PROGETTO

Supervisore:*:* ____Giovanna Musco_____

Titolo/ *Title:* Understanding the molecular details of DFL inhibition activity on HMGB1/CXCL12/CXCR4 axis

Curriculum: _____ Biologia Cellulare e Molecolare

Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento: _____https://research.hsr.it/en/divisions/genetics-and-cell-biology/biomolecular-nuclear-magnetic-resonance/giovanna-musco.html_____

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

HMGB1 is a key molecule that both triggers and sustains inflammation following infection or injury, and is involved in a large number of pathologies, including cancer. HMGB1 participates in the recruitment of inflammatory cells, forming a heterocomplex with the chemokine CXCL12 (HMGB1·CXCL12) (1), thereby activating the G-protein coupled receptor CXCR4. Identification of molecules that disrupt this heterocomplex can and/or interfere with the HMGB1/CXCL12/CXCR4 offer novel pharmacological opportunities to treat inflammation-related diseases.

Work performed in our lab has shown that indeed the HMGB1●CXCL12 heterocomplex is druggable and that the salicylate derivative diflunisal (DFL) is able to target and disassemble the HMGB1●CXCL12 hetero-complex and to block immune cell recruitment (2).

Rationale and hypothesis

The molecular details underlying HMGB1●CXCL12 interaction with CXCR4 and of its pharmacological inhibition through DFL are still unexplored. The complexity and the dynamics of the proteins requires a combination of different techniques to tackle the problem.

Objectives and specific aims

In this project the candidate will study

1. the molecular details of HMGB1●CXCL12●CXCR4 complex
2. investigate whether DFL physically interferes in the interaction of HMGB1●CXCL12 with CXCR4
3. investigate how DFL modulates the HMGB1/CXCL12/CXCR4 axis at cellular level

Expected outcomes

We expect to

1. obtain the first 3D structure of the ternary complex HMGB1●CXCL12•CXCR4
2. to understand the effects of DFL on the ternary complex and give a structural rationale of its inhibitory effect of the HMGB1●CXCL12•CXCR4 axis. This knowledge will create the basis for the design of improved molecules able to interfere with this relevant inflammatory axis.

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

The student will learn: classical techniques for protein expression(in E. Coli and insect cells) and purification, biophysical techniques for structural and thermodynamic characterization of protein-protein interactions (Nuclear Magnetic Resonance, Isothermal titration calorimetry, microscale thermophoresis; cryo-EM Analytical ultracentrifugation). The candidate will be also introduced to cell proliferation, cell migration and proximity ligation assays.

Bibliografia (max. 15)

(1) Mantonico et al. The acidic intrinsically disordered region of the inflammatory mediator HMGB1 mediates fuzzy interactions with CXCL12 Nat. Comm. https://doi.org/10.1038/s41467-024-45505-7

(2) De Leo et al. Diflunisal targets the HMGB1/CXCL12 heterocomplex and blocks immune cell recruitment EMBO Reports (2019)20:e47788