NANOPARTICLE-MEDIATED ENDOCYTOSIS OF MOLECULAR BEACONS AS THERANOSTIC AGENTS IN HUMAN CANCER CELLS

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Polymethylmethacrylate nanoparticles were exploited as nanocarrier of theranostic molecular beacons. Nanoparticle ability to promote internalization of the beacon, involvement of endocytosis in nanoparticle uptake, nanoparticle fate and localization, beacon localization on endoplasmic reticulum were demonstrated.
Keywords: Polymethylmethacrylate nanoparticles; molecular beacon

1. Introduction

A wide variety of nanometric drug-delivery systems have been examined in several fields, including cancer therapy. Among the proposed nanocarriers, polymeric nanoparticles (NPs) are considered effective delivery systems for their stability, handiness and biocompatibility. We have recently demonstrated that polymethylmethacrylate core-shell fluorescent NPs (PMMA-NPs) promote the cellular uptake of a molecular beacon (MB) specific for survivin mRNA, in A549 human lung cancer cell line [1]. MBs constitute potential theranostic agents as they can act at the same time as sensors, able to detect endogenous nucleic acids, and as drug, by silencing the target mRNA.
The aim of this work was to evaluate by confocal microscopy and fluorescence measurements: a) the involvement of endocytosis in NP uptake; b) the NP localization in lysosomes; c) the MB localization on the Endoplasmic Reticulum (ER) where the target mRNA is located.

2. Results

Confocal microscopy images of A549 cells incubated for 30 min with Alexa Fluor® 647 Dextran endocytosis marker alone and with this marker plus PMMA-NPs showed that, the red fluorescent signals are more intense and compact with respect to the red spots observed with the endocytosis marker alone. All these data demonstrate that, in these experimental conditions, PMMA-NPs promote/increase endocytosis.
In addition, green and red fluorescence images of A549 cells incubated with PMMA-NPs for 48 h and then with LysoTracker™ Deep Red for 70 min revealed that, in these specific conditions, PMMA-NPs co-localize with lysosomes.
After the evaluation of the NP localization and fate, further studies were realized to understand the intracellular localization of the survivin MB.
In Figure 1, we show confocal microscopy images of A549 cells incubated with the ER marker for 30 min (Figure 1A) and with the nano-complex consisting of the MB loaded onto non fluorescent PMMA-NPs for 90 minutes (Figure 1B).

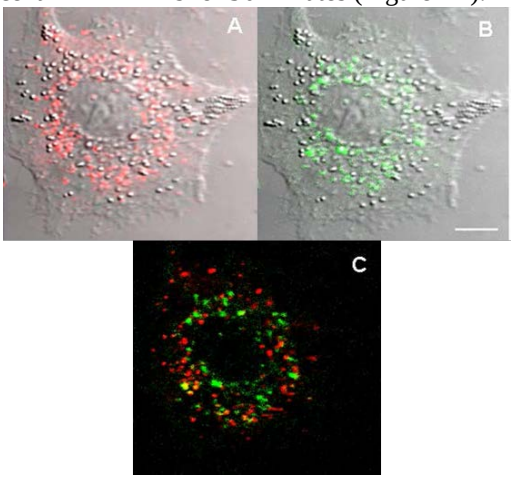

Fig. 1 Confocal images of a single A549 cell treated with ER tracker (Green) and with the MB (Red) loaded onto non-fluorescent PMMA-NPs. A) Merged transmission and Red fluorescence images; B) Merged transmission and Green fluorescence images. C) merged Red and Green fluorescence images. Scale bar 10 µm.

The red fluorescence of the MB is co-localized with the green signal of the ER marker: these data demonstrate that the MB opens up in proximity of the ER where the target mRNA is presumably located.

3. Conclusion

In this work, we have demonstrated that PMMA-NPs promote endocytosis under the described experimental conditions. They co-localize with lysosomes and then they are excreted by lysosomal exocytosis. Furthermore, the opening of the MB occurs in proximity of the ER where it binds with the target survivin mRNA.

References

1. Adinolfi, B., Pellegrino, M., Giannetti, A., et al., *Biosens. Bioelectron.* **88**, 15-24 (2017).