**PROJECT**

**TITLE:** Reversion of the anti-viral status in pancreatic adenocarcinoma

**ABSTRACT:** Pancreatic adenocarcinoma remains an incurable disease. Oncolytic therapy appears as a promising advance in cancer treatment since traditional anticancer therapies are highly toxic for normal cells and for some types of cancer, as pancreatic cancer, still totally, ineffective.

However, our study identified two molecular phenotypes of pancreatic cancer, characterized by a differential expression of interferon-stimulated genes (ISGs) and easily recognized by the expression of the Myxovirus-resistance A protein. We proved that the two phenotypes are characterized by different permissivity to viral vectors used for gene therapy.

In fact cell lines expressing the interferon stimulated gene MxA resisted to Adenovirus 5 mediated lysis and to GFP Ad5 mediated expression in vitro.

The aims of this proposal are to:
1) understand the mechanism that is leading to the upregulation of the ISGs.
2) designing a new therapeutic combinatory strategy to increase the efficacy of oncolytic therapies by reverting the therapy resistant phenotype.

**BACKGROUND:** The incidence and mortality of pancreatic adenocarcinoma (PDAC) almost coincide and novel therapeutic approaches are needed for this deadly disease. Gene therapy aimed at the delivery of gene functions capable of enhancing cancer cell immunogenicity [1] or inducing oncolysis is a promising approach [2-4].

Viral vectors well suit the purpose of gene therapy and adenoviruses are commonly used gene-delivery vectors due to the efficiency of their in vivo gene transfer [5]. Since 1993, about 300 clinical trials based on adenoviral vectors have been performed [6]. Physiologically, a viral infection stimulates the synthesis of interferons (IFNs) that are then secreted to activate the innate immune response of uninfected neighbouring cells preventing the viral spread. This endogenous immune response is induced by the recognition of viral components by Toll-like receptors and follows a two-step process, consisting in the induction of type I IFNs followed by the transcriptional activation of hundreds of IFN-stimulated genes (ISGs) [7]. In turn, the activation of ISGs promotes the rapid expression of proteins with direct anti-viral function such as the Myxovirus-resistance-A (MxA) protein that protects infected as well as non-infected bystander cells against a wide variety of viruses including adenovirus [8].

Various cancers including melanoma, breast, head and neck, prostate, lung and glioma display transcriptional profiles that suggest the existence of two subgroups of cancer cells distinguishable according to a characteristic IFN and inflammatory chemokines expression pattern [9-10]. Interestingly, Weichselbaum et al. [16] recently reported that IFN-related DNA damage resistance signatures (IRDS) occur in common human cancers and that the expression pattern of ISGs can predict responsiveness of breast cancer to chemotherapy and radiation therapy.
In our group we identified by transcriptional profiling two ISG-defined phenotypes of pancreatic cancer that are readily recognized by immunohistochemistry according to the expression of MxA as a marker of IFN activity. We univocally demonstrated that this signature is characteristic of cancer cells unrelated to the tumor microenvironment [11]. The two phenotypes display diverse permissivity to adenoviral replication in vitro suggesting the practical implication that these signatures could have a key role in the tumor recurrence after oncolytic therapy initial sensitivity. This phenomenon suggest the clear importance that the clarification of the mechanism could bring to the gene therapy cancer field.

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REFERENCES:
PROPOSED PROJECT FOR PHD THESIS:

TASK I: to understand the mechanism that is leading to the upregulation of the ISGs in tumor cells (12 months)
- Since NFkB is one of the key transcription factor for the inflammatory phenotype and activator of downstream expression of ISG’s we will focus on this particular pathway that in preliminary data already proved to be, when silenced, able to down regulate the endogenously expression of ISG15[11].
- The transcription factor will be silenced in MxA positive pancreatic cancer cell lines and time course will be done in order to see the down modulation of the MxA (marker for the ISGs positive phenotype) and functionally the in vitro reversion of the viral resistance.

TASK II: prove in vivo the importance of the “antiviral phenotype” (12 months)
In order to confirm in vivo that the ISGs signature is associated to a higher resistance we will inoculate nude mice subcutaneously respectively with MxA+ and MxA- pancreatic cancer cell lines: CFPAC, the HPAF (MxA+) and the Panc1 and MiaPaCa2 (MxA-). We will then inject intratumor the AdS oncolytic virus selected and will follow the tumor progression. The cured mice will be then kept alive in order to check the time of relapse (usually experimentally around 100 days, optimisation data not shown).

TASK III: Designing a new therapeutic combinatory strategy to increase the efficacy of oncolytic therapies (12 months)
This third task will combine the finding of the previous two tasks.
Based on the results of Task I we will select appropriate treatments that will target the pathway responsible for the antiviral phenotype.
We will then pre-treated the tumors in vitro and in vivo before the treatment with the oncolytic Adenovirus described in task II.
We will evaluate the rate of response and the disease free survival (time to relapse) of cured mice and evaluate in this way the possible improvement of the therapy.

TUTOR EXPERTISE:
From 1999 to 2004 Dr. Monsurrò joined the National Institutes of Health, Bethesda, USA, as a visiting fellow to work in the field of peptide based melanoma vaccines in Dr Rosenberg group (under the supervision of Dr Franco Marincola). Her research work was focused on the study of the lymphocyte subpopulations and on the characterization of the antigen specific cells generated trough peptide based vaccine in melanoma patients.
From 2004 to 2006 she was hired by COSMO bioscience for directing the immunomonitoring section for a gene therapy antigen specific based prostate cancer vaccine.
She is now coordinating from 2006 a new research group at University of Verona focused on the study of tumor microenvironment. She recently published the characterisation of a new antiviral phenotype in pancreatic cancer and her laboratory is now focused on the understanding and the clinical translation of this new finding with the aim to design new and more effective anticancer clinical trials.

She is author of several publications in peer review journal, book chapters and member of the editorial board of international journals.