Lipid Composition of Cancer-Derived Extracellular Vesicles and its Potential for the Identification of Body Fluid-Based Biomarkers for Breast Cancer diagnosis

Erika Dorado[1], M Luisa Doria[1], Anika Nagelkerke[2], James McKenzie[1], Stefania Maneta-Stavrakaki[1], Alvaro Perdones-Montero[1], Ulrike Kauscher[2], R Charles Coombes[1], Jeremy Nicholson[1], Molly M Stevens[2], Zoltan Takats[1]

[1]Faculty of Medicine, Division of Systems Medicine and [2]Faculty of Engineering, Department of Materials

1. Introduction

Extracellular vesicles (EVs), including exosomes and microvesicles, are found in high concentrations in body fluids of cancer patients. Cancer-derived EVs play an important role in cancer development, progression, and metastasis. EVs have great potential for the identification of body fluid-based biomarkers for breast cancer diagnosis, which overcome the limitations associated with breast biopsy for breast cancer confirmation. Currently, the understanding of the lipid composition of cancer EVs is limited and it is unknown for breast cancer EVs.

The aim of this study is to characterise the lipid composition of EVs released by different breast cancer cell lines, as well as their potential as body fluid-based biomarkers for breast cancer diagnosis.

2. Methods

11 cell lines: EVs and Cells
3 biological replicates

Breast cancer cell lines
MDA-MB-436
MDA-MB-468
MDA-MB-231
HCC1937
HS578T
MCF-7
T47D
HCC1954
JIMT-1
Non-cancerous mammary cell lines
MCF-10A
HuMEC

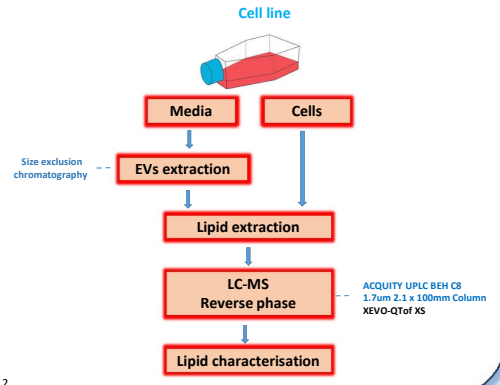
Breast cancer subtypes:

Triple negative (TN) breast cancer ER-/PR-/HER2-

ER+/PR+/HER2- breast cancer

ER-/PR-/HER2+ breast cancer

ER = Estrogen receptor
PR = Progesterone receptor
HER2 = Human epidermal growth factor 2



3. Results and Discussion

LC-MS data distribution

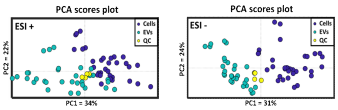


Figure 1. LC-MS data distribution. Principal component analysis (PCA) analysis from positive and negative ionisation modes. PCA shows a separation between EVs and cells, indicating that EVs show a distinct lipid composition due to the fact that EVs do not represent the entirety of cellular components.

Sphingomyelins and ceramides are enriched in breast cancer derived-EVs when compared to their parental cells.

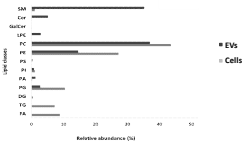


Figure 2. Lipid classes found in breast cancer EVs and cells. Lipid classes: sphingomyelins (SM), ceramides (Cer), galactosylceramides (GalCer), lysophosphatidylethanolamines (LPE), phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylserines (PS), phosphatidylinositols (PI), phosphatidic acids (PA), phosphatidylglycerols (PG), diacylglycerides (DG), triglycerides (TG) and fatty acids (FA).

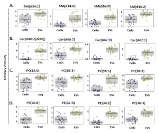
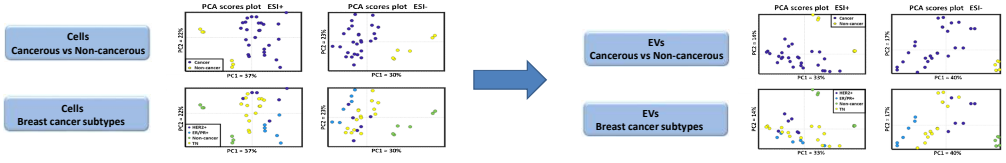


Figure 3. Lipid species found in breast cancer EVs. Examples of sphingolipids and glycerophospholipids found in breast cancer EVs.

EVs can be distinguished between cancerous and non-cancerous EVs based on their lipid content



Mammary epithelial cell lines differ in their lipid content based on their cancerous or non-cancerous origin as well as their ER+/PR+ status.

PC, PE, PI, PS and SM were enriched in breast cancer-derived EVs when compared to non-cancerous EVs.

PI are enriched in EVs from ER+/PR+ cancer cell lines, while PE and PC are enriched in EVs from ER-/PR- cancer cell lines.

Figure 4. Lipidomic analysis of EVs and their parental cells.

4. Conclusions

- Sphingomyelins and ceramides, as well as lysophosphatidylethanolamines are enriched in breast cancer-derived EVs when compared to their parental cells.
- Breast cancer-derived EVs also have a high abundance of phosphatidylcholines and phosphatidylethanolamines.
- EVs released by breast cancer cells and non-cancerous mammary epithelial cells can be distinguished based on their lipid profile.
- The lipid content of EVs derived from ER+/PR+ cell lines differ from those derived from ER-/PR- breast cancer cell lines.
- These findings demonstrate the potential of the lipid content of breast cancer derived-EVs for the identification of body fluid-based biomarkers for breast cancer diagnosis.

References

1. Colombo, M. et al. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol. 30, 255-89 (2014).
2. Raposo G. and W. Stoorvogel. Extracellular vesicles: Exosomes, microvesicles, and friends. J Cell Biol. 200(4): 373–383 (2013).

Acknowledgments

STRATiGRAD
Stratified Medicine Graduate Training Programme in Systems Medicine and Spectroscopic Profiling