Block Copolymer Nanoparticles for Gene Delivery in Tumor Model

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INTRODUCTION: The common method to deliver DNA is the use of viral vectors, but numerous problems exist. Based on this, we prepared new polymeric micelles as non-viral vectors for DNA/RNA which showed to be much less toxic in vitro and in vivo respect to many of the commercial vectors and highly efficient in complexing, stabilizing and delivering the nucleic acids. Aim of this project is to show the effect of micelles-DNA and micelles-siRNA delivery on the tumor growth using B16F10 murine melanoma as model.

METHODS: PEG-PPS telechelic thiolate was synthesized as previously reported[1]. From this reactive intermediate, stable PEG-b-PPS (**AB**) was formed by reaction with 2,2'-dithiodipyridine. In order to form the PEG-b-PPS-b-PEI triblock-copolymer, the PEG-PPS telechelic thiolate was conjugated by a thiol-disulfide exchange reaction with a linear poly(2-ethyl-2-oxazoline) telechelic pyridyl disulfide block, which had been synthesized as reported in the literature[2]. Following conjugation, the poly(2-ethyl-2-oxazoline) block was deprotected by acid hydrolysis to yield the triblock copolymer containing the poly(ethylene imine) (PEI) block (**ABC**). When suspended in water, the ABC block copolymer easily self-aggregates to form micelles of different diameters, between 100 and 250 nm. If, instead, mixed micelles of AB and ABC block copolymers are suspended in water, the size is dramatically decreased (30 nm). Both of the platforms were high efficiently conjugated with DNA and siRNA without dramatic changes in the size of the micelles. Particularly both micelle systems were conjugated with GFP plasmid or with nucleolin siRNA and the complexes have been transfected into B16F10 cells and MDA cells respectively. GFP expression has been evaluated after 48hr by mean of fluorescent microscope and Flow Cytometry. Nucleolin knockdown in vitro was determined by rtPCR. *In vivo* gene delivery was tested by intratumoral injections of micelles-pOVA (encoding ovalbumin plasmid) in melanoma bearing mice: the tumor tissues were dissected after 48 hours and pOVA expression was determined by rtPCR, comparing the efficiency of micelles conjugated plasmid with naked plasmid. The effect of pOVA delivery on tumor growth was evaluated in tumor bearing mice previously immunized against ovalbumin. Not immunized mice containing melanoma tumor were injected instead with micelles-siRNA, being nucleolin, one of the most important player in tumor proliferation, the target. In both of the cases, injections were repeated every day and the tumor volume was monitored every day as well.

RESULTS: Both the micelle systems have shown high transfection efficiency *in vitro* and *in vivo*, being 70% the amount of GFP positive cells in B16F10 (figure 1a) and being the pOVA expression in tumor up to 10^3 folds higher if driven by the micelles (figure 1b). The tumor treatment in pre-immunized mice with OVA-plasmid carried by micelles, have shown highly reduction of tumor growth respect to the control group (figure 1c). Up to 80% of nucleolin knockdown was obtained *in vitro* by micelles-siRNA transfection in MDA cells; *in vivo* siRNA micelles delivery caused strong inhibition of tumors growth (figure 1d).

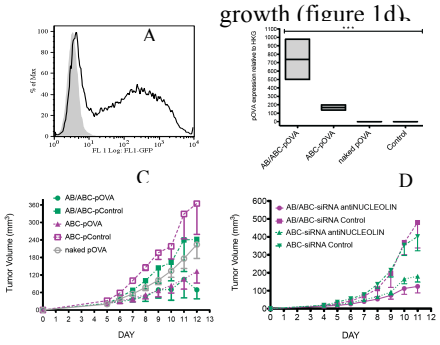

Fig.1.Flow Cytometry analysis (A) of GFP expression in B16F10 mediated by AB-ABC micelles. B) Intratumoral expression of pOVA. Effect of intratumoral pOVA (C) and nucleolin siRNA (D) delivery on tumor growth in mice.

DISCUSSION AND CONCLUSIONS: The DNA and siRNA delivery mediated by ABC and AB-ABC block copolymer micelles has been observed *in vitro* and *in vivo*, being different mouse's organs and tissues targeted by different ways of administration. Among those, interesting results have been obtained in melanoma tissues.

REFERENCES:

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