PROGETTO

Supervisore::	Emilie Venereau
Titolo/*Title*:	Preclinical Study of engineered HMGB1 proteins as Drug Candidates for Regenerative Medicine Applications
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/units/tissue-regeneration-and-homeostasis.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

A fundamental aspect in regenerative medicine is to provide the appropriate environment for cells to successfully repair the injured tissue. Based on our findings, we propose the High mobility group box 1 (HMGB1) protein as a drug candidate to accomplish this essential function. HMGB1 is a nuclear protein released upon tissue injury to signal danger (1-2). We showed that oxidation of HMGB1 switches its activities from tissue regeneration to inflammation, in particular in skeletal muscle both in acute and chronic conditions (3-5). Notably, our recent study revealed that oxidative stress, which contributes to the progression of Duchenne Muscular Dystrophy (DMD), converts HMGB1 into its pro-inflammatory isoform in dystrophic muscle which in turn worsens inflammation and associated muscle degeneration (6). We generated a non-oxidizable mutant of HMGB1 (3S), that improves regeneration in mouse models of acute injury in muscle, liver, bone and hematopoietic compartment but also in DMD and limb-girdle muscular dystrophy (5-7), revealing that this engineered protein represents a promising drug candidate for regenerative medicine. Intriguingly, the fate of HMGB1 after its release upon tissue injury remains completely unknown. but, we recently uncovered its recycling in regenerating muscle (unpublished data).

Rationale and hypothesis

Our findings led us to hypothesize that the recycling of extracellular HMGB1 in injured muscle might limit or potentiate its regenerative properties according to the context. We engineered

different HMGB1 proteins that cannot be recycled by regenerating muscle to determine whether this recycling contributes to its regenerative properties and if these proteins might be possible drug candidates to promote tissue regeneration.

Objectives and specific aims

The objective of the project is to investigate whether HMGB1 recycling process contributes to muscle regeneration in both acute (cardiotoxin injection) and chronic conditions (DMD mouse and rat models) by conducting a preclinical evaluation of our engineered HMGB1 proteins to assess their therapeutic properties on muscle regeneration. The student will integrate *in vivo* investigation exploiting mouse models of muscle injury alongside *ex vivo* experiments on muscle stem cells and isolated myofibers. She/he will take advantage of innovative tools developed in our laboratory, such as HMGB1 engineered proteins, and will employ histological and RNAseq analyses together with functional tests (e.g., rotarod, treadmill) and cutting edge techniques (e.g. IncuCyte, Image Stream).

Expected outcomes

This research may lead to the identification of HMGB1 engineered proteins as promising drug candidates and more in general of the development of strategies targeting HMGB1 recycling to offer innovative therapies for regenerative medicine in skeletal muscle and potentially other tissues in the future.

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

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

Bibliografia (max. 15)

1- Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Scaffidi P, Misteli T, Bianchi ME. Nature. 2002 Jul 11;418(6894):191-5. doi: 10.1038/nature00858.

2- The multifunctional protein HMGB1: 50 years of discovery. Tang D, Kang R, Zeh HJ, Lotze MT. Nat Rev Immunol. 2023 Dec;23(12):824-841. doi: 10.1038/s41577-023-00894-6.
3- Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. Venereau E, Casalgrandi M, Schiraldi M, Antoine DJ, Cattaneo A, De Marchis F, Liu J, Antonelli A, Preti A, Raeli L, Shams SS, Yang H, Varani L, Andersson U, Tracey KJ, Bachi A, Uguccioni M, Bianchi ME. J Exp Med. 2012 Aug 27;209(9):1519-28. doi: 10.1084/jem.20120189. Epub 2012 Aug 6.
4- High-mobility group box 1 protein orchestrates responses to tissue damage via inflammation, innate and adaptive immunity, and tissue repair. Bianchi ME, Crippa MP, Manfredi AA, Mezzapelle R, Rovere Querini P, Venereau E. Immunol Rev. 2017 Nov;280(1):74-82. doi: 10.1111/imr.12601.
5 -High mobility group box 1 orchestrates tissue regeneration via CXCR4. Tirone M, Tran NL, Ceriotti C, Gorzanelli A, Canepari M, Bottinelli R, Raucci A, Di Maggio S, Santiago C, Mellado M, Saclier M, François S, Careccia G, He M, De Marchis F, Conti V, Ben Larbi S, Cuvellier S, Casalgrandi M, Preti A, Chazaud B, Al-Abed Y, Messina G, Sitia G, Brunelli S, Bianchi ME, Vénéreau E. J Exp Med. 2018 Jan 2;215(1):303-318. doi: 10.1084/jem.20160217. Epub 2017 Dec 4.
6- 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.
7- Fully reduced HMGB1 accelerates the regeneration of multiple tissues by transitioning stem cells to G(Alert). Lee G, Espirito Santo AI, Zwingenberger S, Cai L, Vogl T, Feldmann M, Horwood NJ, Chan JK, Nanchahal J. Proc Natl Acad Sci U S A. 2018 May 8;115(19):E4463-E4472.