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Intracellular Sensing by a Survivin Molecular Beacon Coupled to PMMA Nanoparticles in Human Cancer Cells †

Barbara Adinolfi [1,*], Sara Tombelli [1], Mario Pellegrino [2], Cosimo Trono [1], Ambra Giannetti [1], Greta Varchi [3], Giovanna Sotgiu [3], Marco Ballestri [3] and Francesco Baldini [1]

[1] Istituto di Fisica Applicata "Nello Carrara", Consiglio Nazionale delle Ricerche; email1@gmail.com (S.T.); email2@gmail.com (C.T.); email3@gmail.com (A.G.); email4@gmail.com (F.B.)

[2] Istituto Nazionale di Ottica, Consiglio Nazionale delle Ricerche; email5@gmail.com (M.P.)

[3] Istituto per la Sintesi Organica e la Fotoreattività, Consiglio Nazionale delle Ricerche; email6@gmail.com (G.V.); email7@gmail.com (G.S.); email8@gmail.com (M.B.)

* Correspondence: b.adinolfi@ifac.cnr.it

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Abstract: In this work biocompatible polymethylmethacrylate nanoparticles (PMMA-NPs) were used as carrier of a molecular beacon (MB) for sensing survivin mRNA in cancer cells. MBs are oligonucleotide sequences generating a fluorescent signal when they hybridize with their target. They 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. NPs offer numerous advantages over conventional drug delivery approaches, such as the possibility of multiple functionalization for improving the imaging, diagnosis and targeted therapy. In particular, PMMA-NPs used in this study consist of a hydrophobic PMMA core covalently functionalized with fluorescein and an external hydrophilic shell decorated with primary amine groups and quaternary ammonium salts. The aim of the work was to evaluate by confocal microscopy and fluorescence measurements: (a) the ability of PMMA-NPs to promote, in human A549 cancer cells, the internalization of a MB specific for survivin mRNA; (b) the involvement of endocytosis in the NP uptake; (c) the NP fate at different times of cell incubation to verify their localization in lysosomes; (d) the MB localization on the Endoplasmic Reticulum (ER) where the target mRNA is located. The results obtained demonstrated: (a) PMMA-NPs efficiently promote the MB internalization generating a specific fluorescent signal in the presence of survivin mRNA expression; (b) the involvement of endocytosis in the NP uptake; (c) the NP localization in lysosomes at different times of cell incubation and their subsequent release in the cell culture medium; (d) the MB fluorescence localization in proximity of the ER where the target mRNA is presumably located.

Keywords: molecular beacon; nanoparticles; survivin; cancer; sensing