A PLATFORM FOR POLYMERIC NANOPARTICLES AS GENE DELIVERY SYSTEMS IN 2D AND 3D GLIOBLASTOMA MODELS

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Background: Glioblastoma multiforme (GBM) is one of the most aggressive brain tumors classified as grade IV of malignancy by the WHO. Standard-of-care multimodal treatment combines surgical recession, chemotherapy and radiotherapy. However, such interventions result in severe side-effects and only offer a median survival of 15 months from diagnosis. Gene therapy holds great promise due its capacity to target specific pathways within glioblastoma cells by the introduction of exogenous tumor suppressor sequences that are rendered therapeutically effective by using delivery systems. Our research group has been exploring a platform of polymeric nanoparticles (NPs) as gene delivery systems for the treatment of glioblastoma due to an intrinsic ability to encapsulate and protect different nucleic acids, high biocompatibility and delivery efficacy.

Methods: Polymeric NPs with different ratios (8:0:1, 8:4:1, 4:1) were formulated by an ionic complexation method using protamine, polyethylenimine and chitosan as cationic polymers and dextran and a recently synthesized polyphosphazene as anionic elements. The physicochemical and structural properties of the NPs were studied by Photon Correlation Spectroscopy, Laser Doppler Anemometry and Transmission Electron Microscopy. Long- and short-term stability was examined for one month under storage (4ºC) and for 4 hours in physiological conditions (37ºC, pH=7.4), respectively. The association of different nucleic acids to the NPs was studied by agarose gel electrophoresis. *In vitro* cytotoxicity studies were initially optimized using U87MG glioblastoma 2D monolayers and 3D spheroids followed by assessment using a panel of primary patient-derived glioblastoma cells (GIN-8, GIN-28, GCE-28), by proliferation and cell death assays. The 2D and 3D cellular uptake of 4:1 protamine:dextran (Pr:Dx) NPs was studied using fluorescently labelled-nanosystems by confocal microscopy and quantified by Flow Cytometry. Moreover, their transfection with different concentrations of pDNA was carried out with a plasmid encoding the enhanced Green Fluorescent and Luciferase Proteins (pEGFP-Luc) by fluorescence microscopy.

Results: The NPs present spherical morphology, size below 150 nm, positive surface charge, high encapsulation of nucleic acids and stability under storage and physiological conditions. The cell viability studies indicated low/non-toxicity of the NPs in U87MG 2D cells and spheroids, but showed limited toxicity in glioblastoma patient-derived cells, except for the 4:1 Pr:Dx system. The cellular uptake studies of 4:1 Pr:Dx NPs revealed a transfection efficiency of 99%, with internalization of this nanosystem reaching the inner cell compartments. In addition, the transfection assay analyzed by green fluorescence emitted by EGFP showed an efficient capacity of 4:1 Pr:Dx NPs to transfect glioma cells for doses greater than 1 ug/well.

Conclusions: The physicochemical properties of the NPs make them suitable for the association and protection of different genetic cargos. Among all the nanosystems, the 4:1 Pr:Dx NPs presented the lowest cellular toxicity and were efficiently internalized in 2D and 3D cell models. Finally, these NPs promoted an efficient transfection of model pDNA in glioblastoma cells, indicating that this proof-of-concept formulation could be considered a promising gene nanocarrier for glioblastoma treatment by gene therapy.