PROGETTO

Supervisore::	Samuel Zambrnano
Titolo/*Title*:	Role of macrophages NF-kB signaling dynamics in its interaction with tumor cells
Curriculum:	Biologia cellulare e molecolare
Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento:	https://www.unisr.it/en/docenti/z/zambrano-silva-samuel

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

Malignant mesothelioma (MM) is a devastating disease where macrophages (MOs) play a fundamental role (1). Our laboratory has demonstrated that the alarmin High Mobility Group Box 1 (HMGB1) protein plays a central role in this context (2), contributing to MOs recruitment and the formation of the tumor microenvironment. MOs can be polarized towards anti-tumor (M1) or immunosuppressive, pro-tumoral (M2) phenotypes (4); the latter being dominant in MM (1). Our laboratory has demonstrated that BoxA (5), a molecule that antagonizes the activity of HMGB1, induces phagocytosis of tumor cells by MOs, and stimulates the adaptive immune system against the tumor, leading to tumor remission and the development of immune memory. However, the precise molecular mechanisms of this phenotypic switch are unknown.

Rationale and hypothesis

HMGB1 (alone or forming complexes with its ligand, CXCL12) interacts with the TLR4, RAGE and CXCR4 receptors, present both on tumor MM cells and MOs. These receptors are located upstream of the transcription factor NF-κB, a key regulator of chemokine and cytokine expression in the TME (3, 6, 7) and fundamental for MO polarization, antigen presentation, and phagocytosis (8). Recent studies performed on MOs confirm the functional role of NF-κB activation dynamics that we propose (9): NF-κB dynamics determines the specificity of response to stimuli (10) and their epigenetic reprogramming (11). However, how NF-κB dynamics determine MOs behaviour within the tumor is unknown. We hypothesize that NF-κB dynamics is

a key determinant of macrophage behaviour in the TME, fundamental to tilt the balance from tumor progression to response to therapy.

Objectives and specific aims

1) Evaluate the effects of anti-tumor treatments: BoxA and CXCL12 in macrophages from a mouse model that is also a reporter of NF-κB

2) Provide, thanks to a novel reporter model (12) and advanced imaging techniques based on those developed by our group in the past years (13, 14, 15) a dynamic and single cell characterization of the NF-κB activation status for live macrophages directly within tumors, also in response to treatment.

3) Characterize (using 2D and 3D in vitro co-culture models) how the activation of the NF-κB signaling pathway in MOs and interaction with MM cells contributes to their polarization into an immunosuppressive phenotype, and how BoxA and CXCL12 manage to reverse this process.

Expected outcomes

At the moment there are only a few therapeutic options for mesothelioma patients. Deepening our knowledge of how the NF-κB signaling pathway contributes to determining the response of the innate immune system and, in particular, of macrophages which play a key role in tumor progression, would allow new therapeutic approaches and offer patients a therapeutic alternative to current treatments Furthermore, our combination of in-vivo and in-vitro microscopy approaches will put us in a position to develop realistic 3D culture systems further developed as a drug-screening systems.

Competenze che deve acquisire lo studente (Max 600 caratteri spazi inclusi):

Cell culture of MM cell lines.

Mouse handling and administering treatments.

Use of syngeneic tumor models and of bioluminescence systems to monitor tumor growth.

Primary macrophage derivation.

2D culture and 3D culture procedures with spheroids.

Live cell imaging and imaging data analysis in 2D and 3D.

Preparation for intravital imaging.

Generation of RNA-seq data.

Bibliografia (max. 15)

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