UNIVERSITÀ DEGLI STUDI DI MILANO

D-MEM PhD-UNIMI | Doctorate program Milan EXPERIMENTAL MEDICINE

Priming of Mesenchymal Stem/ Stromal Cells to increase their secretome therapeutic potential in counteracting osteoarthritis through EV action

Supervisor Anna Teresa Brini – email: anna.brini@unimi.it

Laboratory of Mesenchymal Stem Cells Pharmacology and Regenerative Medicine Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Università degli Studi di Milano

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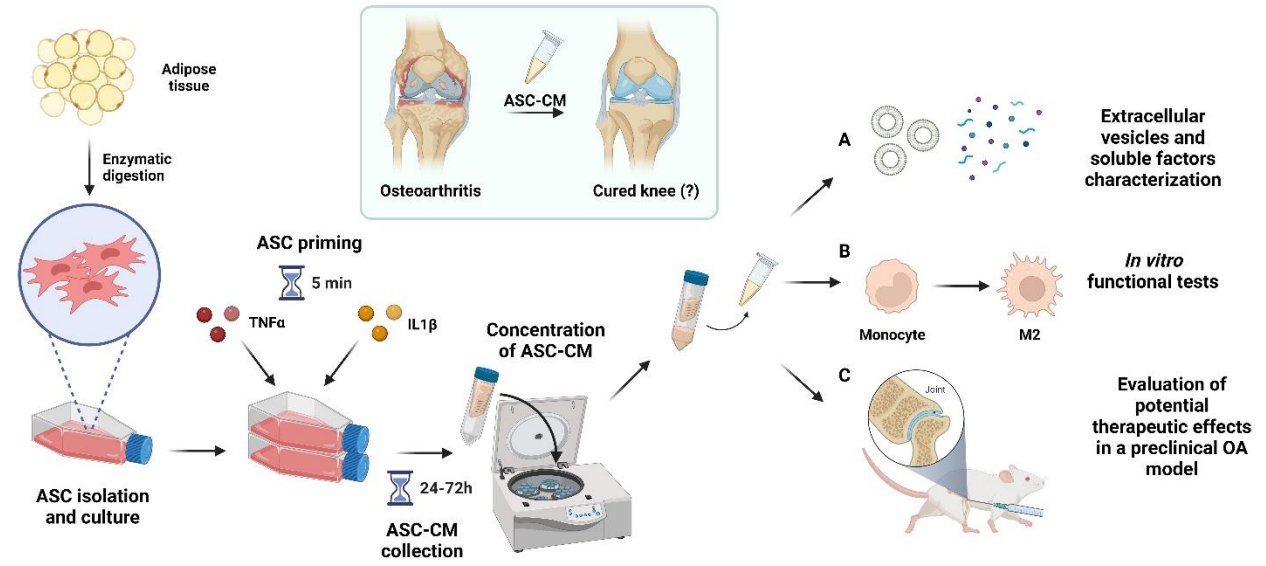
Graphical abstract



Priming of adipose-derived MSC to increase their secretome therapeutic potential in counteracting osteoarthritis through EV action

Laboratory of Biological Applications - Prof. Brini

Project description

Osteoarthritis (OA) is the most common degenerative joint disease. It affects all articular tissues, such as the articular cartilage, the subchondral bone and the synovial membrane. During the progression of OA, the functional and structural properties of these tissues undergo marked alterations.The pathological process of OA exhibits several hallmarks, starting from the hypertrophic shift of articular chondrocytes, whose enhanced proliferation and altered gene expression lead to cartilage degradation. Over time, new bone formation at the joint margins and inflammation occurs. To date, the tissue damage cannot be inhibited nor reverted. Moreover, current strategies for pain and inflammation management are not satisfactory and are often associated with substantial adverse effects.

While searching for novel effective treatments, mesenchymal stem/stromal cells (MSC) and their secretome have been successfully applied to ameliorate pain, reduce inflammation and revert chondrocytes hypertrophy while supporting tissue healing (e.g. in Amodeo et al 2021; doi:10.1016/j.bbi.2021.03.011, Giannasi/Niada 2020; doi: 10.1186/s13287-020-02035-5, Niada/Giannasi 2019; doi: 10.1016/j.scr.2019.101463, D'arrigo et al 2019; doi: 10.3390/jcm8111867). Cell secretome is a particularly convenient cell product since it avoids any possible risk of long term side effect by cell injection.

Despite promising evidences, there are still points to be addressed for conditioned medium optimization:

- differences between MSCs from different donors
- the need for massive in vitro cell expansion;
- the lack of appropriate *in vitro* tests to assess the efficacy of the product

In recent years, MSC priming, which consists in conditioning cells for specific functions by the use of inflammatory cytokines or mediators, hypoxia, pharmacological drugs, chemical agents, biomaterials and different culture conditions, has been largely investigated (Noronha et al 2019; doi: 10.1186/s13287-019-1224-y).

With our project, we aim at identifying specific priming conditions to improve the anti-inflammatory, analgesic and anti-hypertrophic potential of Adipose-derived MSC (ASC) secretome, in the OA context.

Cells will be primed with TNF-alpha and IL-1 beta, two cytokines particularly important in the pathophysiology of OA, while ASC-CM (ASC conditioned medium) will be collected at different time points. Its composition in terms of extracellular vesicles will be investigated by nanotracking analysis and other tecniques. Moreover, the secretome composition will be studied by proteomic and lipidomic analysis and also by investigating specific cytokines and growth factors (such as IL-10, IL-1Ra, HGF eTGF-β1). Appropriate *in vitro* tests, available in the lab, will be performed to investigate ASC_CM action. Human articular chondrocytes will be stimulated by inflammatory cytokines and treated with different ASC-CM preparations. The activity of matrix degrading enzymes (MMPs) and hypertrophic markers will be evaluated (Giannasi/Niada 2020; doi: 10.1186/s13287-020-02035-5, Niada/Giannasi 2019; doi: 10.1016/j.scr.2019.101463).

The effect of ASC-CM on immune cells will be investigated. In particular, we will study whether different CM will be able to induce macrophage M2 polarization (anti-inflammatory phenotype), by evaluating specific M2 and M1 markers (such as CD106, CD165, CD86, CD80 and CCR7). Lastly, the most promising cell products in terms of composition and in vitro activity will be tested in an in vivo model of OA (monoiodoacetate intrarticular injection), associated with chronic and neuropathic pain (Amodeo et al 2021; doi:10.1016/j.bbi.2021.03.011) (collaboration with Prof. P. Sacerdote, University of Milan).

Pain, functional and physical recovery parameters will be evaluated. Moreover, cytokines and other inflammatory markers will be quantified at articular and nervous system levels.
With this project, we expect to unravel the effect of pro-inflammatory cytokines on ASC secretome features while also aiming at identifying priming conditions that might lead to a more powerful therapeutic tool in OA management.

Candidate specific requirements

Strong scientific motivation with a hardworking attitude. Any experience in cell culture and molecular biology is appreciated together with the skill in problem solving. Strong commitment to basic and translational research and good group comunications are welcome.

Most relevant publications for the project

Giannasi C, Mangiavini L, Niada S, Colombo A, Della Morte E, Vismara V, Ambrosanio A, Savadori P, Casati S, Peretti GM, Brini AT. Human Osteochondral Explants as an Ex Vivo Model of Osteoarthritis for the Assessment of a Novel Class of Orthobiologics. Pharmaceutics. 2022 Jun 10;14(6):1231. doi: 10.3390/pharmaceutics14061231. PMID: 35745803; PMCID: PMC9229444.

Giannasi C, Niada S, Della Morte E, Casati S, Orioli M, Gualerzi A, Brini AT. Towards Secretome Standardization: Identifying Key Ingredients of MSC-Derived Therapeutic Cocktail. Stem Cells Int. 2021 Aug 26;2021:3086122. doi: 10.1155/2021/3086122. PMID: 34484347; PMCID: PMC8413055.

Amodeo G, Niada S, Moschetti G, Franchi S, Savadori P, Brini AT, Sacerdote P. Secretome of human adipose-derived mesenchymal stem cell relieves pain and neuroinflammation independently of the route of administration in experimental osteoarthritis. Brain Behav Immun. 2021 May;94:29-40. doi: 10.1016/j.bbi.2021.03.011. Epub 2021 Mar 15. PMID: 33737173.

Giannasi C, Niada S, Magagnotti C, Ragni E, Andolfo A, Brini AT. Comparison of two ASC-derived therapeutics in an in vitro OA model: secretome versus extracellular vesicles. Stem Cell Res Ther. 2020 Dec 3;11(1):521. doi: 10.1186/s13287-020-02035-5. PMID: 33272318; PMCID: PMC7711257.

Niada S, Giannasi C, Gomarasca M, Stanco D, Casati S, Brini AT. Adipose-derived stromal cell secretome reduces TNFα-induced hypertrophy and catabolic markers in primary human articular chondrocytes. Stem Cell Res. 2019 Jul;38:101463. doi: 10.1016/j.scr.2019.101463. Epub 2019 May 15. PMID: 31108390.