Novel application of lipid nanoemulsions in breast cancer

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Metastasis is one of the main causes of cancer death, and consists of a dynamic succession of events involving the dissemination of tumor cells [1]. These cells, called Circulating Tumor Cells (CTCs), are shed from the primary tumor and travel through the blood to colonize distant organs, generating a new metastatic niche [2]. CTCs have relevance as prognostic markers in breast cancer. However, the functional properties of CTCs or their molecular characterization have not been well-studied. Thus, it is essential to culture and study these surviving cells capable of forming such metastases. The main difficult for the analysis of these cells relays in their isolation and culture ex vivo that still represents a big challenge in translational research. The main challenges of CTCs isolation and culture relate to the fact that CTCs are infrequent (1 CTC against 10^6-10^7 of surrounding normal peripheral mononuclear blood cells (PBMCs)) and they are present in a slow proliferative state when isolated [3]. Moreover, CTCs show short rates of survival in the bloodstream because of their exposure to fluid shear stress. At the moment, various emerging methods have been developed for CTC enrichment, in order to improve the amount of viable material that could later be used for downstream analysis. However, robust and reliable isolation and optimized culture conditions for CTCs remain to be established.

Therefore in our laboratory we have formulated oil-in-water (O/W) Nanoemulsions (NEs) using lipids and fatty acids which have reported to influence the metabolism of cancer cells increasing the metabolic activity of cancer cells[4]. We have use them to support the culture of different CTCs samples isolated from metastatic breast cancer patients. These allowed us to perform phenotypic and gene expression analysis using RT-PCR of the cultured CTCs [5]. Finally we are working on the functionalization of these NEs in a way to target CTCs as a future tool for CTCs isolation and culture [6].
In this conference we will present the results showing the synthesis and characterization of NEs and their capability to increase the cell viability of different breast cancer cell lines. Moreover, we will present how we have generated a CTC model from breast cancer mice xenografts, to prove the ability of the NEs to facilitate their culture and expansion. Additionally, we will explain the postulated mechanism of NEs action based on their consumption by cells to use them as energy suppliers, driving proliferation.
We will also show how we use these NEs for culture supporting in isolated viable CTCs from 50 peripheral blood samples obtained from 35 patients with advanced metastatic breast cancer. We found that in 75% of samples the CTC cultures we were able to obtain a success short-term culture were cells lasted more than 23 days. Additionally we observed that the cultivability was able to predict a shorter Progression-Free Survival in these patients, independently of having 5 CTC by Cellsearch®. We also observed that CTCs before and after culture showed a different gene expression profile, concluding that the cultivability of CTCs is a predictive factor. Furthermore, the subset of cells capable of growing ex vivo showed stem or mesenchymal features and may represent the CTC population with metastatic potential in vivo.
Finally we will show our recent data when we have functionalized the nanoemulsions with two peptides, with the aim of using them as a tool for CTC isolation and culture in situ. Therefore, NEs were surface-decorated with the peptides Pep10 and GE11, which act as ligands towards the specific cell membrane proteins EpCAM and EGFR, respectively.

Therefore we will present a new methodology to support the cultivability of CTCs by the use of NEs with specific composition. We will show how the shot-term culture of CTCs could be a tool predictive factor of patient survival and how we can functionalize these NEs for future applications

References

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Figures

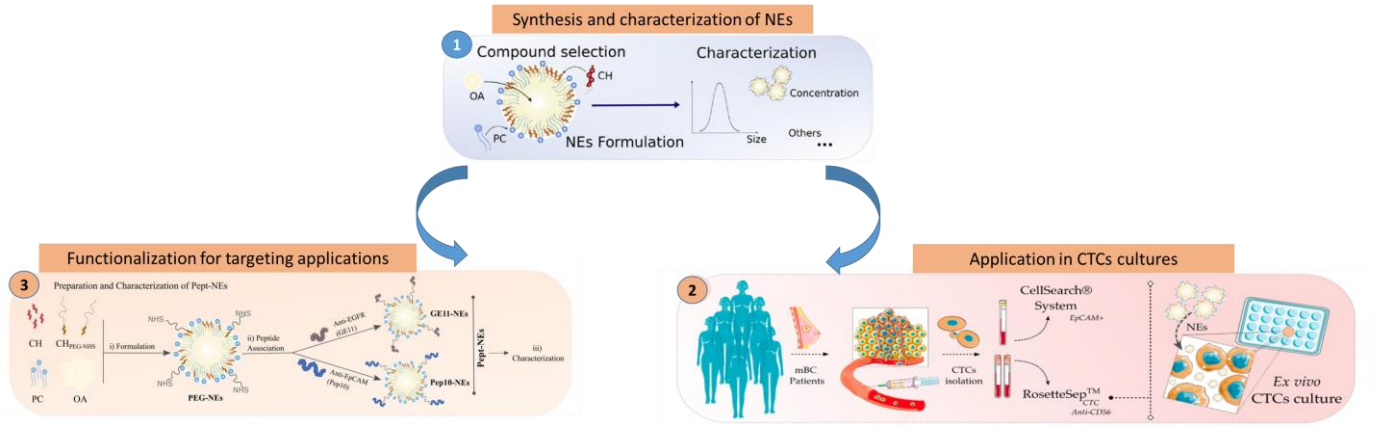

Figure 1. 1. Synthesis and characterizations of NEs formulated from lipid and fatty acids involved in cancer cell metabolism, 2) Use of NEs for CTCs culture and analysis of the cultured CTCs, 3) Functionalization of NEs for CTCs targeting.