Project proposal for the admission to Doctoral programme in Oncology and Experimental Surgery XXXIV cycle, 2018/2019 academic year

Candidate: Ines Ferrara

Title: Stromal microenvironment, DNA Damage Response and immune context: role of a mutual connection on progression of solid and haematological malignancies.

Background

The Extracellular Matrix (ECM) remodelling represents an essential requirement for the maintenance of tissue homeostasis. The immune cells promote tissue regeneration, contributing to ECM deposition, which is characterized by different members: proteins, structural proteins such as collagens, laminin, tenascin, matricellular proteins (*i.e.* SPARC, Osteopontin (OPN)), glycoproteins, proteoglycans, trombospondins. In a tumour context, ECM displays a disorganized architecture. Moreover, further alterations of stroma contribute to an increase of ECM. Based on cancerous origin, grading and differentiation state, ECM deposition varies, fosters cancer progression, proliferative signals and dissemination of cancer cells through collagen fibers as well as acts as a barrier, preventing drug diffusion [1]. Even non-structural, matricellular proteins modulate severe physiological functions, including ECM deposition, cellular proliferation and survival. All these processes are exploited by neoplastic clones to support tumour growth of primary carcinomas and their metastasis. The group of Prof. Tripodo had demonstrated that Osteopontin (OPN) contributed to metastatic progression. Notably, OPN released by malignant clones impeded apoptosis, whereas l'OPN derived from myeloid cells regulated immunosuppressive activity in local metastatic niches [2]. Afterwards, the group of Prof. Tripodo highlighted that Secreted Protein Acidic and Rich in Cysteine (SPARC), another matricellular protein, had an important regulatory function in stromal remodelling [3]. In high- grade, no low grade breast cancer, an high SPARC expression in Extracellular Matrix, were correlated to an increase of epithelial-to-mesenchymal transition (EMT), decreased treatment response and poor prognosis. In addition, SPARC expression favoured an high immunosuppressive microenvironment, composed by infiltrating T regulatory cells, mast cells, and myeloid-derived suppressor cells (MDSCs) [4].

In the lymphoid malignancies, neoplastic B-cell clones are commonly localized within secondary lymphoid organs (SLO). However, malignant clones can reach the bone marrow (BM) within osteoblastic or the vascular niches, based on their germinal center- or extra-follicular-derivation, respectively. The group of Prof. Tripodo speculated the existence of a common mesenchimal and ECM composition between bone marrow and SLO. Intriguingly, they observed that a SPARC-defective stromal microenvironment exerted a remarkable influence on lymphopoietic function of bone marrow and SLO, modulating the equilibrium between the different B-cell precursors and their differentiated population. It could have implications on progression of lymphoid malignancy [5].

Mechanical signals derived from stromal microenvironment may regulate gene expression of surrounding cells. Nowadays, It is unknown how these stimuli are detected and transducted at molecular level. Signals, such as the topology of surrounding extracellular matrix, might be essential for the comprehension of physiological mechanisms and the pathogenesis of the diseases. YAP and TAZ are two transcriptional co regulators. Interacting with enhancer elements, they transduce, at nucleus, biomechanical stimuli into specific-cell and stress biological effects. YAP and TAZ are members of Hippo signalling cascade, involved into cytoplasmic maintenance and/or degradation. In a physiological context, they are inactive and have a subcellular distribution. Their nuclear accumulation is monitored by cell shape, cytoskeleton tension, and ECM topology and rigidity. Tumour stimuli, including changes into inflammation, oncogenic signalling, and regulation of Hippo pathway, activate YAP and TAZ, inducing cell proliferation, metastatic processes and chemo resistance. Therefore, they might represent potential therapeutic targets [6] [7].

As expected, malignant transformation of somatic cells is characterized by genomic instability. The DNA repair processes can actively participate to suppression of cancer and other human pathologies. The genomic DNA can be damaged through different ways, provoking the alteration of genetic information. Sources of DNA Damage can be both exogenous and endogenous to the cell: UV sunlight, ionizing radiation, chemical compounds in food and smoke, but also reactive cellular metabolites, such as the reactive oxygen species, released during aerobic respiration. However, cells have developed several mechanisms to protect genome integrity. DNA repair processes are parts to a set of events, known as “DNA Damage Response”. Briefly, during earlier DDR steps, sensor proteins are recruited to the lesion: MRE11/NBS1/RAD50 (MRN) complex, KU, PARP. After the damage recognition, three key kinase proteins are activated: ATM, DNA-PK, Ataxia Telangiectasia and Rad3-related (ATR). The action of kinases is increased by mediator proteins, such as 53BP1 and MDC1. The three kinases phosphorylate the histone variant H2AX, known as γH2AX, which is recruited in areas flanking the damage. In turn, phospho-γH2AX recalls a series of DDR proteins (DDR focus). Nevertheless, other post-translational modifications (ubiquitination, acetylation) can be involved into DDR. In the subsequent step to DDR activation, the control of progression of cell cycle occurs: ATM and ATR activate CHK1 and CHK2 (phospho-Check1 and phospho-Check1), blocking the progression of cell cycle, until the damage will be solved [8]. Physical and chemical alterations of nuclear envelope (NE) can induce ATR, which, subsequently, promotes the DDR cascade signalling. ATR defective cells have an aberrant chromatin condensation and nuclear envelope breakdown. Nevertheless, until now, most of studies were focused on classic role of ATR (CHK1 phosphorylation, p53 activation, arrest of cell cycle). Hence, the physiological role of ATR in nuclear envelope homeostasis, the mechanisms which foster ATR in the presence of an altered NE, the possible role of ATR in process of mechanosensing (how the mechanical signals derived from cellular microenvironment regulate cellular behaviours), require further detailed studies. Recently, it has been highlighted that, compromising the integrity of NE by deleting Lamin A, a strong reduction of this phospho-ATR signal occurs, suggesting that the NE can influence the function of ATR [9] [10]. Moreover, it seems to be interesting to investigate the role of PREP-1, a protein involved into nuclear envelope stability and the possible correlation between PREP-1 and ATR. In mouse models, a PREP-1 -/- phenotype is connected to an increase of DNA Damage[11]. An altered regulation of DNA Damage Response, induced by physical and chemical modifications of cell and stromal microenvironment, could be responsible of activaction of oncogenic and inflammatory pathways, which promote tumour growth and progression.

Aim

The main aim of the proposed project is to identify the determinants linking stromal heterogeneity, nuclear envelope stability and dna-dmage response profiles with the intra-tumor genetic and transcriptional heterogeneity of malignant clones, and immune context.

Methods

The experimental work of the project will be structured in three years and will be based on the integration of classical pathological histology methods (histochemical stainings and transmitted light optical microscopy), tissue-based immuno-localization (immunohistochemistry in single and double labeling and immunofluorescence in multiple labeling), molecular biology and cytogenetic analysis applied to tissues and in vivo modeling.

Experimental plan

Aim1

At the beginning, the expression of proteins involved into nuclear envelope stability (Lamin A/C, PREP-1, SUN-1/2) and mechanosensing (ATR, YAP-TAZ) will be examined. These patterns will be correlated to expression profiles of DDR markers (pospho-γH2AX, 53BP1, CHK1), stromatogenesis patterns and immune context in solid and hematologic malignancies. About first aim, our work will be focused on pathologies where It is possible to identify gene expression profiles on imposing cohorts of data (Breast cancer, Diffuse large B cell lymphoma). Primary Hypothesis is that different stromal patterns could define compartmentalisations of tumour and immune elements in nuclear stress and that these patterns can play a role in the state of activation of oncogenic and inflammatory pathways.

Aim2

Mechanosensing profiles and DDR markers will be analysed in tertiary and secondary lymphoid tissues. The purpose will be to establish in tertiary and secondary lymphoid organs if the compartmentalisation of T and B lymphoid elements in areas with different stromal composition (Germinal Center, mantle, marginal, inter-follicular) is associated to expression profiles of specific mechanosensing, nuclear enveloper stability and DDR markers. The tissue analysis will be realised on secondary human and murine lymphoid organs (lymph node and spleen) and tertiary lymphoid tissues associated to primary and metastatic cancers. The ideal identification of mechanosensing, nuclear envelope stability and DDR profiles, linked to specific populations involved in immune responses, will be translated into B and T lymphoid malignancies, characterized by variable differentiation: pre-Germinal Center, Germinal Center-related o post-Germinal Center.

Aim3

The goal will be to investigate functionally the identified profiles in the previous aims by adoption and development of constitutive and inducible transgenic mouse models, in contexts of immunologic challenge and/or tumorigenesis.

Conclusions

The existing relationship among stromal patterns, DNA Damage Response and immune context, could bring to the activation of specific oncogenic and inflammatory pathways. This mutual connection might favour the identification of new potential prognostic and therapeutic targets. The feasibility of the project will be ensured by utilization of the research facilities and laboratories of the PROSAMI Department.

References

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Palermo, 19 / 07 / 2018

Candidate's signature

Ines Tinnirello

UNIVERSITÀ DEGLI STUDI DI PALERMO
DIPARTIMENTO DI SCIENZE PER LA PROMOZIONE DELLA SALUTE E MATERNO INFANTILE "GIUSEPPE D'ALESSANDRO"
Direttore Prof.ssa Anna Giammanco

Letter of Acceptance

Ref. Doctoral programme in Oncology and Experimental Surgery XXXIV cycle, 2018/2019 academic year
Applicant: Ines Ferrara
Head of the hosting lab: Prof. Claudio Tripodo
Title of application: "Stromal microenvironment, DNA Damage Response and immune context: role of a mutual connection on progression of solid and haematological malignancies".

I herein certify my willingness to accept Ines Ferrara in my laboratory along the accomplishment of the experimental activity of the proposed research project entitled "Stromal microenvironment, DNA Damage Response and immune context: role of a mutual connection on progression of solid and haematological malignancies".

Hosting Institution
Ines' proposed research activity will be hosted within my research laboratory, the Tumor Immunology Laboratory of the Department of Health Sciences, University of Palermo. The project will be carried out under my personal supervision and the hosting laboratory will provide the necessary lab space and all the required facilities and infrastructures for proper completion of the experimental plan. The hosting lab, which is integrated within the Human Pathology Section of Department of Health Science (PROSAMI), is part of an international network of research institutions characterized by consolidated collaborations in the field of cancer research and, more specifically, of tumor immunology and microenvironment.

Mentoring activities and Complementary skills training
Ines will be encouraged towards building and consolidating independent research collaborations with leading experts in the field of lymphoma and tumour immunology, who frequently visit my lab acting as advisors of our research activities. I will personally mentor her research activities and constantly supervise her advances through monthly lab meetings, data sessions, and one-to-one briefings.

Date
Palermo 19th July, 2018

Prof. C. Tripodo

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