A patient-personalized lipid-based magnetic nanovector for a selective glioblastoma multiforme treatment through oxidative stress induction and metabolic impairment

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Abstract

Glioblastoma multiforme (GBM) is the most aggressive type of brain tumor with limited options for long-term cure. Cases of recurrences are very common, caused by the impossibility of removing all the neoplastic cells even after an invasive surgical resection, followed by radio- and chemotherapy [1]. Finding a more targeted and effective solution for this type of tumor is a challenge that current medicine has not yet addresses. In this context, new nanotechnological solutions seem to offer good hopes. Nanoparticles can deliver anticancer drugs to their destination more effectively; in addition, they can be functionalized to bare the same biological features of the tumor to target. This strategy, named “homotypic targeting”, can be achieved by coating nanoparticles with cell membrane extracts of the target cells, and exploits the well-known abilities of cancer cells to interact with each other [2].
In our work, we developed lipid-based magnetic nanovectors, loaded with the anticancer drug regorafenib (Reg) and with iron oxide nanoparticles in order to combine the action of the drug with the magnetic hyperthermia induced by the proper stimulation with an alternated magnetic field (AMF) [3]. Furthermore, the nanovectors were coated with membranes deriving directly from primary GBM cells in order to provide a selective and patient-personalized targeting of GBM.
In this study, we investigated in details the induction of intracellular damages [3], and in particular, oxidative stress related to intracellular Fenton reaction, related with the production of excessive reactive oxygen species (ROS) [4]. Nanoparticle selective tumor targeting was studied with dynamic flow experiments, focusing also on the particular preference of nanovectors for the specific patient’s source cells. For this experiment, five different primary GBM cell cultures were obtained from GBM surgical samples (ethical permission CER Liguria 341/2019), here called “Pat1”, “Pat2”, “Pat3”, “Pat4”, and “Pat5”. Nanoparticles were coated with “Pat1” cell membrane extracts. The inter-patient targeting experiments were performed in dynamic conditions and evaluated with confocal acquisitions and iron colorimetric assay. Results showed differential uptake among patient cell lines. In particular, the “Pat 1” cells showed a greater uptake with respect to the other primary patient cells (“Pat1”: 33.07 ± 9.50%, “Pat3”: 8.60 ± 6.6%, “Pat4”: 1.00 ± 0.6 %, “Pat5”: 6.70 ± 2.76 %), with the exception of "Pat2"(42.14 ± 14.91%); all % indicate nanoparticle / cell area signal co-localization (Figure 1).
The production of intracellular ROS was investigated by flow cytometry, showing that in presence of nanovectors and nanovectors + AMF there is a significant increase in oxidative stress.
To better understand the effect of the increased intracellular oxidative stress on GBM cells, their metabolic state was investigated by fluorescence lifetime imaging (FLIM), a technique that monitors the decay times of the enzyme-bound and free forms of reduced nicotinamide adenine dinucleotide (phosphate) (NAD(P)H). Fluorescence imaging of NADH is extremely useful for monitoring the metabolism of live cells, since the fluorescence lifetime of NAD(P)H is sensitive to its bound-to-enzyme or free state. In general, more abundance of longer lifetime (e.g., 3.4 ns) are associated to an OX/PHOS oriented metabolism (bound-to-enzyme NAD(P)H), while an abundance of short lifetime values (e.g., 0.4 ns) is associated to an increase of the glycolytic flux [5]. Generally, tumour cells have a higher rate of glucose uptake with respect to healthy cells, and they produce ATP preferentially through aerobic glycolysis (Warburg effect) [6]. Nevertheless, an impairment at the level of the mitochondria or anaerobic environments can further drive cell metabolism towards glycolysis. Our results showed a shift towards free NADH in the nanovectors-treated cells, with and without AMF stimulation compared to the control GBM cells: in these conditions, a further tendency towards glycolysis is observed (Figure 2), suggesting an impairment of mitochondria, induced by the intracellular oxidative stress due to the nanovectors, in agreement with our previous results. This intracellular damage was ultimately demonstrated to induce apoptosis in patient-derived GBM cells, especially after a chronic AMF stimulation.
The obtained results demonstrate that the combined action of magnetic nanovectors + AMF exerts a clear antitumor action based on localized hyperthermia, as previously demonstrated, and that the oxidative stress promoted by iron oxide-induced Fenton reaction and hyperthermia support the tumor cell death, increasing the effect of the drug. In addition, the effective targeting could render this strategy a valid option for personalized nanomedicine against GBM, enhancing the selectivity towards tumor cells and reducing side effects.

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Figures

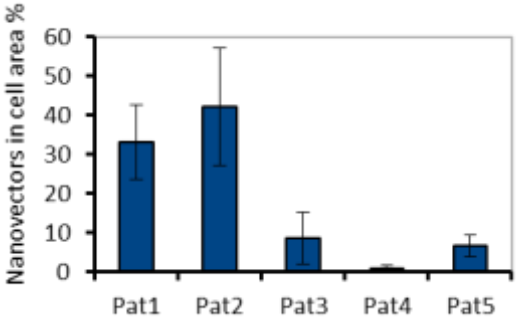

Figure 1. Nanovectors uptake analysis in five primary GBM cultures

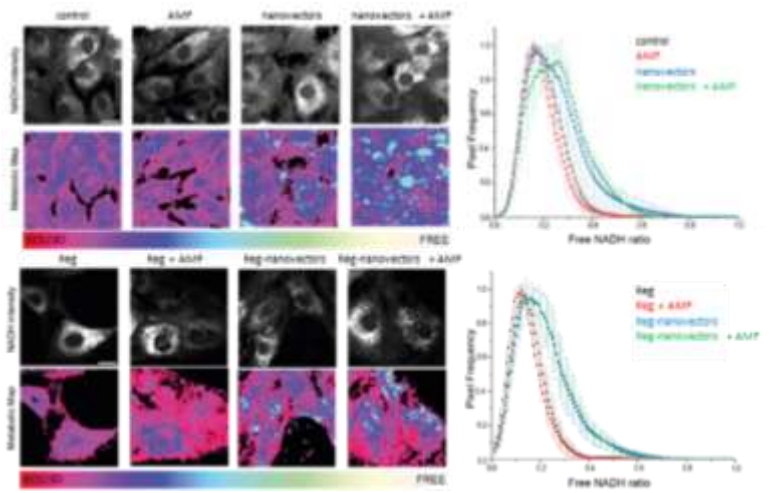

Figure 2. FLIM acquisitions and analysis of primary GBM cells undergoing different treatments.