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‘One way, or another, I'm gonna find ya’: miR-221-3p finds its targets *via* small extracellular vesicles

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In this issue of *Haematologica,* Li *et al.* report yet another mechanism by which the ubiquitously oncogenic miR-221-3p locates its targets, through small extracellular vesicle (sEV)-mediated autocrine and paracrine actions to promote leukemogenesis[1]. Extracellular vesicles (EVs) are lipid-bilayer enclosed structures released by cells into the extracellular (EC) space. EVs are classified into three different subclasses based on size: sEVs, previously known as exosomes, are the smallest, ranging from 30-150 nm in diameter; apoptotic bodies that are 50-5000 nm in diameter; and microvesicles that range from 100-1000 nm in diameter[2]. EV payloads consist of various cellular components including proteins, lipids and DNA, RNA and microRNAs (miRNA) that can participate in intercellular signal transduction.

Recent studies have revealed that the unique payload composition of sEVs can contribute to the progression of Acute Myeloid Leukemia (AML) by promoting intercellular signaling among cells within the bone marrow (BM) niche. In particular, miRNAs transferred from AML cells to non-malignant cells of the BM niche have been demonstrated to promote leukemogenesis through paracrine suppression of normal hematopoiesis by inhibiting hematopoietic stem and progenitor cells (HSPCs), a consequence which contributes to a favourable leukemogenic BM niche[2]. Examples include exosomal mir-150 and mir-155, both found to suppress HSPCs primarily through inhibition of c-myb[3]. Similarly, mir-548ac was found to be transported in AML-sEVs and could suppress normal hematopoiesis by targeting TRIM28 leading to subsequent STAT3 activation[4].

Additionally, sEV-miRNAs can function in an autocrine manner by promoting oncogenic properties of neighboring AML cells[2]. To complicate matters, sEVs can also be derived from bone marrow stromal cells (BMSCs). For instance, in acute lymphoblastic leukemia (ALL), mir-181a was found to be enriched in exosomes derived from both pediatric patient samples and cell lines[5]. Exposure of exosomes containing elevated mir-181a promoted cell proliferation by upregulating PCNA and Ki67, and cell survival by upregulating prosurvival genes (MCL1 and BCL2) and downregulating pro-apoptotic genes (BAX and BAD)[5]. Another study found that BMSCs from AML patients produced sEVs with higher expression of miR-26-5p compared to healthy controls[6]. Exposure of these sEVs to AML cells promoted their proliferation through inhibition of GSKβ and activation of Wnt/β-catenin signaling[6].

In this study, Li *et al.* performed high-throughput profiling of miRNA in sEVs to identify the miRNA that play key roles in intercellular signaling in the BM niche[1]. They identified that miR-221-3p was among the most highly enriched AML-sEVs and was found to promote AML

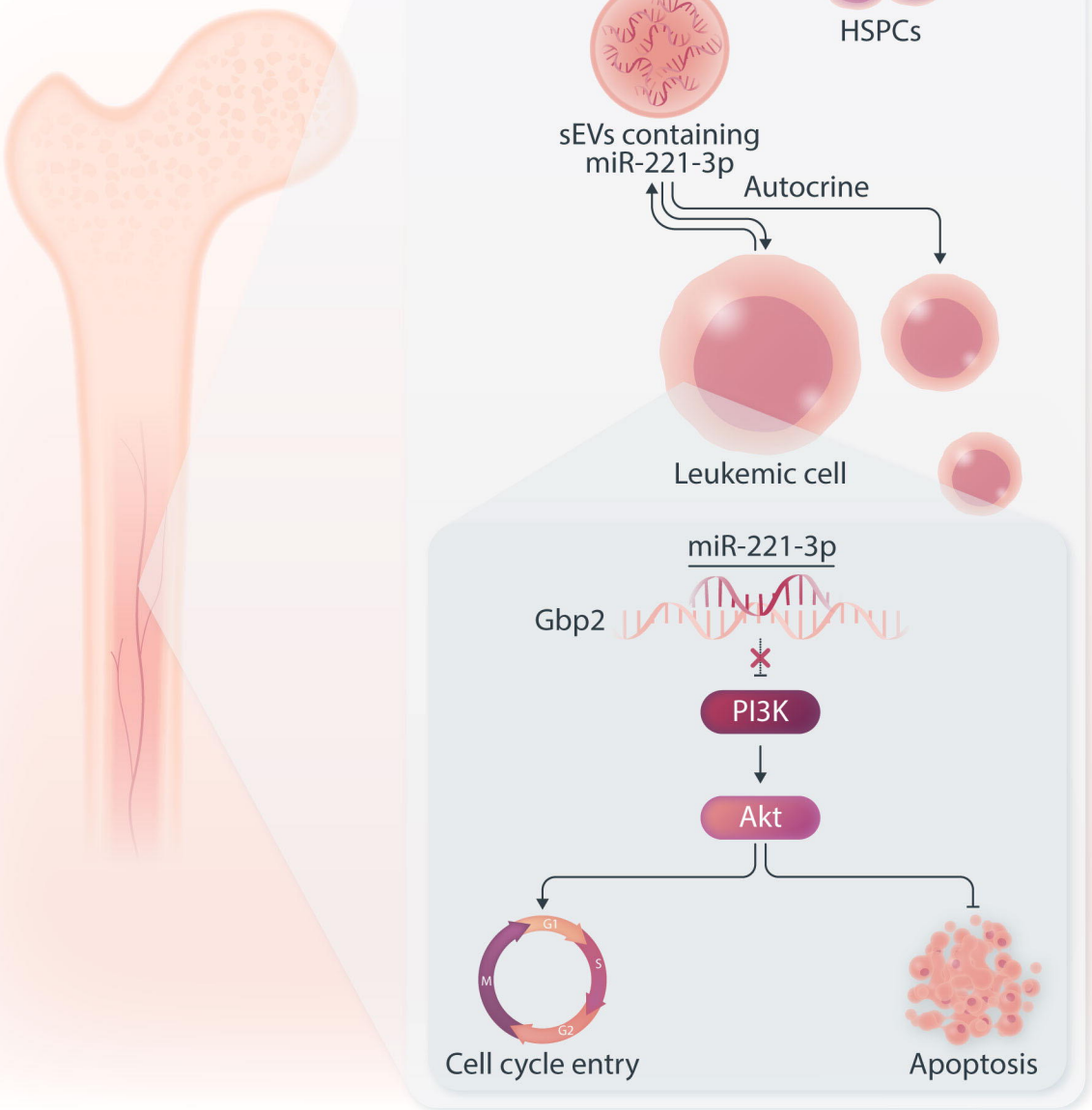
cell growth while simultaneously impairing HSPC cell growth[1]. miR-221 is an established tumor promoting oncomiR[7]; and it has also been demonstrated that miR-221-3p is transported in sEVs derived from BMSCs[8]. Li *et al*. now demonstrate that miR-221-3p is transported in AML derived sEVs and contributes to both autocrine and paracrine signaling. The delivery of miR-221-3p to AML cells via sEVs was observed to induce cell cycle progression while concurrently suppressing apoptosis by inhibiting Gbp2 and regulating PI3K/Akt signaling downstream[1]. Additionally, sEV miR-221-3p exhibited a selective paracrine effect on HSPCs, impeding the erythroid differentiation of normal HSPCs and consequently reducing their colony-forming capacity in both *in vitro* and *ex vivo* settings[1]. While many miRNAs identified in sEVs seem to affect either AML cells or HSPCs exclusively, this study's findings highlight an sEV miRNA capable of impacting both cell types, thereby orchestrating a "two-pronged impact" on the BM niche to promote leukemogenesis. Despite this, the exact molecular mechanism of sEV miR-221-3p mediated suppression of normal hematopoiesis remains unknown. Does the sEV miR-221-3p/Gbp2/PI3K/Akt mechanism also facilitate HSPC suppression, or is there a cell-type dependent mechanism that leads to this "two-pronged impact"? Nonetheless, given these observations, targeting miR-221-3p shows promise as a therapeutic strategy, as its inhibition could not only disrupt its intracellular signaling but also mitigate its intercellular effects within the BM niche.

sEV miR-221-3p holds potential as a clinical biomarker to enhance patient stratification in AML. While the concept of using sEVs as biomarkers is not novel, further refinement of most effective miRNAs is necessary before its practical application. Specifically, miRNAs present in circulating sEVs could serve as minimally invasive indicators of disease prognosis and progression[9]. For instance, elevated serum sEV-mir-10b levels have been identified as an independent predictor of poor prognosis in AML patients, correlating with shorter survival times[10]. Cellular miR-221-3p has previously been suggested as a valuable biomarker across various cancers[7], indicating the significant clinical potential of its sEV counterpart. This study identifies its potential to serve as a novel specific paracrine biomarker. Future studies should focus on determining whether sEVs containing miR-221-3p circulate in the periphery and whether sEV miR-221-3p levels hold prognostic and predictive value. In sum, Li *et al*. provide evidence that the oncogenic function of miR-221-3p can be transmitted in a paracrine manner, thus targeting miR-221-3p harboured in sEVs presents a promising target that can be leveraged theranostically.

References

1. Li M, Sun G, Zhao J, et al. Small extracellular vesicles derived from acute myeloid leukemia cells promote leukemogenesis by transferring miR-221-3p. Haematologica. xxx
2. Izadirad M, Huang Z, Jafari F, et al. Extracellular Vesicles in Acute Leukemia: A Mesmerizing Journey With a Focus on Transferred microRNAs. Front Cell Dev Biol. 2021;9:766371.
3. Hornick NI, Doron B, Abdelhamed S, et al. AML suppresses hematopoiesis by releasing exosomes that contain microRNAs targeting c-MYB. Sci Signal. 2016;9(444):ra88.
4. Zhao C, Zhao Y, Zhao J, et al. Acute myeloid leukemia cell-derived extracellular vesicles carrying microRNA-548ac regulate hematopoietic function via the TRIM28/STAT3 pathway. Cancer Gene Ther. 2022;29(7):918-929.
5. Haque S, Vaiselbuh SR. Silencing of Exosomal miR-181a Reverses Pediatric Acute Lymphocytic Leukemia Cell Proliferation. Pharmaceuticals (Basel). 2020;13(9):241.
6. Ji D, He Y, Lu W, et al. Small-sized extracellular vesicles (EVs) derived from acute myeloid leukemia bone marrow mesenchymal stem cells transfer miR-26a-5p to promote acute myeloid leukemia cell proliferation, migration, and invasion. Hum Cell. 2021;34(3):965-976.
7. Di Martino MT, Arbitrio M, Caracciolo D, et al. miR-221/222 as biomarkers and targets for therapeutic intervention on cancer and other diseases: A systematic review. Mol Ther Nucleic Acids. 2022;27:1191-1224.
8. Zhang X, Xu Y, Wang J, et al. miR-221-3p Delivered by BMMSC-Derived Microvesicles Promotes the Development of Acute Myelocytic Leukemia. Front Bioeng Biotechnol. 2020;8:81.
9. Araújo HV de, Sakamoto LHT, Bacal NS, Epelman S, Real JM. MicroRNAs and exosomes: promising new biomarkers in acute myeloid leukemias? Einstein (Sao Paulo). 2022;20:eRB5954.
10. Fang Z, Wang X, Wu J, Xiao R, Liu J. High serum extracellular vesicle miR-10b expression predicts poor prognosis in patients with acute myeloid leukemia. Cancer Biomark. 2020;27(1):1-9.

Figure 1. sEV miR-221-3p promotes leukemogenesis through autocrine and paracrine signaling in the bone marrow niche. AML derived sEVs containing miR-221-3p can support AML cell growth in an autocrine manner by promoting cell cycle progression and inhibiting apoptosis, in part through inhibiting Gbp2 and regulation of PI3K/Akt signaling. Simultaneously, sEV miR-221-3p can suppress normal hematopoiesis by suppressing HSPCs differentiation in a paracrine manner. These concurrent autocrine and paracrine functions of sEV miR-221-3p consist of a “two-pronged impact” on the BM niche that ultimately favours leukemogenesis.


Paracrine
HSPCs
sEVs containing
miR-221-3p
Autocrine
Leukemic cell
miR-221-3p
Gbp2
PI3K
Akt
G1
S
G2
M
Cell cycle entry
Apoptosis