Unveiling the Promise of Personalized Therapy: Harnessing Cell Membrane-Coated Nanovectors for Targeted Treatment of Glioblastoma Multiforme

Carlotta Pucci[1], Daniele De Pasquale[1], Alessandra Giannoncelli,[2] Valentina Bonanni,[2] Milan Žižić,[2] Gianni Ciofani[1]

[1]Smart Bio-Interfaces, Istituto Italiano di Tecnologia, Pontedera (Pisa), 56025 Italy
[2]Elettra - Sincrotrone Trieste, Strada Statale 149, 34149 Basovizza, Trieste, Italy

carlotta.pucci@iit.it

Precise tumor targeting presents a significant hurdle for emerging antitumor therapies that aim at focusing therapeutic effects on tumor cells while minimizing undesirable side effects. Common targeting strategies rely on simple ligand/receptor interactions. Yet, when dealing with heterogenous tumors, selecting a suitable target receptor becomes complicated due to variations in membrane protein expression among patients.

Glioblastoma multiforme (GBM), a highly aggressive brain tumor, displays high genetic diversity among different patients [1]; thus, it is crucial to devise an innovative targeting strategy to improve patient survival and reduce recurrences. Recently, a novel approach known as "homotypic targeting" has emerged, harnessing cancer cells inherent capability to recognize and interact with each other through specific membrane protein [2]. The nanotechnological implementation of homotypic targeting consists in coating nanoparticles with cell membranes directly extracted from the target cells, thus tailoring the targeting approach to each patient's membrane signature and bypassing challenges posed by tumor heterogeneity. Nevertheless, to ensure an optimal targeting efficacy, it is essential to preserve the presence and the natural conformation of membrane proteins in the coating, and the formation of a protein corona upon interaction with serum proteins should be avoided so that the membrane protein in the coating can be freely exposed to the target cells.

In our work, we introduced lipid-based magnetic nanovectors loaded with iron oxide nanoparticles and coated with cell membrane extracts from patient-derived GBM cells (CDMNVs), as an innovative personalized approach to efficiently target tumor cells and treat GBM with magnetic hyperthermia [3]. We thoroughly demonstrated the effective coating of the nanovectors and quantified protein content using different techniques, including X-ray photoelectron spectroscopy (XPS), Fourier-transform infrared (FTIR) spectroscopy, and the bicinchoninic acid (BCA) assay, while the presence of crucial membrane proteins fundamental in the homotypic targeting mechanism, such as CD44, N-cadherin, neuroplastin-1, and beta-catenin, was verified using Western blotting.

We also demonstrated that coating the nanovectors with cell membrane extracts contributed to improved stability and reduced formation of a hard protein corona when interacting with serum-enriched cell culture medium. Since the correct conformation of proteins is also important to guarantee an effective targeting process, we focused on thoroughly characterizing this important, yet poorly studied, aspect. The structural conformation of membrane proteins in the coating was investigated with a straightforward platform based on several spectroscopic techniques such as fluorescence, FTIR, and Raman spectroscopy. This study confirmed that ultrasonication-based coating procures did not induce protein unfolding; thus, membrane proteins are expected to retain their functionality even when confined on the nanoparticles surface. A more detailed characterization with Raman spectroscopy, however, highlighted that after the coating procedure a slight increase in α-helix structures (≈12%) could be observed. Nevertheless, we demonstrated that this change did not affect the efficacy of homotypic targeting of the coated nanovectors, as they showed preferential uptake by GBM cells compared to healthy brain cells such as neurons, astrocytes, endothelial cells, and pericytes in a home-made fluidic bioreactor.

Finally, to better understand the interaction between the nanovectors and patient-derived GBM cells, we employed label-free techniques to directly detect iron oxide internalized within cells. In 2D cultures (Figure 1) and 3D tumor spheroids, synchrotron X-ray fluorescence (XRF) imaging displayed co-localization of iron signal from CDMNVs with native elements in cells (Na and C), confirming effective internalization. In 3D spheroids, we quantified the depth of CDMNV penetration over time with respect to an analogous nanovector named CD*MNVs, coated with cell membrane extracts previously deprived of membrane proteins. Results showed that CDMNVs were able to interact more efficiently with patient-derived GBM spheroids compared to CD*MNVs, validating the effectiveness of cell membrane coating and unveiling the crucial role of membrane proteins in the targeting process.

This work offers a platform for rapid assessment of cell membrane-coated nanovector functionality, a critical aspect when implementing new formulations and coating procedures. Moreover, it further validates the promising features of this personalized approach for the treatment of GBM.

References

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Figures

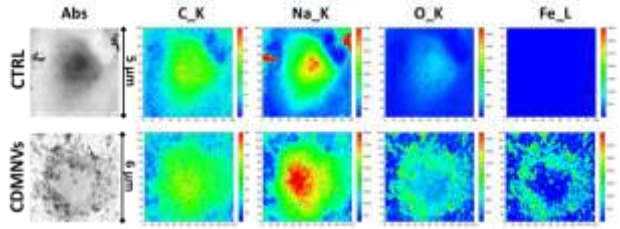

Figure 1. Absorption images and XRF maps of C, Na, O, Fe, (1.3 keV) of patient-derived GBM cells (control cells and cells treated with CDMNVs).