PROJECT

Supervisor:	VENEREAU Emilie
Title:	Exploiting HMGB1-directed strategies to boost the immune response against cancer
Curriculum:	Immunologia e Oncologia di Base e Applicate
Link to the personal page of the University or relevant hospital site website:	https://research.hsr.it/en/divisions/genetics-and-cell-biology/units/tissue-regeneration-and-homeostasis.html

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

Tumor cells control the functions of the cellular and non-cellular components in the microenvironment by redirecting the non-malignant cells to sustain their own benefit (1). Immune escape is a typical example of the tumor's ability to hijack host functions in its favor. Hence, it is essential to deepen our knowledge on the crosstalk between tumor cells and the immune system to identify new targets for therapeutic intervention. In our laboratory, we study a protein, called High Mobility Group Box 1 (HMGB1), that appears to be a promising therapeutic target in cancer. HMGB1 is a highly conserved nuclear protein that binds to DNA and participates in stabilizing nucleosomes, regulating gene expression and DNA repair (2). In the extracellular medium, HMGB1 acts as a danger signal and triggers "sterile" inflammation and tissue regeneration (3-7). In the context of cancer, the role of HMGB1 remains controversial as it can act as a tumor suppressor or an oncogenic factor depending on the context (8, 9). Most importantly, HMGB1 released by dying tumor cells contributes to the activation of the immune response against malignant cells (10, 11). Intriguingly, the fate of HMGB1 after its release in the tumor microenvironment remains completely unknown. Interestingly, we recently uncovered the swapping of HMGB1 protein between tumor cells and their microenvironment (Ruggieri E *et al.*, in revision).

Rationale and hypothesis

Our preliminary data indicate that cancer cells are the primary source of HMGB1 within tumors, and that tumor-derived HMGB1 is taken up by cells in the tumor microenvironment, including immune cells. In addition, we observed that mice lacking HMGB1 in hematopoietic cells display resistance to oxaliplatin, a well-known chemotherapy drug that induces immunogenic cell death with HMGB1 release by tumor cells. Taken together, both published findings and our preliminary data led us to hypothesize that extracellular HMGB1 may be recycled by tumor cells to support their survival and facilitate immune evasion. Accordingly, our aim is to track HMGB1 released by tumor cells following chemotherapy. These experiments will provide a unique opportunity to investigate HMGB1 trafficking *in vivo* within the tumor microenvironment in response to chemotherapy, potentially yielding critical insights into its role in therapeutic response. Furthermore, the project aims to dissect the contribution of HMGB1 to the anti-tumor immune response by identifying its key cellular sources within leukocyte subpopulations, and to assess whether HMGB1 internalization by immune cells influences their phenotype and functional properties.

Objectives and specific aims

The objective of this project is to investigate whether the recycling of HMGB1 contributes to immune escape in a mouse model of lung cancer. To address this question, the student will track HMGB1 released by tumor cells following chemotherapy in wild-type mice, using cutting-edge imaging technologies such as ImageStream, which combines flow cytometry with immunofluorescence imaging, together with novel tools developed in the laboratory (e.g., tumor cells expressing fluorescently tagged HMGB1).

The second objective is to dissect the contribution of HMGB1 to the anti-tumor immune response by identifying its key cellular sources within leukocyte subpopulations. To this end, the student will use conditional transgenic mouse models to determine whether HMGB1 derived from myeloid cells, dendritic cells, and/or $CD8^+$ T cells contributes to the anti-tumor immune response. In addition, RNA-seq analyses will be performed to further characterize the molecular mechanisms underlying HMGB1 recycling in leukocyte populations.

Expected outcomes

By characterizing HMGB1 swapping in the tumor microenvironment upon chemotherapy and by identifying the leukocyte populations delivering HMGB1 to trigger the antitumor response, we aim to shed light on its precise role in the anti-cancer immunity, which might pave the way for exploiting HMGB1 to boost the immune response against cancer.

Skills that the student should acquire (max. 600 characters including spaces):

- Technical skills: cell culture (primary cells and cell lines), molecular biology (e.g. Q-PCR), imaging (e.g. confocal microscopy, ImageStream), flow cytometry, histology, mouse handling (model of cancer, treatments with recombinant protein and chemotherapy), production of recombinant protein, RNAseq analyses.
- IT skills: Microsoft Office, GraphPad Prism, online resources and tools (NCBI), Flow Cytometry Analysis, ImageJ, Photoshop, Inkscape.
- Communication skills: oral/poster presentations (lab meetings, internal seminars, national and international conferences), article writing (research articles and reviews).

References (max. 15)

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7- Rebalancing expression of HMGB1 redox isoforms to counteract muscular dystrophy. Careccia G, Saclier M, Tirone M, Ruggieri E, Principi E, Raffaghello L, Torchio S, Recchia D, Canepari M, Gorzanelli A, Ferrara M, Castellani P, Rubartelli A, Rovere-Querini P, Casalgrandi M, Preti A, Lorenzetti I, Bruno C, Bottinelli R, Brunelli S, Previtali SC, Bianchi ME, Messina G, Vénéreau E. Sci Transl Med. 2021 Jun 2;13(596):eaay8416. doi: 10.1126/scitranslmed.aay8416.

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9- HMGB1 in cancer: good, bad, or both? Rui Kang 1, Qiuhong Zhang, Herbert J Zeh 3rd, Michael T Lotze, Daolin Tang. Clin Cancer Res. 2013 Aug 1;19(15):4046-57. doi: 10.1158/1078-0432.CCR-13-0495. Epub 2013 May 30.
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