MiRNA: Delivery and imaging of miRNAs by multifunctional carbon nanotubes and circulating miRNAs as innovative therapeutic and diagnostic tools for pediatric pulmonary hypertension

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We participate to the applied research project PE-2011-02347026 (2015-2018) financed by the Italian Ministry of Health, in collaboration with the Gene Expression – Microarrays Laboratory, Bambino Gesù Children's Hospital-IRCCS in Rome and the University/British Heart Foundation Centre for Cardiovascular Science, The Queen's Medical Research Institute, University of Edinburgh, UK, devoted to "Delivery and imaging of miRNAs by multifunctional carbon nanotubes and circulating miRNAs as innovative therapeutic and diagnostic tools for paediatric pulmonary hypertension".

Despite the advent of efficacious therapies for pediatric pulmonary hypertension (PH), prevention remains a priority. There are few therapeutic options currently available for children with PH. Response to treatment is less predictable and close monitoring is required in these patients. Therefore, novel therapeutic and diagnostic strategies are needed. The advent of nanotechnology offers novel possibilities for biomedical applications. Carbon nanotubes (CNTs) are promising non-toxic drug delivery vectors owing to their great ability to cross cellular membranes, already employed for the delivery of small interfering RNAs (siRNAs). MicroRNAs (miRNAs), similar to siRNAs, have a crucial role in controlling fundamental pathways underlying PH. Thus, the targeted delivery of miRNAs mimics/inhibitors by CNTs could help to modulate these processes, contributing to limit the outcome of PH. The project assumes that the low toxicity of multifunctional CNTs together with their ability to cross easily cellular membranes can facilitate the safe and targeted delivery of specific genes and/or miRNA mimics/decoys ultimately determining a fine control of PH disease. In fact, many genes and miRNAs have been reported to finely modulate the outcomes of PH. Furthermore, circulating miRNAs (from blood serum) can represent novel biomarkers and a useful tool to diagnose and classify different forms of PH.

Project objectives:

Preparation of soluble multifunctional carbon nanotubes. Pristine and carboxylic carbon nanotubes (CNTs and CNTs-COOH, respectively) will be coated with (or bound to) natural or synthetic polyamine polymers (i.e., chitosan, polyethylenimine, etc.) or polyamidoamine dendrimers (i.e., PAMAM) for gene/miRNA delivery into primary endothelial cells; functionalization with Near-IR probes, contrast agents and/or antibodies/aptamers will confer CNTs additional cellular recognition and imaging properties (multifunctionality).
In vitro and in vivo assessment of gene and/or miRNA delivery by nanotubes and evaluation of transfection efficiency and efficacy. Pulmonary artery endothelial cells and well established mice models of PH (monocrotaline s.c. injection or BMPR2 R899X knock-in mice) will be employed to test: i) citotoxicity, ii) gene/miRNA transfection efficiency by CNTs/CNTs-COOH,
iii) to evaluate the in vivo distribution and targeting abilities of CNTs/CNT-COOH complexes and the gene/miRNAs expression modifications after their transfection, iv) to obtain a functional classification (Gene Ontology) of modulated genes and dissect biological processes related to PH and v) to evaluate in vivo the different PH outcomes (right ventricular systolic pressure, lung vascularization, pulmonary

vessel thickness and vascular cells apoptosis and proliferation) after gene/miRNA transfection by nanotubes.
Evaluation of circulating miRNAs for diagnostic applications. Expression levels of serum miRNAs of a group of at least 20 children affected by secondary PH (compared with 20 patients affected by cardiomyopathy without PH and 20 healthy controls) will be evaluated by extensive profiling and correlated to those miRNAs found deregulated in vitro and in vivo, with the final aim to identify candidate molecular biomarkers of disease and novel potential therapeutic targets.

Technical progress in 2015

We used pristine and carboxy (COOH) functionalized carbon nanotubes (CNTs), obtained following well-established methodologies [1], for preparing functional nanomaterials coated with polyethylenimine (PEI) and polyamidoamine (PAMAM) polymers. The latter have been employed to obtain drug delivery vectors for in vitro and in vivo biomedical applications [2,3].
MicroRNAs (miRNAs) directly regulate gene expression at a post-transcriptional level and represent an attractive therapeutic target for a wide range of diseases. We developed a novel strategy for delivery of miRNAs to endothelial cells (ECs) to regulate angiogenesis, using polymer functionalized carbon nanotubes (CNTs). CNTs were coated with two different polymers, polyethyleneimine (PEI) or polyamidoamine dendrimer (PAMAM), followed by conjugation of miR-503 oligonucleotides as recognized regulators of angiogenesis. Simple physicochemical adsorption allowed us to coat CNTs, whereas polymer-conjugated CNTs have been obtained by amide bonds formation by taking advantage of COOH functionalities. Different functionalized CNTs display different solubility and toxicity properties, as a function of the polymer molecular weight. We evaluated the properties of these functional nanomaterials (i.e. DNA binding ability, toxicity, transfection efficiency) by specific in vitro assays (i.e., gel electrophoresis, cell cultures and fluorescence microscopy) on human primary endothelial cells.
We demonstrated a reduced toxicity and free radical-generation for both polymer-coated CNTs, compared with pristine CNTs or polymers alone. ECs displayed an efficient uptake of miR-503, following treatment with miR-503 bound to polymer-coated CNTs, with PAMAM-coated CNTs showing a higher efficacy of transfection compared to PEI-CNTs. The functionality of PAMAM-CNT-miR- 503 complexes was further demonstrated in ECs in vitro through regulation of the target gene CDC25A, and on cell proliferation and sprouting in a Matrigel assay. Moreover, we demonstrated that PAMAM-CNTs increase the stability of miR-503 oligonucleotides in an in vivo mouse model of angiogenesis, where local release of miR-503 precursors by PAMAM-coated CNTs decreased vessel formation. This comprehensive series of experiments demonstrates that the use of polyamine functionalized CNTs to deliver miRNAs is a novel and effective means to regulate angiogenesis.
[1] Bellucci, S. 2005, Carbon nanotubes: physics and applications. Phys. Stat. Sol. (c). 2: 34–47.
[2] Masotti, A. et al. 2007. Physicochemical and biological study of selected hydrophobic polyethylenimine-based polycationic liposomes and their complexes with DNA. Bioorg Med Chem. 15(3): 1504-15.
[3] Masotti, A. et al. 2008. A novel near-infrared indocyanine dye-polyethylenimine conjugate allows DNA delivery imaging in vivo. Bioconjug Chem. 19(5): 983-7.

List of Conference Talks by LNF Authors in the Year 2015

S. Bistarelli, Innovative therapeutic and diagnostic tools based on delivery and imaging of miRNAs by multifunctional carbon nanotubes, Energy Materials and Nanotechnology (EMN) Meeting, Cancun (Mexico), 8-9 June 2015

S. Bellucci, What Next in Condensed Matter, INFN-LNF, Frascati (Italy), Feb 27, 2015.

S. Bellucci, Research Seminar, Unical Cosenza (Italy), 10 March 2015

S. Bellucci, Carbon nanostructures in biology and medicine, Nanomeeting 2015, Minsk (Belarus), 26-29 May 2015

S. Bellucci, Engineered Nanomaterials Health Effects, Nanoscience and Nanotechnology 2015, INFN-LNF Frascati (Italy), 28 september - 02 October 2015.

S. Bellucci, Regulation of angiogenesis through the efficient delivery of microRNAs into endothelial cells using polyamine-coated carbon nanotubes, Unical Cosenza (Italy), 14 December 2015

Publications by LNF Authors in the Year 2015

Biological interactions of carbon-based nanomaterials: From coronation to degradation, K Bhattacharya, SP Mukherjee, A Gallud, SC Burkert, S Bistarelli, S. Bellucci, M. Bottini, A. Star, B. Fadeel, Nanomedicine: Nanotechnology, Biology and Medicine, Volume 12, Issue 2, February 2016, Pages 333–351

Andrea Masotti, Mark R Miller, Antonella Celluzzi, Lorraine Rose, Federico Micciulla, Patrick WF Hadoke, Stefano Bellucci, Andrea Caporali, Regulation of angiogenesis through the efficient delivery of microRNAs into endothelial cells using polyamine-coated carbon nanotubes, accepted on 16 Feb 2016 for publication in Nanomedicine: Nanotechnology, Biology, and Medicine (Ms. Ref. No.: JN2015522R2)