

mApoE-Functionalized Nanoliposomes Delivering Doxorubicin and Ultrasmall Superparamagnetic Iron Oxide to Glioblastoma Cells Characterized by TEM and Confocal Microscopy

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To overcome the limits of current therapy for glioblastoma (GBM) [1] nanotechnology-based approaches offer attractive and innovative possibilities including improved passage of drugs across the blood brain barrier (BBB) and escaping multidrug resistance by efflux mechanisms.

Nanoliposomes (NLs) covalently coupled with a modified apolipoprotein E peptide (mApoE) have been successfully used to enhance the BBB penetration in the context of neurodegenerative diseases [2]. Here, these mApoE functionalized NLs have been modified to encapsulate the anticancer drug doxorubicin (DOXO) or DOXO and ultrasmall superparamagnetic iron oxide (USPIO) and they have been used to target DOXO to GBM cells. The NLs preparations were characterized by transmission electron microscopy (TEM, Fig. 1) and the role of ApoE on the NLs internalization together with its mechanism have been analyzed by confocal microscopy (CM).

GBM-derived cell lines U87-MG, A172, T98G were incubated for 4 hours with DOXO-mApoE-targeted or DOXO-non targeted NLs. DOXO intracellular uptake was significantly increased in the presence of the ApoE functionalization giving a more pronounced intracellular accumulation of DOXO in cells incubated with DOXO-mApoE-NLs compared to DOXO-NLs (Fig. 2A, B). These results suggested a mApoE-targeted NLs internalization via receptor-mediated endocytosis. The presence in the incubation medium of dynasore, the inhibitor of dynamin, reduced DOXO intracellular accumulation indicating a role of clathrin-dependent endocytosis in DOXO-mApoE-NLs uptake (Fig. 2). The USPIO DOXO-mApoE NLs internalization and intracellular trafficking has been further analyzed by both CM and TEM (Figure 3).

Inhibition of *in vitro* cell growth was assayed by MTT test at 72 hours. DOXO-mApoE-NLs were found to inhibit GBM cell viability in a dose-dependent manner with IC₅₀ values of DOXO comprised between 0.5 and 1.5 µg/ml. No cellular toxicity was observed upon incubation with mApoE-NLs.

Overall the data obtained support the use of mApoE-targeted nanocarriers for the delivery of chemotherapeutics and/or cytotoxic agents to GBM cells. The possibility to load mApoE-NLs with USPIO nanoparticles to exploit the advantages of correlative microscopy to analyze their intracellular trafficking and the potential use of USPIO mApoE-NLs as contrast agents for Magnetic Resonance Imaging will be discussed.

[1] Tanaka et al *Nat Rev Clin Oncol* (2013); 10(1): 14-26.

[2] Re et al. *Nanomedicine* (2011); (5):551-9.

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Figure 1. NLs design and characterization. A) Schematic representation of ApoE- functionalized liposomes encapsulating doxorubicin and USPIO nanoparticles; B) TEM image of ApoE-functionalized liposomes.

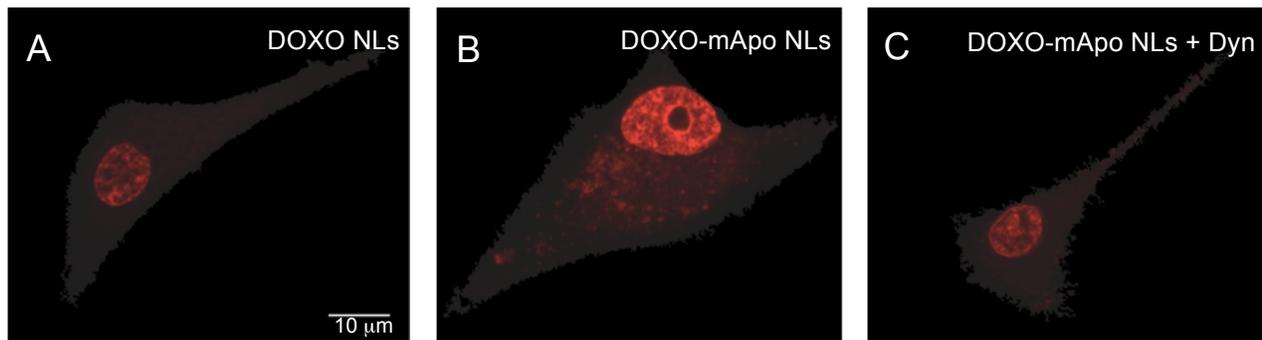


Figure 2. DOXO-mApoE NLs internalization in U87-MG cells analyzed by CM. Confocal optical sections showing internalized doxorubicin (red) and the cell, segmented thanks to the SytoBlue 45® labelling (grey). Cells were incubated with the indicated NLs in the absence (A, B) or in the presence of Dynasore (C).

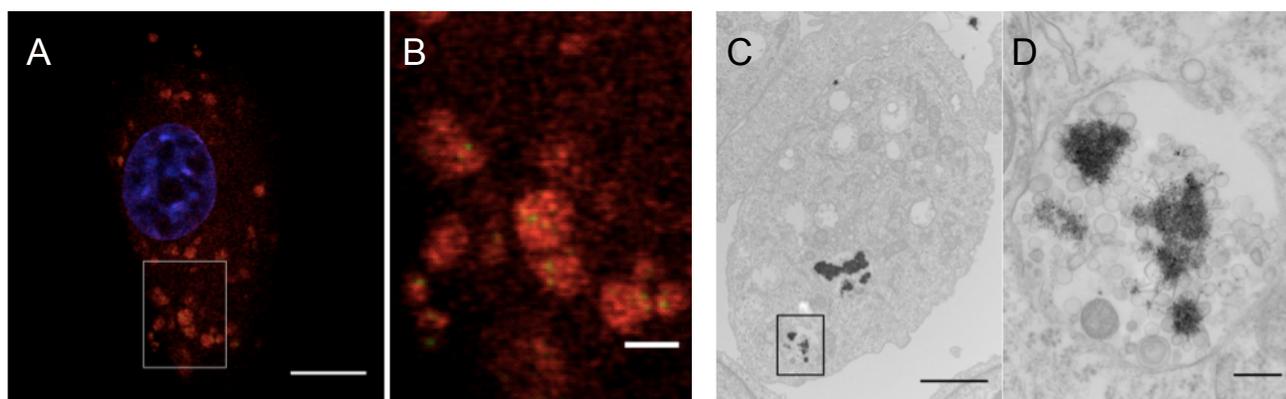


Figure 3. USPIO DOXO-mApoE NLs internalization in U87-MG cells analyzed by CM and TEM. A, B) Confocal optical section of a cell showing internalized doxorubicin (red), USPIO (green) and DAPI (blue). C, D) TEM images of a cell showing internalized NLs and USPIO. B) and D) are the magnified views of A) and C), respectively. Scale bars: 10 μm (A), 2 μm (B, C) and 200 nm (D).