Commentary | Received 2 February 2023; Accepted 15 February 2023; Published 13 October 2023
https://doi.org/10.55092/exrna20230005

Extracellular vesicles from cancer cells are key players of metastatic cell phenotype induction

Ana Lu ísa Teixeira*, Francisca Dias

Molecular Oncology and Viral Pathology Group, Research Center of IPO Porto (CI-IPOP)/Porto Comprehensive Cancer Center (Porto.CCC) & RISE@CI-IPO (Health Research Network), Porto, Portugal

* Correspondence author; E-mail: ana.luisa.teixeira@ipoporto.min-saude.pt.

Wei and colleagues highlight an important health issue associated with colorectal cancer (CRC) management, common to other tumors, that is the high risk of metastases development, especially liver metastases [1]. CRC is the third most frequent cancer worldwide and represents the third most deadly tumor [2]. In fact, it is accepted that approximately 15–25% of patients present metastasis in the moment of diagnosis, being the liver the most common site of metastatic disease. Moreover, it is observed that approximately 50% of the CRC patients develop liver metastases during the course of the disease, which will present a negative impact in patients' overall survival. The literature reports that patients with liver metastases present a poor prognosis, presenting a 5-year survival rate of 12% [3]. Thus, the metastatic disease represents a huge challenge in patient's management, being crucial to understand the molecular mechanisms driving to metastatic disease establishment in CRC, since this issue is the high contributor for cancer-related mortality. A deeper understand of the underlying mechanisms behind the metastization process will create new opportunities for new predictive biomarkers identification, such as circulating biomarkers, that could be quantified through minimally invasive methods and ultimately aid in an improved management of these patients.

The metastatic process is a multistep process, involving invasion, vasculature intravasation, survival in circulation, extravasation, and adaption/growth in secondary site. Nowadays, it is known that tumor cells and multiple elements of the tumor microenvironment (TME) will establish a complex crosstalk that will be essential for the metastatic dissemination. TME is composed by cancer and noncancerous cells, immune cells, mesenchymal stem cells, endothelial cells, niche cells, cancer-associated fibroblasts, and adipocytes that will be involved in cell survival and growth regulation, providing a key niche for cancer progression [4,5]. In TME, cancer cells orchestrated a crosstalk among surrounding stromal and inflammatory cells in order to be established an inflammatory microenvironment that support tumorigenesis, tumor growth and distant metastasis establishment. CRC liver metastasis are typically associated with the interaction between CRC cells and the TME in liver. In fact, in TME occurs a constant cell communication driven by cytokines, chemokines, growth factors,

inflammatory enzymes and extracellular vesicles (EVs) that support tumor growth and inflammation among other elements [6].

Actually, it is accepted that cell-to-cell communication through EVs is one of the most relevant communication mechanisms in the TME, being involved in the crosstalk among the different cell types—cancer, normal, immune, endothelial stromal [7]. During the recent decades, EVs gained attention since they are major intercellular players of the cell communication being involved in physiological and pathological processes. EVs are nano-sized lipid bilayer encapsulated membranes carrying proteins, lipids, nucleic acids and sugars that are shed by the cells in all the three domains of life, representing the first form of cell cell-tocell communication [8,9]. EVs consist of a mixed population of microvesicles with different sizes, shed by cells, that enable cell-to-cell communication through the transport of active biomolecules from one cell to another, with the ability to change the receptor cells [10]. EVs can be released from primary tumor cells, transporting biomolecules that can modulate the biology and the phenotype of neighbor cells, moreover, they can modulate distant organ niches to support the seeding and growth of metastatic cells [11,12]. In fact, they can circulate to distant organ and induce changes in microenvironment to potentiate the future metastatic spread in consequence of their ability to horizontally transfer information, such as nucleic acids or proteins.

Interestingly, EVs application surpass the oncology field as effective vehicles that modulate cellular processes. For example, pioneer works performed by Rozmyslowicz *et al*. described that CD4+/CXCR4-null cell lines 'acquired' CXCR4 receptor after exposure to EVs from platelets and became infected by human immunodeficiency virus (HIV) [13]. More recently, it has been described that EVs are also involved in HIV latency which is a huge challenge to the success of antiretroviral therapies [14]. On the other hand, Gram-positive and Gram-negative bacteria can also secrete EVs being involved in acquisition of nutrients, adhesion to hosts, delivery of virulence factors and immune modulation [15]. Bacteria EVs can carry β-lactamases resulting in enhanced antibiotic resistance to β-lactam antibiotics [15–18]. Moreover, EVs secreted by Staphylococcus aureus are enriched in penicillin-binding proteins, which bind to β-lactam antibiotics and contribute to methicillin resistance [15–17]. Thus nowadays scientists from basic and clinical research areas look at EVs with great enthusiasm due to their potential to be used as clinical biomarkers useful for disease diagnosis, prognosis, patient's follow-up or even as therapeutic agents.

It is now known that EVs could promote several processes involved in CRC metastases formation affecting cell proliferation, invasion and migration capacity, epithelialmesenchymal transition, tumor angiogenesis, premetastatic niche establishment, inflammation and immunosuppression status, organotropism, treatment resistance, among others [19].

Interestingly, Wei *et al.*, found that CRC-derived EVs presented the ability to activate NOD1 in macrophages and initiate the secretion of inflammatory cytokines and chemokines, being these activated- macrophages involved in the promotion tumor cell migration [1]. In fact, the authors show that CRC-derived EVs carry a high cargo of CD42, that were transported to macrophages and mediate the NOD1 activation, triggering downstream nuclear factor-κB (NF-κB) and p38-mitogen-activated protein kinase (MAPK)-dependent inflammatory cytokines and chemokines, which ultimately promote tumor cell migration. Therefore, tumor cells induce IL-6, CCL1 and CCL2 macrophages production creating a TME favorable to tumor cell migration and metastasis formation. On the other hand, the authors found that patients with liver metastases CRC present higher expression of EVs-related CDC42

than heathy individuals, being these EVs involved in NOD1 activation in human blood mononuclear cells. Taken together, the authors suggested the NOD1 as a potential target for CRC liver metastases treatment.

Several works have shown the ability of tumor cells to secrete EVs with different cargo types that when uptaked by surrounding cells, induce a change in their phenotype and affect the metastatic microenvironment modeling. For example, Shao and co-workers showed that CRC-EVsrelated-microRNA-21 (miR-21) is essential for establish a proinflammatory liver microenvironment, through TLR7 activation in macrophages, being this step crucial for liver metastases [20]. Recently, Zhao *et al.* found that metastatic CRC cells release high levels of EVs-relatedmiR-181a-5p than low metastatic cells. In fact, the EVsrelated-miR-181a-5p can activated the hepatic stellate cells and consequently the IL6-STAT3. Moreover, activated hepatic stellate cells could secrete CCL20 and also activate a CCL20/CCR6/ERK1/2/Elk-1/miR-181a-5p positive feedback loop, resulting in reprogramming of the TME and the formation of pre-metastatic niches in liver [21]. Zhang *et al.* identified that CRC-EVs cause HSPC11 upregulation in hepatic stellate cells affecting lipid metabolism in cancerassociated fibroblasts via up-regulation of acetyl-CoA, which ultimately lead to CXCL5 expression [22]. Recently, we also observed that the mRNA LAT1 (amino acid transporter) can be transported by CRC-EVs and is able to modulate the cellular phenotype of recipient cells *in vitro*, inducing a higher cell migration capacity and proliferation in liver and kidney cell lines as well as to induce changes in the transcriptome and protein levels of these cells, suggesting that this mRNA could be an interesting biomarker but also opening a new idea to develop a new therapeutic approach to target the LAT1 mRNA [23]. Regarding the application of EVs in cancer treatment approaches, our research group is currently studying a new strategy to block the expression of an onco-RNA involved CRC progression using microRNAs mimics delivery by EVs, that present the advantages to cross natural barriers with prolonged circulation time with less toxicity and less immunogenicity [24].

The application of EVs analysis could open a new field for CRC diagnosis and tumor progression monitoring through minimally invasive strategies allowing the real application of the liquid biopsy concept. The analysis of EVs-cargo associated with CRC could allow early diagnosis, as well as a real-time patient monitoring, with a positive impact in the early detection of tumor progression and metastases development. The EVs could allow the analysis of multiple types of material that can be submitted to genomic, transcriptomic, proteomic and lipidomic analysis.

In fact, one of the most exciting possibilities of EVs applications is their use as treatment agents, due to their compatibility with the host immune system and their ability to be engineered as targeted drug delivery systems or vaccines, which was the case of COVID-19 mRNA vaccines. In the future, anti-cancer drugs, small RNAs and antiinflammatory agents could be loaded and delivery through EVs, EVs from stem cells could be used to direct repair damage tissues and EVs from bacteria could be applied for vaccine development due to their immunogenicity and adjuvanticity.

Conflicts of Interests

Francisca Dias has a junior researcher contract funded by UIDP/00776/2020-4B. The other author has no conflicts of interest to declare.

Ethical Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

[1] Wei X, Ye J, Pei Y, Wang C, Yang H, *et al.* Extracellular vesicles from colorectal cancer cells promote metastasis via the NOD1 signalling pathway. *J. Extracell Vesicles* 2022, 11:e12264.

[2] Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Jesus-Rodriguez-Yoldi M. Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer. *Int. J. Mol. Sci.* 2017, 18:197.

[3] Yin W, Xu J, Li C, Dai X, Wu T, *et al.* Circular RNA circ_0007142 Facilitates Colorectal Cancer Progression by Modulating CDC25A Expression via miR-122-5p. *OncoTargets Ther.* 2020, 13:3689-3701.

[4] Cacho-Díaz B, García-Botello DR, Wegman-Ostrosky T, Reyes-Soto G, Ortiz-Sánchez E, *et al.* Tumor microenvironment differences between primary tumor and brain metastases. *J. Transl. Med.* 2020, 18:1.

[5] Mantovani A, Allavena P, Marchesi F, Garlanda C. Macrophages as tools and targets in cancer therapy. *Nat. Rev. Drug Discov.* 2022, 21:799-820.

[6] Pereira JFS, Jordan P, Matos P. A Signaling View into the Inflammatory Tumor Microenvironment. *Immuno*. 2021, 1:91-118.

[7] Cavallari C, Camussi G, Brizzi MF. Extracellular Vesicles in the Tumour Microenvironment: Eclectic Supervisors. *Int. J. Mol. Sci.* 2020, 21:6768.

[8] Gill S, Catchpole R, Forterre P. Extracellular membrane vesicles in the three domains of life and beyond. *FEMS. Microbiol. Rev.* 2019, 43:273-303.

[9] Hopkin K. Core Concept: Extracellular vesicles garner interest from academia and biotech. *Proc. Natl. Acad. Sci. USA.* 2016, 113:9126-9128.

[10] Teixeira AL, Dias F, Gomes M, Fernandes M, Medeiros R. Circulating biomarkers in renal cell carcinoma: the link between microRNAs and extracellular vesicles, where are we now? *J. Kidney Cancer VHL* 2014, 1:84-98.

[11] Peinado H, Zhang H, Matei IR, Costa-Silva B, Hoshino A, *et al.* Pre-metastatic niches: organ-specific homes for metastases. *Nat. Rev. Cancer.* 2017, 17:302-317.

[12] Costa-Silva B, Aiello NM, Ocean AJ, Singh S, Zhang H, *et al.* Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat. Cell Biol.* 2015, 17:816-826.

[13] Rozmyslowicz T, Majka M, Kijowski J, Samuel LM, Dareus OC, *et al.* Platelet- and megakaryocyte-derived microparticles transfer CXCR4 receptor to CXCR4-null cells and make them susceptible to infection by X4-HIV. *AIDS.* 2003, 17:33-42.

[14] Okoye I, Xu L, Oyegbami O, Shahbaz S, Pink D, *et al.* Plasma Extracellular Vesicles Enhance HIV-1 Infection of Activated CD4(+) T Cells and Promote the Activation of Latently Infected J-Lat10.6 Cells via miR-139-5p Transfer. *Front. Immunol.* 2021, 12:697604.

[15] Chen J, Zhang H, Wang S, Du Y, Wei B, *et al.* Inhibitors of Bacterial Extracellular Vesicles. *Front. Microbiol.* 2022, 13:835058.

[16] Lee EY, Choi DY, Kim DK, Kim JW, Park JO, *et al.* Gram-positive bacteria produce membrane vesicles: proteomics-based characterization of Staphylococcus aureus-derived membrane vesicles. *Proteomics.* 2009, 9:5425-5436.

[17] Uddin MJ, Dawan J, Jeon G, Yu T, He X, *et al.* The Role of Bacterial Membrane Vesicles in the Dissemination of Antibiotic Resistance and as Promising Carriers for Therapeutic Agent Delivery. *Microorganisms* 2020, 8:670.

[18] Schaar V, Nordström T, Mörgelin M, Riesbeck K. Moraxella catarrhalis outer membrane vesicles carry β-lactamase and promote survival of Streptococcus pneumoniae and Haemophilus influenzae by inactivating amoxicillin. *Antimicrob. Agents Chemother.* 2011, 55:3845-3853.

[19] He X, Zhong X, Hu Z, Zhao S, Wei P, *et al.* An insight into small extracellular vesicles: Their roles in colorectal cancer progression and potential clinical applications. *Clin. Transl. Med.* 2020, 10:e249.
[20] Shao Y, Chen T, Zheng X, Yang S, Xu K, *et al.* Colorectal cancer-derived small extracellular vesicles establish an inflammatory premetastatic niche in liver metastasis. *Carcinogenesis* 2018, 39:1368-1379.
[21] Zhao S, Mi Y, Zheng B, Wei P, Gu Y, *et al.* Highly-metastatic colorectal cancer cell released miR-181a-5p-rich extracellular vesicles promote liver metastasis by activating hepatic stellate cells and remodelling the tumour microenvironment. *J. Extracell Vesicles* 2022, 11:e12186.
[22] Zhang C, Wang XY, Zhang P, He T, Han J, *et al.* Cancer-derived exosomal HSPC111 promotes colorectal cancer liver metastasis by reprogramming lipid metabolism in cancerassociated fibroblasts. *Cell Death Dis.* 2022, 13:57.
[23] Almeida C, Teixeira AL, Dias F, Machado V, Morais M, *et al.* Extracellular Vesicles Derived-LAT1 mRNA as a Powerful Inducer of Colorectal Cancer Aggressive Phenotype. *Biology (Basel)* 2022;11:145.
[24] Huyan T, Li H, Peng H, Chen J, Yang R, *et al.* Extracellular Vesicles – Advanced Nanocarriers in Cancer Therapy: Progress and Achievements. *Int. J. Nanomedicine* 2020, 15:6485-6502.