global cancer control

Section C: Project Description

1. **Project description** - This is the **most important** component of your application. It should be prepared by yourself and have the approval of your home and host supervisors. The description *must not exceed* 2 pages. It must address in *sufficient* scientific, medical and/or technical detail all of the following points:

a. Purpose:

The ultimate goal of the research project is to exploit a novel and recently discovered mechanism to inhibit growth and survival of bladder cancer cells. Human bladder cancer is one of the most commonly diagnosed malignancies in Western societies and its incidence and prevalence are rising. Dysregulation of epidermal growth factor (EGF) and src signaling have been strongly correlated with the transition from superficial to invasive bladder cancer. Therefore, inhibition of EGF signaling represents the main focus of current therapy for this cancer. Recently, we found that PTPD1, an important activator of the src proto-oncogene, regulates mitochondrial respiration and plays a central role in EGF-dependent proliferation and motility of many mammalian cell types, including human bladder cells. We have also discovered that PTPD1 levels are markedly increased in most of the human bladder cancer tissues that we analyzed. Thus, the experiments are designed to selectively interfere with the signaling of PTPD1 and evaluate the effects of this genetic manipulation on growth and progression of human bladder cancer *in vivo*.

The principal aims of this project are the following:

AIM 1. Role of PTPD1 in cancer growth *in vivo*.

AIM 2. Role of PTPD1 in cancer metastases.

b. Background

Bladder cancer is among the most common cancers in Western countries and contributes significantly to overall cancer mortality. The probability of recurrence is in excess of 50%, and stage/grade progression occurs in 10% to 50% of cases (Dinney et al., 2994). Treatment of invasive urothelial carcinomas is ineffective. 50% of patients die from metastases within 2 years of diagnosis, and the 5-year survival rate for metastatic bladder cancer is 6%. Low-grade papillary tumors, which rarely become muscle-invasive, and high-grade invasive tumors, which generally become metastatic, appear to arise by different mechanisms. The former carry mutations that activate the receptor tyrosine kinase-Ras pathway, whereas the latter suffer of loss-of-function mutations in the p53 and Rb pathways. Dysregulation of epidermal growth factor (EGF) signaling has been strongly correlated with the transition from superficial to invasive bladder cancer. Correcting these signaling defects has been proposed as a possible treatment (Oottamasathien et al., 2006). Protein tyrosine phosphatase D1 (PTPD1) is a cytosolic non-receptor tyrosine phosphatase expressed in several tissues (Moller et al., 2004). PTPD1 activates src by dephosphorylating the inhibitory phosphate at Y527, and up-regulates EGF receptor phosphorylation and downstream ERK1/2 signaling. Recent evidence indicates that PTPD1, using distinct molecular modules, forms a stable complex with actin, src tyrosine kinase and FAK (Focal Adhesion Kinase), a scaffold protein kinase enriched at adhesion plaques. Localization of PTPD1 at actin cytoskeleton and adhesion sites is required for cell scattering and migration (Livigni et al., 2006; Carlucci et al., 2008).

c. Methods & Technologies:

AIM 1. To understand whether altered expression of PTPD1 is causally associated with tumour growth *in vivo*, we will examine the consequences of knocking-down PTPD1 in human bladder cancer cells *in vivo* in a cell recombination assay (Oottamasathien S. *et al*. 2006, Gao *et al* 2006). In particular, PTPD1 will be depleted using a lentiviral-RNAi vector in bladder epithelial cells (RT4, derived from a transitional cell papilloma.) followed by recombination with embryonic bladder mesenchyme from embryos of pregnant rats. The recombinants will be mixed with 40 µl collagen mix (5:1 ratio of collagen to setting solution 0.2M NaHCO3, 0.05 M NaOH) and plated individually on dishes. Following overnight incubation at 37°C, cell recombinants will be surgically implanted under the kidney capsule of nude mice and grown for 8 to 10 weeks. We will monitor tumour growth under the renal capsule *in vivo* and we will check the effects on the size of the tumour grafts of $PTPD1_{RNAi}$. One to two months after implantation, we will check by immunohistochemical analysis the morphology and the grade of uroepithelial tumour lesions in RT4 control cells, compared to RT4 where PTPD1 has been knocked-down by RNAi.

AIM 2. Our preliminary results showed that transfection of mouse 3LL cells with a catalytic-inactive mutant of PTPD1 ($PTPD1_{C1108S}$) significantly reduced lung metastases after tail vein injection (Carlucci et al., 2009 in preparation). We wish to extend these studies to human bladder tumour cell lines (RT4 and J82), using nude mice as a model system. Lung metastases will be monitored in nude mice after tail vein or subcutis injection. Tail vein injection was effective in monitoring the effects of PTPD1 on 3LL metastases, and we will initially use this route of administration. However, tail vein injection measures cancer cell extravasation and establishment of a metastatic colony. It does not provide information on the ability of the cells to intravasate from a solid tumour. Accordingly, we will inject treated and control bladder cancer cells subcutis and follow the growth of the tumor and metastases to lung. Cytostatic effects of PTPD1 down-regulation may be evidenced by slower growth of a subcutaneous tumor. We expect to interfere significantly with PTPD1 signaling *in vivo*, reducing blood vessel invasion and lung metastases of bladder tumours.

What transferable skills do you hope to gain and how will you apply these after the Fellowship: The use of lentiviral-RNAi approach targeting PTPD1 in a novel mouse model of human bladder cancer already available at ICR (Columbia University, NY), represents a valuable tool to inhibit bladder cancer growth and progression *in vivo*. My stage at the Institute of Cancer Research, Columbia University, will be a great opportunity to learn these experimental approaches and transfer these procedures to my laboratory in Naples, Italy. Furthermore, the acquisition of such expertise and molecular tools will be of enormous help for our understanding of the molecular mechanisms underlying the human bladder cancer.

d. Relevance of the project to the cancer problem in your country:

In all countries, including Italy, bladder cancer is a major health problem and represents a prevalent cause of death. The morbidity and prevalence of bladder cancer has wide impact in terms of general health and welfare. Thereby, a means of inhibiting carcinogenesis onset and/or progression is a subject of great interest to scientists and physicians world wide. Early cancer detection, targeted therapy, and finally, increased patient survival depend upon an expansion of our understanding of the molecular mechanism underlying to cellular growth and tumor formation. My research project will provide further insights into the mechanism by which PTPD1 signaling regulates growth and progression of human bladder cancer. Inhibiting PTPD1 signaling *in vivo* is expected to provide a valuable and novel tool to be potentially used against bladder cancer.

e. Relevance and potential benefit to activities in the home institute:

My stage at Institute of Cancer Research, Columbia University, will give me the opportunity to learn new experimental approaches aimed to interfere with the growth and progression of malignant bladder tumours *in vivo*. The acquisition of such expertise and molecular tools will be of great help for our research project in Naples, and it will provide important acknowledge for the development of novel molecular tools potentially relevant for detection and therapy of bladder cancer.

f. Reason(s) for choice of host institute:

We have identified a novel gene (PTPD1) whose product regulates the growth factor-dependent proliferation of most mammalian cells and recent evidence in our laboratory indicates that this gene is essential for cell respiration and energy production. Thus, the experiments are designed to selectively interfere with PTPD1 expression/activity and evaluate the effects of this genetic manipulation on growth and progression of bladder cancer. The experiments will be carried out in the laboratory of Dr Max Gottesman, Director of the Institute of Cancer Research (ICR), Columbia University. Dr Gottesman and I have been collaborating for several years in this research project. During this stage at Columbia, as Fellow of UICC, We expect to develop novel tools that selectively inhibit growth and progression of bladder cancer. PTPD1 will be the favorable target molecule of such studies. The ICR at Columbia University provides efficient mouse models of bladder cencer and it is fully equipped to carry out the proposed experiments. The presence of a good working environment and a cooperative atmosphere at ICR will contribute to the success of my stage in Dr's Max Gottesman laboratory.

g. Facilities available upon return to continue the work, apply and disseminate the newly acquired skills:

Our Department of Mooelcular and Cellular Pathology, University of Naples Italy, is adequately equipped to perform most of the biochemical, molecular and cellular studies, as well as it has an animal facility that fullfills all the international control standards. Therefore, upon return to my laboratory in Naples, I will be able to successfully continue the work as well as to disseminate the acknowledge acquired at ICR to collegueas, collaborators and students.

h. References to recent publications in the project field:

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Moller, N.P., Moller, K.B., Lammers, R., Kharitonenkov, A., Sures, I., and Ullrich, A. (1994). Src kinase associates with a member of a distinct subfamily of protein-tyrosine phosphatases containing an ezrin-like domain. Proc. Natl. Acad. Sci. USA 91, 7477-7481.

Oottamasathien S, Williams K, Franco OE, Thomas JC, Saba K, Bhowmick NA, Staack A, Demarco RT, Brock JW 3rd, Hayward SW, Pope JC 4th. (2006) Bladder tissue formation from cultured bladder urothelium. Dev Dyn. 235, 2795-801.

1. **Justification of project duration:** The project will be developed in the following steps: **1.** Set up the experimental conditions for optimal RNAi lentiviral infection of cultured bladder tumour cells (2 weeks); **2.** Infection with RNAi lentiviral infection and recombinant of bladder tumour cells with mesenchime from rat embryos(1 week); **3.** IV or subcutis injection of RNAi infected bladder cells into mice and *in vivo* analysis of tumour growth and metastases (8-10 weeks)