Survivin-directed molecular beacon as potential 'theranostic agent' in melanoma cells

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The intracellular transport of sensors and drugs by nanoparticles is one of the new frontiers in biomedical nanotechnology (Rimessi et al., 2009) and may have a strong impact in the field of theranostics, the integration of therapy and diagnostics (Choi et al., 2012). The use of an antisense oligonucleotide (which acts as molecular beacon) able to generate a fluorescent signal when it hybridizes with the target mRNA, may represent an innovative strategy that conjugates the ability of imaging with the pharmacological silencing activity.
Survivin is a multifunctional protein that may play a role in melanoma development and progression (McKenzie et al., 2012). Survivin is overexpressed in cancer cells while it is undetectable in most healthy tissues. In the current study, we investigated the pharmacological activity of molecular beacon-oligodeoxynucleotide (MB-ODN) targeting survivin mRNA, as potential anticancer strategy against human melanoma.
Experiments were performed on the human melanoma A375 cell line, using human monocytes as negative control. RT-PCR and western blot were used to analyse survivin mRNA and protein expression, respectively. MB-ODN was firstly transfected by the classical lipid agent, lipofectamine, and experiments on the effect induced by MB-ODN delivered by polymethylmethacrylate nanoparticles (PMMA NPs) are in progress. The fluorescence signal of MB-ODN delivered by lipofectamine and nanoparticles conjugated with fluoresceine in living cells was evaluated by confocal microscopy at different time points. Cell viability was determined by colorimetric method (WST-1 assay). All experiments were performed in triplicate. Our findings demonstrated a fluorescence increase in the cytoplasm 1 h after beginning the transfection without evidence of fluorescence signals in the extracellular environment. Moreover, no fluorescence was observed in transfected cells that did not express survivin. Cytoplasmic blebbing typical of the apoptotic process became evident after 3 h exposure to MB-ODN. MB-ODN induced cytotoxicity in A375 cells with the maximal effect observed after 6 h (reduction in cell viability of 71.6%, as compared to control; $p<0.05$), while transfection of non-silencing control siRNA and ODN did not affect cell viability. Western blot analysis demonstrated that MB-ODN significantly decreased survivin expression in A375 cells, thus confirming the pharmacological silencing activity of MB-ODN. Experiments performed on human lung carcinoma A549 cells provided preliminary evidence of the subcellular distribution of PMMA nanoparticles in living cells and further experiments in A375 cells by using the MB adsorbed onto PMMA nanoparticles are in progress. In conclusion, our data indicate that MB-ODN may represent a specific molecular probe for survivin detection in melanoma living cells and a potential novel pharmacological strategy for preventing, overcoming or reversing drug resistance in human cancer.

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