UNIVERSITÀ DEGLI STUDI DI MILANO

SCUOLA DI DOTTORATO
Scienze Biochimiche

DIPARTIMENTO DI MEDICINA VETERINARIA

CORSO DI DOTTORATO DI RICERCA
IN SCIENZE BIOCHIMICHE
Ciclo XXX

TESI DI DOTTORATO DI RICERCA

Analysis of mesenchymal stem cells (MSCs) secretome from mouse models and human patients to characterize their immunomodulatory properties: a proteomic approach.

Dott.ssa Fabiana SANTAGATA
MATRICOLA: R10884

TUTOR: prof.ssa Gabriella TEDESCHI

COORDINATORE DEL DOTTORATO: prof. Sandro SONNINO

Anno Accademico 2016 - 2017

AIM of WORK / SUMMARY

Mesenchymal stem cells (MSC) are multipotent progenitor cells with self-renewable capacity and the potential to differentiate into various cell types, especially of the mesodermal lineages. They have immunomodulatory properties and, in particular when exposed to pro-inflammatory cytokines, they acquire immunosuppressive and anti-inflammatory properties due in part to an array of soluble mediators. The characterization of the totality of soluble mediators, also indicated as “secretome”, could be useful to clarify the mechanism of MSC activity and, eventually, to design strategies to modulate their properties for the design of rational therapy design or improvement of existing therapies. However, until now, a thorough characterization of pro-inflammatory primed MSC secretome is still lacking, being its characterization *in vivo* very difficult, so a commonly used approach is the analysis of media conditioned by cells in culture. The aim of this investigation is the proteomic characterization of bone marrow derived cultured MSC secretome following stimulation with pro-inflammatory cytokines. We performed the study using a protocol set up in our laboratory and published in *Nonnis et al, 2016*, using two different models: murine and human. The chapter 3 of this dissertation, that concerns results obtained from the application of the previously mentioned protocol, is divided into three main parts: the first one describes results obtained from the proteomic characterization of murine MSC secretome; the second one those from human MSC secretome, and the last one is the comparison between the results from the two models, in order to define a unique molecular mechanism for MSC activity. Despite important differences among human and mouse, secreted proteins in both models are associated with inflammation and angiogenesis. In particular, the attention was focused on two proteins: CSF1 and TIMP1, which are present in conditioned medium of stimulated MSC (st MSC-CM) of both species and play a key role in immunity/inflammation and angiogenesis, respectively. This work allows to confirm the potential therapeutic role of MSC secretome and to design pre-clinical experiments and clinical trials. Results reported in this phD thesis have been in part published in:

Mouse mesenchymal stem cells inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1 L.Zanotti, R.Angioni, B.Cali, C.Soldani, C.Ploia, F.Moalli, M.Gargesha, G.D’Amico, S.Elliman, G.tedeschi, E.Maffioli, A.Negri, S. Zacchigna, A.Sarukhan, JV Stein and A. Viola, Leukemia (2016) 30, 1143–1154 and in **Proteomic analysis of the secretome of human bone marrow-derived**

Mesenchymal Stem Cells primed by pro-inflammatory cytokines E.Maffioli, S.Nonnis, R. Angioni, F.Santagata, B.Calì, L. Zanotti, A.Negri, A.Viola, G.Tedeschi, Journal of proteomics (2017) 166, 115-126.

INTRODUCTION

1.1 Stem cells

1.1.1 Definition and origin

The human body comprises over 200 different cell types that are organized into tissues and organs to provide all the functions required for viability and reproduction. Historically, biologists have been interested primarily in the events that occur prior to birth. The second half of the twentieth century was a golden era for the developmental biology, since the key regulatory pathways that control specification and morphogenesis of tissues were defined at the molecular level [1]. The origins of stem cell research lie in a desire to understand how tissues are maintained in adult life, rather than how different cell types arise in the embryo. Stem cells are unspecialized cells found in multicellular organisms, characterized by the ability to self-renew by mitosis while in undifferentiated state, and to give rise to various differentiated cell types by cell differentiation [Fig.1], [2]. For definition, a stem cell possesses two important properties:

- **self-renewal**: the ability to go through numerous cycles of cell division while maintaining the undifferentiated state [3];
- **potency**: the capacity to differentiate into specialized cell types [3].

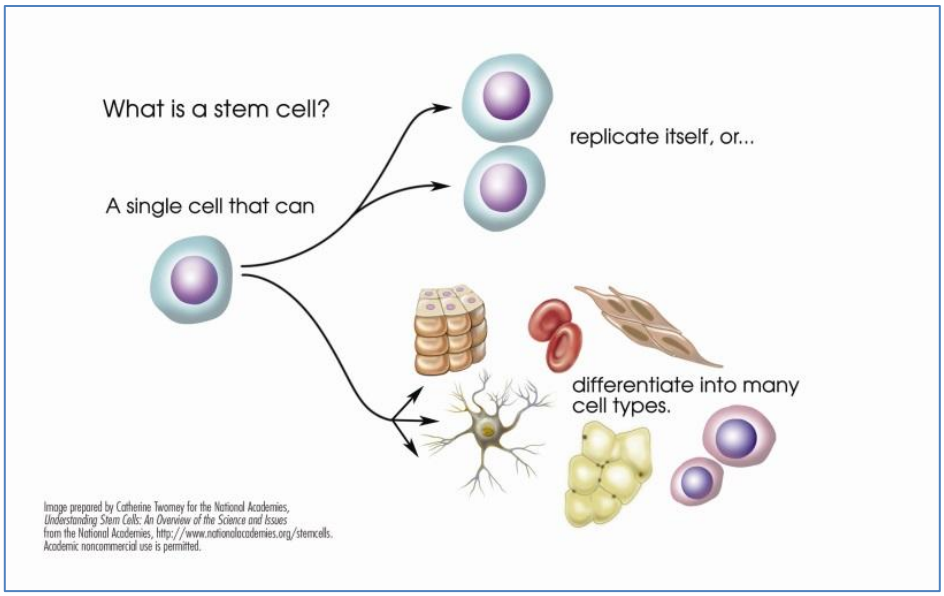


Fig.1 The two fundamental properties of a stem cell: self-renewal and potency. Self-renewal is the cell's ability to replicate itself, and potency is the capacity to differentiate into many cell types [3].

1.1.1.1 Self-renewal

To ensure self-renewal, stem cells undergo two types of cell division: s*ymmetric division,* which gives rise to two identical daughter cells both endowed with stem cell properties; and a*symmetric division*, which produces only one stem cell and a progenitor cell with limited self-renewal potential. Progenitors can go through several rounds of cell division before terminally differentiating into a mature cell [Fig. 2], [4].

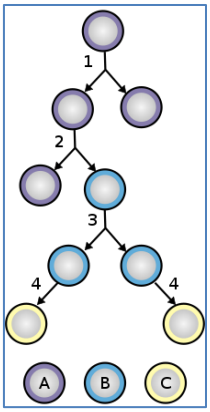


Fig.2 A stem cell division and differentiation. A: stem cell; B: progenitor cell; C: differentiated cell; 1: symmetric stem cell division; 2: asymmetric stem cell division; 3: progenitor division; 4: terminal differentiation [4].

1.1.1.2 Potency

In terms of the capacity to differentiate into different cell types, stem cells can be divided in [Fig.3]:

- *totipotent stem cells:* produced from the fusion of an egg and sperm cell, they can differentiate into embryonic and extraembryonic cell types. Such cells can construct a complete, viable organism [5];
- *pluripotent stem cells:* descendants of totipotent cells, these cells can differentiate into nearly all cells [5], for example, cells derived from any of the three germ layers [7];
- *multipotent stem cells:* these cells can differentiate into a number of cell types, but only those of a closely related family of cells [5];
- *oligopotent stem cells*: these cells can differentiate into only a few cell types (such as lymphoid or myeloid stem cells) [5];

- *unipotent cells:* they can produce only one cell type, their own, but have the property of self-renewal, which distinguishes them from non-stem cells (e.g. progenitor cells, which cannot self-renew) [5].

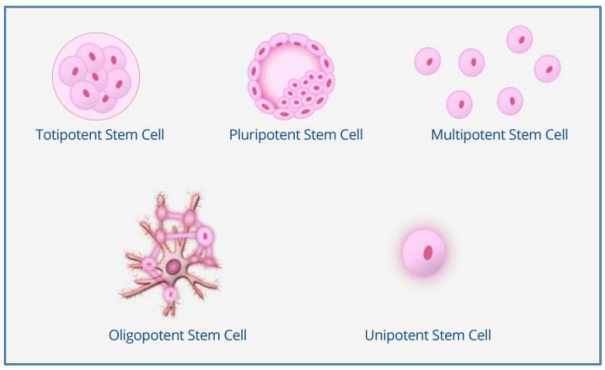


Fig. 3 Classification of stem cells about their potency. Totipotent, pluripotent, multipotent, oligopotent and unipotent stem cells [8].

1.1.2 Types of stem cells

In mammals, there are two broad types of stem cells: *embryonic stem cells* [9], which are isolated from the inner cell mass of blastocysts, and *adult stem cells* [11], which are found in various tissues [Fig.4]. In a developing embryo, stem cells can differentiate into all the specialized cells (ectoderm, endoderm and mesoderm) but can also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing adult tissues.

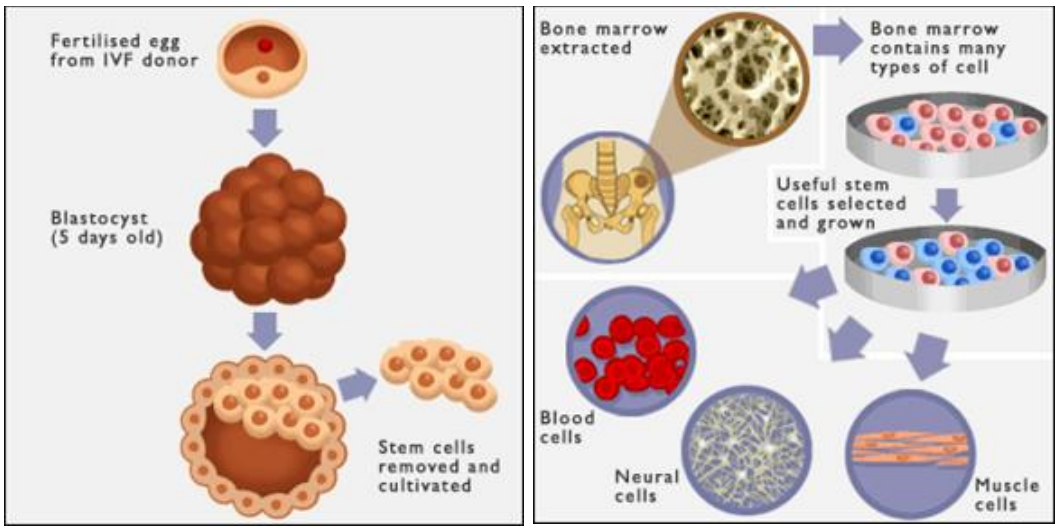
1.1.2.1 Embryonic stem cells

Embryonic stem cells (ES) are the cells of the inner cell mass of a blastocyst, an early-stage embryo [9]. Human embryos reach the blastocyst stage 4–5 days post fertilization, at which time they consist of 50–150 cells. ES cells are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. In other words, they can develop into each of the more than 200 cell types of the adult body when given sufficient and necessary stimulation for a specific cell type. They do not contribute to the extra-embryonic membranes or the placenta. A human embryonic stem cell is defined by the expression of several transcription factors and cell surface proteins. Among the transcription factors, Oct-4, Nanog, and Sox2 form the core regulatory network that ensures the suppression of genes leading to differentiation and maintenance of pluripotency [10]; while the cell surface antigens most commonly used to

identify human ES cells are the glycolipids stage specific embryonic antigen 3 and 4 and the keratan sulfate antigens Tra-1-60 and Tra-1-81[10].

1.1.2.2 Adult stem cells

Adult stem cells, also called somatic stem cells, are stem cells which maintain and repair the tissue in which they are found [11]. They are present in children, as well as adults [12]. Pluripotent adult stem cells are rare and generally small in number, but they can be found in umbilical cord blood and other tissues [13]. Bone marrow is a rich source of adult stem cells [14], which have been used in treating several conditions including liver cirrhosis [15], chronic limb ischemia [16] and end stage heart failure [17]. The quantity of bone marrow stem cells declines with age and is greater in males than females during reproductive years [18]. DNA damage accumulates with age in both stem cells and in the cells that comprise the stem cell environment. This accumulation is considered to be responsible, at least in part, for increasing stem cell dysfunction with aging [19]. Most adult stem cells are lineage-restricted (multipotent) and are generally referred to by their tissue origin (mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, dental pulp stem cell, etc.) [10].



Embryonic stem cells Adult stem cells

Fig.4 Types of stem cells. A. Embryonic stem cells are obtained from the inner mass of blastocyst and can be removed and cultivated for different uses. B. Adult stem cells are stem cells which maintain and repair the tissue in which they are found. They can be extracted, selected, grown and under stimulation, also differentiated into a specific cell type [21].

1.1.2.3 Other types: fetal and amniotic stem cells

The primitive stem cells located in the organs of fetuses are referred to as fetal stem cells. They can be *fetal proper stem cells* and e*xtraembryonic fetal stem cells* [22] The fetal proper stem cells come from the tissue of the fetus, and are generally obtained after an abortion, they are not immortal but have a high level of division and are multipotent [22]; the extraembryonic fetal stem cells*,* from extraembryonic membranes, are not distinguished from adult stem cells [22]. These stem cells are acquired after birth; they are not immortal but have a high level of cell division and are pluripotent [23]. Multipotent stem cells are also found in amniotic fluid, and are known also as amniotic stem cells. These stem cells are very active, expand extensively without feeders and are not tumorigenic; they can differentiate in cells of adipogenic, osteogenic, myogenic, endothelial, hepatic and neuronal lines [24] and represent a topic of active research.

1.1.3 Clinical application/Therapeutic use

Stem cell therapy is the use of stem cells to treat or prevent a disease or pathological condition exploiting their capacity to differentiate in various cells type**.** Stem cells are used in neurodegenerative diseases, diabetes, heart disease, and other conditions [25], but also for research purpose to further understand human development, organogenesis, and diseases [26]. For example, by using human embryonic stem cells to produce specialized cells in the lab, scientists can gain access to adult human cells without taking tissue from patients. They can then study these specialized adult cells in detail to catch complications of diseases, or to study cells reactions to potentially new drugs. Adult stem cells have limitations in their potency since they are not able to differentiate into cells from all three germ layers. However, a genetic reprogramming allows for the creation of pluripotent cells, called *induced pluripotent stem cells* (iPSCs), [Fig.5]. It is important to note that these are not adult stem cells, but adult cells (e.g. epithelial cells) reprogrammed to give rise to cells with pluripotent capabilities [27, 28, 29] and they are also different from embryonic stem cells (ESs). Importantly, the chromatin of iPSCs appears to be more "closed" or methylated than that of ESs [30, 31] and similarly, the gene expression pattern is different from ESs or even among iPSCs sourced from different origins [30].

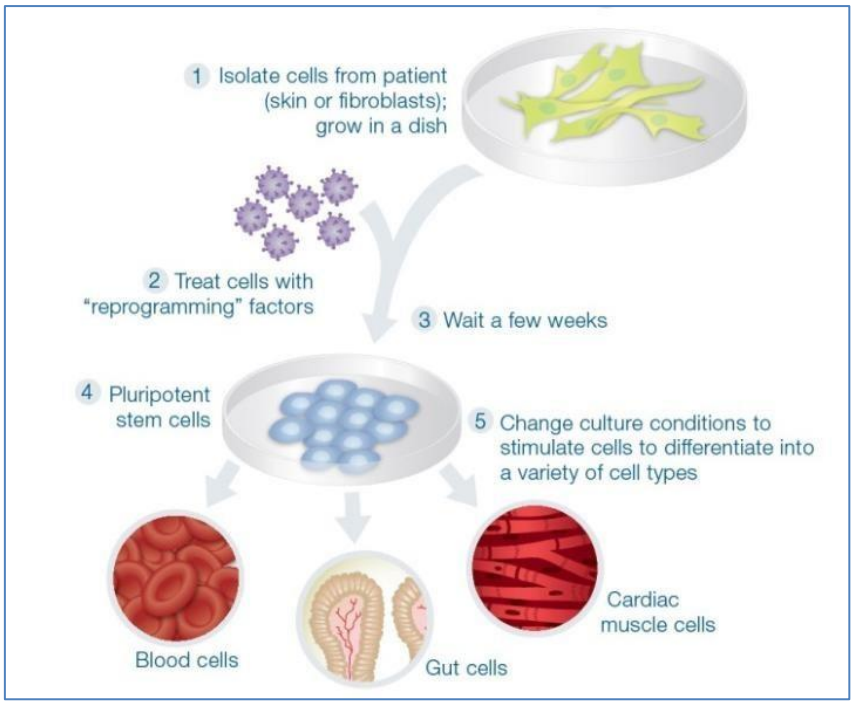


Fig.5 Induced pluripotent stem (iPS) cells. They are created artificially in the lab by "reprogramming" a patient's own cells. These cells can be made from readily available cells including fat, skin, and fibroblasts (cells that produce connective tissue) [32].

Adult stem cell treatments have been successfully used for many years to treat leukemia and related bone/blood cancers through bone marrow transplants [33]. They are also used in veterinary medicine to treat tendon and ligament injuries in horses [34].

1.1.4 Potential risks of the use of stem cells

The use of stem cells presents also some disadvantages:

1. it is difficult to obtain the exact cell type needed, because not all cells in a population differentiate uniformly and undifferentiated cells can create tissues other than the desired one [23];
2. some stem cells form tumors after transplantation [81];
3. stem cell treatments may require immunosuppression by radiation before the transplant to remove the person's previous cells and to avoid the targeting of stem cells by patient's immune system. One approach to avoid the second possibility is to use stem cells from the same patient who is being treated [23].

1.2 Mesenchymal stem cells

1.2.1 Definition

The term mesenchymal stem cells (MSCs) [Fig.6] is referred to stem cells present in the hematopoietic microenvironment of bone marrow that can differentiate into different tissues developing from the mesoderm. It was first used to refer to a hypothetical postnatal, multipotent and self-renewing precursor derived from an original embryonic MSC, the function of which was to maintain the turnover of skeletal tissues in homeostasis or tissue repair during adulthood.

Fig.6 Mesenchymal Stem Cells [36].

MSCs refer to cultivated cells that are used in research and in the clinic. When cultivated, these cells are a mix of cells ranging from stem cells to mature stromal cells; in this case, MSCs refer to multipotent mesenchymal stromal cells [37]. Although MSCs were first described in bone marrow, they have been found in all tissues and are present within the pericyte population in the vasculature wall [38]. Many studies have further reported mesenchymal stromal cell differentiation into multiple other cell types of mesodermal and non-mesodermal origin, including endothelial cells [39], cardiomyocytes [40], hepato cytes [41] and neural cells [42].

1.2.1.1 MSC classification

The International Society of Cellular Therapy defines MSCs or multipotent stromal cells by three main characteristics [37]:

1. their adhesion to plastic;
2. their expression of a specific set of membrane molecules (CD73, CD90, CD105), together with lack of expression of the hematopoietic markers CD14, CD34 and CD45 and human leucocyte antigen-DR (HLA-DR);
3. their ability to differentiate within three main pathways -- osteoblastic, chondrogenic and adipocytic.

Although these main characteristics can be applied to all cultivated MSCs, some differences might depend on the tissue origin [43]. An additional important consideration is

that mesenchymal stromal cells derived from various postnatal or embryonic tissues using identical culture conditions display significant differences in colony morphology, differentiation potential and gene expression [44, 45]. Data suggest that mesenchymal stromal cell cultures may originate from an array of tissue-specific multipotent precursor cells that are present in native tissues and have diverse degrees of plasticity and self-renewal.

1.2.2 Studying MSCs *in vivo*

After years of investigating MSCs out of their native context, little has been learned regarding the identity and function of their precursors in vivo. It is important to note that the fundamental biological properties of mesenchymal stromal cells are likely to be altered by culture conditions and thus should not be directly ascribed to their presumed in vivo counterpart. Progress in our understanding of bona fide MSCs largely relies on having the capacity to recognize progenitor cells in situ, prospectively isolate them and finally assay their multi- potency and self-renewal capacity *in vivo* [170].

1.2.2.1 Perivascular localization *in vivo*

A key task for assessing the function of MSCs *in vivo* is to define their micro-anatomical localization *in situ* in diverse organs. Efforts to track the identity of tissue-resident MSCs have consistently suggested that these cells lie adjacent to blood vessels. Evidence for such association came from initial observations that pericytes (also known as Rouget cells or mural cells), which are defined by their perivascular location and morphology, display MSC-like features [46], [Fig.7].

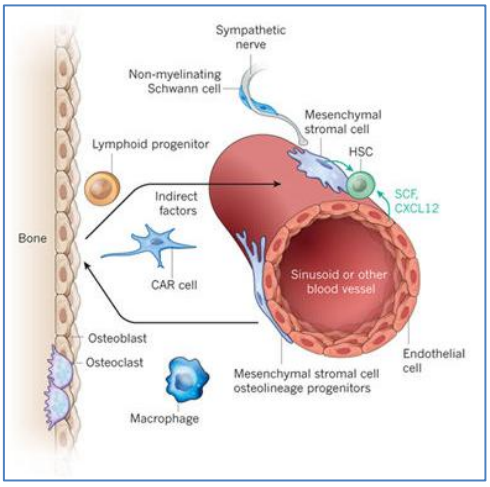


Fig. 7 *In vivo* perivascular localization of MSC. MSCs lie adjacent to blood vessel, such as pericyte, that are contractile cells that wrap around the endothelial cells that line the capillaries and venules and that display MSC-like features [47]**.**

Pericyte-derived cultures are similar to mesenchymal stromal cell cultures in terms of morphology and cell-surface antigen expression, and can be induced to differentiate into not only osteoblasts, chondrocytes and adipocytes but also smooth muscle cells and myocytes under appropriate conditions [48, 49].

1.2.3 MSCs: potential for differentiation

MSCs can differentiate *in vitro* to osteoblasts or chondrocytes and should be able to differentiate *in vivo* as well. The transplantation and differentiation of MSCs to functional osteoblasts was demonstrated *in vivo* in an animal model and in humans, which led to treatment for osteogenesis imperfecta and bone loss [43]. MSCs participate in stem cell niches such as the hematopoietic niche, and recently, bone marrow sub-endothelial cells expressing CD146, a population of MSC, were found to reconstitute the hematopoietic microenvironment [44]. The multipotent character of MSCs and their plasticity within mesodermic lineages is supported by lineage priming [45]. However, the differentiation towards other lineages originating from the mesoderm, such as skeletal and myocardial muscle cells, remains questionable and could depend on the origin of MSCs; for example, adipose-tissue-derived MSCs are able to differentiate to cardiomyocytes [46].

1.2.4 MSCs: immune privilege of MSCs

In addition to their stem/progenitor properties, MSCs have also been shown to possess broad immunoregulatory abilities and are capable of influencing both adaptive and innate immune responses. Recent findings have demonstrated that MSCs actively interact with components of the innate immune system and that, through these interactions; they display both **anti-inflammatory** and **pro-inflammatory** effects [50].

1.2.4.1 MSC as “sensor of inflammation”

Inflammation serves as a localized or systemic protective response elicited by infection, injury or tissue destruction to eliminate pathogens and preserve host integrity. Within hours after the onset of an inflammatory response, molecules expressed by pathogens or associated with tissue injury are recognized by Toll-like receptors (TLRs) present on innate effector cells. TLR ligation triggers phagocytosis and the release of inflammatory mediators, which may initiate innate immune responses that provide a first line of nonspecific defence, mainly through the activation of phagocytic cells, including macrophages and neutrophils [51]. TLR ligation may not only activate phagocytic cells but also stromal cells, including MSCs, thus creating an inflammatory environment [52, 53]. Human and mouse MSCs dynamically express a number of distinct and overlapping TLRs

in culture. Moreover, *in vitro* stimulation of specific TLRs affects the subsequent immune modulating responses of MSCs [54, 55, 56]. Under hypoxic culture conditions, stimulation of MSCs with the pro-inflammatory cytokines IFN-γ, TNF, IFN-α, and IL-1β upregulates expression of a subset of TLRs, thus increasing the sensitivity of MSCs to the inflammatory milieu [57]. However, prolonged stimulation with TLR ligands causes downregulation of TLR2 and TLR4 [58], most likely as a self-regulatory mechanism to prevent overactive skewing of the immune response. To direct appropriate immune responses to a diversity of pathogenic insults, the different TLRs are activated by specific endogenous or pathogen-associated molecules, including lipopolysaccharide (LPS) from Gram-negative bacteria (TLR4) and double strand RNA (dsRNA) carried by some viruses (TLR3) [53]. This has suggested that MSCs may polarize into two distinctly acting phenotypes following specific TLR stimulation, resulting in different immune modulatory effects and distinct secretomes. The TLR4-primed MSC population exhibits **a pro-inflammatory profile (MSC1)** and the TLR3-primed MSC population delivers **anti-inflammatory signals (MSC2**), [Fig.8].

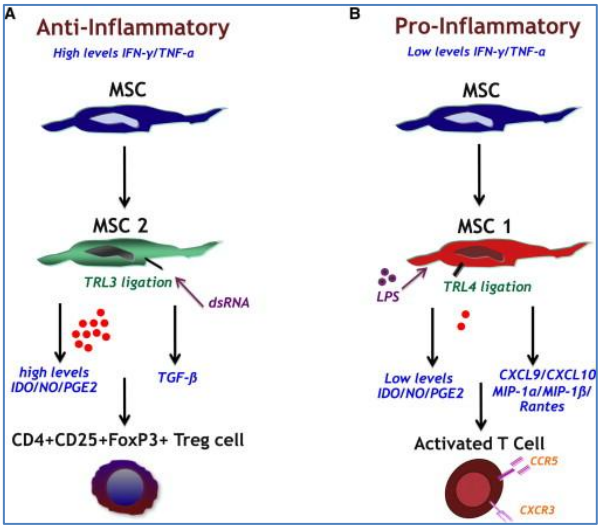


Fig. 8 The polarization of MSCs into a Pro-inflammatory and Anti- Inflammatory Phenotype. (A) In the presence of an inflammatory environment, MSCs become activated and adopt an immune-suppressive phenotype (MSC2) by secreting high levels of soluble factors that suppress T cell proliferation. (B) In the absence of an inflammatory environment, MSCs may adopt a pro-inflammatory phenotype (MSC1) and enhance T cell responses by secreting chemokines that recruit lymphocytes to sites of inflammation [59].

In particular, besides their potential for differentiation, MSCs can exert an immunosuppressive effect *in vitro* and *in vivo* [60, 61] by acting on all immune effectors [62]. In fact, MSCs are unable to induce considerable alloreactivity because of a number of

unique characteristics that protect MSCs from alloreactive natural killer (NK)-cell-mediated lysis [63]. However, some controversial data have been obtained with animal-derived MSCs. For example, *in vitro* and *in vivo*, murine allogeneic MSCs can elicit an immune response in immunocompetent mice [64, 65].

1.2.4.1.1 MSC interaction with immune cells (T cells, B cells, NK cells and dendritic cells)

As “sensor of inflammation”, MSCs interact with cells involved in the process [Fig.9]. About T-cells, MSC may inhibit their proliferation induced by several stimuli both *in vitro* and *in vivo* [66, 67, 68], arresting activated T-cells in the G0/G1 phase of the cell cycle [69] without inducing apoptosis [66, 68] and the same is for B-cells [70]. Moreover, MSCs can also modulate B-cell migration and production of IgM, IgA and IgG, without inducing apoptosis [70, 71]. MSCs may also inhibit both IL-2 and IL-15 induced NK proliferation [72, 73] and may interfere also with dendritic cells (DC) function and support the development of tolerogenic antigen-presenting cells (APCs). *In vivo* results indicate that MSCs actively interact with cells of the innate immune system and modulate their function to establish a fine balance between pathogen elimination and repair processes, aiming at controlling inflammation, preventing organ failure, and preserving tissue homeostasis. Therefore, the further elucidation of mechanisms that trigger a functional switch between MSC phenotypes remains an important research goal for future studies [74].

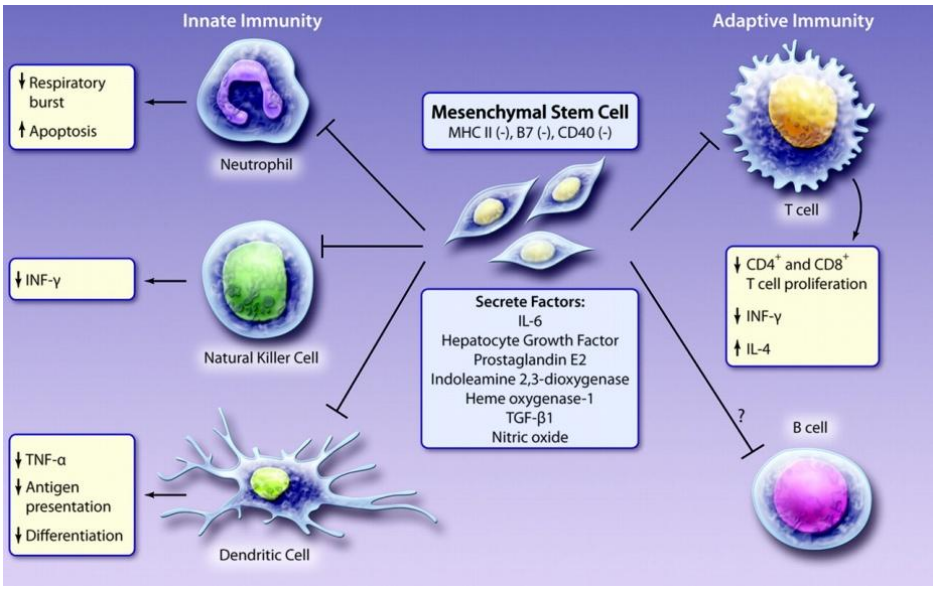


Fig. 9 MSC interactions with immune cells. MSCs are immune privileged cells that inhibit both innate (neutrophils, dendritic cells and natural killer cells) and adaptive (T cells and B cells) immune cells (INF- indicates interferon; TNF- indicates tumor necrosis factor) [74].

1.2.5 Clinical application/ Therapeutic use

The wide range of *in vivo* effects of MSCs, from cell replacement and immunosuppression to trophic effects, drives their increasing use in regenerative medicine and immune intervention. Whatever the use, MSCs must be produced according to good manufacturing practices (GMPs), with relevant controls, to obtain efficient and safe cell therapy [75]. The ability of MSCs to adopt a different phenotype in response to sensing an inflammatory environment is crucial for understanding their therapeutic potential in immune-mediated disorders. The available evidence suggests that responses to MSC treatment may be independent of the MSC donor or dose of the immune-suppressive treatment employed. This heterogeneity in response might be related to the presence or absence of the appropriate environment in the patient capable of activating MSCs.

1.2.5.1 Regenerative medicine: stem cell transplantation

Stem cell transplantation is a procedure that replaces unhealthy blood-forming cells with healthy cells arising from stem cells. There are two different types of stem cell transplantation: *autologous* and *allogenic*. In the first one stem cells come from patient own body, instead in the allogenic one cells are from a healthy person (the donor). In this last case, an important medical complication is the *graft versus host disease* (GvHD) that consist in the fact that immune cells in the donated tissue (the graft) recognize the recipient (the host) as foreign (nonself); so the transplanted immune cells then attack the host's body cells. It has been observed [76] that, because of the profound immunomodulatory effect of MSCs *in vivo* and *in vitro*, use of *ex vivo*-expanded MSCs for treatment or prophylaxis of steroid-resistant GvHD was recommended. Another important potential application of MSCs in allogeneic stem cell transplantation is for enhancement of engraftment. In fact, since MSCs secrete many growth factors stimulating hematopoiesis, provide a scaffold for hematopoiesis and support primitive progenitor cells *in vivo*, they might enhance engraftment after stem cell transplantation [77].

1.2.5.2 Immune Intervention

Under the effect of inflammatory cytokines, MSCs are capable to migrate to inflamed tissues and modulate the local inflammatory reactions thanks to the effects on both innate and adaptive immunity [67, 78]. In addition, MSCs may recruit and support local autologous stem cells inside the injured tissues, thus promoting cell survival and tissue repair [75]. Therefore, MSC-based treatments may represent a novel strategy against systemic autoimmunity and inflammation, in diseases such as rheumatoid arthritis, multiple sclerosis and type-I diabetes. *In vitro* and *in vivo* studies in animal model [79, 80] suggest

the use of MSCs for managing autoimmune and inflammatory diseases. In addition, no evidence exists of systemic immunosuppression and increased risk of infections as side-effects of MSC infusion in immunocompetent individuals, which suggests that the immune-regulatory effects of MSCs are restricted to inflamed tissues. For these reasons, MSC-based immunotherapy may become suitable in the future for many severe inflammatory diseases [171]:

1. Bone and cartilage repair
2. Heart and vessels
3. Epithelium
4. Central nervous system

1.2.6 Potential risks in using MSCs

The use of MSCs could imply different risks, because of the differentiation potentials, their immunosuppressive properties and some possible immortalisation/transformation during long-term culture. Recent concerns have been expressed about the potential transformation of MSCs during the culture process, as shown recently in describing the transformation of human MSCs in cells cultured for a long time. In fact, although they can be managed safely during the standard *ex vivo* expansion period (6-8 weeks), human mesenchymal stem cells can undergo spontaneous transformation following long-term *in vitro* culture (4-5 months) [81]. Human cells have two control points that regulate their life span *in vitro*, the senescence and crisis phases. Senescence is associated with moderate telomere shortening and is characterized by cell cycle arrest and positive β-galactosidase staining at pH 6 [82]. If cells bypass this stage, they continue to grow until telomeres become critically short and cells enter crisis phase, characterized by generalized chromosome instability that provokes mass apoptosis [83]. Human cells immortalize at low frequency seem resistant to spontaneous transformation, instead MSC in long-term cultures immortalize at high frequency and undergo spontaneous transformation [81] supporting recent cautionary speculation that “mutant stem cells may seed cancer” [84].

1.2.7 Animal Models – different biological and functional properties of MSCs in mouse and man

Animal models are of critical importance for translating *in vitro* immune regulatory properties of MSCs into therapeutic application and dissecting mechanisms of efficacy. However, it’s important to note that murine MSCs are intrinsically different from human cells. Although murine and human MSCs share properties such as multi lineage

differentiation capacity, they are also distinct with respect to other properties. *Ex vivo* expansion with murine cells is slower than with human cells, and murine MSCs require weeks before entering a linear growth rate [85]. At this stage, murine MSCs undergo transformation and immortalization in culture. Several reports have indicated that transformed murine MSCs have an increased proliferation rate, display an altered morphology, carry cytogenetic abnormalities, and form tumors following injection into syngeneic mice. Murine BM-derived MSCs in long-term culture gradually exhibit increased telomerase activity and proceed to a malignant state, resulting in sarcoma formation i*n vivo* [86, 87]. This susceptibility to malignant transformation may be attributed to the high degree of chromosomal instability in genetically unstable inbred mice, characterized by the development of both structural and numerical aberrations even at early culture passages. Therefore, culture-expanded murine MSCs should be regarded as transformed cells, even in the absence of a malignant phenotype. These differences should be taken into consideration when interpreting data. The dissimilarities between MSCs isolated from murine and human species require a careful evaluation when choosing animal models to test MSCs in preclinical studies and so, when interpreting *in vivo* effects of murine MSCs, especially in light of efforts to look at clinical application of MSCs.

1.3 Study of MSC secretome

Mesenchymal stem cells represent a promising therapeutic approach and they action is due mainly to secreted mediators that often acts through chemotactic signaling [88], that are referred as "*secretome*". This term was first used by Tjalsma et al. [89] to include all proteins that are synthesized and processed by the secretary pathway and proteins located in the secretion machinery, although, the term was recently limited to include only the set of secreted or extracellular proteins in a species. For the use of MSC, it's very important to know as much as possible the identity of molecules responsible for their activities. Since the secretome plays a direct role in the biological activities of MSCs, the qualitative and quantitative analysis of the protein component of MSC secretome is a fundamental step in order to identify key players in the control and regulation of the many biological processes influenced by these cells [90]. Previously, studies used to compare plasma/serum from cancer patients with those from normal controls because, as suggested by Liotta, *"the blood contains a treasure trove of previously unstudied biomarkers that could reflect the ongoing physiologic state of all tissues"*, and the latter,

therefore, appears to be more attractive [91]. However, the prospects of blood proteomics are challenged by the fact that blood is a very complex body fluid, comprising an enormous diversity of proteins and protein isoforms with a large dynamic range of at least 9–10 orders of magnitude. The abundant blood proteins, such as albumin, immunoglobulin, fibrinogen, transferrin, haptoglobin and lipoproteins, may mask the less abundant proteins, which are usually potential markers [92]. So, studies redirected to the "secretome" although, only few studies have characterized the cellular secretome *in vivo*, assuming the idea that cells grown *in vitro* and stimulated using specific factors to which cells are exposed under certain conditions *in vivo,* present *in vitro* a secretion phenotype similar to one *in vivo* [93,94]. In particular, for this purpose an optimized protocol set up in our laboratory was used to allow the collection of the secretome of human bone marrow mesenchymal stem cells (BM-MSC) in order to detect the differential expression of secreted protein induced by exposing cells to specific stimulation conditions.

1.3.1 Proteomic characterization of MSC secretome

The proteomic approach for the characterization of MSC secretome, described in this thesis, is a shotgun label free approach to allow direct identification and quantification of «all detectable» proteins in the secretome of stimulated (MIX) vs unstimulated (CTR) MSC, both in mouse and human. The shot gun approach is a "bottom-up" protein analysis which allow the characterization of all the proteins present in a sample without any previous purification before digestion and nano LC-MS/MS analysis. For many years 2D-PAGE/MS, which is an overall, comparative, quantitative proteomic technique, was the gold standard for analysis of protein expression and biomarker discovery. However, there are several disadvantages associated with gel-based proteomic techniques. For example, any 2D approach is subjected to the restrictions imposed by the gel method, which include limited dynamic range, difficulty in handling hydrophobic proteins, and difficulty in detecting proteins with extreme molecular weights and pI values [95]. Another negative aspect is that spots on a 2D gel often contain more than one protein, making quantification ambiguous; throughput is low and gel-to-gel reproducibility can be a challenge [95]. Furthermore, co-migration of proteins can cause problems during the excision and identification steps as there may be more than one protein present in the gel spot excised. Moreover, low-abundance proteins may be masked in the gel by high-abundance housekeeping proteins. Therefore, in more recent years, there has been a move towards gel-free MS methods for proteome analysis. The gel-free methods are based on the high-throughput ‘‘shotgun" analysis of peptides from a digested complex protein sample using

an on-line high performance liquid chromatography (HPLC) method, prior to identification using MS [96]. Label-free approaches have been developed for quantitative shotgun proteomic and are methods that don't use chemical tags for quantification. In this method, each sample is processed separately and individually analysed through LC-MS/MS [95].

The basic steps of all the label-free techniques are:

- sample preparation including protein extraction, reduction, alkylation, and digestion;
- sample separation by liquid chromatography (LC or LC/LC) and analysis by MS/MS;
- data analysis including peptide/protein identification, quantification, and statistical analysis.

Shotgun proteomic has provided powerful tools for studying large scale protein expression and characterization in complex biological systems [99, 100]. This proteomic strategy converts a complex protein mixture to an even more complicated peptide mixture. For this reason, to resolve complex peptide mixtures, high-resolution HPLC separations are necessary to maximize peptide separation for acquisition of tandem mass spectra. The liquid chromatography step separates the peptides and elutes them directly into the ESI ionizer of the mass spectrometer.

There are two different kind of measurement used for the quantification (Fig.10): the first one calculates ion intensity changes as peptide peak areas or heights in chromatography, while the second is based on the spectral counting of identified proteins after tandem mass analysis. The two measures are calculated for each LC-MS/MS or LC/LC-MS/MS runs, so it is possible to point out differences in protein abundance comparing the results of different analyses. The relative quantification by peak intensity is based on extracting the ion chromatogram of the peptides and comparing them across range (Fig.10 A). In LC-MS, an ion with a specific m/z and intensity is detected and recorded at a certain time and it has been observed that its signal intensity from electrospray ionization (ESI) correlates with the concentration [101]. As reported in [101], it was experimentally observed that the chromatographic peaks area of each peptide, increases with the increase of the ion's concentration and the sum of the peak's area of all the peptides identified correlates linearly with the protein concentration. The strong correlation between chromatographic peak areas and the peptide/protein concentration was still observed when the when the protein was spiked into a complex mixture and its digests were detected by LC-MS/MS [102, 103]. Although these early studies showed that the relative quantification of the peptides could be achieved via direct comparison of peak intensity of each peptide ion in

multiple LC-MS datasets, applying this method for the analysis of changes in protein abundances in complex biological samples had some practical constraints. First, even the same sample can result in differences in the peak intensities of the peptides from run to run. These differences are caused by experimental variations such as differences in sample preparation and sample injection. Normalization is required to account for this kind of variation. Second, any experimental drifts in retention time and m/z will significantly complicate the direct, accurate comparison of multiple LC-MS datasets. Chromatographic shifts may occur as a result of multiple sample injections onto the same reverse-phase LC column. Unaligned peak comparison will result in large variability and inaccuracy in quantification. Thus, highly reproducible LC-MS and careful chromatographic peak alignment are required and critical in this comparative approach [104 – 109]. In the spectral counting approach, relative protein quantification is achieved by comparing the number of identified MS/MS spectra from the same protein in each of the multiple LC-MS/MS or LC/LC-MS/MS datasets (Fig.10 B). This is possible because an increase in protein abundance typically results in an increase in the number of its proteolytic peptides, and vice versa. This increased number of (tryptic) digests then usually results in an increase in protein sequence coverage, the number of identified unique peptides, and the number of identified total MS/MS spectra (spectral count) for each protein [110]. Liu et al. [111] studied the correlation between relative protein abundance and sequence coverage, peptide number, and spectral count. It was demonstrated that among all the factors of identification, only spectral count showed strong linear correlation with relative protein abundance with a dynamic range over 2 orders of magnitude [111]. Therefore, spectral count can be used as a simple but reliable index for relative protein quantification. Spectral counting-based quantification is proved more reproducible and has a larger dynamic range than the peptide ion chromatogram-based quantification [112]. In contrast to the chromatographic peak intensity approach, which requires delicate computer algorithms for automatic LC-MS peak alignment and comparison, no specific tools or algorithms have been developed specially for spectral counting due to its ease of implementation. However, normalization and statistical analysis of spectral counting datasets are necessary for accurate and reliable detection of protein changes in complex mixtures. Relative quantification by spectral count has been widely applied in different biological complex [113, 114, 115] and it is the type of measurement used in this work.

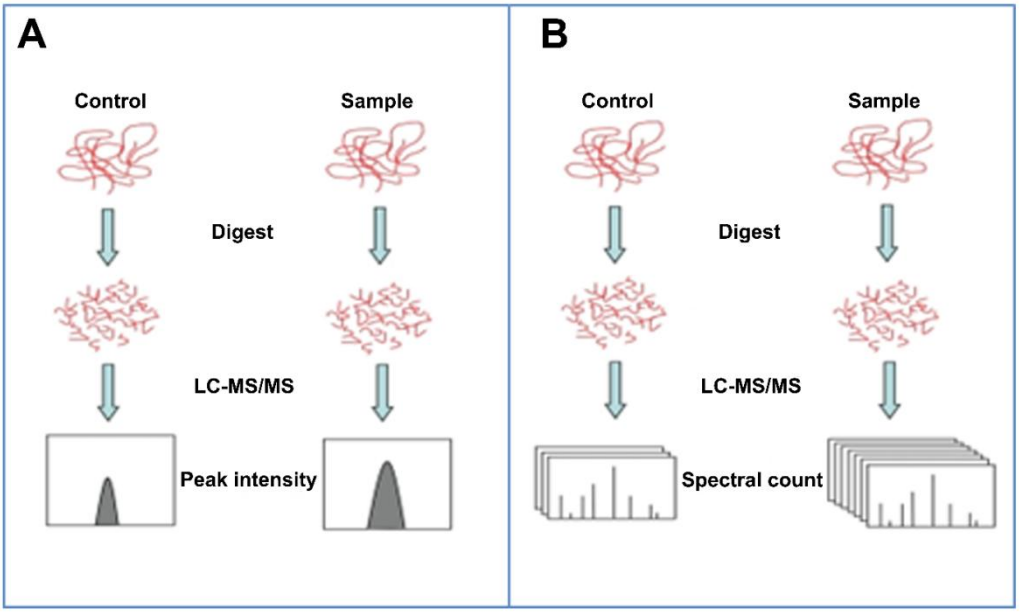


Fig.10 Label-free quantitative proteomics. Control and sample are subject to individual LC-MS/MS analysis. Quantification is based on the comparison of peak intensity of the same peptide (A) or the spectral count of the same protein (B) [95].

MATERIAL AND METHODS

2.1 Isolation of murine MSC

C57BL/6J mice were purchased from Charles River Laboratories (Calco, Italy). All mice used as primary cell donors or recipients were between 8 and 12 weeks of age. Procedures involving animals and their care conformed to institutional guidelines in compliance with national (4D.L. N.116, G.U., suppl. 40, 18-2-1992) and international (EEC Council Directive 2010/63/UE; National Institutes of Health Guide for the Care and Use of Laboratory Animals) law and policies. The protocol was approved by the Italian Ministry of Health on 18 June 2007 and modified by Protocol 162/2011-B. All efforts were made to minimize the number of animals used and their suffering. In all the experiment, the mice were sex and age matched, no further randomization was applied. MSC were isolated as described [116] by flushing the femurs and tibias from 8 week-old, C57Bl/6 female mice and cultured in 25 cm^2 tissue culture flasks at a concentration of 2X106 cells/cm^2 using complete Dulbecco modified Eagle medium low glucose (DMEM, Lonza, Braine-L'Alleud, Belgium) supplemented with 20% heat inactivated fetal bovine serum (Biosera, Ringmer, United Kingdom), 2mM glutamine (Lonza), 100 U/ml penicillin/streptomycin (Lonza). Cells were incubated at 37°C in 5% CO_2. After 48 hours, the non-adherent cells were removed. After reaching 70–80% confluence, the adherent cells were trypsinized (0.05% trypsin at 37°C for 3 minutes), harvested and expanded in larger flasks. MSC at passage 10 were screened by flow cytometry for the expression of CD106, CD45, CD117, CD73, CD105, MHC-I, SCA-1 and CD11b and used to perform experiments (BD Pharmingen, Oxford, UK).

2.2 Isolation of human MSC

MSC were provided by Orbsen Therapeutics Ltd (Galway, Ireland). Ethical approvals are granted from the NUIG Research Ethics Committee and the Galway University Hospitals Clinical Research Ethics Committee (CREC). Briefly, bone marrow was harvested from volunteers, and the cell culture was set up as previously described [117]. MSC were characterized according to international guidelines [118]. All samples were obtained with informed consent. Procurement of the sample conformed to European Parliament and Council directives (2001/20/EC; 2004/23/EC).

2.3 Collection of conditioned medium (CM)

2.3.1 Collection of conditioned medium (CM) of murine

MSCMSC were plated and let grow until confluence in ventilated cap flask. Growth medium was substituted with DMEM low glucose supplemented with 10% FBS, 2mM glutamine, 100 U/ml penicillin/streptomycin, with or without 25 ng/ml mIL1b, 20 ng/ml mIL6, 25 ng/ml mTNFa for 24 hours. Then this medium was changed with DMEM low glucose supplemented with 2mM glutamine, 100 U/ml penicillin/streptomycin for the following 18 hours. Conditioned medium was harvested and centrifuged at 4000 rpm for 10 min.

2.3.2 Collection of conditioned medium (CM) of human MSC

MSC were plated in with MEM Alpha with Glutamax supplemented with 10% FBS, 2 mM glutamine, 100 U/mL penicillin/streptomycin and let grow until confluence in in a humidified incubator with 5% CO_2 and 37°C. At the moment of the confluence, medium was substituted with MEM Alpha with Glutamax supplemented with 2% FBS, 2 mM glutamine, 100 U/mL penicillin/streptomycin, with (st hMSC) or without (unst hMSC) 25 ng/mL hIL1b, 20 ng/mL hIL6, 25 ng/mL hTNFa. 24 hours later, after three washes in MEM Alpha with Glutamax, the medium was changed with MEM Alpha with Glutamax supplemented with 2 mM glutamine, 100 U/mL penicillin/streptomycin for the following 18 hours. Conditioned medium was harvested and centrifuged at 4000 rpm for 10 min. Both isolation and collection, were performed in collaboration with the lab of prof.ssa Antonella Viola from University of Padua.

2.4 Proteomic analysis

Proteomic methods used are the same for both mouse and human samples.

2.4.1 Sample preparation

The protein concentration of MSC secretome was determined using the Bradford assay and then, proteins (150 ug for mouse samples, in the range 135-200 ug for human samples) were precipitated with 10 % tricholoracetic acid (TCA) for 2 hours on ice.

Prior to proteolysis, proteins were subjected to reduction with dithiothreitol (10 mM DTT in 50mM NH_4HCO_3), for 30 minutes at 56°C; and to alkylation with iodoacetamide (200mM IAA in 1M NH_4HCO_3), for 30 minutes at room temperature in the dark. Then protein were

digested with sequence-grade trypsin (Roche) for 16 hours at 37°C using a protein:trypsin ratio of 50:1. The reaction was stopped by acidification with 98% formic acid and the pellet was then desalted using Zip-Tip C18 (Millipore) before mass spectrometric (MS) analysis. The following protocol was applied:

Equilibrate the ZipTip for Sample Binding:

1) pre-wet the tips with 50% CH_3CN 3 times (3 x 100 µl);
2) wash the tips with TFA 0.1% 3 times (3 x 100 µl).

Bind and Wash the Peptides:

1) bind the sample to ZipTip pipette tip. Aspirate and dispense the material 10-13 cycles for maximum binding of complex mixtures;
2) wash the tips with 5% CH_3CN/0.1% TFA at least once.

Elute the Peptides:

1) elute the sample with 50% CH_3CN in HCOOH 1%, 3 times (3 x 100 µl), into a clean vial, for mass spectrometry analysis.

2.4.2 LC-ESI-MS/MS

Analysis was performed on a DionexUltiMate 3000 HPLC System with a PicoFritProteoPrep C18 column (200 mm, internal diameter of 75 µm) (New Objective, USA). Gradient: 1% ACN in 0.1 % formic acid for 10 min, 1-4 % ACN in 0.1% formic acid for 6 min, 4-30% ACN in 0.1% formic acid for 147 min and 30-50 % ACN in 0.1% formic for 3 min at a flow rate of 0.3 µl/min. The eluate was electrosprayed into an LTQ OrbitrapVelos (Thermo Fisher Scientific, Bremen, Germany) through a Proxeon nanoelectrospray ion source (Thermo Fisher Scientific). The LTQ-Orbitrap was operated in positive mode in data-dependent acquisition mode to automatically alternate between a full scan (m/z 350-2000) in the Orbitrap (at resolution 60000, AGC target 1000000) and subsequent CID MS/MS in the linear ion trap of the 20 most intense peaks from full scan (normalized collision energy of 35%, 10 ms activation). Isolation window: 3 Da, unassigned charge states: rejected, charge state 1: rejected, charge states 2+, 3+, 4+: not rejected; dynamic exclusion enabled (60 s, exclusion list size: 200). Five technical replicate analyses of each biological sample were performed. Data acquisition was controlled by Xcalibur 2.0 and Tune 2.4 software (Thermo Fisher Scientific).

2.4.3 Data processing

Mass spectra were analyzed using MaxQuant software (version 1.3.0.5) [119]. The initial maximum allowed mass deviation was set to 6 ppm for monoisotopic precursor ions and 0.5 Da for MS/MS peaks. Enzyme specificity was set to trypsin, defined as C-terminal to arginine and lysine excluding proline, and a maximum of two missed cleavages were allowed. Carbamidomethylcysteine was set as a fixed modification, N-terminal acetylation and methionine oxidation as variable modifications. The spectra were searched by the Andromeda search engine against the mouse Uniprot sequence database (release 29.05.2013 for mouse, release 2014_01 for human). Protein identification required at least one unique or razor peptide per protein group. Quantification in MaxQuant was performed using the built in XIC-based label free quantification (LFQ) algorithm [125] using fast LFQ. The required false positive rate was set to 1% at the peptide and 1% at the protein level, and the minimum required peptide length was set to 6 amino acids.

2.4.4 Statistical analysis

Statistical analyses were performed using the Perseus software (version 1.4.0.6, www.biochem.mpg.de/mann/tools/ [119]). Only proteins present and quantified in at least 3 out of 5 technical repeats were considered as positively identified in a sample and used for statistical analyses. Proteins were considered differentially expressed if they were present only in unstimulated (unst-) or stimulated (st-) MSC-CM (both in human and mouse) or showed significant t-test difference (cut-off at 1% permutation-based False Discovery Rate) in both biological replicates.

2.4.5 Bioinformatic analysis

Proteins were considered secreted or involved in inflammation/angiogenesis according to the following databases/datasets: Geno Ontology [121], NextProt [122], UniProt [123], Gene Cards [124], datasets [125, 126] and manual literature mining. Bioinformatic analyses were carried out by DAVID software (release 6.7) in order to cluster enriched annotation groups of Biological Function within the set of identified secretome proteins [124]. GOBP and groups were filtered for significant terms (modified Fisher exact EASE score p value <0.05 and at least five counts). Networks of up-regulated proteins in st hMSC-CM involved in inflammation or angiogenesis was performed using String [128] **(**active interactions: text mining, experiments, databases).

The mass spectrometry proteomics data, for human samples, have been deposited to the ProteomeXchange Consortium via the PRIDE [129] partner repository with the dataset identifier PXD005746.

RESULTS AND DISCUSSION

3.1 Proteomic characterization of murine MSC (mMSC) secretome

The secretome of MSC stimulated (st-MSC-CM) or not (unst-MSC-CM) with inflammatory cytokines was analyzed by a shotgun label free proteomic approach. The analysis allowed the identification of 1613 and 1630 proteins in the secretome of control (CTR) and stimulated (MIX) MSC, respectively. Proteins were considered differentially expressed if: (a) a protein was present only in MSC-CM or in control; (b) the LFQ (label free quantification) intensity resulted statistically significant as calculated by Perseus (t-test cut off at 1% permutation-based false discovery rate).

According to this analysis, 7.6% or 8.3% of the proteins detected in the secretome of control or stimulated MSC, respectively, were differentially expressed, either upregulated or downregulated, as shown in the Venn diagram reported in Fig.11.

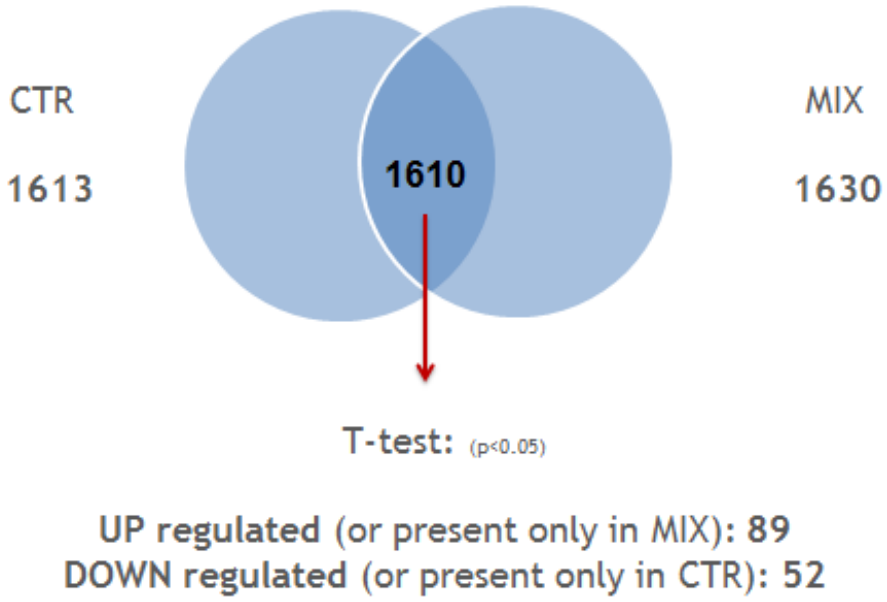


Fig.11 Venn diagram. Description of the comparison between unstimulated MSC in conditioned medium and the stimulated one. 1613 and 1630 proteins were identified in the secretome of control (CTR/unstimulated, unst-) and treated (MIX/stimulated, st-) MSC respectively. Among these, 1610 proteins were present in both samples st- and unst- MSC-CM. Applying a t-test with $p<0.05$,in total 89 resulted significantly UP regulated or present only in stimulated MSC-CM, and 52 significantly DOWN regulated in stimulated or present only in unstimulated MSC-CM.

These proteins were clustered according to their functions using the DAVID platform [130] filtered for significant Gene Ontology Biological Process (GOBP) terms using a P-value of ≤ 0.05 and at least five gene counts for each category.

3.1.1 Proteins up-regulated in stimulated murine MSC-CM

The differential expression analysis identified 141 proteins that were either up (Tab.1) or down (Tab.2) regulated in stimulated MSC.

We focused our attention mainly on the proteins up-regulated or exclusively expressed in MSC. These proteins are listed in Tab.1. A GO enrichment analysis was performed as shown in Fig.12. In detail, in Fig.12A the bar chart shows the fold enrichment of the top 26 most enriched GO terms in MSC versus unstimulated MSC-CM, while in Fig.12B, the histograms report the GOBP categories related to angiogenesis or inflammation. Interestingly 18% and 30% of the proteins, listed in Tab.1, belong to these two categories and are shown in bold in Tab.1, while concerning the 52 proteins that were significantly down-regulated or present only in the secretome of unstimulated MSC [Tab.2], the GO analysis revealed that most terms are related to metabolic processes [Fig.13]. The presence of an 'angiogenesis-related' signature among up-regulated proteins was also confirmed by the analyses of human MSC secretome, reported in the following chapter, which reveals that all the 16 up-regulated proteins in stimulated MSC secretome common to human and mouse are modulators of angiogenesis [Tab.3].

Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels [131]. It is a normal and vital process in growth and development, although, it is also involved in the process of transition of tumors from a benign state to a malignant one [132]. The process involves a complex and dynamic interaction between endothelial cells and the corresponding extracellular environment. Its chemical stimulation is performed by various angiogenic proteins, including several growth factors [133] that are also involved in inflammation processes [134]. Critical, in all phases of the process, are proteolysis and remodeling of the extracellular matrix (ECM) that affect endothelial cells migration, invasion into the perivascular tissue and morphologic formation of luminal structures. In these two events very important is the role taken on matrix metalloproteinase (MMP), a family of proteases that degrade ECM proteins and are critical in vascular remodeling, cellular migration, and sprout formation [135]. The activities of these metalloproteinases are precisely regulated under physiological conditions at the levels of transcription, zymogen activation and inhibition by endogenous inhibitors. Disruption of the balance between the production of active enzymes and their inhibition

may result in diseases associated with uncontrolled ECM turnover, inflammation, cell growth and migration, such as arthritis, cardiovascular disease, cancer, pulmonary disease, nephritis, neurological disorders and tissue ulceration [136]. Tissue inhibitors of metalloproteinases (TIMPs) are endogenous inhibitors of these metalloproteinases and are consequently important regulators of ECM turnover, tissue remodelling and cellular behaviour, so the balance between MMPs and their natural inhibitors is very critical. Interestingly, the proteomic analysis of the murine MSC secretome indicated that the cells activated by inflammatory cytokines, upregulate the expression of several proteins potentially involved in angiogenesis and inflammation through multiple pathways and, in particular, among these proteins, the attention was focused on the tissue inhibitors of metalloproteinases TIMP-1, because of its well-known anti-angiogenic properties [137]. We thus used the tube formation assay to analyze the effect of MSC-derived TIMP-1 on angiogenesis. Although the blocking anti-TIMP-1 antibody had no effect on the ability of endothelial cells to form tubes when cultured in the supernatants of unstimulated MSC, it totally reverted the anti-angiogenic properties of the supernatant from stimulated MSC [Fig. 14a], indicating that, at least in this *in vitro* setting, TIMP-1 is one of the key MSC-secreted molecules targeting the endothelium. In an *in vivo* setting, in the lab of our collaborator in Padua, the injection of neutralizing anti-TIMP-1 antibody [138] 1 day after MSC transplantation reverted the MSC-induced reduction of endothelial cell numbers and high endothelial venules (HEV) in lymph nodes (dLNs) [Fig. 14b-d], suggesting that TIMP-1 may be directly responsible for the anti-inflammatory effects of MSC on LNs. To confirm this hypothesis, they used a siRNA approach to knock down TIMP-1 expression in MSC [Fig. 15]. Again, the absolute cell numbers of endothelial cells and HEV in dLN were reduced by MSC transfected with the scramble siRNA control but not by MSC with TIMP-1 siRNA [Fig. 14 e-g]. On the basis of these results, we speculated that overexpression of TIMP-1 might be sufficient to mimic the effects of MSC transplantation, in terms of inhibition of angiogenesis in the inflamed lymph nodes. TIMP-1 overexpression by AAV9-mediated gene transfer [139] in mice immunized with CFA/OVA [Fig.16a] inhibited the inflammatory reaction in the draining LNs, as indicated by the reduced total cellularity [Fig.16b], which was due to a decreased number of both CD45+ cells [Fig.16c] and endothelial and HEV cells [Fig.16d, e].

Tab.1 Proteins up-regulated in stimulated-MSC vs unstimulated-MSC, or present only in stimulated-MSC secretome in both biological replicates. Proteins categorized as modulators of angiogenesis according to GOBP analysis (see Fig.12a and b) are shown in bold. Legend: (a) Uniprot accession ID, (b) percentage of the protein sequence that is coverage by the identified peptides, (c) number of unique and razor peptides associated with the protein, (d) –log p-value t-test for biological replicate 1, (e) –log p-value t-test for biological replicate 2, (f) not detected in un-stimulated MSC secretome.

Gene names	Protein Ids [a]	Seq. cov. [%][b]	Raz. + uniq. Pep. [c]	*t-test* -logP_R1 [d]	*t-test* -logP_R2 [e]	Protein names
Adamts7	Q68SA9	2,3	2	only in stimulated-MSC		A disintegrin and metalloproteinase with thrombospondin motifs 7
Aebp1	Q640N1	33,5	27	3,131	6,375	Adipocyte enhancer-binding protein 1
Agrn	**M0QWP1**	**3,0**	**4**	**3,035**	**4,952**	**Agrin**
Angptl4	Q9Z1P8	39,5	9	2,966	5,238	Angiopoietin-related protein 4
B2m	P01887	64,7	5	4,801	3,841	Beta-2-microglobulin
Bgn	P28653	50,9	15	4,188	6,370	Biglycan
C1ra	Q8CG16	57,0	26	6,050	6,539	Complement C1r-A subcomponent
C1s	E9Q6C2	53,6	24	4,007	5,683	Complement C1s-A subcomponent
C1sb	Q8CFG8	21,5	1	4,057	3,594	Complement C1s-B subcomponent
C3	**P01027**	**62,4**	**75**	**6,788**	**6,564**	**Complement C3**
Calr	**P14211**	**51,2**	**13**	**4,465**	**2,672**	**Calreticulin**
Calu	O35887	60,0	3	2,935	4,600	Calumenin
Ccdc43	E9Q2S9	11,7	1	only in stimulated-MSC [f]		Coiled-coil domain-containing protein 43
Ccl3	P10855	41,3	2	only in stimulated-MSC [f]		C-C motif chemokine 3
Ccl5	P30882	26,4	3	only in stimulated-MSC [f]		C-C motif chemokine 5
Ccl7	Q03366	21,6	2	3,054	7,462	C-C motif chemokine 7
Ccl9	P51670	17,2	3	2,568	4,870	C-C motif chemokine 9
Cp	E9PZD8	40,0	27	6,235	8,698	Ceruloplasmin
Creg1	O88668	13,2	2	2,860	2,941	Protein CREG1
Csf1	**P07141**	**18,7**	**9**	**5,764**	**5,213**	**Macrophage colony-stimulating factor 1**
Ctps	P70698	29,9	11	3,375	4,543	CTP synthase 1
Ctsb	P10605	46,6	9	2,566	5,692	Cathepsin B
Ctsd	P18242	39,3	10	4,334	3,669	Cathepsin D
Ctsl1	P06797	50,6	10	4,249	8,609	Cathepsin L1
Ctsz	Q9WUU7	27,8	4	2,648	2,673	Cathepsin Z
Cx3cl1	**O35188**	**9,1**	**3**	**7,532**	**8,700**	**Fractalkine**
Cxcl1	P12850	45,8	3	6,516	7,345	Growth-regulated alpha protein
Cxcl10	**P17515**	**12,2**	**1**	**only in stimulated-MSC [f]**		**C-X-C motif chemokine 10**
Cxcl5	P50228	26,5	3	only in stimulated-MSC [f]		C-X-C motif chemokine 5
Dtd1	Q9DD18	30,6	3	only in stimulated-MSC [f]		D-tyrosyl-tRNA(Tyr) deacylase 1

Dync1h1	Q9JHU4	30,4	90	5,677	2,678	Cytoplasmic dynein 1 heavy chain 1
Ecm1	Q61508	52,1	1	5,541	5,793	Extracellular matrix protein 1
Eef1a1	P10126	54,5	17	2,804	2,518	Elongation factor 1-alpha 1
Eif3a	P23116	22,2	23	2,560	3,022	Eukaryotic translation initiation factor 3 subunit A
Eprs	Q8CGC7	30,1	27	3,291	2,959	Bifunctional glutamate/proline--tRNA ligase
Ext1	P97464	9,8	4	4,912	4,066	Exostosin-1
Ext2	P70428	12,0	5	3,249	6,039	Exostosin-2
Flnb	Q80X90	64,5	99	6,812	4,598	Filamin-B
Fstl1	Q62356	64,7	17	2,447	4,032	Follistatin-related protein 1
Gars	Q9CZD3	36,6	18	3,325	3,916	Glycine--tRNA ligase
Gbp2	Q9Z0E6	10,9	3	only in stimulated-MSC [f]		Interferon-induced guanylate-binding protein 2
Gm13646	Q8CGP2-2	23,9	3	3,427	3,206	Histone H2B
Gpc1	Q9QZF2	37,7	13	4,578	3,819	Glypican-1
H2-D1	P01899	20,7	6	7,299	5,107	H-2 class I histocompatibility antigen, D-B alpha chain
H3f3c	P02301	23,5	1	only in stimulated-MSC [f]		Histone H3.3C
Hexb	P20060	11,2	4	3,303	6,383	Beta-hexosaminidase subunit beta
Hist1h2ab	P22752	26,9	3	4,538	3,967	Histone H2A type 1
Hist1h3b	P84228	23,5	1	only in stimulated-MSC [f]		Histone H3.2
Hist1h4a	P62806	48,5	6	4,735	3,063	Histone H4
Hk2	O08528	5,6	1	only in stimulated-MSC [f]		Hexokinase-2
Hmox1	**P14901**	**59,9**	**10**	**5,557**	**3,425**	**Heme oxygenase 1**
Hp	Q61646	47,8	17	8,530	7,108	Haptoglobin
Hspa5	P20029	42,7	22	4,425	2,664	78 kDa glucose-regulated protein
Hspg2	E9PZ16	45,2	104	5,257	7,416	
Ifnar2	O35664-3	9,7	2	2,799	6,573	Interferon alpha/beta receptor 2
Igfbp7	F8WH23	57,1	13	4,351	7,034	Insulin-like growth factor-binding protein 7
Il6	**P08505**	**45,5**	**6**	**only in stimulated-MSC [f]**		**Interleukin-6**
Iqgap1	Q9JKF1	53,8	60	3,839	2,561	Ras GTPase-activating-like protein IQGAP1
Lama5	**Q61001**	**7,0**	**15**	**3,714**	**3,101**	**Laminin subunit alpha-5**
Lamb1	**E9QN70**	**21,2**	**24**	**5,999**	**5,236**	**Laminin subunit beta-1**
Lamc1	F8VQJ3	28,6	25	6,874	4,970	Laminin subunit gamma-1

Lcn2	P11672	42,0	8	only in stimulated-MSC [f]		Neutrophil gelatinase-associated lipocalin
Lgals3bp	Q07797	44,4	17	3,452	8,915	Galectin-3-binding protein
Lox	**P28301**	**63,5**	**15**	**6,640**	**7,780**	**Protein-lysine 6-oxidase**
Mfge8	**P21956**	**28,9**	**8**	**3,208**	**2,663**	**Lactadherin**
Mgat5	Q8R4G6	4,3	2	3,025	3,319	Alpha-1,6-mannosylglycoprotein 6-beta-N-acetylglucosaminyltransferase A
Mmp13	P33435	70,1	25	10,006	8,003	Collagenase 3
Mmp9	P41245	15,8	6	only in stimulated-MSC [f]		Matrix metalloproteinase-9
Mvp	E9Q3X0	25,7	13	2,606	3,401	Major vault protein
Nid1	P10493	36,5	26	6,439	2,611	Nidogen-1
Nit1	Q8VDK1	12,1	2	only in stimulated-MSC [f]		Nitrilase homolog 1
Nos2	**P29477**	**20,5**	**12**	**only in stimulated-MSC [f]**		**Nitric oxide synthase, inducible**
Npc2	Q9Z0J0	32,2	4	2,500	4,317	Epididymal secretory protein E1
P4ha2	Q5SX75	28,7	9	4,720	4,039	Prolyl 4-hydroxylase subunit alpha-2
P4hb	P09103	56,6	23	2,921	5,326	Protein disulfide-isomerase
Pfkl	P12382	27,6	11	2,712	2,753	6-phosphofructokinase, liver type
Pla1a	Q8VI78	15,8	4	only in stimulated-MSC [f]		Phospholipase A1 member A
Plod2	E9Q718	11,7	5	4,693	4,598	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2
Plod3	Q9R0E1	30,4	14	5,994	3,766	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3
Prl2c2	**P04095**	**24,6**	**4**	**4,026**	**4,441**	**Prolactin-2C2**
Rps10	P63325	32,1	4	2,621	4,764	40S ribosomal protein S10
Saa3	P04918	41,8	7	only in stimulated-MSC [f]		Serum amyloid A-3 protein
Serpine1	**G5E899**	**52,2**	**16**	**6,729**	**7,471**	**Plasminogen activator inhibitor 1**
Shisa5	Q9D7I0	13,2	2	only in stimulated-MSC [f]		Protein shisa-5
Snd1	Q78PY7	52,9	30	3,042	4,672	Staphylococcal nuclease domain-containing protein 1
Srsf5	Q9D8S5	8,9	2	only in stimulated-MSC [f]		Serine/arginine-rich splicing factor 5
Timp1	**P12032**	**55,6**	**9**	**7,125**	**9,443**	**Metalloproteinase inhibitor 1**
Tnc	Q80YX1-2	32,4	36	4,026	7,013	Tenascin
Vcam1	P29533	39,9	20	8,128	5,358	Vascular cell adhesion protein 1

Tab.2 Proteins down-regulated in stimulated-MSC vs unstimulated-MSC, or present only in unstimulated-MSC secretome in both biological replicates. Legend: (a) Uniprot accession ID, (b) percentage of the protein sequence that is coverage by the identified peptides, (c) number of unique and razor peptides associated with the protein, (d) –log p-value t-test for biological replicate 1, (e) –log p-value t-test for biological replicate 2, (f) not detected in un-stimulated MSC secretome.

Gene names	Protein Ids [a]	Seq. cov. [%][b]	Raz. + uniq. Pep.[c]	*t-test* -logP_R1 [d]	*t-test* -logP_R2 [e]	Protein names
Abhd14b	E9QN99	36,2	6	3,25	2,92	Abhydrolase domain-containing protein 14B
Acaa2	Q8BWT1	37,5	9	4,68	2,86	3-ketoacyl-CoA thiolase, mitochondrial
Acadm	P45952	8,3	8	3,15	5,36	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial
Aco2	Q99KI0	46,5	24	4,49	3,68	Aconitate hydratase, mitochondrial
Acot2	Q9QYR9	28,5	10	3,59	3,50	Acyl-coenzyme A thioesterase 2, mitochondrial
Agt	Q3UTR7	41,5	14	6,29	3,12	Angiotensinogen
Ahnak	E9Q616	65,8	175	7,28	7,08	Protein Ahnak
Ak3	Q9WTP7	32,2	5	2,76	3,26	GTP:AMP phosphotransferase, mitochondrial
Akap12	Q9WTQ5	41,9	36	5,41	7,36	A-kinase anchor protein 12
Alyref	O08583	17,3	3	2,97	2,73	THO complex subunit 4
Ciapin1	Q8WTY4	17,5	2			Anamorsin
Cirbp	P60824	26,7	3	3,30	3,04	Cold-inducible RNA-binding protein
Col6a1	Q04857	52,8	35	7,30	5,71	Collagen alpha-1(VI) chain
Col6a2	Q02788	37,1	23	6,14	5,36	Collagen alpha-2(VI) chain
Col6a3	E9PWQ3	51,3	111	4,76	6,81	Protein Col6a3
Exosc8	Q9D753	13,0	2			Exosome complex component RRP43
Fabp5	Q05816	57,8	6	3,77	3,01	Fatty acid-binding protein, epidermal
Fhl1	A2AEX6	32,7	6	2,54	3,21	Four and a half LIM domains protein 1
Fn1	P11276	60,1	95	7,20	4,17	Fibronectin
Ftl1	Q9CPX4	60,1	7	3,18	4,44	Ferritin
Got2	P05202	56,0	17	3,87	3,17	Aspartate aminotransferase, mitochondrial
Gsn	P13020	47,9	27	2,55	3,56	Gelsolin
Gstm2	P15626	59,6	13	3,58	3,33	Glutathione S-transferase Mu 2
Hmgn5	Q9JL35	5,9	1			High mobility group nucleosome-binding domain-containing protein 5
Idh1	O88844	50,0	19	3,08	3,86	Isocitrate dehydrogenase [NADP] cytoplasmic
Ldhb	P16125	56,0	12	3,86	3,82	L-lactate dehydrogenase B chain
Lmna	P48678	54,0	31	2,72	3,68	Prelamin-A/C
Lmnb1	P14733	31,0	14	3,98	4,14	Lamin-B1
Matn2	O08746	23,0	13	3,13	3,11	Matrilin-2
Mdh2	P08249	56,2	14	2,88	4,20	Malate dehydrogenase, mitochondrial

Myof	E9Q390	8,8	13	2,89	4,62	Myoferlin
Nov	Q64299	35,0	7	4,31	4,02	Protein NOV homolog
Nras	P08556	13,2	1			GTPase Nras
Nucks1	Q80XU3	28,2	4	3,80	5,88	Nuclear ubiquitous casein and cyclin-dependent kinase substrate 1
Ogn	Q62000	43,3	12	7,05	6,01	Mimecan
Pck2	Q8BH04	42,7	19	4,30	2,90	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial
Pcna	P17918	73,6	11	2,74	2,63	Proliferating cell nuclear antigen
Pik3ip1	Q7TMJ8	17,8	3			Phosphoinositide-3-kinase-interacting protein 1
Pls3	B1AX58	59,6	29	2,58	4,07	Plastin-3
Prdx3	P20108	36,2	6	3,97	5,14	Thioredoxin-dependent peroxide reductase, mitochondrial
Ptk7	Q8BKG3	29,4	19	3,02	5,58	Inactive tyrosine-protein kinase 7
Puf60	Q3UEB3	28,4	9	3,53	2,65	Poly(U)-binding-splicing factor PUF60
Samhd1	F8WJE0	43,0	24	3,83	3,36	SAM domain and HD domain-containing protein 1
Serpinc1	P32261	15,7	6	2,65	2,77	Antithrombin-III
Smc1a	Q9CU62	1,7	2			Structural maintenance of chromosomes protein 1A
Sparcl1	P70663	4,2	2			SPARC-like protein 1
Sptan1	P16546-2	47,6	80	6,08	6,57	Spectrin alpha chain, brain
Sptbn1	Q62261	36,9	57	4,05	4,87	Spectrin beta chain, brain 1
Tagln	P37804	46,8	8	6,02	3,47	Transgelin
Ube2h	P62257	21,9	3	3,52	2,81	Ubiquitin-conjugating enzyme E2 H
Vcl	Q64727	52,3	42	5,25	4,52	Vinculin
Ywhae	P62259	67,5	18	3,67	2,72	14-3-3 protein epsilon

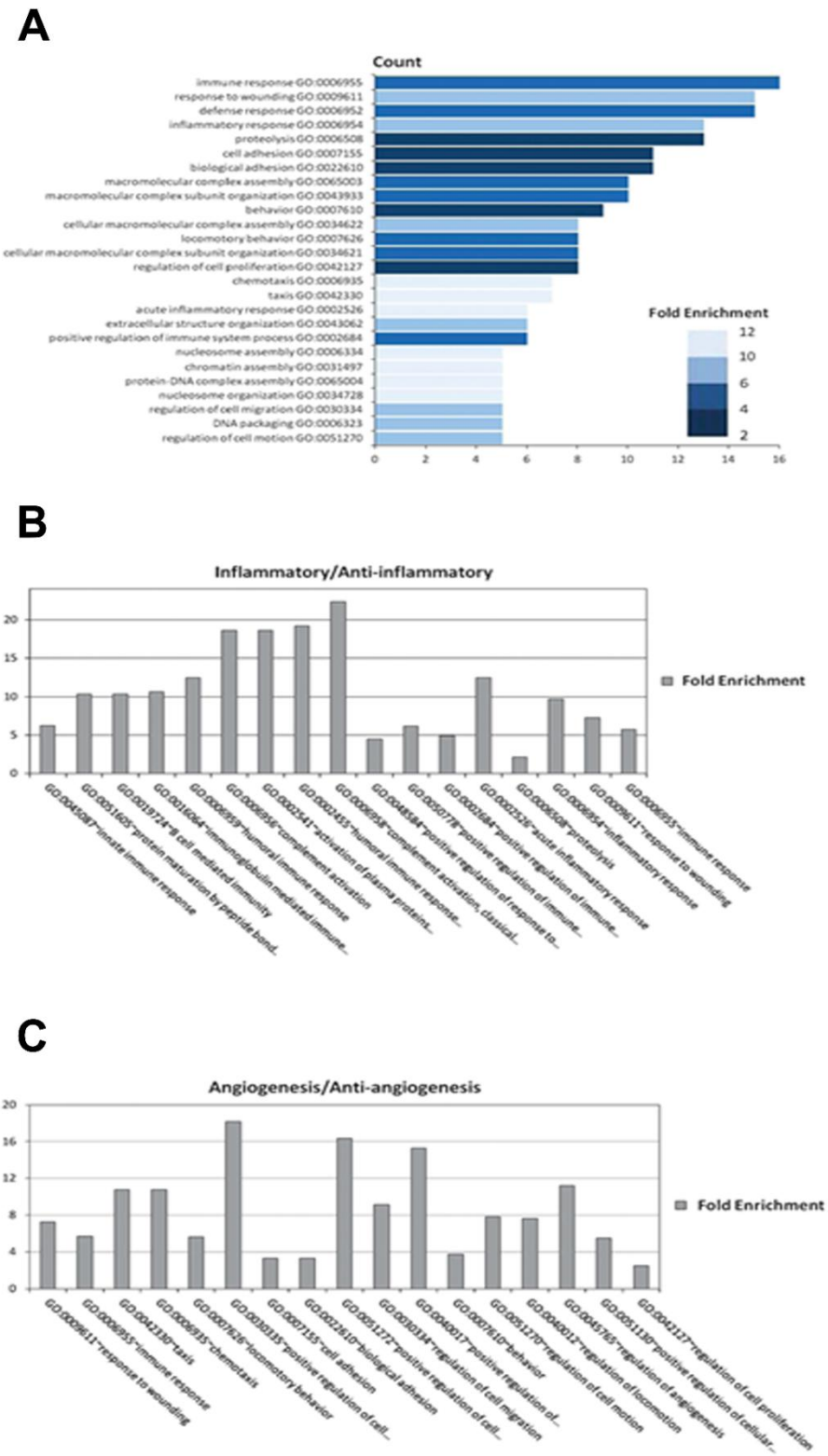


Fig.12 Distribution into biological processes of the proteins upregulated in MSC-CM. The proteins that were significantly upregulated or present only in MSC-CM were classified into different biological processes according to the GO classification system. (a) The bar chart shows the count of the top 26 most-enriched GO terms in MSC-CM versus unstimulated MSC-CM. Color coding indicates the fold enrichment. (b) Proteins were categorized as modulators involved in inflammation processes and/or angiogenesis. The histograms report the GOBP groups related to these categories.

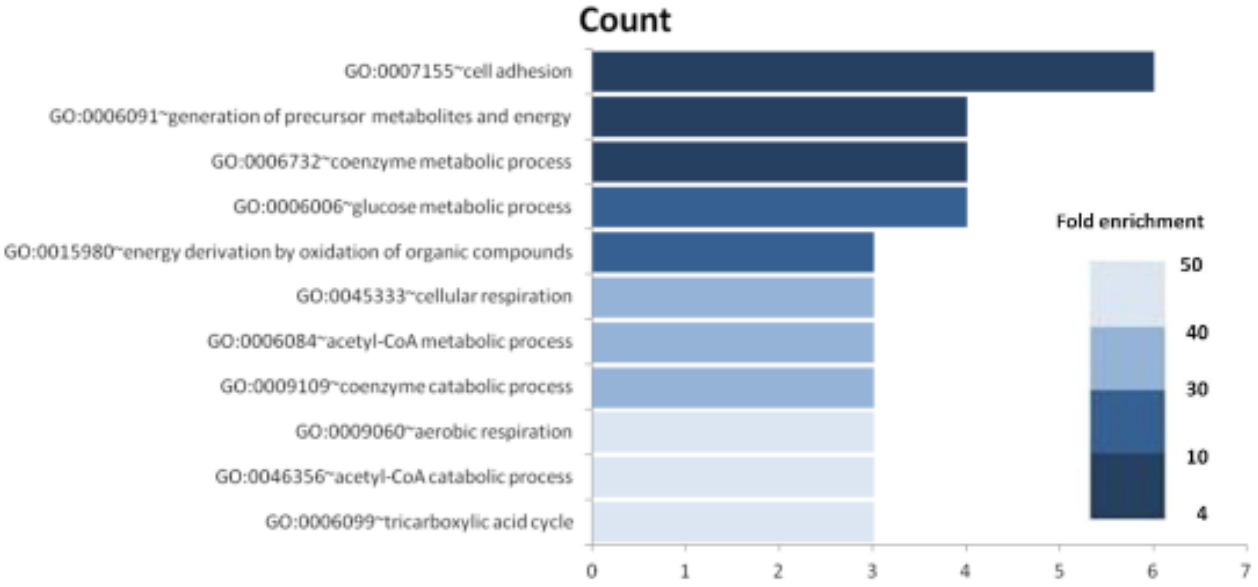


Fig.13 Distribution into biological processes of the proteins downregulated in MSC-CM. The proteins that were significantly downregulated or present only in unstimulated secretome MSC-CM were classified into different biological processes according to the GO classification system. Analysis reveal that most term are related to metabolic processes.

Mouse Protein Id	Human Protein Id	Gene name	Protein name	Role in angiog
M0QWP1	O00468-6	AGRN	Agrin	X
P10605	P07858	CTSB	Cathepsin B	X
P50228	P42830	CXCL5	C-X-C motif chemokine 5	X
E9Q6C2	P09871	C1S	Complement C1s subcomponent	
P01027	P01024	C3	Complement C3 fragment	
Q62356	Q12841	FSTL1	Follistatin-related protein 1	X
E9PZ16	P98160	HSPG2	Perlecan	X
F8WH23	Q16270	IGFBP7	Insulin-like growth factor-binding protein 7	X
Q07797	Q08380	LGALS3BP	Galectin-3-binding protein	X
P10493	P14543	NID1	Nidogen-1	X
G5E899	P05121	SERPINE1	Plasminogen activator inhibitor 1	X
P12032	P01033	TIMP1	Metalloproteinase inhibitor 1	X
Q80YX1	P24821	TNC	Tenascin	X
P29533	P19320	VCAM1	Vascular cell adhesion protein 1	X

Tab.3 Common proteins up regulated in stimulated- vs- unstimulated- mouse and human secretome. Proteins involved in angiogenesis are indicated in detail.

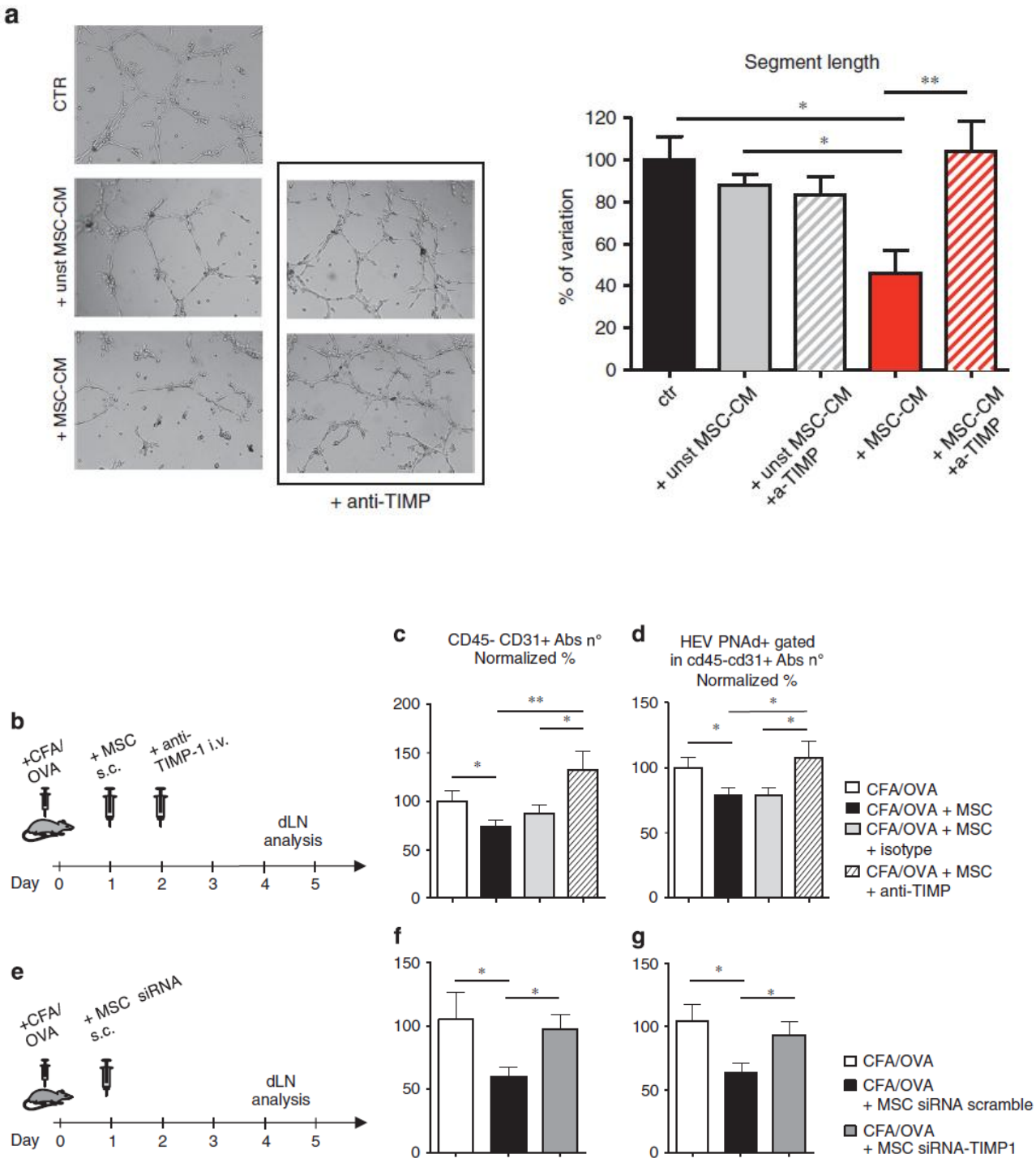


Fig.14 TIMP-1 mediates the anti-angiogenic effect of MSC-CM *in vitro* and the anti-inflammatory effect of MSC *in vivo*. SVEC4-10 network formation in matrigel in the presence of MSC-CM or unst MSC-CM and anti-TIMP-1 blocking antibody.

(a) anti-mTIMP1 blocking antibody restores SVEC4-10 network formation in matrigel in the presence of MSC-CM. Representative images at 6 h (left) and segment length quantification as percentage of variation (right) are shown. Data are expressed as mean±s.e.m. (*P<0.05, **P<0.01; one-way ANOVA). (b) Diagram of the experimental protocol designed to block the TIMP-1 activity during the anti-inflammatory effects of

MSC. Mice were immunized in the dorsal region with CFA/OVA on day 0 and, on day 1, three groups of animals received subcutaneous injection of 106 MSC in the lumbar region. Eighteen hours after MSC transplantation, goat polyclonal anti-TIMP-1 IgG or isotype-matched goat IgG was i.v. administrated. On day 4,*-3 brachial LNs were collected, processed and analyzed by flow cytometry; (c, d) the graphs show the absolute number of CD45−CD31+ cells and HEV PNAd+ cells per single LN, expressed as normalized percentage on CFA/OVA (t-test). (e) Diagram of the experimental protocol designed to investigate the contribution of MSC-derived TIMP-1 on dLN endothelium. Mice were immunized in the dorsal region with CFA/OVA on day 0. The day after, two groups of animals received in the lumbar region subcutaneous injection of 106 MSC transfected with either scramble control siRNA or siRNA specific for TIMP-1, respectively. On day 4, brachial LNs were collected, processed and analyzed by flow cytometry; (f, g) graphs showing the absolute number of CD45−CD31+ cells and HEV PNAd+ cells per single dLN. Data are expressed as normalized percentage on CFA/OVA (Mann–Whitney test) (*P<0.05; **P<0.01).

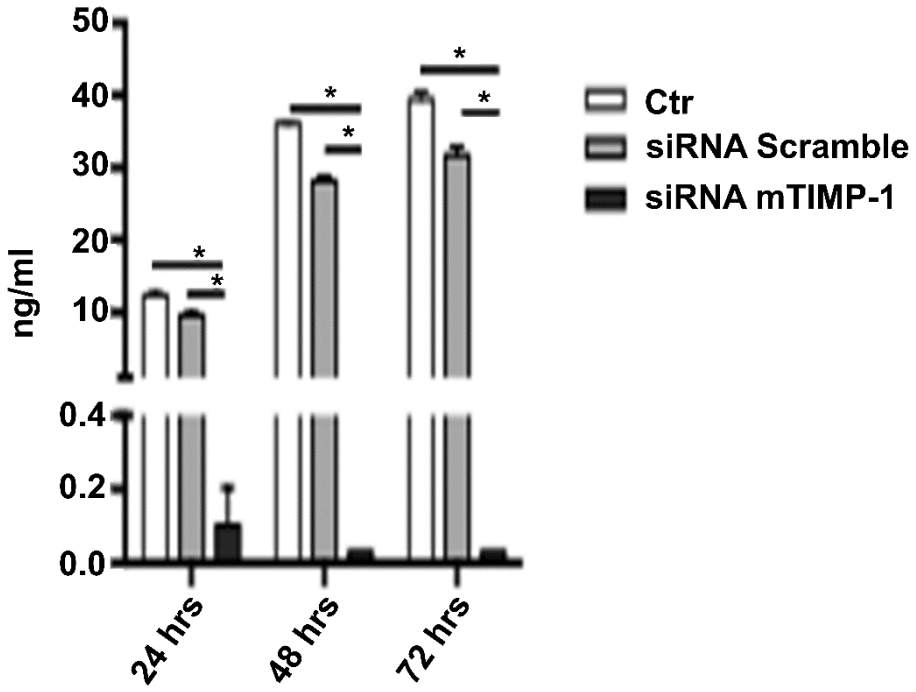


Fig.15 siRNA approach to knock down TIMP-1 expression in MSC. Histograms represent levels of mTIMP-1 production after 24, 48 and 72 hours after sRNA silencing.

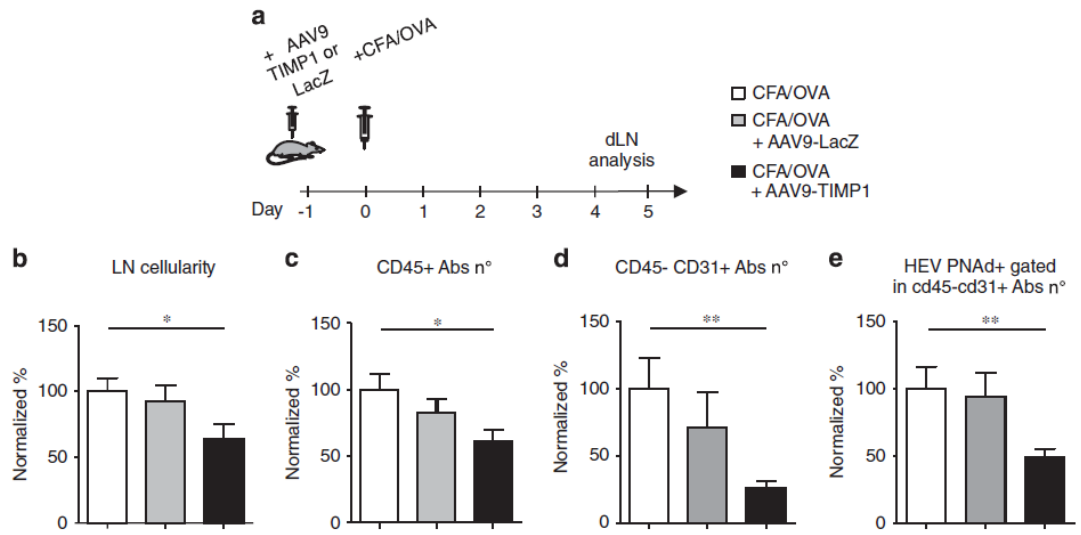


Fig. 16. TIMP-1 overexpression *in vivo* mimics MSC transplantation. (a) Diagram of the experimental protocol designed to overexpress TIMP-1 in immunized mice. One day after AAV9-TIMP-1 or AAV9-LacZ administration (day 0), mice were immunized with CFA/OVA. Brachial dLNs were collected 4 days after immunization and processed for flow cytometry. The graphs show the absolute number of total cells (b), CD45+ cells (c), CD45−CD31+ (d) and HEV PNAd+ (e) cells per single LN, expressed as normalized percentage on CFA/OVA. Error bars represent standard error (*Po0.05; **Po0.01; Mann–Whitney test).

3.2 Proteomic characterization of human MSC (hMSC) secretome

To the best of our knowledge, the present study reports for the first time a quantitative proteomic characterization of the secretome of human bone marrow-derived MSC primed with pro-inflammatory cytokines. Proteomic analyses were conducted under exactly the same conditions used in the previous investigation on mMSC in order to avoid variations with methodology, allowing direct comparative analysis between the results obtained with the two organisms. The proteomic characterization of human MSC secretome was performed on samples from two different patients, indicated as “donors H30” and “donors H34”, before (CTR condition/ unstimulated human MSC conditioned medium) and after stimulation (MIX condition/ stimulated human MSC conditioned medium). Fig.17 summarizes the results of the proteomic characterization of secretome of hMSC before (unst-) and after (st-) stimulation with inflammatory cytokines. Only the 497 proteins present in at least 3 out of 5 technical repeats in both biological replicates (donors H30 and H34) were considered for further analysis: these proteins are listed in Tab. Suppl.

together with their main identification parameters. Fig.17 highlights the number of proteins detected in stimulated human MSC conditioned medium (465 in st hMSC-CM) and unstimulated human MSC conditioned medium (457 in unst hMSC-CM). As shown in Fig.17, 32 proteins and 39 proteins were exclusively identified in unst- and st- cells respectively. Amongst the 465 proteins identified in st hMSC-CM (proteins in groups 1, 2, 4 and 5 of Tab.S1.), 133 are listed as cytokine or chemokine or functionally related to these classes of compounds according to the NextProt database [122].

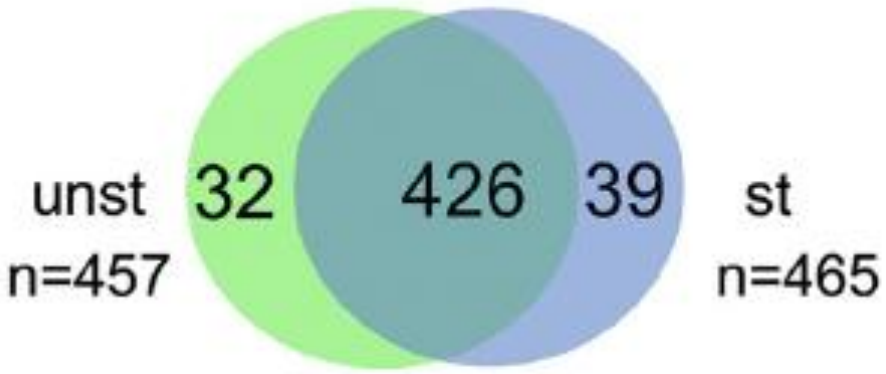


Fig.17 Summary of the results obtained in the proteomic characterization of hMSC-CM. Venn diagram showing proteins detected in at least 3 out of 5 technical replicas in both patients in stimulated hMSC-MC and unstimulated hMSC-CM

3.2.1 Proteins up-regulated in stimulated human MSC-CM

Since MSC enhance their therapeutic efficacy following priming by cytokines [59, 140], analyses were focused on proteins over-expressed or present only in stimulated human MSC compared to unstimulated human MSC secretome. In particular 39 proteins are present only in stimulated hMSC-CM, while 426 are common to stimulated and unstimulated hMSC-CM [Fig.17]. The statistical analysis of the common proteins indicates that 57 proteins are up-regulated in stimulated hMSC-CM (t-test difference, cut-off at 1% permutation-based False Discovery Rate). A Pearson correlation coefficient R=0.73 was calculated by comparing the log(2) t-test difference value in the two biological replicates [Fig.18]. Overall, 96 proteins are up-regulated or present only in stimulated hMSC-CM. These proteins, listed in Tab. 5, are predicted to be potentially secreted, included in exosomes according to annotations in Gene Ontology [141], NextProt [122], UniProt [142], Gene Cards [124], in datasets [125, 143] or from manual literature mining. A subsequent bioinformatics analysis, performed with the aim of evaluating enriched proteins, showed

that 70% and 64% are involved in inflammation or angiogenesis, respectively [Tab. 4, Tab. 7] and to evaluate the extended network of interaction, amongst inflammation or angiogenesis related proteins up-regulated in stimulated hMSC-CM, a STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) [128] analysis was used. Fig.19, represents the graphical expression of the network considered, showing the proteins involved in the processes of inflammation (A) and angiogenesis (B), respectively. Red symbols are proteins present only in stimulated hMSC secretome, yellow symbols indicate proteins with protease or protease inhibitor activity. Moreover, a number of proteases (BMP1, C1R, C1S, CFB, CTSB, MMP1, MMP2, MMP3, MMP10 MMP13, PSMA5, PSME2, QPCT) and protease inhibitors (C3, COL7A1, FBLN1, FN1INHBA, ITIH2, SERPINB2, SERPINE1, TIMP1) are up-regulated in stimulated hMSC secretome, strengthening our suggestion, based on the results obtained in mouse MSC secretome, that a fine but complex tuning of proteolytic activity is a key mechanism regulating MSC effects on angiogenesis and tissue remodeling [144]. MMPs are presently considered not only effectors but also regulators of a number of biological processes since they can activate, inactivate or antagonize the biological functions of growth factors, cytokines and chemokines by proteolytic processing and thus either promote or suppress inflammation and angiogenesis [145, 146]. Notably several protease/protease inhibitors listed above are amongst the proteins showing large quantitative differences in stimulated vs unstimulated hMSC-CM [Tab.4, Fig.18, Fig.19]. In conclusion, the proteomic analysis of human model reveal that pro-inflammatory cytokines have a strong impact on the secretome of human bone marrow-derived MSC and that the large majority of cytokine-induced proteins are involved in inflammation and, or angiogenesis.

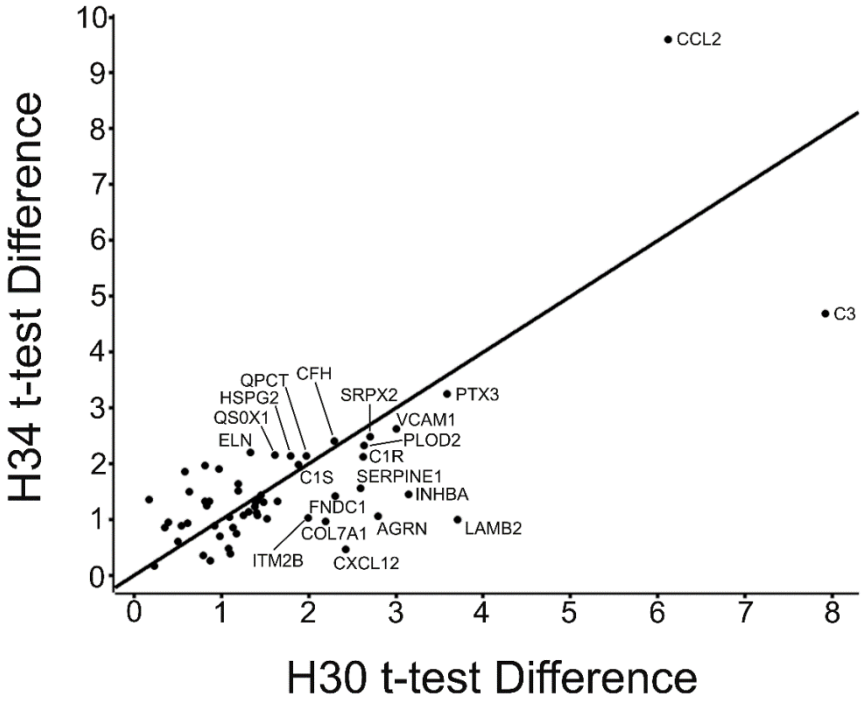


Fig. 18 Pearson correlation from 2 databases: H30 and H34. T-test difference (difference of log(2) mean intensity of a protein in stimulated and unstimulated hMSC-CM replicas, [120]) observed in the two patients for the 57 proteins present in stimulated and unstimulated hMSC-MC and significantly overrepresented in stimulated hMSC-MC according to t-test p-value (cut-off at 1% permutation-based False Discovery Rate). Pearson correlation coefficient R = 0.73. Complete protein identities and detailed values are reported in Tab.4.

Tab.4 Proteins overrepresented or present only in st hMSC-CM. Legend: (a) t-test diff: difference of log(2) mean intensity of a protein in technical replicas of st- *versus* unst hMSC-CM from t-test analysis using Perseus [120] as detailed in the text; (b) proteins related to angiogenesis or inflammation according to criteria detailed in “Materials and methods”.

Gene names	Protein names	Protein ID	H30 -log P t-test	H30 t-test Diff[a]	H34 -log P t-test	H34 t-test Diff[a]	Angiogenesis[b]	Inflammation[b]
ABI3BP	Target of Nesh-SH3	D3YTG3		only in stimulated				
AGRN	Agrin	O00468	5.339	2.799	3.970	1.066	x	x
ALCAM	CD166 antigen	Q13740		only in stimulated			x	x
ARHGAP1	Rho GTPase-activating protein 1	Q07960		only in stimulated				
BMP1	Bone morphogenetic protein 1	P13497	3.745	1.175	4.642	0.751	x	x
C1R	Complement C1r subcomponent	P00736	8.619	2.624	6.801	2.132		x
C1S	Complement C1s subcomponent	P09871	6.886	1.883	6.503	1.992		x
C3	Complement C3	P01024	10.267	7.920	5.001	4.683	x	x
CA12	Carbonic anhydrase 12	O43570		only in stimulated			x	x
CCL2	C-C motif chemokine 2	P13500	9.381	6.120	10.273	9.600	x	x
CDC37	Hsp90 co-chaperone Cdc37	Q16543		only in stimulated				x
CFB	Complement factor B	B4E1Z4		only in stimulated			x	x
CFH	Complement factor H	P08603	5.941	2.293	6.526	2.405		x
CHI3L1	Chitinase-3-like protein 1	P36222	6.932	1.415	6.918	1.353	x	x
CLSTN1	Calsyntenin-1	O94985	4.674	0.615	3.190	0.932		
COL16A1	Collagen alpha-1(XVI) chain	A6NDR9	5.166	1.459	4.026	1.439		x
COL3A1	Collagen alpha-1(III) chain	P02461	7.283	0.588	10.347	1.865		x
COL5A2	Collagen alpha-2(V) chain	P05997	1.555	0.170	7.749	1.353		
COL7A1	Collagen alpha-1(VII) chain	Q02388	5.682	2.193	2.828	0.961		x
CSF1	Macrophage colony-stimulating factor 1	P09603		only in stimulated			x	x
CTHRC1	Collagen triple helix repeat-cont prot 1	Q96CG8	5.339	1.314	4.189	1.134	x	
CTSB	Cathepsin B	P07858	3.149	0.636	5.472	1.500	x	x
CXCL1	Growth-regulated alpha protein	P09341		only in stimulated			x	x
CXCL12	Stromal cell-derived factor 1	P48061	5.665	2.429	1.652	0.470	x	x

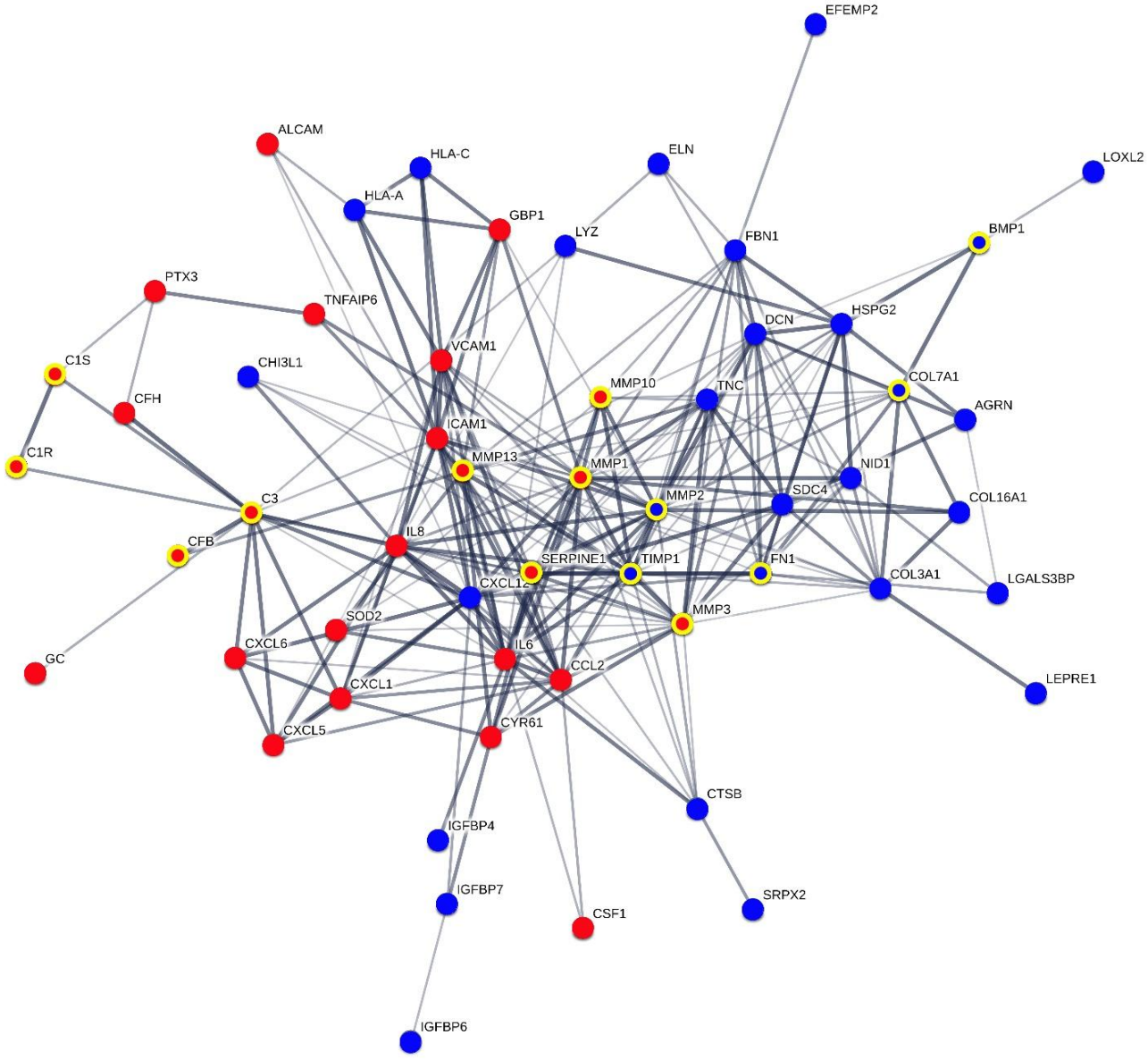
CXCL5	C-X-C motif chemokine 5	P42830		only in stimulated			x	x
CXCL6	C-X-C motif chemokine 6	P80162		only in stimulated			x	x
CYR61	Protein CYR61	O00622		only in stimulated			x	x
DCN	Decorin	P07585	5.699	0.835	5.760	1.253	x	x
EFEMP2	EGF-cont fibulin-like extrac matrix prot 2	O95967	3.013	1.193	5.790	1.640		x
EIF6	Eukaryotic translation initiation factor 6	P56537		only in stimulated			x	x
ELN	Elastin	F8WAH6	4.693	1.332	5.715	2.206	x	x
EXT1	Exostosin-1	Q16394	3.775	1.085	2.266	0.485		
EXT2	Exostosin-2	Q93063		only in stimulated				
FBLN1	Fibulin-1	P23142	4.242	0.867	6.450	1.323	x	
FBN1	Fibrillin-1	P35555	6.035	0.969	6.557	1.909	x	x
FKBP1A	Peptidyl-prolyl cis-trans isomerase	P62942		only in stimulated				x
FN1	Fibronectin	P02751	5.787	0.793	5.421	0.358	x	x
FNDC1	Fibronectin type III domain-cont prot 1	Q4ZHG4	5.245	2.301	7.588	1.418	x	x
FSTL1	Follistatin-related protein 1	Q12841	3.823	0.817	7.039	1.968	x	x
GALNT2	Polypeptide N-acetylgalactosaminyltransferase 2	Q10471	2.914	0.542	2.860	0.895	x	
GBP1	Interferon-induced guanylate-binding prot 1	P32455		only in stimulated			x	x
GC	Vitamin D-binding protein	P02774		only in stimulated				x
HLA-A	HLA class I histocompatibility antigen, A-24 alpha chain	P05534	5.130	1.393	3.813	1.312		x
HLA-C	HLA class I histocompatibility antigen, Cw-7 alpha chain	A2AEA2	3.071	1.381	3.304	1.231		x
HSPG2	Base membr-spec hepar sulf proteoglycan core prot	P98160	8.735	1.796	9.468	2.141	x	x
HYOU1	Hypoxia up-regulated prot 1	Q9Y4L1		only in stimulated			x	x
ICAM1	Intercellular adhesion molecule 1	P05362		only in stimulated			x	x
IGFBP4	Insulin-like growth factor-binding prot 4	P22692	4.346	1.526	3.986	1.009	x	x
IGFBP6	Insulin-like growth factor-binding prot 6	P24592		only in stimulated			x	x
IGFBP7	Insulin-like growth factor-binding prot 7	Q16270	2.725	0.397	4.650	0.947	x	x
IL6	Interleukin-6	P05231		only in stimulated			x	x
IL8	Interleukin-8	P10145		only in stimulated			x	x
INHBA	Inhibin beta A chain	P08476	7.879	3.140	5.875	1.448	x	
ITIH2	Inter-alpha-trypsin inhibitor heavy chain H2	P19823	3.742	1.104	1.276	0.382		x
ITM2B	Integral membrane protein 2B;BRI2	Q9Y287	3.573	1.994	5.351	1.035		
KRT6B	Keratin, type II cytoskeletal 6B	P04259		only in stimulated				
LAMA4	Laminin subunit alpha-4	Q16363	2.027	0.234	1.925	0.173	x	

LAMB2	Laminin subunit beta-2	P55268	5.174	3.708	4.936	1.006		
LEPRE1	Prolyl 3-hydroxylase 1	Q32P28		only in stimulated			x	x
LGALS3BP	Galectin-3-binding protein	Q08380	8.011	1.487	6.749	1.314	x	x
LOXL2	Lysyl oxidase homolog 2	Q9Y4K0	6.219	1.195	5.732	1.521	x	x
LYZ	Lysozyme C	P61626	3.547	0.507	1.328	0.607		x
MAN1A1	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA	P33908		only in stimulated			x	x
MANBA	Beta-mannosidase	O00462		only in stimulated				
MMP1	Interstitial collagenase	P03956		only in stimulated			x	x
MMP10	Stromelysin-2	P09238		only in stimulated			x	x
MMP13	Collagenase 3	G5E971		only in stimulated			x	x
MMP2	72 kDa type IV collagenase	P08253	6.555	1.090	7.061	1.043	x	x
MMP3	Stromelysin-1	P08254		only in stimulated			x	x
NID1	Nidogen-1	P14543	3.840	0.925	3.450	0.890	x	x
NID2	Nidogen-2	Q14112	4.384	1.133	3.823	0.855		
NUCB1	Nucleobindin-1	Q02818	5.878	0.985	3.867	0.703		
PLOD1	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	B4DR87	3.944	0.876	2.180	0.264		
PLOD2	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	O00469	5.967	2.636	5.402	2.331	x	
PSMA5	Proteasome subunit alpha type-5	P28066		only in stimulated				
PSME2	Proteasome activator complex subunit 2	Q9UL46		only in stimulated				
PTX3	Pentraxin-related protein PTX3	P26022	7.000	3.580	8.516	3.256	x	x
PXDN	Peroxidasin homolog	Q92626	6.359	1.401	5.119	1.122		
QPCT	Glutaminyl-peptide cyclotransferase	Q16769	3.070	1.972	4.593	2.140		x
QSOX1	Sulfhydryl oxidase 1	O00391	5.232	1.616	9.152	2.155	x	
RNASE4	Ribonuclease 4	P34096		only in stimulated			x	x
SDC4	Syndecan-4	P31431	1.696	0.810	3.199	1.321	x	x
SDF4	45 kDa calcium-binding prot	Q9BRK5	2.014	0.353	3.547	0.862	x	
SERPINB2	Plasminogen activator inhibitor 2	P05120		only in stimulated				x
SERPINE1	Plasminogen activator inhibitor 1	P05121	8.172	2.596	6.111	1.557	x	x
SLC39A14	Zinc transporter ZIP14	Q15043		only in stimulated				
SLC3A2	4F2 cell-surface antigen heavy chain	P08195		only in stimulated				
SOD2	Superoxide dismutase [Mn]	P04179		only in stimulated			x	x
SRGN	Serglycin	P10124	5.249	1.412	3.353	1.084	x	
SRPX2	Sushi repeat-containing prot SRPX2	O60687	7.209	2.708	3.423	2.482	x	x

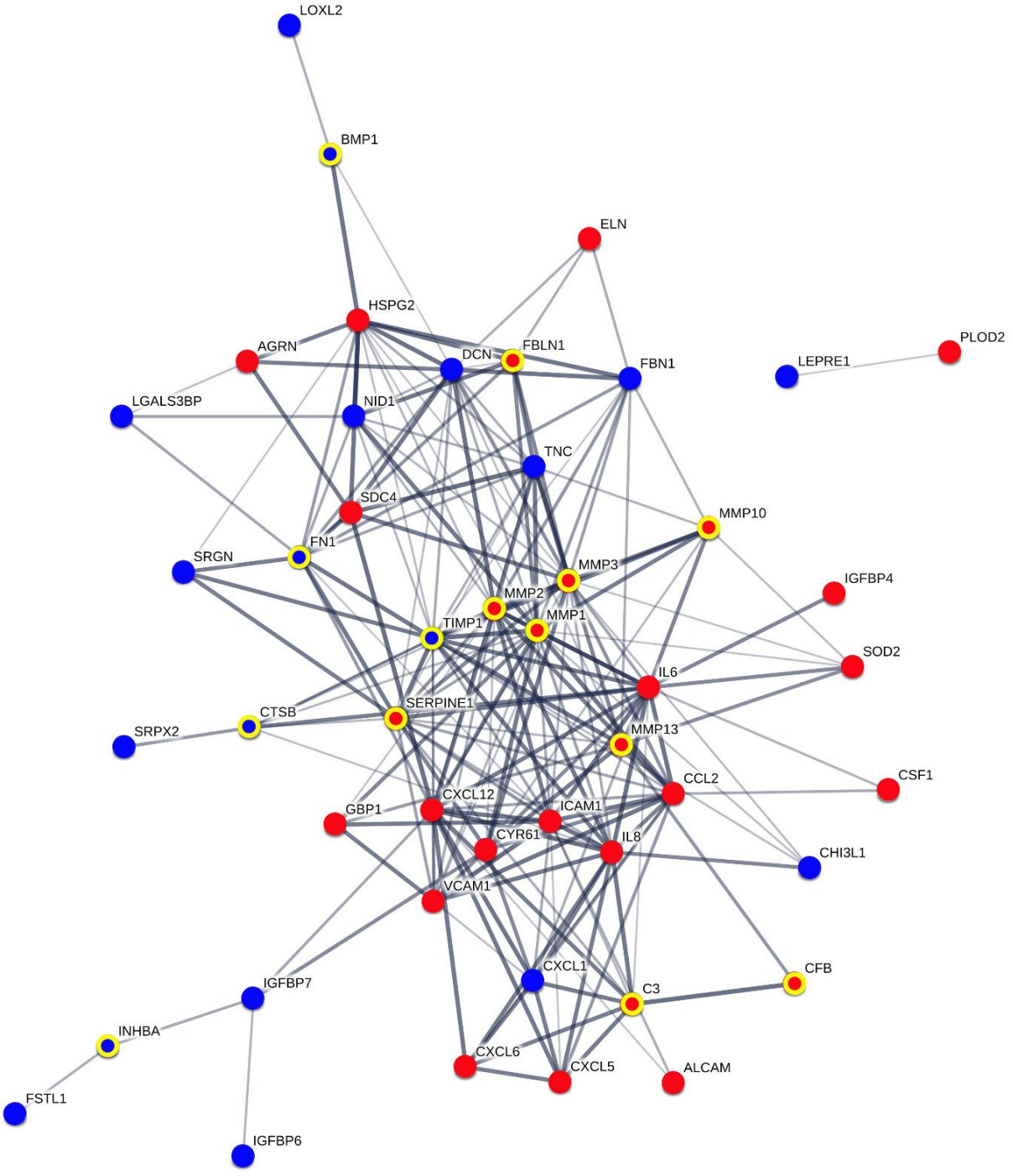
SSB	Lupus La protein	P05455		only in stimulated				
STC2	Stanniocalcin-2	O76061		only in stimulated			x	
TIMP1	Metalloproteinase inhibitor 1	P01033	2.940	1.254	5.763	1.081	x	x
TNC	Tenascin	P24821	6.040	1.645	7.257	1.329	x	x
TNFAIP6	Tumor necrosis factor-inducible gene 6 protein	P98066		only in stimulated				x
VCAM1	Vascular cell adhesion protein 1	P19320	5.307	3.003	5.227	2.623	x	x

Fig. 19 Network interactions of overrepresented proteins in stimulated hMSC-CM involved in inflammation or in angiogenesis. Overrepresented proteins in stimulated hMSC-CM involved in inflammation (A) or angiogenesis (B), respectively, according to targeted accurate literature mining as reported in Tab.4, have been searched for possible interactions using **String** [128]. Active interactions: text mining, experiments, databases; edges thickness indicates "confidence". Red symbols: proteins present only in stimulated hMSC secretome or showing high t-test difference according to Fig.18. Yellow edges indicate proteins with proteases/protease inhibitors activity.

A



B


LOXL2
BMP1
ELN
HSPG2
AGRN
DCN
FBLN1
FBN1
PLOD2
LEPRE1
LGALS3BP
NID1
TNC
SDC4
MMP10
FN1
SRGN
MMP3
IGFBP4
MMP2
TIMP1
MMP1
SOD2
IL6
SERPINE1
CTSB
SRPX2
MMP13
CSF1
CCL2
CXCL12
GBP1
ICAM1
CYR61
IL8
CHI3L1
VCAM1
CXCL1
CFB
IGFBP7
C3
INHBA
CXCL6
CXCL5
ALCAM
FSTL1
IGFBP6

3.2.3 Comparison between different stimulation conditions

Since it has been established that tissue origin, growth and stimulation conditions may influence the type and quantity of proteic components of MSC secretome [147], we compared the list of up-regulated proteins in st hMSC-CM with those reported in recent studies performed using a similar mass spectrometry based quantitative proteomic approach on human MSC. Tab. 5 confirms that different stimulation conditions lead to up-regulation of largely different sets of proteins. Notably, 24 proteins (25%) detected as up-regulated in our study were up-regulated also in TNF-α stimulated MSC deriving from a different adult tissue (adipose tissue) [148]. This finding gives experimental evidences at molecular level, supporting the idea that the type of stimulus influences the MSC secretome.

Tab5. Comparison of up-regulated proteins in st hMSC-CM according to the present study with those reported in previous mass spectrometry based proteomic studies on stimulated human MSC-CM. Stimulation condition: (a) Dexamethasone 10 $^{-3}$M, retinoic acid 10^{-7}M, ascorbic acid 10 uL/mL, transferrin 10 uL/mL, TGF-beta310 ng/mL, FBS 15%; (b) 20%FBS, 10 mM beta-glycerophosphate, 50 uM ascorbate-2-phosphate and 100 nM dexamethasone; (c) hTNF-alpha 10 ng/mL; (d) 10 mMbeta-glycerophosphate, 50ug/mL 2-phosphate ascorbate, 10 nM dexamethasone, 10 nM 1.25 dihydroxyvitamin D3; (e) 100 ng/mL recombinant human GF-II; (f) proteins related to angiogenesis or inflammation, accordind to criteria detailed in materials and methods.

Proteins over-represented also in TFN-alpha stimulated MSC form adipose tissue are highlited

						Angiogenesis	Inflammation
MSC tissue origin	Bone marrow	Bone marrow	Adipose tissue	Bone marrow	Bone marrow		
Stimulation conditions	(a)	(b)	(c)	(d)	(e)	(f)	(f)
Total number of up-regulated proteins	34	177	118	185	64		
ABI3BP			**X**	X	X	x	x
AGRN						x	x
ALCAM		X	**X**				
ARHGAP1						x	x
BMP1				X			x
C1R			**X**		X		x
C1S			**X**			x	x
C3				X		x	x
CA12						x	x
CCL2			**X**				x
CDC37						x	x
CFB			**X**	X			x
CFH				X		x	x
CHI3L1							
CLSTN1							x
COL16A1			**X**				x
COL3A1				X			
COL5A2				X			x
COL7A1						x	x
CSF1						x	
CTHRC1				X		x	x
CTSB				X	X	x	x
CXCL1						x	x
CXCL12						x	x

MSC tissue origin	Bone marrow	Bone marrow	Adipose tissue	Bone marrow	Bone marrow	Angiogenesis	Inflammation
Stimulation conditions	(a)	(b)	(c)	(d)	(e)	(f)	(f)
Total number of up-regulated proteins	34	177	118	185	64		
CXCL5			**X**			x	x
CXCL6			**X**			x	x
CYR61				X	X	x	x
DCN							x
EFEMP2						x	x
EIF6						x	x
ELN							
EXT1							
EXT2						x	
FBLN1				X		x	x
FBN1				X	X		x
FKBP1A		X				x	x
FN1					X	x	x
FNDC1						x	x
FSTL1	X			X	X	x	
GALNT2						x	x
GBP1							x
GC							x
HLA-A							x
HLA-C			**X**	X		x	x
HSPG2			**X**		X	x	x
HYOU1						x	x
ICAM1						x	x
IGFBP4	X			X	X	x	x
IGFBP6	X					x	x
IGFBP7	X				X	x	x

MSC tissue origin	Bone marrow	Bone marrow	Adipose tissue	Bone marrow	Bone marrow	Angiogenesis	Inflammation
Stimulation conditions	(a)	(b)	(c)	(d)	(e)	(f)	(f)
Total number of up-regulated proteins	34	177	118	185	64		
IL6			**X**			x	x
IL8			**X**			x	
INHBA			**X**				x
ITIH2							
ITM2B			**X**				
KRT6B						x	
LAMA4				X			
LAMB2				X		x	x
LEPRE1						x	x
LGALS3BP			**X**		X	x	x
LOXL2			**X**		X		x
LYZ						x	x
MAN1A1							
MANBA						x	x
MMP1			**X**			x	x
MMP10						x	x
MMP13						x	x
MMP2				X	X	x	x
MMP3						x	x
NID1				X			
NID2				X			
NUCB1							
PLOD1				X		x	
PLOD2							
PSMA5				X			
PSME2						x	x

MSC tissue origin	Bone marrow	Bone marrow	Adipose tissue	Bone marrow	Bone marrow	Angiogenesis	Inflammation
Stimulation conditions	(a)	(b)	(c)	(d)	(e)	(f)	(f)
Total number of up-regulated proteins	34	177	118	185	64		
PTX3	X		**X**	X	X		
PXDN				X			x
QPCT						x	
QSOX1			**X**			x	x
RNASE4						x	x
SDC4						x	
SDF4					X		x
SERPINB2			**X**			x	x
SERPINE1			**X**	X	X		
SLC39A14							
SLC3A2						x	x
SOD2				X		x	
SRGN				X		x	x
SRPX2				X			
SSB						x	
STC2	X		**X**	X		x	x
TIMP1	X				X	x	x
TNC	X			X	X		x
TNFAIP6			**X**			x	x
VCAM1							

3.3 Proteomic based comparison between mouse and human MSC-CM

Murine models allowed to elucidate the molecular pathway involved in the effects of mMSC on the complex crosstalk between inflammation and angiogenesis [156], but, because it is widely accepted that significant differences exist between mouse and human MSC [59, 149], and because of the tremendous relevance of inflammation-induced angiogenesis in human diseases, we focused our attention on comparing mouse and human MSC secretome.

Tab.S1 lists the 286 proteins (out of the 465 proteins identified in stimulated hMSC-CM, (62%) present in stimulated hMSC-CM that have been detected also in stimulated mMSC-CM. The number of proteins significantly up-regulated or present only in the secretome of stimulated MSC is similar in the two species: 89 in mouse (Tab.1 and 96 in human, respectively [Tab.4]).

A comparative analysis of GO_BP category enrichment of over-represented proteins in human and/or mouse [Fig.20] suggests that:

a) proteins up-regulated in the secretome of stimulated MSC from both organisms are, for the most part, involved in similar biological processes, mainly related to defense, immune and inflammatory response, chemotaxis and extracellular matrix remodeling;

b) there are clear important differences among human and mouse. Thus, only stmMSC-CM is enriched in proteins involved in chromatin structure assembly, cell proliferation regulation and related processes. On the contrary, complement activation, leukocyte migration, bone development and metabolic processes specifically related to collagen are amongst the statistically enriched GO functional categories in human but not in mouse.

Such differences are confirmed by the observation that only 23 proteins are up-regulated or present only in stimulated MSC-CM both in mouse and human [Tab.6]; this again points to a fine species-related tuning of the overall effects of secretome from the two organisms; interestingly, our analysis indicates that 74 % and 83% of the common up-regulated proteins are associated with angiogenesis or inflammation, respectively.

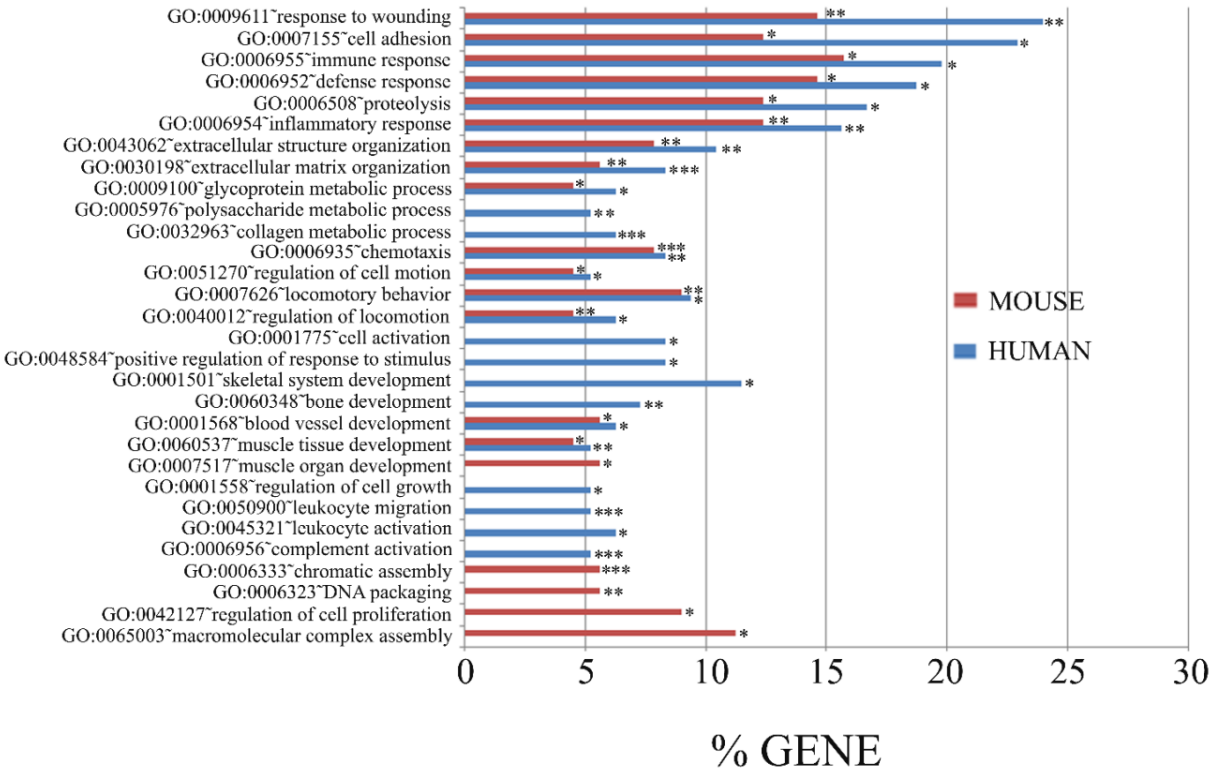


Fig.20 Distribution into biological processes of the proteins overrepresented in stimulated hMSC-CM in human and mouse. The proteins that were significantly up-regulated or present only in stimulated MSC-CM (Table 1 and [156]) were classified into different biological processes according to the Gene Ontology classification system (GOBP) using DAVID software [150]; confidence level: medium; only categories showing modified Fisher exact EASE score p value<0.05 and at least 5 counts in hMSC are represented. The bars represent the percentage of proteins involved in a category out of the total number of overrepresented proteins in human (96) or mouse (89) secretome. Asterisks indicate Fold Enrichment range for each category: * 1-5, ** 6-10, ***>10.

Gene Names	Protein Names	Angiogenesis[a]	Inflammation[a]
AGRN	Agrin	x	x
C1R	Complement C1r subcomponent		x
C1S	Complement C1s subcomponent		x
C3	Complement C3	x	x
CSF1	Macrophage colony-stimulating factor 1	x	x
CTSB	Cathepsin B	x	x
CXCL1	Growth-regulated alpha protein	x	x
CXCL5	C-X-C motif chemokine 5	x	x
EXT1	Exostosin-1		
EXT2	Exostosin-2		
FSTL1	Follistatin-related protein 1	x	x
HSPG2	Basem membr-spec heparan sulfate proteoglycan core prot	x	x
IGFBP7	Insulin-like growth factor-binding protein 7	x	x
IL6	Interleukin-6	x	x
LAMB2	Laminin subunit beta-2		
LGALS3BP	Galectin-3-binding protein	x	x
MMP13	Collagenase 3	x	x
NID1	Nidogen-1	x	x
PLOD2	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	x	
SERPINE1	Plasminogen activator inhibitor 1	x	x
TIMP1	Metalloproteinase inhibitor 1	x	x
TNC	Tenascin	x	x
VCAM1	Vascular cell adhesion protein 1	x	x

Legend:
[a] proteins involved in angiogenesis or inflammation in both organisms according to criteria detailed in "Materials and methods"

Tab.6 Proteins overrepresented or present only in st MSC-CM common to mouse and human

3.3.1 Functional evidence of human and mouse MSC secretome similarities or differences

Our proteomic results indicate that the majority of secreted proteins from both human and mouse MSC are associated with inflammation and angiogenesis (Tab.4 and [156]). To identify specific functional analogies or differences of human and mouse MSC in the regulation of these two important processes, we focused on two proteins, M-SCF/CSF1 and TIMP1, which are present in st MSC-CM of both species and play a key role in immunity/inflammation and angiogenesis, respectively [151, 152].

3.3.1.1 Macrophage colony-stimulating factor (M-CSF)

M-CSF is a growth factor secreted by a large variety of cells including macrophages, endothelial cells, fibroblast and lymphocytes. Locally produced M-CSF in the vessel wall contributes to the development and progression of atherosclerosis [153]. By interacting with its membrane receptor (CSF1R or M-CSF-R), it stimulates the survival, proliferation, and differentiation of monocytes and macrophages [154, 155].

Our proteomic data indicated that M-CSF (CSF1) is up-regulated in the secretome of both human and mouse MSC upon stimulation by inflammatory cytokines [Tab.S1, Tab.4, Fig.19]. As reported in Tab.S1 and Fig.20, M-CSF is amongst the proteins showing the highest increase in stimulated human secretome according to mass spectrometric analysis.

3.3.1.2 TIMP-1

Concerning angiogenesis, it has been analysed the effect of mMSC-CM on *in vitro* angiogenesis exploiting the tube formation assay [156]. Interestingly, in the case of human cells, MSC-CM was able to inhibit tube formation even when MSC had not been primed by cytokines. However, pre-activation with pro-inflammatory cytokines strengthened the anti-angiogenic effects of hMSC-CM, thus supporting our hypothesis that, during an inflammatory response, MSC target angiogenesis and thus dampen the inflammatory response [156].

The anti-angiogenic effect of MSC is mediated by TIMP-1 [156], according to many studies based on *in vitro* and *in vivo* approaches.

Because the proteomic analyses indicate that TIMP-1 is one of the proteins up-regulated in both human and mouse stMSC-CM [Tab.4], we compared the results obtained by blocking TIMP-1 in SVEC4-10 cells incubated in the presence of mMSC-CM [Fig.12a] with those generated using HUVEC cells and hMSC-CM [Fig.12b]. By inhibiting TIMP-1 activity with a specific blocking antibody, we observed the complete recovery of HUVEC cell ability to form tubes even in the presence of st hMSC-CM, indicating that TIMP1 is one of the key secreted molecules targeting endothelial cells in both mouse and human MSC. TIMP-1 concentration was measured by ELISA in st and unst, human and mouse MSC-CM (Fig. 21a for mouse and b for human). In accordance with our data of tubulogenesis showing that unst mMSC-CM has no effect on angiogenesis (Figs. 22 and Fig.23a), the concentration of TIMP-1 in mMSC-CM was about 5 times higher when cells had been primed by pro-inflammatory cytokines. Thus, in mouse MSC, the anti-angiogenic phenotype is acquired only after licensing with pro-inflammatory cytokines. In human MSC, however, the basal high level of secreted TIMP1 may explain the partial anti-angiogenic effect of the unst hMSC-CM (Figs. 22, 23b). In fact, in support of this hypothesis, TIMP-1 blockade restored the formation of the endothelial network in the presence of unst or st hMSC-CM. Again, proteomic data fully agree with functional assays and ELISA results for human TIMP1. As reported in Fig. 2 and Table 1, this protein is listed amongst those over-represented in st hMSC but showing relative lower level increase following stimulation.

Additional bioinformatics analyses of proteomic data further support the observation that even relatively small changes in the level of TIMP1 can result in very significant modulation of secretome properties. First of all, TIMP-1 concentration will greatly influence the proteolytic potential of the secretome and, consequently, the overall activity of a number of secretome components, including proteins which level is not increased following stimulation and proteins not directly involved in inflammation and angiogenesis; secondly, but not less importantly, TIMP1 is functionally related to a number of overrepresented proteins in stimulated secretome besides proteases [Fig. 19**]**, like cytokines and structural proteins (such as IL6, IL8, CCL2 CXCL12, COL3A1). The complete list of the 54 proteins of stimulated hMSC-CM functionally correlated to TIMP1 according to String [171] is reported Tab. 8.

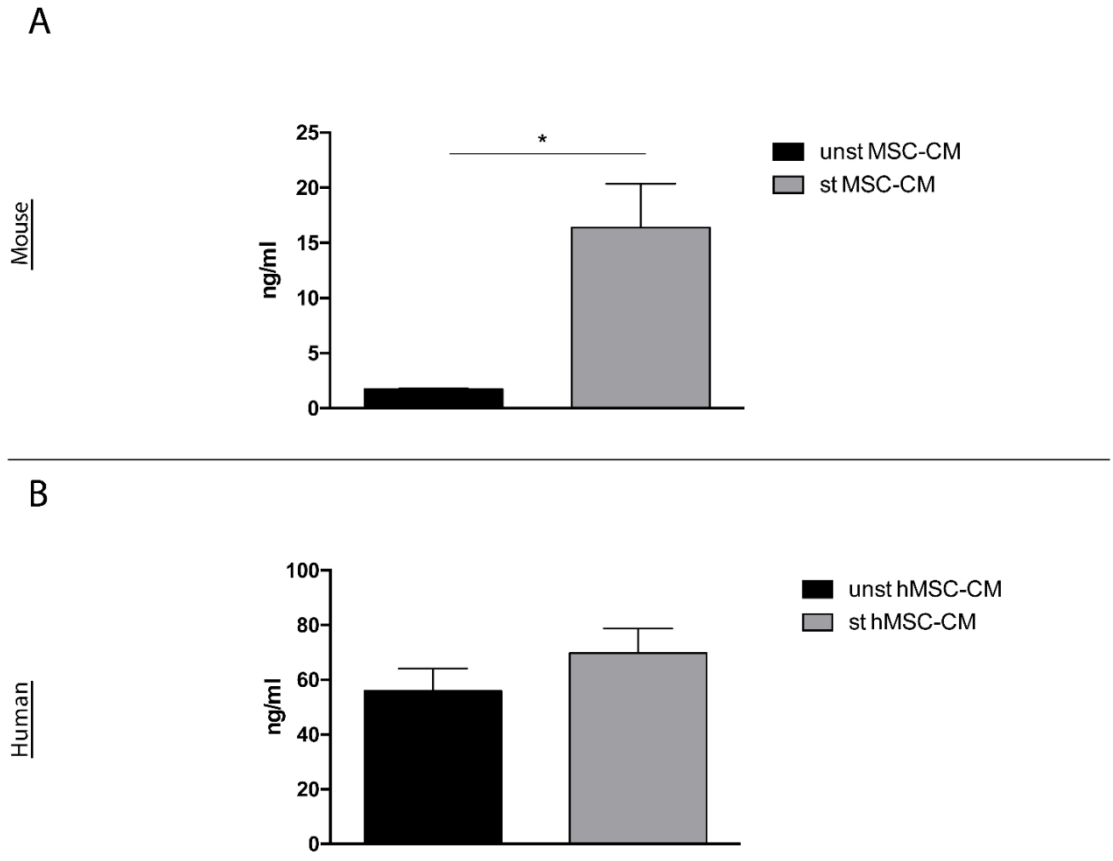


Fig.21 Mouse and human MSC-derived TIMP-1 quantification. MSC-derived TIMP-1 concentration in A) mouse and B) human unstimulated or stimulated MSC conditioned medium was measured with ELISA. Data are expressed as mean ± SEM (*p b 0.05, parametric t-test), 2 independent experiments.

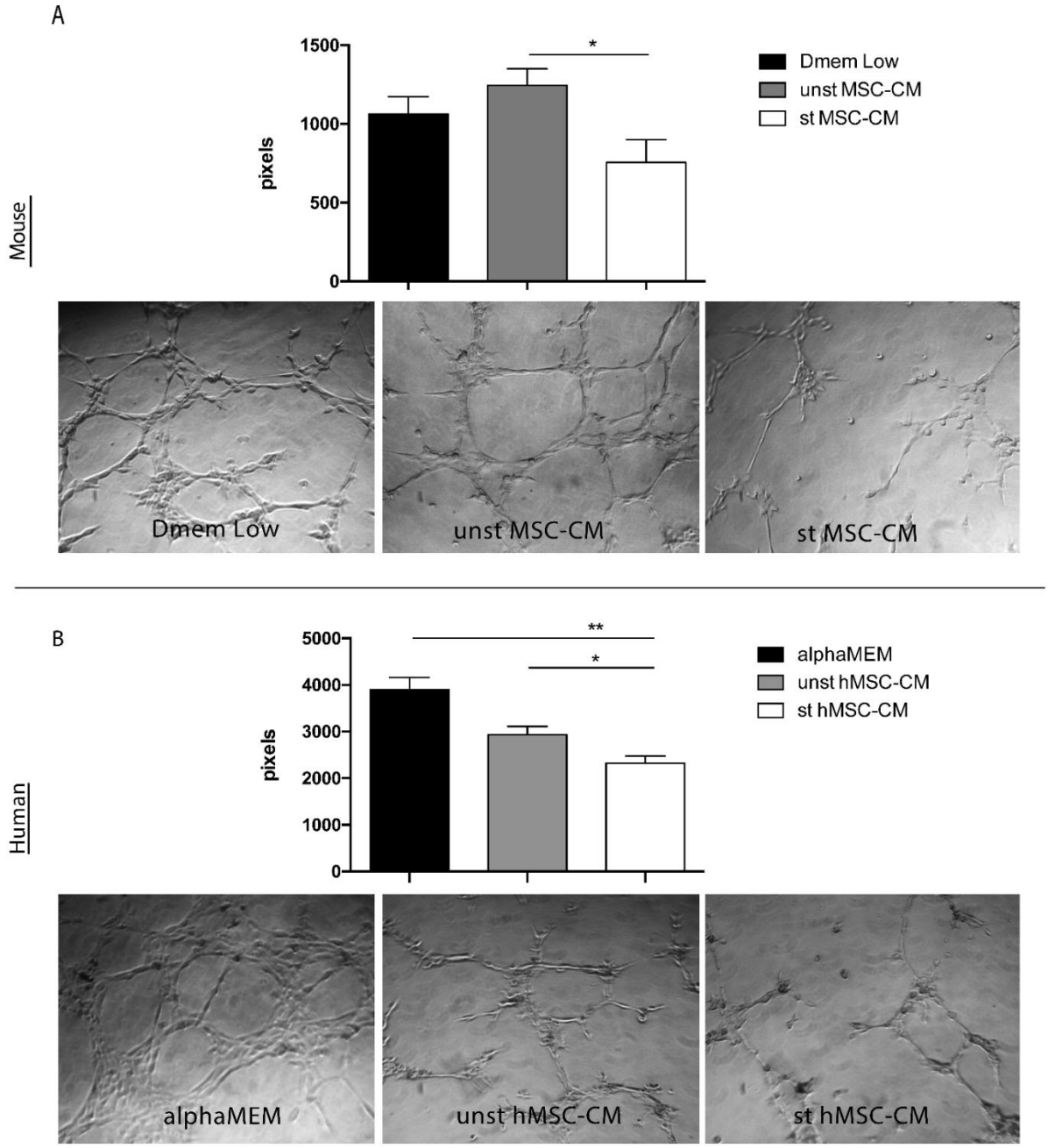


Fig.22 Effect of human or mouse MSC conditioned medium on tube formation assay. The effect of unstimulated or stimulated MSC media on endothelial cells was determined by a tube formation assay. Cells were seeded on the top of a matri-gel phase in the presence of unstimulated or stimulated A)mouse, B) human MSC-CM. 6 h later, images were acquired with a phase contrast inverted microscope at 4× objective magnification. Analysis was performed with ImageJ Angiogenesis Analyzer. A) SVEC4-10 network formation; quantification of the tube segment length (expressed in pixel number) and representative images at 4 h. B) Huvec network formation; quantification of the tube segment length and representative images (expressed in pixel number) at 4 h. 3 independent experiments, data are expressed as mean ± SEM (*p b 0.05, **p b 0.01, One way ANOVA

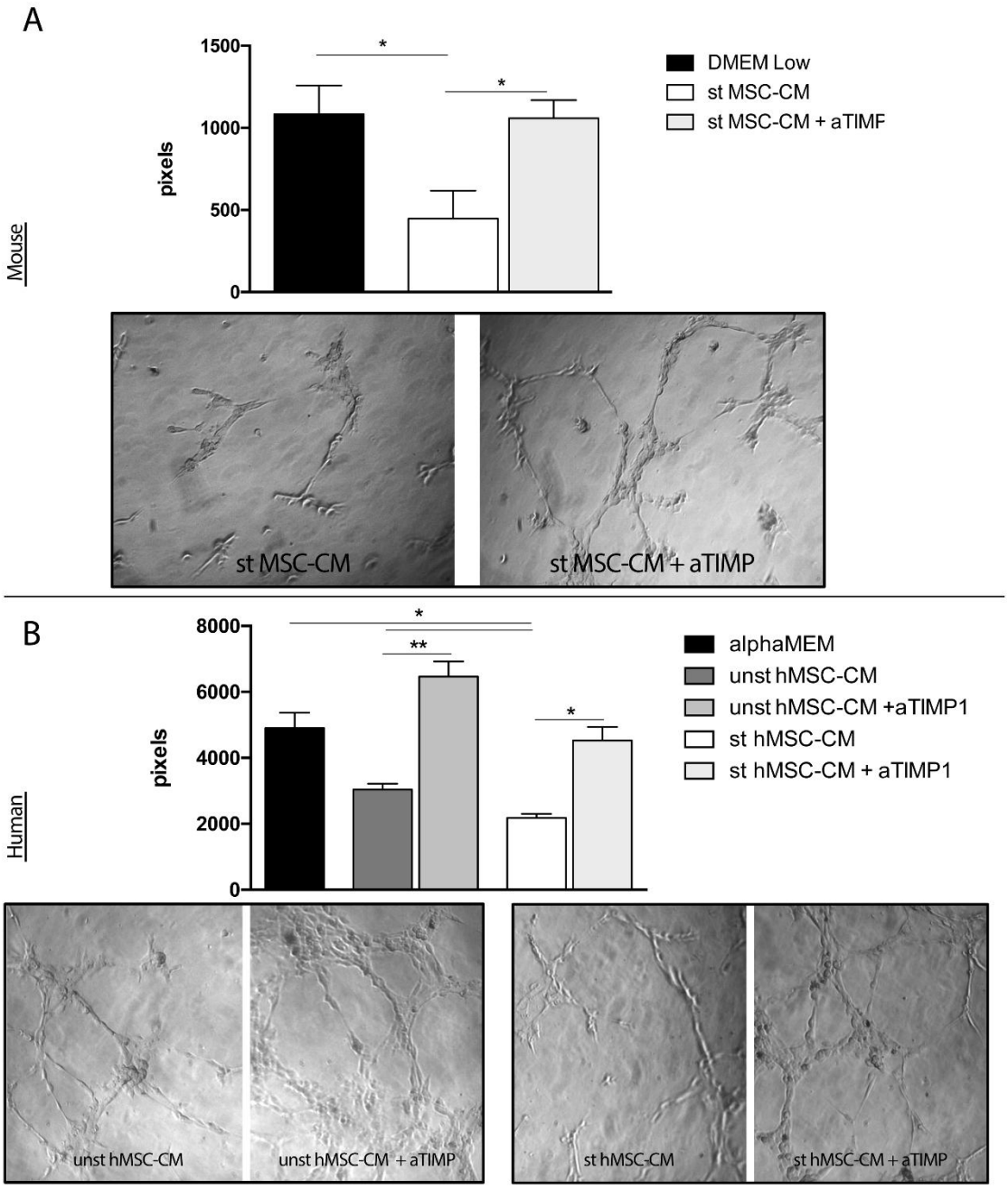


Fig. 23: Timp-1 blocking reverts the anti-angiogenic effect of mouse and human MSC conditioned media. In order to investigate the role of MSC-derived TIMP-1 on angiogenesis, the tube formation assay was performed in the presence of **A) mouse or B) human** TIMP-1 blocking antibody. Representative images of **A)** SVEC4-10 cell line or **B)** Huvec cells are taken with a phase contrast inverted microscope at 4× objective magnifications. Graphs show the quantification of the tube segment length measured with ImageJ Angiogenesis Analyzer. Data are expressed as mean ± SEM (*p<0.05, **p<0.01; One way Anova), 3 independent experiments.

Tab.7: TIMP1 interactions with other proteins in st hMSC-CM according to String

Node1	Node2	Node1_string_internal_id	Node2_string_internal_id	Node1_external_id	Node2_external_id	Experimentally_determined_interaction	Database annotated	Automated textmining	Combined score	Up regulated [a]
ACTA2	TIMP1	1843003	1842705	9606.ENSP00000224784	9606.ENSP00000218388	0	0	0,418	0,418	
ACTN1	TIMP1	1858193	1842705	9606.ENSP00000377941	9606.ENSP00000218388	0	0,9	0,125	0,908	
ACTN4	TIMP1	1844152	1842705	9606.ENSP00000252699	9606.ENSP00000218388	0	0,9	0,137	0,91	
ADAM9	TIMP1	1861419	1842705	9606.ENSP00000419446	9606.ENSP00000218388	0	0	0,448	0,448	
ALB	TIMP1	1847637	1842705	9606.ENSP00000295897	9606.ENSP00000218388	0	0,9	0,643	0,962	
ALDOA	TIMP1	1851945	1842705	9606.ENSP00000336927	9606.ENSP00000218388	0	0,9	0,08	0,904	
APP	TIMP1	1846936	1842705	9606.ENSP00000284981	9606.ENSP00000218388	0	0,9	0,197	0,916	
CCL2	TIMP1	1843055	1842705	9606.ENSP00000225831	9606.ENSP00000218388	0	0	0,532	0,532	X
CD44	TIMP1	1860096	1842705	9606.ENSP00000398632	9606.ENSP00000218388	0	0	0,86	0,859	
CDH2	TIMP1	1846066	1842705	9606.ENSP00000269141	9606.ENSP00000218388	0	0	0,414	0,414	
CHI3L1	TIMP1	1844377	1842705	9606.ENSP00000255409	9606.ENSP00000218388	0	0	0,473	0,473	X
CLU	TIMP1	1849761	1842705	9606.ENSP00000315130	9606.ENSP00000218388	0	0,9	0,415	0,938	
COL1A1	TIMP1	1843064	1842705	9606.ENSP00000225964	9606.ENSP00000218388	0	0	0,594	0,594	
COL1A2	TIMP1	1847845	1842705	9606.ENSP00000297268	9606.ENSP00000218388	0	0	0,543	0,543	
COL3A1	TIMP1	1848637	1842705	9606.ENSP00000304408	9606.ENSP00000218388	0	0	0,548	0,548	X
CST3	TIMP1	1856578	1842705	9606.ENSP00000366124	9606.ENSP00000218388	0	0	0,506	0,506	
CTGF	TIMP1	1854698	1842705	9606.ENSP00000356954	9606.ENSP00000218388	0	0	0,7	0,7	
CTSB	TIMP1	1852546	1842705	9606.ENSP00000342070	9606.ENSP00000218388	0	0	0,539	0,539	X
CTSD	TIMP1	1843439	1842705	9606.ENSP00000236671	9606.ENSP00000218388	0	0	0,45	0,45	
CXCL12	TIMP1	1858332	1842705	9606.ENSP00000379140	9606.ENSP00000218388	0	0	0,511	0,511	X
FBN1	TIMP1	1850745	1842705	9606.ENSP00000325527	9606.ENSP00000218388	0	0	0,407	0,407	X
FN1	TIMP1	1853098	1842705	9606.ENSP00000346839	9606.ENSP00000218388	0	0,9	0,503	0,948	X
GAPDH	TIMP1	1843185	1842705	9606.ENSP00000229239	9606.ENSP00000218388	0	0	0,658	0,658	
GAS6	TIMP1	1851398	1842705	9606.ENSP00000331831	9606.ENSP00000218388	0	0,9	0,103	0,906	
HSPG2	TIMP1	1856167	1842705	9606.ENSP00000363827	9606.ENSP00000218388	0	0	0,459	0,459	X
ICAM1	TIMP1	1845628	1842705	9606.ENSP00000264832	9606.ENSP00000218388	0	0	0,636	0,636	X
IGFBP3	TIMP1	1857331	1842705	9606.ENSP00000370473	9606.ENSP00000218388	0	0	0,472	0,472	
IL6	TIMP1	1844631	1842705	9606.ENSP00000258743	9606.ENSP00000218388	0	0	0,937	0,937	X
IL8	TIMP1	1848852	1842705	9606.ENSP00000306512	9606.ENSP00000218388	0	0	0,926	0,926	X
ITGB1	TIMP1	1848520	1842705	9606.ENSP00000303351	9606.ENSP00000218388	0	0	0,837	0,838	
LAMB2	TIMP1	1848923	1842705	9606.ENSP00000307156	9606.ENSP00000218388	0	0	0,472	0,472	X

LOX	TIMP1	1843271	1842705	9606.ENSP00000231004	9606.ENSP00000218388	0	0	0,504	0,504	
LUM	TIMP1	1845897	1842705	9606.ENSP00000266718	9606.ENSP00000218388	0	0	0,407	0,407	
MMP1	TIMP1	1850481	1842705	9606.ENSP00000322788	9606.ENSP00000218388	0,958	0	0,938	0,997	X
MMP10	TIMP1	1846633	1842705	9606.ENSP00000279441	9606.ENSP00000218388	0,906	0	0,769	0,977	X
MMP13	TIMP1	1844775	1842705	9606.ENSP00000260302	9606.ENSP00000218388	0,107	0	0,961	0,964	X
MMP2	TIMP1	1842719	1842705	9606.ENSP00000219070	9606.ENSP00000218388	0,605	0	0,995	0,998	X
MMP3	TIMP1	1848108	1842705	9606.ENSP00000299855	9606.ENSP00000218388	0,958	0	0,994	0,999	X
NID1	TIMP1	1845497	1842705	9606.ENSP00000264187	9606.ENSP00000218388	0	0	0,512	0,512	X
SERPINB2	TIMP1	1848072	1842705	9606.ENSP00000299502	9606.ENSP00000218388	0	0	0,472	0,472	X
SERPINE1	TIMP1	1842959	1842705	9606.ENSP00000223095	9606.ENSP00000218388	0	0,9	0,765	0,975	X
SERPINE2	TIMP1	1861176	1842705	9606.ENSP00000415786	9606.ENSP00000218388	0	0	0,553	0,553	
SERPING1	TIMP1	1846563	1842705	9606.ENSP00000278407	9606.ENSP00000218388	0	0,9	0,191	0,915	
SPARC	TIMP1	1843274	1842705	9606.ENSP00000231061	9606.ENSP00000218388	0	0,9	0,472	0,944	
SRGN	TIMP1	1843628	1842705	9606.ENSP00000242465	9606.ENSP00000218388	0	0,9	0,198	0,916	X
TGFB1	TIMP1	1842869	1842705	9606.ENSP00000221930	9606.ENSP00000218388	0	0,9	0,939	0,993	
THBS1	TIMP1	1844779	1842705	9606.ENSP00000260356	9606.ENSP00000218388	0	0	0,917	0,917	
THBS2	TIMP1	1854448	1842705	9606.ENSP00000355751	9606.ENSP00000218388	0	0	0,483	0,483	
TIMP1	DCN	1842705	1842218	9606.ENSP00000218388	9606.ENSP00000052754	0	0	0,583	0,583	
TIMP1	MIF	1842705	1842503	9606.ENSP00000218388	9606.ENSP00000215754	0	0	0,415	0,414	
TMSB4X	TIMP1	1857259	1842705	9606.ENSP00000370007	9606.ENSP00000218388	0	0,9	0,099	0,906	
TNC	TIMP1	1845696	1842705	9606.ENSP00000265131	9606.ENSP00000218388	0	0	0,562	0,562	X
UBC	TIMP1	1852861	1842705	9606.ENSP00000344818	9606.ENSP00000218388	0,854	0	0,16	0,872	
VCAM1	TIMP1	1847502	1842705	9606.ENSP00000294728	9606.ENSP00000218388	0	0	0,516	0,516	X
VCAN	TIMP1	1845684	1842705	9606.ENSP00000265077	9606.ENSP00000218388	0	0	0,507	0,507	
VTN	TIMP1	1843077	1842705	9606.ENSP00000226218	9606.ENSP00000218388	0,107	0	0,708	0,728	

(a) only in st-hMSC-CM according to Table 5

CONCLUSION

MSC have been studied across a range of clinical indications and represent a promising therapeutic approach in many diseases in view of their potent immunomodulatory properties. To design better therapeutic protocols and define the clinical endpoints, it is important to identify the specific targets of MSC anti-inflammatory action *in vivo*. Recently, it was demonstrated that MSC have a potent stabilizing effect on the vascular endothelium, having the capacity of inhibiting endothelial permeability after traumatic brain injury [156] and in hemorrhagic shock [157]. The data obtained from mouse MSCs suggest that the effects of MSC are all mediated by soluble factors released by MSC [158]. This is in agreement with another study showing an antiangiogenic activity for soluble factors present in media derived from MSC/glioma co-cultures. Moreover, the proteomic analysis of hMSC-CM and mMSC-CM confirms that exposure to pro-inflammatory cytokines results in significantly higher secretion of a number of immunomodulatory and angiogenesis-related proteins by MSC from both species. Notably, 62% of the proteins identified in st hMSC-CM were also identified in st mMSC-CM, clearly highlighting the existence of a common signature in the secretome of human and mouse MSC. However, although human and mouse MSC show a similar proteomic signature in response to stimulation by pro-inflammatory cytokines, our data indicate that they may induce different biological responses. Thus, even if M-CSF is up-regulated in both human and mouse MSC-CM, only hMSC-CM induce macrophage differentiation efficiently because of its high concentration of M-CSF. In both species, several up-regulated proteins are associated with angiogenesis. This process requires degradation of the vascular basement membrane and remodelling of the extracellular matrix to allow endothelial cells migration and invasion into the surrounding tissue [159]. The extended network of interactions amongst inflammation and angiogenesis-related proteins in stimulated hMSC-CM makes it extremely difficult to assess the *in vivo* physiological importance of each factor. The angiogenesis requires the action of matrix metalloproteinases (MMPs) that degrade both matrix and non-matrix proteins and have central roles in morphogenesis, wound healing, tissue repair and in progression of chronic diseases [159] and, in particular, the presence of a number of protease and protease inhibitors implies the possibility of additional self-modulation of the properties of the various components of the secretome [146]. The balance between MMPs and their natural inhibitors, the TIMPs, is critical for extracellular matrix remodelling and angiogenesis. The TIMP family comprises four protease inhibitors: TIMP-1, TIMP-2, TIMP-3 and TIMP-4. With the exception of TIMP-4 [160], all three TIMPs inhibit angiogenesis *in*

vivo [137], although through diverse mechanisms. MSC secrete both MMPs and their inhibitors, and thus contribute to the regulation and protection of the perivascular niche [161]. Using both in vitro and in vivo assays, we identified the metalloproteinase inhibitor TIMP-1 as the molecule responsible for the anti-angiogenic effects of MSC. TIMP-1 is known to inhibit endothelial cells migration by MMP-dependent and MMP independent mechanisms [162, 163]. The latter involve regulation of various biological processes such as cell growth, apoptosis and differentiation through the CD63 receptor [164, 165]. In addition, TIMP-1 was shown to induce secretion of soluble VEGFR-1 by human endothelial cells, leading to a decrease of bioavailable VEGF and of blood vessel growth [166]. TIMP-3 has also been identified as a soluble factor produced by MSC with beneficial effects on endothelial cell function in a mouse model of traumatic brain injury [167]; however, we did not find evidence for TIMP-3 upregulation in the mouse or human MSC secretomes. It is likely that, in vivo, other soluble factors in addition to TIMP-1 contribute to MSC-mediated immune regulation: MSC are also known to produce prostaglandin E2 and thus inhibit the activation of macrophages [168], which are a source of multiple growth factors that enhance endothelial cell proliferation and survival [169]. Therefore, by identifying TIMP-1 as a critical effector of the anti-inflammatory properties of MSC, and by observing its anti-angiogenic role, both in mouse and in human, we can confirm the important role of TIMP-1 as key secreted molecule targeting endothelial cells. The identification of TIMP-1 as potential effector molecule responsible for the anti-angiogenic properties of MSC, both in mouse and in human, allow to confirm that MSC exert specific effect by secretion of a broad range of bioactive molecules and allow to design pre-clinical experiments and clinical trials, with the aim of exploit their potentially therapeutic role.

Moreover, these data could be also a starting point for further analysis aimed to understand important differences between human and mouse also in a pharmacological field. In fact, although mouse and rats are still considered the animal models of choice for applied pharmacological research and drug test, up to now, many important biochemistry pathways involved in life processes, in both species, have not been yet clearly understood, often precluding the possibility to translate results in humans without leading to errors. Indeed, there are few studies in literature focused on this particular aspect, therefore the data reported in this thesis represent a source for new studies evaluating similarities and differences between human and mouse models in pharmacological field.

The results reported in this phD thesis have been partially published in the papers listed below:

Mouse mesenchymal stem cells inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1 Leukemia (2016) 30, 1143-1154.

Proteomic analysis of the secretome of human bone marrow-derived Mesenchymal Stem Cells primed by pro-inflammatory cytokines Journal of proteomics (2017) 166, 115-126.

And in following poster presentation:

S. Nonnis, E. Maffioli, **F. Santagata**, F. Grassi Scalvini, S. Morelli, A. Negri, A. Viola, G. Tedeschi. **Proteomic analysis of bone marrow derived human MSC secretome stimulated with pro- inflammatory cytokines**. SIB 2017, 20-22 settembre 2017, Caserta.

G. Tedeschi, L. Zanotti, **F. Santagata**, E. maffioli, S. Nonnis, A. Negri, A. Viola. **Human mesenchymal stem cells secretome before and after treatment with pro inflammatory cytokines investigated by a label free proteomic approach.** XI ItPA national Congress, 16 – 19 maggio 2016, Perugia.

S. Nonnis, L. Zanotti, **F. Santagata**, E. Maffioli, A. Negri, A. Viola, G. Tedeschi. **Proteome characterization of human stromal mesenchymal stem cells secretome before and after treatement with pro inflammatory cytokines.** Proteine 2016, 30 marzo-1aprile 2016, Bologna.

S. Nonnis, L. Zanotti, E. Maffioli, **F. Santagata**, A. Negri, L. Chiesa, A. Viola, G. Tedeschi. **Human mesenchymal stem cells secretome investigated by using a label free proteomic approach**. 58th National Meeting of the Italian society of biochemistry and molecular biology (SIB), 14-16 settembre 2015, Urbino.

S. Nonnis, L. Zanotti, E. Maffioli, **F. Santagata**, , A. Negri, A. Viola, G. Tedeschi. **Proteomic analysis of the mesenchymal stem cells secretome using a label free approach**. EupA IX Annual Congress, 23-28 giugno 2015, Milano.

SUPPLEMENTARY MATERIALS

Tab.S1 Proteins identified and quantified in hMSC secretome in at least 3 out of 5 technical replicas in at least one stimulation condition in both patients. Notes (a) cytokine or chemokine or proteins functionally related to these classes of compounds according to the nextProt database [122]; (b) proteins present also in mouse stimulated MSC-CM; (c) number of razor+unique peptides used to identified the protein in patients H30 or H34; (d) posterior error probability score calculated for the identification of protein; (e) percentage of the protein sequence that is covered by the identified peptides.

Proteins are grouped as follows:

Group 1: proteins present only in st hMSC-CM

Group 2: proteins present in higher abundance in st hMSC-CM compared to unst hMSC-CM according to t-test (filtered for FDR 1%)

Group 3: proteins present only in unst hMSC-CM

Group 4: proteins present in lower abundance in st hMSC-CM compared to unst hMSC-CM according to t-test (filtered forFDR 1%)

Group 5: proteins present in the same quantity in st hMSC-CM and unst hMSC-CM according to t-test (filtered for FDR 1%)

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
1			11	11	D3YTG3	195,32	1,2173E-79	9,5	ABI3BP
4		X	5	5	P53396	120,84	4,3369E-11	6,6	ACLY
5			1	1	P62736	42,009	2,2516E-125	25,2	ACTA2
4			1	1	P60709	41,736	3,8597E-302	62,4	ACTB
4		X	2	2	P68032	42,019	6,2584E-145	25,2	ACTC1
4			15	16	P63261	41,792	0	62,4	ACTG1
4		X	14	15	P12814-4	107,14	6,941E-250	35,9	ACTN1
4	x	X	28	28	O43707	104,85	5,9344E-227	39,7	ACTN4
3			3	3	P61163	42,613	2,1649E-09	14,6	ACTR1A
5		X	5	8	P61158	47,371	0	33,5	ACTR3
3			3	3	O43184	99,541	3,7224E-10	6,6	ADAM12
5	x	X	2	2	Q13443	90,555	4,9351E-07	2,0	ADAM9
5		X	3	3	O95450	134,75	5,4068E-28	4,0	ADAMTS2
4		X	10	12	Q8IUX7	130,93	7,8012E-41	13,9	AEBP1
2		X	27	27	O00468-6	214,84	1,4008E-168	18,8	AGRN
4		X	35	35	Q09666	629,09	2,9098E-98	20,7	AHNAK
5		X	4	4	P02768	69,366	2,9631E-18	5,6	ALB
1			4	4	Q13740	65,102	1,2248E-12	12,2	ALCAM
5			8	8	P04075-2	45,26	3,2802E-83	33,5	ALDOA
5			1	2	P09972	39,455	6,4277E-99	7,1	ALDOC
3			1	2	Q92688	28,787	0,000057757	10,8	ANP32B
4	x		4	6	P15144	109,54	2,0524E-16	8,0	ANPEP

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
4	x	X	12	14	P04083	38,714	0	50,0	ANXA1
5	x		19	22	P07355	40,411	0	57,4	ANXA2
4		X	13	14	P08758	35,936	5,0793E-84	49,7	ANXA5
4		X	13	15	P08133	75,872	8,8256E-47	29,3	ANXA6
5	x		1	1	P02647	30,777	0,00050492	6,0	APOA1
5	x	X	4	5	P05067	86,942	6,4852E-09	8,3	APP
5			2	2	P05089-2	35,664	0,000021449	9,1	ARG1
1		X	2	2	Q07960	50,435	0,0012091	5,5	ARHGAP1
5		X	2	3	J3QQX2	25,831	6,616E-13	26,8	ARHGDIA
4		X	1	2	O15145	20,546	3,8051E-13	13,5	ARPC3
5		X	1	1	P15289	53,588	0,0027636	3,4	ARSA
5		X	3	3	P31939	64,615	5,0734E-08	9,0	ATIC
4		X	4	4	P06576	56,559	2,4032E-09	12,1	ATP5B
5		X	1	2	Q15904	52,025	0,00084483	5,7	ATP6AP1
5			4	4	P25311	34,258	1,7363E-19	20,5	AZGP1
5	x	X	2	2	F5H6I0	13,972	1,575E-27	28,7	B2M
5		X	5	5	P15291	43,92	3,1029E-92	25,1	B4GALT1
5		X	3	3	P80723	22,693	8,3791E-17	35,7	BASP1
5		X	16	16	P21810	41,654	0	49,7	BGN
2	x	X	7	7	P13497	111,25	0	9,8	BMP1
3			2	2	M0QYN0	20,387	0,000012101	11,1	C19orf10
2			18	1	P00736	73,731	8,0979E-240	36,9	C1R
5			1	18	H0YFH3	80,118	1,9282E-269	37,4	C1R
2			17	17	P09871	76,684	0	34,0	C1S
2	x	X	59	59	P01024	187,15	0	47,9	C3
1			2	2	O43570	39,45	0,000028224	10,2	CA12
4		X	8	8	Q05682	93,23	4,1829E-91	12,9	CALD1
5			2	2	H0Y7A7	20,762	5,6239E-76	16,0	CALM2
3			2	2	Q9NZT1	15,892	4,3805E-08	24,7	CALML5
5		X	6	8	P27797	48,141	1,0075E-35	29,3	CALR

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
5		X	12	12	O43852-3	38,05	4,7152E-63	44,9	CALU
3			3	3	Q86VP6	136,37	1,8716E-08	3,4	CAND1
4		X	1	3	B4DGP8	71,502	0,00042945	6,5	CANX
4		X	3	3	Q01518	51,901	2,747E-17	8,8	CAP1
3			3	3	P52907	32,922	3,06E-12	15,0	CAPZA1
5			6	6	P31944	27,679	0	27,7	CASP14
4		X	3	3	P20810-6	84,942	1,161E-07	6,3	CAST
5			2	2	P16152	30,375	2,4016E-09	15,5	CBR1
4		X	17	18	Q76M96-2	109,49	1,2796E-251	23,6	CCDC80
2	x	X	1	1	P13500	11,025	3,3088E-07	11,1	CCL2
4		X	4	5	P78371	57,488	5,4358E-11	16,1	CCT2
4		X	3	3	P49368	60,533	2,6916E-06	8,3	CCT3
5		X	4	4	P40227	58,024	3,9311E-13	14,9	CCT6A
5		X	1	1	Q99832	59,366	0,00013165	4,1	CCT7
4		X	3	3	P50990	59,62	4,3306E-09	7,1	CCT8
5	x	X	3	4	Q6YHK3	161,69	4,0838E-11	4,6	CD109
5	x	X	2	2	P16070	81,537	1,9746E-08	3,8	CD44
5			1	1	E9PR17	14,529	0,000021381	9,2	CD59
1	x	X	1	1	Q16543	44,468	0,00037317	5,6	CDC37
4		X	2	3	P55287	87,964	6,3973E-22	5,0	CDH11
5			7	7	P19022	99,808	4,3623E-26	13,1	CDH2
5			1	1	G8JLG2	51,607	0,00084614	3,4	CDSN
1			11	11	B4E1Z4	140,94	2,6051E-142	12,5	CFB
2	x		15	15	P08603	139,09	5,2825E-102	19,7	CFH
4	x	X	8	8	E9PK25	22,728	5,3478E-269	52,5	CFL1
2	x		10	11	P36222	42,625	3,6425E-58	39,2	CHI3L1
4			5	5	Q07065	66,022	4,0301E-11	14,1	CKAP4
5	x		6	6	Q9Y240	35,694	2,9499E-37	21,1	CLEC11A
4		X	3	4	O00299	26,922	1,5209E-42	35,3	CLIC1
5		X	4	4	Q9Y696	28,772	2,6953E-12	28,9	CLIC4

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
2		X	10	10	O94985	109,79	5,4014E-55	13,8	CLSTN1
4		X	1	1	P09496-2	23,662	0,0019797	9,6	CLTA
4		X	11	14	Q00610	191,61	3,0898E-34	14,9	CLTC
5	x		12	12	P10909-2	57,832	1,0393E-214	26,1	CLU
5		X	3	3	B4DUT8	35,943	4,4091E-13	13,3	CNN2
5		X	1	3	F8W031	29,224	0,000082411	17,9	CNPY2
5			1	3	P12107-2	182,42	1,814E-15	4,0	COL11A1
4		X	99	106	Q99715	333,14	0	46,8	COL12A1
2			6	6	A6NDR9	157,67	2,3711E-19	7,3	COL16A1
5	x	X	60	60	P02452	138,94	0	65,8	COL1A1
5	x	X	58	58	P08123	129,31	0	69,5	COL1A2
2	x	X	32	33	P02461	138,56	0	33,0	COL3A1
4	x	X	4	6	P02462	160,61	2,6473E-18	7,4	COL4A1
4		X	18	26	P08572	167,55	1,1433E-112	26,6	COL4A2
5		X	23	2	P20908	23,534	8,2125E-140	36,2	COL5A1
5		X	2	23	H7BY82	183,56	0	23,1	COL5A1
2		X	32	32	P05997	144,91	1,263E-244	38,0	COL5A2
5		X	36	36	P12109	108,53	0	49,3	COL6A1
5		X	24	24	P12110	108,58	0	32,9	COL6A2
5		X	113	113	P12111	343,67	0	46,3	COL6A3
2			18	18	Q02388	295,22	9,1653E-63	11,3	COL7A1
5		X	11	11	P27658	73,363	2,0038E-33	24,6	COL8A1
5			3	3	P49747	82,86	1,263E-60	5,7	COMP
3			2	2	P53618	107,14	0,0019246	3,3	COPB1
4		X	1	1	P35606	102,49	0,00052126	2,1	COPB2
4		X	4	4	Q9ULV4-3	58,947	1,899E-38	10,8	CORO1C
5		X	3	2	Q14019	15,945	5,4185E-19	30,3	COTL1
5			1	1	Q8N4T0-3	22,889	0,014243	9,6	CPA6
5	x		1	1	P52943-2	30,221	2,2516E-06	11,3	CRIP2
1	x	X	7	7	P09603	60,179	2,4288E-86	13,5	CSF1

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
4		X	3	3	P21291	20,567	5,359E-08	28,0	CSRP1
5	x	X	2	3	P01034	15,799	1,2877E-24	30,1	CST3
5			2	2	P01040	11,006	1,3088E-18	39,8	CSTA
5	x		1	2	P29279	38,091	0,0031205	7,4	CTGF
2			4	5	Q96CG8	26,224	3,6588E-41	22,6	CTHRC1
2	x	X	7	7	P07858	37,821	5,219E-44	30,1	CTSB
5	x	X	3	4	P07339	44,552	3,3571E-10	14,3	CTSD
5		X	1	1	P07711	37,564	0,000026438	3,6	CTSL1
5		X	2	2	Q9UBR2	33,868	8,5205E-14	9,2	CTSZ
4		X	1	1	O60888-2	20,925	0,0033772	7,1	CUTA
1	x	X	4	4	P09341	11,301	4,7499E-60	38,3	CXCL1
2	x	X	2	2	P48061-4	15,495	7,652E-09	18,7	CXCL12
1	x	X	3	3	P42830	11,972	1,3196E-37	25,4	CXCL5
1	x		3	3	P80162	11,897	4,8991E-74	21,9	CXCL6
1	x		3	3	O00622	42,026	1,412E-49	15,2	CYR61
3			2	2	Q14118	97,44	5,444E-07	3,5	DAG1
5			5	5	P81605	11,284	7,7115E-78	37,3	DCD
2			11	11	P07585	39,746	6,4075E-75	36,5	DCN
3			1	1	O95865	29,644	0,00067307	7,4	DDAH2
4			4	4	F8VQ10	50,745	1,1336E-13	12,4	DDX39B
5		X	4	4	F6SYF8	39,95	1,7346E-10	20,3	DKK3
3			1	1	P11532	426,74	0,0012018	0,3	DMD
4		X	3	3	Q16555	62,293	2,1662E-08	9,4	DPYSL2
4	x	X	5	5	Q14195-2	73,91	4,8742E-17	11,0	DPYSL3
5			5	5	Q08554	99,986	2,8298E-10	8,1	DSC1
4			8	8	Q02413	113,75	1,2022E-236	11,4	DSG1
4			27	27	P15924	331,77	1,5003E-172	14,4	DSP
5		X	1	2	P60981	18,506	6,1594E-07	13,9	DSTN
4		X	6	7	Q16610-4	63,563	1,0421E-40	19,6	ECM1
5			8	12	O43854	53,764	7,3153E-40	37,5	EDIL3

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
4	x		8	9	P68104	50,14	3,5691E-57	32,0	EEF1A1
3			1	3	E9PRY8	76,569	0,0007596	7,0	EEF1D
4		X	4	4	P26641	50,118	1,149E-13	10,3	EEF1G
4		X	16	16	P13639	95,337	3,7141E-152	21,8	EEF2
5			10	14	Q12805	54,64	1,8381E-68	42,0	EFEMP1
2		X	7	7	O95967	49,405	1,6239E-36	25,5	EFEMP2
4			6	7	P60842	46,153	6,3872E-38	25,4	EIF4A1
5		X	3	3	I3L504	20,503	1,1597E-06	19,4	EIF5A
1		X	1	1	P56537	26,599	0,0015202	9,8	EIF6
2	x		5	5	F8WAH6	68,992	2,198E-49	14,1	ELN
5		X	14	14	Q9Y6C2	106,67	2,6898E-76	24,9	EMILIN1
5	x		1	1	P17813	70,577	0,00045922	2,7	ENG
4	x	X	14	14	P06733	47,168	0	46,3	ENO1
5			2	6	P22413	104,92	0,000036242	11,9	ENPP1
4		X	1	1	P84090	12,259	2,4683E-08	10,6	ERH
2		X	2	2	Q16394	86,254	1,8112E-14	5,6	EXT1
1		X	4	4	Q93063-3	85,814	4,1958E-19	9,2	EXT2
3	x		1	1	P15311	69,412	2,03E-10	7,0	EZR
5		X	4	4	Q01469	15,164	8,84E-54	39,3	FABP5
5		X	3	3	Q96TA1	84,137	1,2074E-06	6,6	FAM129B
5		X	4	4	P49327	273,42	7,6621E-08	3,1	FASN
2	x	X	17	17	P23142	77,213	3,3194E-199	37,4	FBLN1
5	x	X	3	3	B1AHL2	78,329	1,9178E-143	32,5	FBLN1
5		X	47	60	P35555	312,24	0	31,3	FBN1
5			2	2	P35556	314,77	6,6202E-06	1,2	FBN2
5			1	1	Q9Y613	126,55	0,002695	0,9	FHOD1
5		X	6	6	Q96AY3	64,244	4,4895E-53	12,2	FKBP10
1	x		1	1	P62942	11,951	1,4001E-84	13,0	FKBP1A
5			2	3	Q5D862	248,07	1,4325E-15	2,7	FLG2
4		X	64	65	P21333	280,74	0	39,9	FLNA

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
4		X	6	10	O75369-8	281,63	1,6185E-30	8,9	FLNB
4		X	13	13	Q14315	291,02	2,91E-72	9,7	FLNC
2	x	X	111	111	P02751	262,62	0	61,3	FN1
5	x	X	1	111	P02751-11	205,56	1,3378E-214	61,3	FN1
2			21	24	Q4ZHG4	54,529	1,0207E-21	16,9	FNDC1
5	x	X	5	5	Q16658	54,529	1,0207E-21	16,2	FSCN1
5			1	1	P19883	38,007	1,2565E-10	4,9	FST
2		X	12	12	Q12841	34,985	0	41,9	FSTL1
3			2	1	H3BPE7	53,496	0,000078972	4,6	FUS
4		X	2	3	Q13283	52,164	2,7023E-06	10,3	G3BP1
2		X	4	4	Q10471	64,732	7,4295E-38	9,1	GALNT2
5			4	4	Q7Z7M9	106,26	1,2669E-10	4,3	GALNT5
5		X	7	7	Q14697-2	109,44	8,9282E-24	11,5	GANAB
4	x		10	10	P04406	36,053	2,2718E-202	41,8	GAPDH
4	x		12	18	J3KP07	79,686	3,8615E-31	38,9	GAS6
3			1	2	Q04446	80,473	0,0033043	4,1	GBE1
1	x		1	1	P32455	67,93	2,5342E-08	2,0	GBP1
1			1	1	P02774-3	55,123	3,8539E-15	4,5	GC
5		X	10	10	E7EU23	51,18	1,0541E-36	30,5	GDI2
5			2	2	Q92820	35,964	1,1429E-13	8,5	GGH
5	x	X	2	2	P04899-4	41,548	7,1546E-07	8,5	GNAI2
5	x	X	1	1	Q9UBI6	8,0061	0,00081527	22,2	GNG12
5		X	4	4	P35052	61,68	7,6846E-11	12,7	GPC1
5			2	4	Q9Y625	62,735	0,000011526	12,7	GPC6
4	x		2	2	P06744-2	64,324	0	5,6	GPI
4		X	6	6	P06396	85,696	1,872E-53	12,3	GSN
4	x		7	7	P09211	23,356	1,3922E-90	48,1	GSTP1
4			2	2	P16104	15,144	2,0889E-27	19,6	H2AFX
4			2	12	P10915	40,165	4,6436E-07	44,4	HAPLN1
5		X	1	1	P12081	57,41	0,00105	4,3	HARS

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
5			2	3	P69905	15,257	2,343E-14	28,2	HBA1
5			1	1	P02042	16,055	0,0031855	6,8	HBD
5		X	5	5	H3BP20	61,999	7,8509E-17	11,9	HEXA
4		X	6	6	P62805	11,367	9,898E-32	48,5	HIST1H4A
4			2	2	B4DR52	18,041	1,8541E-17	14,5	HIST2H2BF
2	x		3	3	P05534	40,688	1,5534E-123	18,9	HLA-A
2	x		4	4	A2AEA2	41,301	2,8476E-59	17,7	HLA-C
5			7	7	Q96RW7	613,38	5,235E-17	1,6	HMCN1
4			7	7	P09651	38,746	1,1501E-75	19,6	HNRNPA1
4		X	8	7	P22626	37,429	7,4645E-30	21,2	HNRNPA2B1
3			1	1	Q99729-2	35,967	0,00098977	4,2	HNRNPAB
4			3	3	P07910	33,67	3,3197E-09	12,1	HNRNPC
5	x	X	2	2	Q14103	38,434	0,000092604	7,6	HNRNPD
4			2	1	P52597	45,671	1,0433E-07	8,2	HNRNPF
3			1	1	P31942	36,926	0,00094624	4,9	HNRNPH3
4	x		4	6	P61978-2	51,028	6,5625E-16	20,9	HNRNPK
3			2	3	Q00839	90,583	0,000011316	6,1	HNRNPU
5			2	4	Q86YZ3	282,39	3,6127E-25	8,7	HRNR
4		X	7	21	P07900-2	98,16	1,425E-171	28,6	HSP90AA1
4	x	X	16	9	P08238	83,263	1,6351E-226	38,5	HSP90AB1
5			1	1	Q58FF8	44,348	6,9506E-40	13,4	HSP90AB2P
4	x	X	20	24	P14625	92,468	8,2902E-184	38,5	HSP90B1
4	x	X	3	3	P34932	94,33	8,5632E-07	5,6	HSPA4
4	x	X	19	21	P11021	72,332	0	40,6	HSPA5
4		X	14	14	P11142	70,897	6,4947E-111	35,1	HSPA8
4	x		5	5	P04792	22,782	1,0748E-19	40,0	HSPB1
4	x	X	2	4	P10809	61,054	0,00015791	12,6	HSPD1
4		X	2	2	P61604	10,932	8,5774E-11	25,5	HSPE1
2		X	107	107	P98160	468,83	0	44,3	HSPG2
4			11	11	Q92743	51,286	4,0219E-58	35,2	HTRA1

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
1		X	2	2	Q9Y4L1	111,33	0,00011375	3,8	HYOU1
1	x		4	4	P05362	57,825	2,9554E-15	10,3	ICAM1
3			1	1	O75874	46,659	0,00073021	4,1	IDH1
4	x	X	6	6	P18065	34,814	1,3593E-30	29,5	IGFBP2
4	x		5	9	P17936-2	32,222	4,2374E-19	36,0	IGFBP3
2	x	X	5	5	P22692	27,934	7,3409E-46	29,8	IGFBP4
1	x		2	3	P24592	25,322	5,1402E-58	23,8	IGFBP6
2		X	11	13	Q16270	29,13	7,7067E-216	49,3	IGFBP7
3			3	3	P01876	37,654	8,1679E-07	11,9	IGHA1
1	x	X	8	8	P05231	23,718	9,6068E-184	43,4	IL6
1	x		1	1	P10145-2	11,338	1,1376E-32	15,7	IL8
3			2	2	Q12905	43,062	0,00039782	8,7	ILF2
2	x		13	13	P08476	47,442	8,908E-200	36,2	INHBA
4		X	4	4	P46940	189,25	7,0721E-10	4,6	IQGAP1
5			8	8	O14498	45,997	8,5878E-64	29,2	ISLR
4	x		1	2	P06756	116,04	0,0008026	3,1	ITGAV
5	x	X	5	5	P05556-3	91,619	5,4582E-10	10,1	ITGB1
5			2	5	O95965	53,921	7,0008E-09	20,6	ITGBL1
2		X	4	4	P19823	106,46	6,4646E-11	5,4	ITIH2
2		X	1	2	Q9Y287	30,338	5,2179E-32	6,8	ITM2B
5			11	2	P14923	66,35	5,8756E-67	17,1	JUP
5			2	11	F5GWP8	81,744	1,9806E-47	22,1	JUP
4		X	5	5	Q14974	97,169	8,1441E-40	8,1	KPNB1
5			3	4	Q5T749	64,135	0,000032643	12,8	KPRP
4	x	X	31	34	P04264	66,038	0	53,3	KRT1
5			26	26	P13645	58,826	0	47,3	KRT10
5			4	5	P13646	49,588	3,496E-114	21,2	KRT13
5			9	10	P02533	51,561	0	42,8	KRT14
5			19	21	P08779	51,267	0	50,1	KRT16
5	x		1	1	Q04695	48,105	7,4727E-120	16,7	KRT17

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
5			27	27	P35908	65,432	0	56,8	KRT2
4			5	6	P19013	57,285	2,4897E-45	15,4	KRT4
5			10	10	P13647	62,378	0	32,4	KRT5
1			2	3	P04259	60,066	0	40,6	KRT6B
5			15	15	P48668	60,024	0	39,9	KRT6C
5			1	1	Q86Y46	58,923	1,6567E-114	6,1	KRT73
5			2	2	Q7Z794	61,901	6,7925E-128	7,8	KRT77
4		X	6	6	Q8N1N4	56,865	2,3703E-24	13,5	KRT78
4			31	36	P35527	62,064	0	74,2	KRT9
2			33	33	Q16363	202,52	1,8974E-204	27,3	LAMA4
5		X	25	25	G3XAI2	200,48	0	20,8	LAMB1
2			9	9	P55268	195,98	3,5826E-30	9,6	LAMB2
5		X	34	34	P11047	177,6	0	31,6	LAMC1
5		X	2	2	Q14847	29,717	0,000013343	10,7	LASP1
5		X	15	15	P00338-3	39,837	4,4103E-261	49,3	LDHA
4		X	8	10	P07195	36,638	1,8783E-76	40,1	LDHB
1			1	1	Q32P28-3	90,615	0,0026747	1,2	LEPRE1
5	x	X	6	6	P09382	14,716	1,5893E-203	57,0	LGALS1
2		X	13	13	Q08380	65,33	7,8245E-148	30,4	LGALS3BP
5	x		2	2	P47929	15,075	0,000090438	22,1	LGALS7
5		X	1	1	Q99538	49,411	2,7541E-07	3,9	LGMN
4			13	18	P02545	74,139	2,1168E-86	35,4	LMNA
5		X	5	9	P28300	46,944	1,84E-33	24,9	LOX
5		X	5	6	Q08397	63,109	1,612E-35	18,6	LOXL1
2			10	10	Q9Y4K0	86,724	2,4493E-212	20,9	LOXL2
5	x	X	5	7	Q07954	504,6	5,8194E-14	20,4	LRP1
5	x	X	11	18	Q14766-4	186,87	3,6992E-90	18,2	LTBP1
5	x		20	25	Q14767	195,05	0	22,4	LTBP2
5	x		3	3	P02788	78,181	1,4402E-21	5,6	LTF
5		X	10	10	P51884	38,429	0	34,9	LUM

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
2			2	2	P61626	16,537	1,5988E-82	12,2	LYZ
1		X	7	7	P33908	72,968	1,669E-25	18,5	MAN1A1
1			1	2	O00462	100,89	0,0018495	3,8	MANBA
5		X	1	1	P55145	20,7	0,00090496	8,2	MANF
4		X	16	16	P46821	270,63	9,2501E-56	11,3	MAP1B
4			4	4	E7EVA0	245,44	9,1425E-10	3,0	MAP4
5		X	1	2	P29966	31,554	1,9663E-11	15,4	MARCKS
4		X	3	4	P40926	35,503	2,4104E-09	20,4	MDH2
5		X	10	14	Q08431	43,122	7,291E-90	43,9	MFGE8
4	x	X	2	2	P14174	12,476	2,5891E-23	17,4	MIF
1	x		21	21	P03956	54,006	0	48,0	MMP1
1			1	1	P09238	54,151	1,2036E-145	6,7	MMP10
1	x	X	15	15	G5E971	55,802	5,0983E-191	39,5	MMP13
2	x	X	31	31	P08253	73,881	0	57,6	MMP2
1	x	X	22	22	P08254	53,977	6,3791E-289	39,4	MMP3
5		X	2	2	Q9UBG0	166,67	8,1963E-07	1,8	MRC2
5		X	12	12	P26038	67,819	1,0932E-94	23,1	MSN
4		X	1	1	Q13126-2	38,356	0,0011824	5,2	MTAP
4		X	8	8	Q14764	99,326	7,029E-26	15,5	MVP
4	x	X	39	39	P35579	226,53	0	27,0	MYH9
5			6	6	J3QRS3	20,457	5,761E-139	49,2	MYL12B
4			7	7	B7Z6Z4	26,707	4,4088E-73	31,5	MYL6
3			3	3	E9PAV3	205,42	8,6504E-76	2,0	NACA
4		X	1	3	P55209	45,374	0,00013359	11,5	NAP1L1
3			2	2	F5HFY4	44,078	0,000011275	11,7	NAP1L4b
4	x	X	10	13	P19338	76,613	3,5174E-20	22,7	NCL
4			4	10	Q8TB73	64,672	4,3441E-08	25,5	NDNF
4			4	4	Q92859	160,01	2,6805E-09	5,0	NEO1
2		X	17	17	P14543	136,38	1,1114E-91	20,7	NID1
2		X	23	23	Q14112	151,25	1,0003E-131	23,8	NID2

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
5	x		6	6	Q32Q12	32,642	1,4811E-13	43,5	NME1-NME2
5	x	X	5	9	P06748	32,575	2,534E-29	42,9	NPM1
5	x	X	3	3	O14786	103,13	2,1912E-14	4,9	NRP1
5			2	5	P21589	63,367	5,1722E-08	13,9	NT5E
2		X	16	16	Q02818	53,879	5,7719E-206	39,0	NUCB1
5	x	X	7	7	P80303	50,195	7,5298E-76	22,1	NUCB2
3	x		2	2	Q9H1E3	27,296	9,0124E-10	8,2	NUCKS1
4		X	11	11	Q9NRN5	46,01	2,0093E-214	33,0	OLFML3
5		X	2	5	P13674	61,049	7,8338E-07	11,8	P4HA1
5		X	13	15	P07237	57,116	8,0232E-255	33,5	P4HB
5		X	2	3	P19021-5	108,4	6,2099E-12	4,8	PAM
4	x	X	4	4	Q99497	19,891	1,9265E-11	32,8	PARK7
5		X	18	18	Q15113	47,972	0	51,2	PCOLCE
3			3	3	Q13442	20,63	7,5466E-07	25,4	PDAP1
3			1	1	O14737	14,285	0,000017624	10,4	PDCD5
4	x	X	13	13	P30101	56,782	1,2278E-48	32,7	PDIA3
5		X	1	1	H7BZJ3	13,519	2,9624E-164	19,5	PDIA3
5		X	7	11	P13667	72,932	8,7324E-137	20,8	PDIA4
5		X	3	3	Q15084-2	53,9	1,5934E-09	11,2	PDIA6
4	x	X	5	5	P30086	21,057	6,9516E-66	52,9	PEBP1
4		X	6	6	P07737	15,054	6,7657E-242	55,7	PFN1
5			4	5	P18669	28,804	6,6112E-15	32,3	PGAM1
4		X	10	10	P00558	44,614	3,0388E-44	34,5	PGK1
5			1	1	P01833	83,283	0,0018878	2,5	PIGR
5			5	5	P12273	16,572	6,0064E-35	36,3	PIP
4		X	25	24	P14618	57,936	0	64,2	PKM2
4		X	14	29	Q15149	531,78	2,3512E-33	9,6	PLEC
2		X	22	22	B4DR87	88,271	0	36,0	PLOD1
2		X	8	8	O00469-2	87,097	4,9703E-99	15,4	PLOD2
3		X	38	33	Q15063-3	90,423	0	50,3	POSTN

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
5		X	1	38	B1ALD8	87,253	0	53,4	POSTN
4		X	9	10	P62937	18,012	2,9506E-30	50,3	PPIA
5		X	11	11	P23284	23,742	5,1199E-49	47,7	PPIB
5	x	X	5	7	Q06830	22,11	7,2542E-16	33,7	PRDX1
5	x	X	3	3	P32119	21,892	2,1983E-11	19,2	PRDX2
5		X	3	3	P30044	22,086	2,4871E-07	16,8	PRDX5
4		X	6	6	P30041	25,035	1,668E-16	41,5	PRDX6
5		X	2	4	K7ELL7	60,192	0,000026711	10,3	PRKCSH
5	x	X	1	1	P04156	27,661	0,0018303	4,7	PRNP
5		X	1	1	E7EQ64	28,123	8,751E-63	3,8	PRSS1
5		X	2	3	P25787	25,898	0,00017956	26,5	PSMA2
1			3	3	P28066	26,411	0,000001107	18,7	PSMA5
5		X	3	3	G3V5Z7	28,147	4,9208E-11	17,1	PSMA6
4			4	5	O14818	27,887	8,7658E-16	30,6	PSMA7
5			2	2	P49720	22,949	2,1632E-07	16,6	PSMB3
5		X	3	3	P62191	49,184	1,0653E-07	12,7	PSMC1
3			2	2	P17980	49,203	0,00041949	7,3	PSMC3
4		X	2	2	P62333	44,172	0,00024838	7,5	PSMC6
5		X	2	2	Q06323	28,723	0,000034987	13,7	PSME1
1		X	2	2	Q9UL46	27,401	1,7113E-08	12,1	PSME2
5	x		1	2	F8W7I2	24,39	0,0011554	14,7	PTGDS
4		X	2	4	Q6NZI2	43,476	3,3817E-07	17,7	PTRF
2	x	X	10	10	P26022	41,975	0	36,7	PTX3
2	x	X	38	38	Q92626	165,27	0	39,4	PXDN
2			3	3	Q16769	40,876	9,6559E-30	13,6	QPCT
2		X	25	25	O00391	82,577	0	41,9	QSOX1
5		X	1	1	P61026	22,541	5,5581E-07	5,5	RAB10
5	x	X	1	1	A6NIZ1	20,925	0,000072541	6,5	RAP1B
4	x		2	2	P38159	42,331	4,5996E-11	7,2	RBMX
5		X	4	4	Q15293	38,89	1,6837E-17	18,1	RCN1

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
5		X	5	5	Q96D15	37,493	1,3013E-64	26,2	RCN3
1		X	1	1	P34096	16,84	0,000032706	8,8	RNASE4
4		X	2	3	P62906	24,831	5,8605E-11	15,7	RPL10A
4			3	4	P30050	17,818	2,757E-15	33,9	RPL12
3			1	1	P50914	23,432	7,4848E-10	5,6	RPL14
4		X	1	1	B7Z4C8	15,118	0,00070111	10,8	RPL31
4		X	2	4	P46777	34,362	5,7572E-06	15,5	RPL5
4			2	5	Q02878	32,728	0,00022824	24,0	RPL6
4		X	2	4	P18124	29,225	3,7478E-09	21,8	RPL7
4			3	3	P62424	29,995	3,9904E-14	15,0	RPL7A
4			5	8	P05388	34,273	4,3875E-16	38,2	RPLP0
5		X	1	2	P05386	11,514	2,2287E-13	66,7	RPLP1
4		X	5	5	P05387	11,665	4,3567E-56	81,7	RPLP2
4			1	1	P04843	68,569	0,0001149	2,5	RPN1
5			2	2	Q9NQ39	20,12	1,7362E-11	8,5	RPS10P5
5			3	3	P08708	15,55	9,7446E-18	33,3	RPS17
4			4	4	P15880	31,324	6,4217E-13	16,0	RPS2
5		X	2	2	P60866-2	16,005	9,8066E-06	16,2	RPS20
4		X	1	1	P62854	13,015	0,00056193	13,0	RPS26
4		X	1	2	P62857	7,8409	9,0016E-07	30,4	RPS28
5		X	4	4	P23396-2	28,486	2,1182E-16	23,2	RPS3
5		X	1	2	M0R0R2	25,333	6,9624E-07	14,2	RPS5
4		X	2	4	P62753	28,68	0,00073328	22,5	RPS6
5		X	3	3	P62241	24,205	4,2644E-15	18,8	RPS8
4			5	5	A6NE09	32,909	1,3393E-38	27,5	RPSAP58
4		X	4	4	Q9P2E9	152,47	3,9709E-12	5,4	RRBP1
5		X	2	2	Q9NQC3	129,93	0,000015088	10,2	RTN4
5	x	X	3	3	P31949	11,74	5,8601E-145	34,3	S100A11
5	x		3	3	P05109	10,834	4,5815E-38	31,2	S100A8
5	x		4	4	P06702	13,242	1,9591E-126	43,9	S100A9

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
5			3	3	Q6UWP8	60,54	1,0407E-10	10,2	SBSN
2	x	X	1	1	P31431	21,641	6,3266E-55	7,1	SDC4
2		X	6	6	Q9BRK5	41,806	2,6947E-41	18,8	SDF4
5		X	3	4	Q15436	86,16	2,2549E-07	7,5	SEC23A
5	x		4	4	O75326	74,823	7,3273E-13	9,3	SEMA7A
4			4	4	Q15019-2	44,965	3,5298E-06	14,1	SEPT2
5		X	2	2	Q8NC51	46,596	4,0177E-07	7,6	SERBP1
1			3	3	P05120	44,564	0,000012768	10,4	SERPINB2
5			2	4	P29508	45,059	0	14,4	SERPINB3
2	x	X	19	19	P05121	44,002	0	63,4	SERPINE1
5			20	23	P07093	45,266	0	55,0	SERPINE2
5	x	X	11	11	P36955	46,312	8,349E-148	34,2	SERPINF1
5			6	6	B4E1F0	55,768	4,4506E-17	15,0	SERPING1
5	x	X	16	16	P50454	46,44	3,1949E-301	49,5	SERPINH1
5		X	3	3	Q01105	33,488	5,3382E-17	12,1	SET
5		X	2	2	Q9H299	10,438	6,972E-26	20,4	SH3BGRL3
1			1	1	Q15043	54,212	7,6098E-08	4,1	SLC39A14
1	x	X	1	1	P08195-4	71,122	0,0020916	2,1	SLC3A2
5		X	2	3	Q7KZF4	102	0,000079667	7,0	SND1
5	x	X	2	2	P00441	15,936	1,9898E-11	32,5	SOD1
1	x	X	2	2	P04179	24,722	4,0574E-134	12,6	SOD2
5		X	13	1	P09486	17,47	0	59,7	SPARC
5		X	1	14	F5GY03	34,632	0	47,9	SPARC
5			4	6	Q08629	49,124	6,9296E-14	13,7	SPOCK1
5			5	5	A6NG51	284,94	1,1402E-10	3,3	SPTAN1
4	x	X	2	4	Q01082	274,61	0,000013503	2,9	SPTBN1
2	x		3	4	P10124	17,652	1,4458E-09	26,6	SRGN
4			2	3	P78539	51,571	7,7367E-10	7,3	SRPX
2			5	5	O60687	52,971	5,1095E-22	11,4	SRPX2
4			2	2	P84103	19,329	0,000092522	14,0	SRSF3

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
1		X	2	2	P05455	46,836	0,00019615	6,1	SSB
1			3	3	O76061	33,248	3,9107E-07	19,5	STC2
5			1	1	A8MU27	16,956	5,6951E-15	8,2	SUMO3
5			1	1	Q15431	114,19	0,0010523	1,3	SYCP1
4	x	X	4	5	O60506	69,602	3,3833E-12	11,4	SYNCRIP
4		X	11	12	Q01995	22,611	2,9945E-184	61,2	TAGLN
4			9	9	P37802	22,391	3,0984E-227	54,3	TAGLN2
3			1	2	P17987	60,343	0,0018515	7,7	TCP1
5	x		2	2	P01137	44,341	0,000030247	10,0	TGFB1
4			20	20	G8JLA8	74,693	0	41,6	TGFBI
5			4	4	Q08188	76,631	1,6293E-20	9,7	TGM3
4	x	X	27	43	P07996	129,38	3,0836E-285	48,6	THBS1
4		X	30	30	P35442	129,99	0	35,0	THBS2
2	x	X	7	7	P01033	23,171	3,9875E-193	54,1	TIMP1
5	x	X	9	9	P16035	24,399	1,6028E-99	40,0	TIMP2
4		X	5	6	P29401-2	68,813	7,0954E-97	17,3	TKT
4		X	19	19	Q9Y490	269,76	2,8117E-71	13,9	TLN1
4			1	1	P63313	5,0256	0,000010354	31,8	TMSB10
4	x		3	3	P62328	5,0526	1,264E-19	61,4	TMSB4X
2	x	X	70	70	P24821	240,85	0	45,7	TNC
5	x	X	2	2	P24821-4	230,86	0	47,2	TNC
1	x		4	4	P98066	31,203	3,57E-115	20,2	TNFAIP6
5		X	9	9	P60174	30,791	4,015E-164	45,8	TPI1
4		X	9	9	P09493	32,708	0	23,9	TPM1
4		X	3	4	P07951-2	32,989	0	28,5	TPM2
5			7	7	P06753-2	29,032	6,6061E-186	29,0	TPM3
5		X	4	4	P67936	28,521	1,801E-70	27,0	TPM4
5	x	X	1	1	Q5W0H4	21,525	0,0024708	7,4	TPT1
4			13	15	P68363	50,151	1,3285E-98	54,1	TUBA1B
4			14	14	P07437	49,67	7,7556E-165	47,1	TUBB

Groups	Cyt/Chem (a)	Mouse (b)	Razor + unique peptides H30 (c)	Razor + unique peptides H34 (c)	Protein IDs	Mol. weight [kDa]	PEP (d)	Sequence coverage [%] (e)	Gene names
3			2	5	Q13509	50,432	2,8564E-102	40,2	TUBB3
5		X	2	2	P04350	49,585	1,1541E-117	47,7	TUBB4A
4			2	16	P68371	49,83	2,0638E-122	53,0	TUBB4B
5	x	X	3	3	P10599	11,737	6,6824E-17	31,4	TXN
5		X	3	3	Q8NBS9	47,628	3,7447E-07	6,7	TXNDC5
4		X	9	9	P22314	117,85	4,452E-56	13,8	UBA1
5	x		3	3	P0CG48	77,038	4,7073E-74	40,7	UBC
5			2	2	D6R956	26,839	6,8563E-07	14,5	UCHL1
5	x	X	4	4	Q6EMK4	71,712	8,8934E-63	8,2	VASN
2	x	X	7	7	P19320	81,275	7,1081E-57	16,8	VCAM1
4		X	14	15	P13611	372,82	2,051E-120	5,8	VCAN
4	x	X	14	14	P18206	123,8	5,9369E-82	19,0	VCL
4	x	X	13	17	P55072	89,321	6,4039E-39	30,8	VCP
4	x	X	29	32	P08670	53,651	0	57,9	VIM
5			1	1	P04004	54,305	8,0359E-11	3,1	VTN
5			4	4	Q6PCB0	46,804	5,5956E-24	17,1	VWA1
4		X	9	9	O75083	66,193	2,7227E-22	25,7	WDR1
4			2	4	H0Y449	41,905	4,1197E-08	26,2	YBX1
5	x	X	2	2	P31946	28,082	6,4766E-107	22,8	YWHAB
5		X	4	4	P62258	29,174	1,0932E-98	28,6	YWHAE
5		X	2	2	P61981	28,302	3,0238E-82	20,6	YWHAG
3			1	1	Q04917	28,218	3,1271E-66	15,0	YWHAH
5		X	6	4	P27348	27,764	2,7827E-84	33,1	YWHAQ
5		X	3	6	E7EX29	28,036	1,3285E-119	32,9	YWHAZ
5		X	3	3	Q15942	61,277	6,3542E-06	11,4	ZYX

REFERENCES

1. AM. Arias, *Drosophila melanogaster and the development of biology in the 20th century*, Methods in Molecular Biology, 2008, 420, 1–25.

2. http://www.biology-online.org/dictionary/Stem_cell

3. http://nas-sites.org, *Understanding stem cells: an over view of the science and issues from the National academies.*

4. https://en.wikipedia.org/wiki/Stem_cell

5. HR. Schöler, *The Potential of Stem Cells: An Inventory*, In: N. Knoepffler, D. Schipanski, SL. Sorgner, editors, Human biotechnology as Social Challenge, 2007, p. 28.

7. F. Ulloa-Montoya, CM. Verfaillie, WS. Hu, *Culture systems for pluripotent stem cells*, Journal of Bioscience and Bioengeneering, 2005, 100, 12–27.

8. www.bioinformant.com

9. JA. Thomson, J. Itskovitz-Eldor, SS. Shapiro, MA. Waknitz, JJ. Swiergiel, VS. Marshall, JM. Jones, *Blastocysts Embryonic Stem Cell Lines Derived from Human*, Science, 1998, 282, 1145–47.

10. LA. Boyer, TI. Lee, MF. Cole, SE. Johnstone, SS. Levine, JP. Zucker, MG. Guenther, RM. Kumar, HL. Murray, RG. Jenner, DK. Gifford, DA. Melton, R. Jaenisch, RA. Young, *Core transcriptional regulatory circuitry in human embryonic stem cells*, Cell, 2005, 122, 947–56.

11. Mayo foundation for medical education and research “Stem cells” Mayo Clinic, Web. March 23, 2013.

12. Y. Jiang et al., *Pluripotency of mesenchymal stem cells derived from adult marrow*, Nature, 2002, 418, 41-9.

13. MZ. Ratajczak, B. Machalinski, W. Wojakowski, J. Ratajczak, M. Kucia, *A hypothesis for an embryonic origin of pluripotent Oct-4(+) stem cells in adult bone marrow and other tissues*, Leukemia, 2007, 21, 860–7.

14. SD. Narasipura, JC. Wojciechowski, N. Charles, JL. Liesveld, MR. King, *P-Selectin coated microtube for enrichment of CD34+ hematopoietic stem and progenitor cells from human bone marrow,* Clinical Chemistry, 2008, 54, 77–85.

15. S. Terai, T. Ishikawa, K. Omori, K. Aoyama, Y. Marumoto, Y. Urata, Y. Yokoyama, K. Uchida, T. Yamasaki, Y. Fujii, K. Okita, I. Sakaida, *Improved liver function in patients with liver cirrhosis after autologous bone marrow cell infusion therapy*, Stem Cells, 2006, 24, 2292–8.

16. R. Subrammaniyan, J. Amalorpavanathan, R. Shankar et al., *Application of autologous bone marrow mononuclear cells in six patients with advanced chronic critical limb ischemia as a result of diabetes: our experience*, Cytotherapy, 2011, 13, 993–9.

17. N. Madhu Sankar, K.Vaidyanathan, *Use of Bone Marrow derived Stem Cells in Patients with Cardiovascular Disorders*, Journal of Stem Cells and Regenerative Medicine, 2007, 3, 28-29.

18. VD. Dedeepiya, YY. Rao, GA. Jayakrishnan, JK. Parthiban, S. Baskar, SR. Manjunath, R. Senthilkumar, SJ. Abraham, *Index of CD34+ Cells and Mononuclear Cells in the Bone Marrow of Spinal Cord Injury Patients of Different Age Groups: A Comparative Analysis*, Bone Marrow Research, 2012, 1-8.

19. B. Barrilleaux, DG. Phinney, DJ. Prockop, KC. O' Connor, *Review: ex vivo engineering of living tissues with adult stem cells*, Tissue Engineering, 2006, 12, 3007–19.

20. JM. Gimble, AJ. Katz, BA. Bunnell, *Adipose-derived stem cells for regenerative medicine*, Circulation Research, 2007, 100, 1249–60.

21. http://slideplayer.com/slide/9129448

22. A. Bongso, L. EngHin, *Stem cells: their definition, classification and sources, Stem Cells: From Benchtop to Bedside.* World Scientific, 2005, p. 5. ISBN 981-256-126-9.

23. K.L. Moore, T.V.N. Persaud, A.G. Torchia, *Before We Are Born: Essentials of Embryology and Birth Defects.* Philadelphia, PA: Saunders, Elsevier, 2013.

24. P. De Coppi, G. Bartsch, MM. Siddiqui, T. Xu, CC. Santos, L. Perin, G. Mostoslavsky, AC. Serre, EY. Snyder, JJ. Yoo, ME. Furth, S. Soker, A. Atala, *Isolation of amniotic stem cell lines with potential for therapy*, Nature Biotechnology, 2007, 25, 100–106.

25. T. Bubela, MD. Li, M. Hafez, M. Bieber, H. Atkins, *Is belief larger than fact: Expectations, optimism and reality for translational stem cell research*, BMC Medicine, 2012, 10, 133.

26. M. Ader, EM. Tanaka, *Modeling human development in 3D culture*. Current Opinion in Cell Biology, 2014, 31, 23-28.

27. *Making human embryonic stem cells*, The Economist, 2007-11-22.

28. M. Brand, J. Palca, A. Cohen, *Skin Cells Can Become Embryonic Stem Cells*, 2007, National Public Radio.

29. *Breakthrough Set to Radically Change Stem Cell Debate*, News Hour with Jim Lehrer, 2007.

30. EA. Kimbrel, R. Lanza, *Pluripotent stem cells: The last 10 years*, Regenerative Medicine, 2016, 11, 831–847.

31. K. Takahashi, S. Yamanaka, *Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors*, Cell, 2006, 126, 663–76.

32. http://learn.genetics.utah.edu/content/stemcells/quickref

33. "Bone Marrow Transplant".ucsfchildrenshospital.org

34. E. Kane, *Stem-cell therapy shows promise for horse soft-tissue injury*, DVM Newsmagazine, 2008.

35. K.L. Moore, T.V.N. Persaud, A.G. Torchia, *Before We Are Born: Essentials of Embryology and Birth Defects.* Philadelphia, PA: Saunders, Elsevier, 2013

36. http://www.sigmaaldrich.com

37. EM. Horwitz, K. Le Blanc, M. Dominici, I. Mueller, I. Slaper-Cortenbach, FC. Marini, RJ. Deans, DS. Krause, A. Keating, *International Society for Cellular Therapy: Clarification of the nomenclature for MSC: the International Society for Cellular Therapy position statement*, Cytotherapy, 2005, 7, 393–399.

38. M. Crisan, S. Yap, L. Casteilla, CW. Chen, M. Corselli, TS. Park, G. Andriolo, Sun B, Zheng B, Zhang L, Norotte C, Teng PN, Traas J, Schugar R, Deasy BM, Badylak S, Bühring HJ, Giacobino JP, Lazzari L, Huard J, Peault B: *A perivascular origin for mesenchymal stem cells in multiple human organs*, Cell Stem Cell 2008, 3, 301–313.

39. J. Oswald, et al., *Mesenchymal stem cells can be differentiated into endothelial cells in vitro*, Stem Cells, 2004, 22, 377–384.

40. S. Makino, et al., *Cardiomyocytes can be generated from marrow stromal cells in vitro,* Journal of clinic investigations, 1999, 103, 697-705.

41. S. Snykers, et al., *In vitro differentiation of embryonic and adult stem cells into hepatocytes: state of the art*, Stem Cells, 2009, 27, 577-605.

42. A. Arthur, et al., *Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues*, Stem Cells, 2008, 26, 1787-95.

43. DA. De Ugarte, K. Morizono, A. Elbarbary, Z. Alfonso, PA. Zuk, M. Zhu, JL. Dragoo, P. Ashijan, B. Thomas, P. Benhaim, I. Chen, J. Frazer, MH. Hedrick, *Comparison of multilineage cells from human adipose tissue and bone marrow*, Cells Tissues Organs, 2003, 174:101–109.

44. S. Meirelles, L. Chagastelles, NB. Nardi, *Mesenchymal stem cells reside in virtually all postnatal organs and tissues*, Journal of cells science, 2006, 119, 2204–2213.

45. N. Kaltz, et al. *Novel markers of mesenchymal stem cells defined by genome-wide gene expression analysis of stromal cells from different sources*, Experimental Cell Research, 2010, 316, 2609–2617.

46. M. Corselli, CW. Chen, M. Crisan, L. Lazzari, B. Peault, *Perivascular ancestors of adult multipotent stem cells*, Arteriosclerosis, Thrombosis and vascular biology, 2010, 30, 1104–1109.

47. http://endonovo.com/cytotronics-platform.

48. C. Farrington-Rock, et al., *Chondrogenic and adipogenic potential of microvascular pericytes*, Circulation, 2004, 110, 2226–2232.

49. MJ. Doherty, et al., *Vascular pericytes express osteogenic potential in vitro and in vivo*, Journal of Bone and Mineral Research, 1998, 13, 828–838.

50. A. Keating, *Mesenchymal stromal cells: new directions*, Cell stem cell, 2012, 10, 709-16.

51. S. Gordon, A. Mantovani, *Diversity and plasticity of mononuclearphagocytes*, European Journal of immunology, 2011, 41, 2470–2472.

52. A. Mantovani et al., *Macrophage plasticity and polarization in tissue repair and remodelling*, The Journal of pathology, 2013, 229, 176-85.

53. Waterman et al., *A new mesenchymal stem cells (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype*, PlOsOne, 2010, 5, 4.

54. K. Nemeth et al., *Bone marrow stromal cells use TGF-β to suppress allergic responses in a mouse model of ragweed-induced asthma*, PNAS, 2010, 107, 5652–5657.

55. SL. Tomchuck et al., *Toll-like receptors on human mesenchymal stem cells drive their migration and immunomodulating responses*, Stem cells, 2008, 26, 99-107.

56. Delarosa et al., *Toll-like receptors as modulators of mesenchymal stem cells*, Frontiers in Immunology, 2012, 3, 182.

57. Raicevic et al., *Inflammation modifies the pattern and the function of Toll-like receptors expressed by human mesenchymal stromal cells*, Human Immunology, 2010, 71-235.

58. IF. Mo et al., *Prolonged exposure to bacterial toxins downregulated expression of toll-like receptors in mesenchymal stromal cell-derived osteoprogenitors*, BMC Cell Biology, 2008, 9, 52.

59. ME. Bernardo, EW. Fibbe, *Mesenchymal Stromal Cells: Sensors and Switchers of Inflammation*, Cell Stem Cell, 2013, 13.

60. AJ. Nauta, WE. Fibbe, *Immunomodulatory properties of mesenchymal stromal cells*, Blood, 2007, 110, 3499–3506.

61. A. Bartholomew, C. Sturgeon, M. Siatskas, K. Ferrer, K. McIntosh, S. Patil, W. Hardy, S. Devine, D. Ucker, R. Deans, A. Moseley, R. Hoffman, *Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo*, Experimental Hematology, 2002, 30, 42–48.

62. A. Tyndall, UA. Walker, A. Cope et al, *Immunomodulatory properties of mesenchymal stem cells:* a review based on an interdisciplinary meeting held at the Kennedy Institute of Rheumatology Division, London, UK, 31 October 2005, Arthritis Research and Therapy, 2007, 9:301.

63. I. Rasmusson, O. Ringden, B. Sundberg, K. Le Blanc, *Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells*, Transplantation, 2003, 76, 1208–1213.

64. N. Eliopoulos, J. Stagg, L. Lejeune, S. Pommey, J. Galipeau, *Allogeneic marrow stromal cells are immune rejected by MHC class I and II mismatched recipient mice*, Blood, 2005, 106, 4057–4065.

65. AJ. Nauta, G. Westerhuis, AB. Kruisselbrink, EG. Lurvink, R. Willemze, WE. Fibbe, *Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a non myeloablative setting*, Blood, 2006, 108, 2114–2120.

66. WT. Tse, JD. Pendleton, WM. Beyer, MC. Egalka, EC. Guinan, *Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation,* Transplantation, 2003, 75, 389–397.

67. S. Aggarwal, MF. Pittenger, *Human mesenchymal stem cells modulate allogeneic immune cell responses,* Blood, 2005, 105, 1815–1822.

68. M. Di Nicola, C. Carlo-Stella, M. Magni, M. Milanesi, PD. Longoni, P. Matteucci, S. Grisanti, AM. Gianni, *Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli*, Blood, 2002, 99, 3838–3843.

69. S. Glennie, I. Soeiro, PJ. Dyson, EW. Lam, F. Dazzi, *Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells,* Blood, 2005, 105, 2821–2827.

70. A. Corcione, F. Benvenuto, E. Ferretti, D. Giunti, V. Cappiello, F. Cazzanti, M. Risso, F. Gualandi, GL. Mancardi, V. Pistoia, A. Uccelli, *Human mesenchymal stem cells modulate B-cell functions*, Blood, 2006, 107, 367–372.

71. I. Rasmusson, K. Le Blanc, B. Sundberg, O. Ringden: *Mesenchymal stem cells stimulate antibody secretion in human B cells*, Scandinavian Journal of Immunology, 2007, 65, 336–343.

72. M. Krampera, L. Cosmi, R. Angeli, A. Pasini, F. Liotta, A. Andreini, V. Santarlasci, B. Mazzinghi, G. Pizzolo, F. Vinante, P. Romagnani, E. Maggi, S. Romagnani, F. Annunziato,

Role of the IFN-γ in the immunomodulatory activity of human mesenchymal stem cells, Stem cells, 2005, 24, 386–398.

73. PA. Sotiropoulou, SA. Perez, AD. Gritzapis, CN. Baxevanis, M. Papamichail, *Interactions between human mesenchymal stem cells and natural killer cells*, Stem Cells, 2006, 24, 74–85.

74. AR. Williams, JM. Hare, S. Dimmeler, D. Losordo, *Mesenchymal Stem Cells: Biology, Pathophysiology, Translational Findings, and Therapeutic Implications for Cardiac Disease*, Circulation Research, 2011, 109, 923-40.

75. DG. Phinney, DJ. Prockop, *Concise review: mesenchymal stem/multipotent stromal cells: the state of trans differentiation and modes of tissue repair*, Stem cells, 2007, 25, 2896-2902.

76. K. Le Blanc, I. Rasmusson, B. Sundberg, C. Gotherstrom, M. Hassan, M. Uzunel, O. Ringden, *Treatment of severe acute graftversus- host disease with third party haploidentical mesenchymal stem cells*, Lancet, 2004, 363, 1439–1441.

77. G. Behre, S. Theurich, T. Weber, M. Christopeit, Reply to *'The correlation between cotransplantation of mesenchymal stem cells and higher recurrence rates in hematologic malignancy patients: outcome of a pilot clinical study'* by Ning et al., Leukemia, 2009, 23,178–180.

78. SM. Devine, C. Cobbs, M. Jennings, A. Bartholomew, R. Hoffman, *Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates*, Blood, 2003, 101, 2999–3001.

79. F. Djouad, V. Fritz, F. Apparailly, P. Louis-Plence, C. Bony, J. Sany, C. Jorgensen, D. Noël, *Reversal of the immunosuppressive properties of mesenchymal stem cells by tumor necrosis factor alpha in collagen-induced arthritis*, Arthritis Rheum, 2005, 52, 1595–1603.

80. VS. Urban, J. Kiss, J. Kovacs, E. Gócza, V. Vas, E. Monostori, F. Uher, *Mesenchymal stem cells cooperate with bone marrow cells in therapy of diabetes*, Stem Cells, 2008, 26, 244–253.

81. D. Rubio, J. Garcia-Castro, MC. Martin, R. De La Fuente, JC. Cigudosa, AC. Lloyd, A. Bernard, *Spontaneous human adult stem cell transformation*, Cancer Research, 2005, 65, 3035–3039.

82. AC. Lloyd, *Limits to lifespan*, Nature Cell Biology, 2002, 4.

83. L. Chin, SE. Artandi, Q. Shen, *p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis*, Cell, 1999, 97, 527–38.

84. J. Marx, Cancer research, *Mutant stem cells may seed cancer*, Science, 2003, 301, 1308–10.

85. Phinney et al., *Plastic adherent stromal cells from the bone marrow of commonly used strains of inbred mice: variations in yield, growth, and differentiation*, Biochemical journal, 1999, 72, 570–585.

86. Miura et al., *Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation*, Stem Cells, 2006, 24, 1095–1103.

87. Tolar et al., *Sarcoma derived from cultured mesenchymal stem cells*, Stem Cells, 2007, 25: 371–379.

88. P.R. Amable, M.V. Teixeria, R.B. Carias, J.M. Granjeiro, R. Borojevic, *Protein synthesis and secretion in human mesenchymal cells derived from bone marrow, adipose tissue and Wharton's jelly*, Stem cells Research and Therapy, 2014, 53 – 66.

89. H. Tjalsma, A. Bolhuis, J.D., Jongbloed et al., *Signal peptide-dependent protein transport in Bacillus subtilis: a genome-based survey of the secretome*, Microbiology and Molecular Biology Review, 2000, 64 , 515 – 547.

90. S. Nonnis, E. Maffioli, L. Zanotti, F. Santagata, A. Negri, A. Viola, S. Elliman, G. Tedeschi, *Effect of fetal bovine serum in culture media on MS analysis of mesenchymal stromal cells secretome*, EuPA Open Proteomics, 2016, 28-30.

91. LA. Liotta, M. Ferrari, E. Petricoin, *Clinical proteomics: written in blood*, Nature, 2003, 425, 905-10.

92. GS. Omenn, DJ. States, M. Adamski, TW. Blackwell, R. Menon, H. Hermjakob, R. Apweiler, BB. Haab, RJ. Simpson, JS. Eddes, *Overview of the HUPO Plasma Proteome Project: results from the pilot phase with 35 collaborating laboratories and multiple*

analytical groups, generating a core dataset of 3020 proteins and a publicly-available database, Proteomics, 2005, 5, 3226-45.

93. J.E. Celis, P. Gromov, T. Cabezon, J.M. Moreira, N. Ambartsumian, K. Sandelin, F.Rank, I. Gromova, *Proteomic characterization of the interstitial fluid perfusing the breast tumor microenvironment: a novel resource of for biomarkers and therapeutic target discovery,* Molecular and Cellular Proteomics, 2004, 3, 327-344.

94. Y. Shi, CA. Elmets, JW. Smith, YT. Liu, YR. Chen, CP. Huang, W. Zhu, HN. Ananthaswamy, RL. Gallo, CM. Huang, *Quantitative proteomes and in vivo secretomes of progressive and regressive UV-induced fibrosarcoma tumor cells: mimicking tumor microenvironment using a dermis-based cell-trapped system linked to tissue chamber*, Proteomics, 2007, 7, 4589–4600.

95. W. Zhu, JW. Smith, CM. Huang, *Mass Spectrometry-Based Label-Free Quantitative Proteomics,* Journal of Biomedicine and Biotechnology, 2010.

96. CC. Wu, MJ. Mac Coss, *Shotgun proteomics: tools for the analysis of complex biological systems*, Current Opinion in Molecular Therapeutics, 2002, 4, 242– 250.

97. EI. Chen, JR. Yates, *Cancer proteomics by quantitative shotgun proteomics*, Molecular Oncology, 2007, 1,144–159.

98. SP. Gygi, B. Rist, SA. Gerber, F. Turecek, MH. Gelb, R. Aebersold, *Quantitative analysis of complex protein mixtures using isotope coded affinity tags*, Nature Biotechnology, 1999, 17, 994–999.

99. A. Motoyama, JR. Yates, *Multidimensional LC separations in shotgun proteomics,* Analytical Chemistry, 2008, 7187-7193.

100. B. Domon, R. Aebersold, *Mass spectrometry and protein analysis*, Science, 2006, 212-217.

101. RD. Voyksner, H. Lee, *Investigating the use of an octupole ion guide for ion storage and high-pass mass filtering to improve the quantitative performance of electrospray ion trap mass spectrometry*, Rapid Communications in Mass Spectrometry, 1999, 1427–1437.

102. D. Chelius, PV. Bondarenko, Quantitative profiling of proteins in complex mixtures using liquid chromatography and mass spectrometry, *Journal of Proteome Research*, 2002, 4, 317–323.

103. PV. Bondarenko, D. Chelius, TA. Shaler, *Identification and relative quantitation of protein mixtures by enzymatic digestion followed by capillary reversed-phase liquid chromatography-tandem mass spectrometry*, Analytical Chemistry, 2002, 74, 4741–4749.

104. J. Lengqvist, J. Andrade, Y. Yang, G. Alvelius, R. Lewensohn, and J. Lehtiö, *Robustness and accuracy of high speed LC-MS separations for global peptide quantitation and biomarker discovery*, Journal of Chromatography B, 2009, 13, 1306–1316.

105. N. Fatima, D. Chelius, B. T. Luke et al., *Label-free global serum proteomic profiling reveals novel celecoxib-modulated proteins in familial adenomatous polyposis patients*, Cancer Genomics and Proteomics, 2009, 6, 41–49.

106. S. K. Huang, MM. Darfler, MB. Nicholl et al., *LC/MS-based quantitative proteomic analysis of paraffin-embedded archival melanomas reveals potential proteomic biomarkers associated with metastasis*, PLoS ONE, 2009.

107. Y. Levin, E. Schwarz, L. Wang, F. M. Leweke, and S. Bahn, *Label-free LC-MS/MS quantitative proteomics for large-scale biomarker discovery in complex samples*, Journal of Separation Science, 2007, 30, 2198–2203.

108. JPC. Vissers, JI. Langridge, and JMFG. Aerts, Analysis and quantification of diagnostic serum markers and protein signaturs for Gaucher disease, Molecular and Cellular Proteomics, 2007, 6, 755–766.

109. JTJ. Huang, T. McKenna, C. Hughes, F. M. Leweke, E. Schwarz, and S. Bahn, *CSF biomarker discovery using label-free nano-LC-MS based proteomic profiling: technical aspects*, Journal of Separation Science, 2007, 30, 214–225.

110. MP. Washburn, D. Wolters, JR. Yates, *Large-scale analysis of the yeast proteome by multidimensional protein identification technology,* Nature Biotechnology, 2001, 19, 242–247.

111. H. Liu, R. G. Sadygov, JR. Yates, *A model for random sampling and estimation of relative protein abundance in shotgun proteomics*, Analytical Chemistry, 2004, 76, 4193–4201.

112. B. Zybailov, MK. Coleman, L. Florens, MP. Washburn, *Correlation of relative abundance ratios derived from peptide ion chromatograms and spectrum counting for quantitative proteomic analysis using stable isotope labeling*, Analytical Chemistry, 2005, 77, 6218–6224.

113. JX. Pang, N. Ginanni, AR. Dongre, SA. Hefta, GJ. Opiteck, *Biomarker discovery in urine by proteomics*, Journal of Proteome Research, 2002, 1, 161–169.

114. PV. Rao, AP. Reddy, X. Lu et al., *Proteomic identification of salivary biomarkers of type-2 diabetes*, Journal of Proteome Research, 2009, 8, 239–245.

115. M. Asara, HR. Christofk, LM. Freimark, LC. Cantley, *A label-free quantification method by MS/MS TIC compared to SILAC and spectral counting in a proteomics screen*, Proteomics, 2008, 8, 994–999.

116. L. Zanotti, A. Sarukhan, E. Dander, M. Castor, J. Cibella, C. Soldani et al., *Encapsulated mesenchymal stem cells for in vivo immunomodulation*, Leukemia, 2013, 27, 500-3.

117. DF. Mc Auley, GF. Curley, UI. Hamid, JG. Laffey, J. Abbott, DH. McKenna, X. Fang, MA. Matthay, JW. Lee, *Clinical grade allogeneic human mesenchymal stem cells restore alveolar fluid clearance in human lungs rejected for transplantation*, American Journal of Physiology, Lung Cellular and Molecular Physiology, 2014, 306, 809-15.

118. M. Dominici, K. Le Blanc, I. Mueller, I. Slaper-Cortenbach , F. Marini, D. Krause, R. Deans, A. Keating, E. Horwitz, *Minimal criteria for defining multipotent mesenchymal stromal cells*, The International Society for Cellular Therapy position statement, Cytotherapy, 2006, 8, 315-7.

119. J. Cox, M. Mann, *Max Quant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification*, Nature Biotechnology, 2008, 26, 1367–72.

120. S. Tyanova, T. Temu, P. Sinitcyn, A. Carlson, MY. Hein, T. Geiger, M. Mann, J. Cox, *The Perseus computational platform for comprehensive analysis of (prote) omics data*, Nature Methods, 2016, 13, 731-40.

121. The Gene Ontology Consortium, *Gene Ontology Consortium: going forward*, Nucleic Acids Research, 2015, 43.

122. P. Gaudet, PA. Michel, M. Zahn-Zabal, I. Cusin, PD. Duek, O. Evalet, A. Gateau, A. Gleizes, M. Pereira, D. Teixeira, Y. Zhang, L. Lane, A. Bairoch, The neXtProt knowledgebase on human proteins: current status, Nucleic Acids Res, 2015, 43, 764-70.

123. The UniProt Consortium, *UniProt: a hub for protein information*, Nucleic Acids Research, 2015.

124. G. Stelzer, R. Rosen, I. Plaschkes, S. Zimmerman, M. Twik, S. Fishilevich, T. Iny Stein, R. Nudel, I. Lieder, Y. Mazor, S. Kaplan, D. Dahary, D. Warshawsky, *The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analysis,* Current Protocol in Bioinformatics, 2016, 54,1.

125. B. Rocha, *Secretome analysis of human mesenchymal stem cells undergoing chondrogenic differentiation*, Journal of Proteome Research, 2014, 13, 1045-54.

126. SP. Kristensen, L. Chen, M. Overbeck Nielsen, DW. Qanie, I. Kratchmarova, Kassem M, Andersen JS, *Temporal Profiling and Pulsed SILAC Labeling Identify Novel Secreted Proteins During ex vivo Osteoblast Differentiation of Human Stromal Stem Cells*, Molecular and Cellular Proteomics, 2012, 11, 989-1007.

127. DW. Huang, BT. Sherman, RA. Lempicki, *Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists*, Nucleic Acids Research, 2009, 37,1-13.

128. D. Szklarczyk, A. Franceschini, S. Wyder, K. Forslund, D. Heller, J. Huerta-Cepas, M. Simonovic, A. Roth, A. Santos, KP Tsafou, M. Kuhn, P. Bork, LJ Jensen, C. von Mering, *STRING v10: protein-protein interaction networks, integrated over the tree of life,* Nucleic Acids Research, 2015, 43.

129. JA., Vizcaíno, A. Csordas, N. del-Toro, JA. Dianes, J. Griss, I. Lavidas, G. Mayer, Y. Perez-Riverol, F. Reisinger, T. Ternent, QW. Xu, R. Wang, H. Hermjakob, *2016 update of the PRIDE database and related tools*, Nucleic Acids Research, 2016, 44.

130. W. Huang, BT. Sherman, RA Lempicki, *Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources*, Nature Protocols, 2009, 4,44-57.

131. *Angiogenesis insights from a systematic overview*, New York: Nova Science, 2013.

132. N. Naoyo, Y.Hirohisa et al., *Angiogenesis in Cancer*, Vascular Health and Risk Management, 2006, 2, 213–219.

133. AA. Ucuzian, AA. Gassman, AT. East, *Molecular Mediators of Angiogenesis*, Journal of Burn Care and Research, 2010, 31, 58-75.

134. SM. Wahl, H. Wong, NJ. McCartney-Francis, *Role of growth factors in inflammation and repair*, Cell Biochemistry, 1989, 40, 193-9.

135. R. Visse, H. Nagase, *Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry*, Circulation Research, 2003, 92, 827-39.

136. K. Brew, H. Nagase, *The tissue inhibitors of metalloproteinases (TIMPs): An ancient family with structural and functional diversity*, Biochimica et Biophysica Acta, 2010, 1803, 55–71.

137. E. Lambert, E. Dasse, B. Haye, E. Petitfrere, *TIMPs as multifacial proteins*, Critical Reviews in Oncology/Hematology, 2004, 49,187–198.

138. SJ. Crocker, RF. Frausto, JK. Whitmire, N. Benning, R. Milner, JL. Whitton *Amelioration of coxsackievirus B3-mediated myocarditis by inhibition of tissue inhibitors of matrix metalloproteinase-1*, American Journal Pathology, 2007, 171, 1762–1773.

139. S. Zacchigna, L. Pattarini, L. Zentilin, S. Moimas, A. Carrer, M. Sinigaglia et al., *Bone marrow cells recruited through the neuropilin-1 receptor promote arterial formation at the sites of adult neo-angiogenesis in mice*, Journal of Clinical Investigation , 2008, 118, 2062–2075.

140. ME. Groh, B. Maitra, E. Szekely, ON. Koç, *Human mesenchymal stem cells require monocyte-mediated activation to suppress alloreactive T cells*, Experimental Hematology, 2005, 33:928-34.

141. Gene Ontology Consortium*, Gene ontology consortium: going forward*, Nucleic Acids Research, 2015, 43, D1049–56.

142. UniProt Consortium, *UniProt: a hub for protein information*, Nucleic Acids Research, 43, 2015, D204–12.

143. S.P. Kristensen, L. Chen, M. Overbeck Nielsen, D.W. Qanie, I. Kratchmarova, M. Kassem, J.S. Andersen, *Temporal profiling and pulsed SILAC labeling identify novel secreted proteins during ex vivo osteoblast differentiation of human stromal stem cells*, Molecular and Cellular Proteomics, 2012, 989–1007.

144. L. Zanotti, R. Angioni, B. Calì, C. Soldani, C. Ploia, F. Moalli, M. Gargesha, G. D'amico, S. Elliman, G. Tedeschi, E. Maffioli, A. Negri, S. Zacchigna, A. Sarukhan, JV Stein, A. Viola, *Mouse mesenchymal stem cells inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1*, Leukemia, 2016, 30, 1143-54.

145. L. Nissinen, VM. Kahari, *Matrix metalloproteinases in inflammation*, Biochimica and Biophysica Acta, 2014, 2571-80.

146. D. Rodríguez, CJ. Morrison, CM. Overal, *Matrix metalloproteinases: What do they not do? New substrates and biological roles identified by murine models and proteomics*, Biochimica and Biophysica Acta, 2010,1803, 39-54.

147. A. Clabaut, C. Grare, T. Léger, P. Hardouin, O. Broux, *Variations of secretome profiles according to conditioned medium preparation: The example of human mesenchymal stem cell-derived adipocytes*, Electrophoresis, 2015, 36,2587-93.

148. MJ. Lee, J. Kim, MY. Kim, YS. Bae, SH. Ryu, TG. Lee, JH. Kim, *Proteomic analysis of tumor necrosis factor-alpha-induced secretome of human adipose tissue-derived mesenchymal stem cells*, Journal of Proteome Research, 2010, 9,1754-62.

149. R. Romieu-Mourez, DL. Coutu, J. Galipeau, *The immune plasticity of mesenchymal stromal cells from mice and men: concordances and discrepancies*, Frontiers in Bioscience, 2012, 4, 824-37.

150. D.W. Huang, B.T. Sherman, R.A. Lempicki, *Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists*, Nucleic Acids Research, 2009, 37, 1-13.

151. JA. Hamilton, *Colony-stimulating factors in inflammation and autoimmunity*, Nature Reviews Immunology, 2008, 8, 533-44.

152. Q. Xiang, A. Sang, *Complex role of matrix metalloproteinases in angiogenesi*s, Cell Research, 1998, 8,171-7.

153. T. Rajavashisth , JH Qiao, S. Tripathi, J. Tripathi, N. Mishra, M. Hua, XP Wang, A. Loussararian, S. Clinton, P. Libby, A. Lusis , *Heterozygous osteopetrotic (op) mutation reduces atherosclerosis in LDL receptor- deficient mice*, The Journal of Clinical Investigation, 1998, 101, 2702–10.

154. N. Tojo, E. Asakura, M. Koyama, T. Tanabe, N. Nakamura, *Effects of macrophage colony-stimulating factor (M-CSF) on protease production from monocyte, macrophage and foam cell in vitro: a possible mechanism for anti-atherosclerotic effect of M-CSF*, Biochimica and Biophysica Acta, 1999, 1452, 275-84.

155. P. Fixe, V. Praloran, *Macrophage colony-stimulating-factor (M-CSF or CSF-1) and its receptor: structure-function relationships*, European Cytokine Network, 1997, 125-36.

156. S. Pati, AY. Khakoo, J. Zhao, F. Jimenez, MH. Gerber, M. Harting et al., *Human mesenchymal stem cells inhibit vascular permeability by modulating vascular endothelial cadherin/beta-catenin signalling*, Stem Cells and Development, 2011, 20, 89–101.

157. S. Pati, MH. Gerber, TD. Menge, KA. Wataha, Y. Zhao, JA. Baumgartner et al., *Bone marrow derived mesenchymal stem cells inhibit inflammation and preserve vascular endothelial integrity in the lungs after hemorrhagic shock*, PLoS One, 2011.

158. IA. Ho, HC. Toh, WH. Ng, YL. Teo, CM. Guo, KM. Hui et al., *Human bone marrow derived mesenchymal stem cells suppress human glioma growth through inhibition of angiogenesis*, Stem Cells, 2013, 31, 146–155.

159. P. Lu, K. Takai, VM. Weaver and Z.Werb, *Cold Extracellular Matrix Degradation and Remodeling in Development and Disease*, Spring Harbor Perspect Biology, 2011, 3, 12.

160. CA. Fernandez, MA. Moses, *Modulation of angiogenesis by tissue inhibitor ofmetalloproteinase-4,* Biochemical and Biophysical Research Communication, 2006, 345, 523–529.

161. TP. Lozito, RS. Tuan, *Mesenchymal stem cells inhibit both endogenous and exogenous MMPs via secreted TIMPs*, Journal of Cellular Physiology, 2011, 226, 385–396.

162. MJ. Reed, T. Koike, E. Sadoun, EH. Sage, P. Puolakkainen, *Inhibition of TIMP1 enhances angiogenesis in vivo and cell migration in vitro*, Microvascular Research, 2003, 65, 9–17.

163. T. Akahane, M. Akahane, A. Shah, CM. Connor, UP. Thorgeirsson, *TIMP-1 inhibits microvascular endothelial cell migration by MMP-dependent and MMP independent mechanisms*, Experimental Cell Research, 2004, 301,158–167.

164. KK. Jung, Liu XW, R. Chirco, R. Fridman, HR. Kim, *Identification of CD63 as a tissue inhibitor of metalloproteinase-1 interacting cell surface protein*, EMBO J, 2006, 25, 3934–3942.

165. WG. Stetler-Stevenson, *Tissue inhibitors of metalloproteinases in cell signaling: metallo proteinase-independent biological activities*, Science Signal, 2008, 8, 1, 27.

166. E. Bruegmann, R. Gruemmer, J. Neulen, K. Motejlek, *Regulation of soluble vascular endothelial growth factor receptor 1 secretion from human endothelial cells by tissue inhibitor of metalloproteinase 1*, Molecular Human Reproduction, 2009, 15, 749–756.

167. T. Menge, M. Gerber, K. Wataha, W. Reid, S. Guha, CS. Cox et al., *Human mesenchymal stem cells inhibit endothelial proliferation and angiogenesis via cell-cell contact through modulation of the VE-Cadherin/beta-catenin signaling pathway*, Stem Cells and Development, 2013, 22, 148–157.

168. K. Nemeth, A. Leelahavanichkul, PS. Yuen, B. Mayer, A. Parmelee, K. Doi et al., *Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent re-programming of host macrophages to increase their interleukin-10 production*, Nature Medicine, 2009, 15, 42–49.

169. C. Baer, ML. Squadrito, ML. Iruela-Arispe, M. De Palma, *Reciprocal interactions between endothelial cells and macrophages in angiogenic vascular niches*, Experimental Cell Research, 2013, 319, 1626–1634.

170. C. Arrieta, J. Ritz, L. Silbersten, *The elusive nature and function of MSC*, Nature reviews and molecular cell biology, 2011, 12, 126-131.

171. N. Kim, SG. Cho, *Clinical applications of mesenchymal stem cells*, Korean Joournal of Intern Medicine, 2013, 28, 387-402.