sources (Wharton Jelly MSC (WJMSC)). MSC are a very interesting tool for tissue engineering because of their differentiation properties and for cell therapy because of their immunomodulation properties. Indeed, MSC secrete soluble factors which can modulate the immune response.

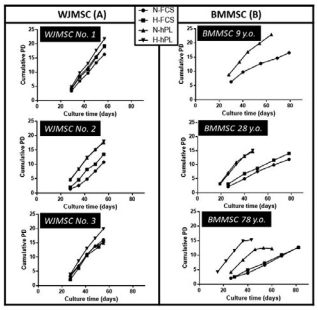
Before their clinical use, an *in vitro* expansion step must be performed in order to obtain the sufficient dose. However, this expansion step could affect MSC quality and need to be controlled in order to increase the therapeutic efficacy.

The first challenge was to optimize the culture conditions and to choose the most appropriate media for the MSC culture to ensure that they retain their functional properties and thus their therapeutic potential. The second challenge was to know the impact of *in vitro* expansion on MSC functional properties.

Methods, Results & Conclusion We firstly studied the impact of culture conditions (normoxia (N), hypoxia (H), fetal calf serum (FCS) supplement, human platelet lysate (hPL) supplement on the behavior of MSC. Our results showed that better proliferation properties were obtained for MSC expanded with human platelet lysate (hPL) in hypoxia for WJMSC and in normoxia for BMMSC. Moreover, we observed that WJMSC and BMMSC expanded with hPL have better clonogenicity and are less senescent. Using neutralizing antibodies, we confirmed the involvement of high concentration of growth factors (PDGF, EGF,...) in hPL in WJMSC proliferation properties. The main surface markers and differentiation capacities were found to be equivalent for WJMSC and BMMSC for the different culture conditions.

We secondly studied the impact of *in vitro* expansion on MSC immunomodulation properties. We showed that WJMSC and BMMSC expanded in hypoxia have better immunosuppressive properties when they are co-cultivated with CD4+ T cells and that senescence and interferon-γ (IFN-γ) stimulation could affect the MSC immunomodulatory properties.

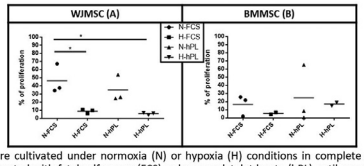
To conclude MSC expansion with hPL gives better clonogenicity properties and better proliferation properties in normoxia for BMMSC and in hypoxia for WJMSC without affecting surface markers and differentiation properties. Moreover, WJMSC expanded in hypoxia with hPL with low senescence are more immunosuppressive. This property can be improved by IFN-γ stimulation.



WJMSC and BMMSC proliferation

WJMSC (**A**) and BMMSC (**B**) were cultivated under normoxia (N) or hypoxia (H) conditions in complete medium supplemented with fetal calf serum (FCS) or human platelet lysate (hPL). Cellular expansion growth rate was evaluated by number of cumulative population doubling (PD) in functions of time (in days) for 3 WJMSC samples (No. 1-2 and 3) and 3 BMMSC samples (9-28 and 78 years old (y.o.)). PD was calculated via the formula: PD=log(Nx/Ni) / log2 with Ni = Initial number of cell seeded and Nx = Number of cells detached.

WJMSC and BMMSC immunomodulatory properties



MSC were cultivated under normoxia (N) or hypoxia (H) conditions in complete medium supplemented with fetal calf serum (FCS) or human platelet lysate (hPL) until passage 3 for WJMSC (**A**) or passage 2 for and BMMSC (**B**) . 50 000 MSC were co-cultivated either with 50 000 CD4+ T cells (ratio 1:1) activated by mouse anti-human CD3 and mouse anti-human CD28 like an allogenic context for immunosuppression properties in normoxia. CD4+ T cells were labelled with cell proliferation dye efluor ®450 proliferation before. CD4+ T cells proliferation was analysed by flow cytometry (Gallios, Beckman Coulter) for WJMSC (**A**) and BMMSC (**B**). Results are expressed as mean ± SD, p <0,05 (*). One-way ANOVA analysis followed by Tukey's multiple comparison test was used for statistical analyses (GraphpadPrism).

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Cardiac function evaluation of bone marrow mesenchymal stromal cells intracoronary transplantation in acute myocardial infarction Lee-Sung mini-PIG model

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Background & Aim Acute myocardial infarction (AMI) is the single most common cause of death worldwide. Mesenchymal stromal cells (MSC) exert strong immunomodulatory effects and angiogenesis property. MSC cell therapy in AMI therefore showed some beneficial effects in several studies and clinical trials. Here we isolated, expanded and characterized human bone marrow MSC in serum free condition under GTP regulation and evaluated the efficacy of these MSC in porcine AMI model after intracoronary injection.

Methods, Results & Conclusion These MSC also showed strong immunosuppression, T cell suppression, immunomodulation and anti-inflammatory activity by MLR, T cell proliferation, IDO activity and INF-r TNF-a expression assay. AMI was induced in Lee-Sung mini pigs by inflating an angioplasty balloon for 90 mins in the mid-left anterior descending artery. After reperfusion for 90min, MSC or buffer were intracoronary injected through the central lumen of the balloon catheter. Fifteen animals were randomly divided into three groups: shame control; AMI-M (MSCs injection); AMI-B (Buffer injection). After 90 min balloon occlusion, the left ventricular ejection fraction (LVEF) by echocardiography of three groups were reduced from 62.94±4.28% (sham), 62.46±2.10% (AMI-B), 61.01±3.21% (AMI-M) to 63.08±5.69 % (sham), 37.44±4.27% (AMI-B), 39.92±2.67% (AMI-M), respectively. ST-segment elevation were observed by electrocardiography. Three months after AMI, LEVF of sham group was 60.98±3.82 %. LVEF of AMI-B group remained low at 36.68±3.12 % while AMI-M group rose to 53.71±4.46 %. The concentration of cardiac troponin I (cTnI) and cardiac troponin T (cTnT) of three groups were 4.6, 19.5 pg/mL (sham); 149.2, 388.6 pg/mL (AMI-B) and 21.9, 46.1 pg/mL (AMI-M) after 24 hours of AMI, respectively. Those biomarker of AMI-B was higher than ULRF while AMI-M group was reduced to lower than the MI reference value. It suggested that the MSC transplantation may suppress the myocardial necrosis caused by AMI. LEVF and MRI images as well as blood concentration of cTnT I/T were analyzed during five-month follow-up. After 5 months of observation, the status of tissue necrosis, fibrosis or angiogenesis were assayed by tissue analysis including TCC staining; hematoxylin-eosin staining; Masson Trichrome staining; immunohistochemistry staining with cTnT, cTnI, vWF and isolectin-B4. In conclusion, our bone marrow MSCs showed promising potential for cardiac function improvement after intracoronary transplantation into AMI Lee-Sung mini-pig.

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Basic Fibroblast Growth Factor and Epithelial Growth Factor could Induce Trans-differentiation of Mesenchymal Stem Cell into Neural Stem Cell

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Background & Aim Background Stem cell therapy has been considered as a potential strategy in regenerative for the patient with neurological deficits. Mesenchymal Stem/Stromal Cells (MSC) have been extensively investigated as a treatment in stroke. However, the MSC lost its potency for neural repair in aged patients due to the limited

numbers of Neural Stem Cell (NSC). NSC is essential for neurogenesis of the injured brain.

In vitro studies have shown that MSCs which were cultured in the laboratory has the capability to transform into different cells. Adding stimulants such as growth factors into the culture media could induce the trans-differentiation of MSC into NSC. However, to produce NSC in the clinical setting on a large scale is still a challenge.

Objective In this study, we like to evaluate the use of epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), heregulin B27, and N2 the trans-differentiation mechanism of umbilical cord MSC (UC-MSC) into NSC.

Methods, Results & Conclusion Method MSC was transferred into a 24-well plate at 2×10^4 cells per well. Induction of NSC is done using DMEM containing N2/B27 supplement, 20 ng/ml epidermal growth factor (EGF), and 10 ng/ml basic fibroblast growth factor (bFGF). Identification of success trans-differentiation is analyzed using NeuroFlour CDr3 antibody.

Result and Discussion MSC transdifferentiates into NSC as an important source of cell in therapy for neurological diseases. UC-MSC was induced using N2 to helps the cell to commit to differentiation and its survival. B27 is used for the long term survival of neurons cells and helps the NSC to grow and proliferates. bFGF beliefs as pre-inductive molecules that enhance neuronal differentiation and self-renewal. Upon bFGF stimulation, NGFβR and NRP1 will upregulate and promotes differentiation. On the contrary, EGF induces differentiation at lower concentrations. EGF is used for inducing the expression of the neuron and glial marker.

To confirm the successful transdifferentiating process, the expression of CDr3 in the cell culture is analyzed. CDr3 positive result as the membrane-permeable fluorescent probe was shown in Figure 1. NeuroFluor CDr3 binds to human fatty acid-binding protein 7 (FABP7) which use to confirm the neural induction from human stem cells.

Conclusion UC-MSC could be cultured and differentiate into NSC in vitro when supplemented with N2/B27, bFGF, and EGF, which may produce NSC in the clinical setting on a large scale.

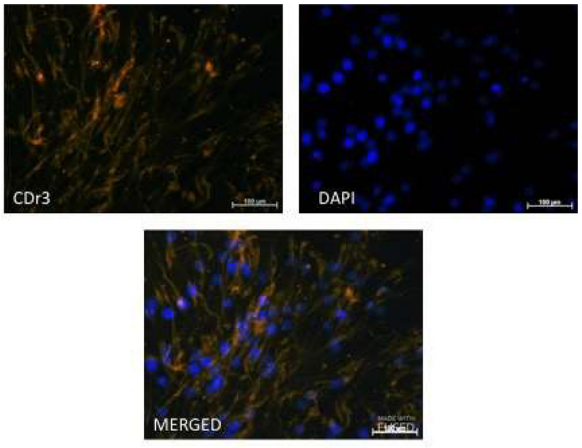

Figure 1. Immunofluorescence of Transdifferentiated NSC from UC-MSC.

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Bone marrow mesenchymal stromal cells retain their tumor-educated phenotype after treatment of multiple myeloma

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Background & Aim Multiple myeloma (MM) is a type of blood cancer characterized by abnormal plasma cells (PC) accumulation in the bone marrow. Disease development is strongly affected by interactions between MM and tumor microenvironment (TME) composed of various cell types including mesenchymal stromal cells (MSC), immune cells, endothelium and others. Influenced by tumor cells, MSC aquire the tumor-associated phenotype and play a key role in TME. TME MSC contribute to survival of residual tumor clone and thus to early relapse.

The aim of the study was to compare the MSC from patients with different types of response to treatment and MSC from healthy donors (HD).

Methods, Results & Conclusion Three HD and twelve patients (49-71 years) after standard bortezomib-based treatment followed by autologous hematopoietic stem cell transplantation were enrolled in the study: 3 non-respondes (NR) and 9 patients with partial or complete response (PoCR). Trepanobiopsy samples were studied for PC and microvessels density by immune histochemistry. Primary cultures of the MSC were obtained from BM and studied for the proliferation rate, osteogenic differentiation and the presence of TME markers (α-smooth muscle actin, senescence-associated β-galactosidase, non-coding DNA transcripts) by cell counting, immunofluorescent staining, specific chromogenic staining and FISH.

Increased microvessel density was revealed in MSC from all MM patients and correlated with PC number in the myelogram. MSC from all NR and 37,5% PoCR patients had decreased proliferation rate. The osteogenic potential was decreased more in NR than in PoCR patients and HD. Expression of markers of cancer-associated MSC was also increased in cultures from NR to a greater extent than in cultures from PoCR patients and HD.

Thus, the MSC phenotype of MM patients differed from the one of HD and displayed features of cancer-associated fibroblasts phenotype. The impairment of hematopoietic niche may be the cause of the incurability of MM.

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In vitro pro-angiogenic effects of human adipose derived multipotent mesenchymal stromal cells: effect of donor's age

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Background & Aim Multipotent mesenchymal stromal cells (MSCs) play a pivotal role in regulating blood vessel formation and promoting vascular homeostasis. These effects are primarily mediated through trophic factors secreted by MSCs (MSC secretome). Those include angiogenic, anti-apoptotic, and immunomodulatory factors that are essential for vascular network remodeling. We have previously shown that adipose derived MSCs from elderly subjects have impaired immunomodulatory properties compared to those from young donors. A higher content of proinflammatory factors (e.g., IL-6, IL-8/CXCL8, and MCP-1/CCL2) in the secretome of elderly-MSCs mediates their reduced immunopotency. It is unclear if age-related changes in the MSC secretome impact the pro-angiogenic effects of MSCs.

Methods, Results & Conclusion Methods: Human adipose derived MSC at passage 4-5 (P4-P5) from six adult (mean age: 66.5±11.6 years) and six pediatric (mean age: 16.5±3.2) healthy donors were evaluated. The effect of MSCs or MSC conditioned media (MSC-CM)