could be relevant for therapeutic intervention. However, before lncRUNX2-AS1 can be considered as a potential therapeutic target for MM-associated bone lesions, evidence of its transfer and function *in vivo* needs to be obtained. Indeed, administration of GW4869 to mice, as performed by the authors, indiscriminately affects exosome release from all cell types, and can induce the release of EV subpopulations budding from the plasma membrane (Menck et al., 2017). Thus, while suggestive of a role for exosome communication in MM bone disease, this approach falls short in demonstrating a specific role for exosomal lncRUNX2-AS1 transfer in bone lesions. This point could be addressed by showing that selective inactivation of the lncRNA in tumor cells rescues bone formation *in vivo*.

Arguably, many other cell types in the bone environment including osteoblasts, osteoclasts, but also endothelial, hematopoietic and mature immune cells can be influenced by the noncoding RNA cargo of cancer exosomes, and different signals might be involved in the development of osteoblastic, osteolytic or mixed lesions (Li et al., 2018a; Maeder and Gersbach, 2016). However, the mechanisms by which tumor-secreted lncRNAs perturbate bone homeostasis are still largely unexplored.

2. Concluding remarks

The exceptional tissue- and tumor-specificity of many lncRNAs (Arun et al., 2018), together with the recent success of RNAi-based therapeutics (Adams et al., 2018), provides unprecedented opportunities to explore lncRNAs as novel candidate targets for cancer therapy. If the physiological relevance and the expression of lncRUNX2-AS1 in human tumors are confirmed, the work from Li and colleagues could have important clinical implication for the treatment of bone lesion not only in MM, but in all cancer types that develop in or disseminate to the bone. Finally, detection of lncRNAs in circulating plasma exosomes could provide a unique opportunity for the development of a liquid biopsy approach for bone disease monitoring in cancer.

Conflictof interest statement

The authors declare no conflict of interest.

Authors' contributions

SRB and NL conceived and wrote this commentary.

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