Insights in Stem Cells

Extended Abstract
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Stem Cell Research 2017-Regenerative Secretoma of Adipose-Derived Stem Cells from Ischemic Patients- Ilker Uçkay- Geneva University Hospitals

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Introduction

An important number of elderly and/or diabetic patients have advanced chronic ischemic limb disease with severe and chronic ulcers. In the absence of correct wound healing, these ulcers become a risk of permanent pain, infection, and amputation. Vascular surgery or angioplasties are not always feasible, especially with a long-standing evolution and microangiopathy. Thus, alternative strategies other than mechanical neo-vascularization are needed. Most of the prior innovative studies used angiogenic growth factors alone and reported a limited efficacy. This prompted many author groups to investigate cell-based therapies. The local injection of unselected bone marrow or peripheral blood-derived mononuclear cells was encouraging, but often not sufficiently efficacious for complete revascularization; probably due to the insufficient delivery of regenerative factors from a too complex mixture of cell populations. Other researchers investigated the autologous bone marrow cell transplantation to increase limb perfusion, with low success. Mesenchymal stem cells of adult fat tissues, called Adipose-derived Stem Cells (ASC), are particularly attractive for the local treatment of ischemic ulcers due to their easy accessibility with minimal invasiveness and ethical concerns, and their ability to produce most of the healing/angiogenic factors. However, the proof of feasibility in ischemic patients is almost lacking. There is a general consensus about the potential for clinical treatment of ischemic ulcers. For ASC isolation, superficial subcutaneous fat is usually harvested by resection or lipoaspiration. The viability, yield and growth characteristics of ASC are influenced by the type of harvesting procedure, as well as by many biochemical and biophysical stimuli in the in vivo microenvironment, such as fluid shear stress, hydrostatic pressure, and produced factors. Mesenchymal stem cells isolated from diabetic patients yielded dysfunctions in oxidative stress, autophagy healing properties in mice and VEGF expression. However, this observation is controversial and reports from diabetic rats or patients still retain the ability to promote wound healing. We thus describe a proof-of-principle pilot study regarding the feasibility of amplifying ASC from patients with longstanding ischemia and/or diabetes mellitus from various origins for a theoretical future autologous use for chronic ulcer regeneration. HS420 embryonic stem cell line was cultured on matrigel (corning) -coated tissue culture flasks in nutristem medium (Biological Industries) until 50% of confluency. Then, 10 M rock inhibitor Y27632 was added in the nutristem culture media. After 24h, cells were detached with Accutase (Thermofisher) and aggregated into spheroids of 1000 cells through by using Aggrewell-400 (STEMCELL technologies). 24h after aggregation, spheroids were cultured for 10 days in differentiation medium in suspension under constant agitation in the differentiation medium. Then, spheroids were plated in matrigel-coated dishes and cultured in adherence for an additional 11 days in differentiation medium. Differentiation medium: KnockOut DMEM (Thermofisher), 15% KnockOut serum replacement (Thermofisher), 2 mM L-glutamine (Sigma-Aldrich), 1% Penicillin/ Streptomycin solution (Life Technologies), 1% of MEM Non-Essential Amino Acids

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Solution (Life Technologies), 0.1 mM β-mercaptoethanol (Sigma Aldrich).

Culture of fibroblasts

Human foreskin fibroblasts (ATCC CCD-1112SK) were cultured in DMEM culture media (Life Technologies) supplemented with 10% fetal calf serum (Gibco) and 1% penicillin-streptomycin (Gibco). Cells were harvested for experiments at 90% confluence.

Flow cytometry and differentiation of ASC

We analyzed the ASC phenotype with the human stem cell verification kit (R and D Systems). Briefly, cells were incubated with fluorochrome-labeled antibodies for 30 minutes at 4°C in binding buffer, prior to washing them with PBS and analyzing them using the BD AccuriTM-B6 flow cytometer (BD Biosciences). We differentiated ASC into chondrocytes, adipocytes or osteocytes by using culture additives and procedures according to the functional mesenchymal stem cell verification kit (R and D Systems).

Immunocytochemistry, cytokines measurements and microarrays

We cultured ASC on glass coverslips prior to fixation with paraformaldehyde 0.5%; for 15 minutes at room temperature. The cells were incubated overnight at 4°C in PBS containing 0.3% Triton X-100 and 0.5% bovine serum-albumin with primary antibodies. We incubated the secondary anti-mouse IgM-Alexa 555 antibodies for one hour at +4°C in the same PBS solution. The cells were again incubated with DAPI 1 µg/ml for 10 minutes, prior to final washing and mounting. Supernatants from ASC cultures were collected after three days. We assessed the cytokines in culture supernatants according to the manufacturer's instructions, by using the human cytokine base kit A (R and D Systems) combined with a magnetic Luminex assay (Bioplex 200, Biorad) and performed microarrays targeting 21 448 mRNA chips on the extracted RNA (Complete GeneChip® Instrument System, Affymetrix® , www.affymetrix.com). The heatmap was done on RMA normalized data produced with the software TAC4.0.1.36 (Biosystems®) using the R package pheatmap (https://cran.rproject.org/web/packages/pheatmap/index.html) default parameters. The data was used without transformation.

Conclusion

In conclusion, ASC form ischemic patients show a secretome in favor of healing promotion. These regenerative properties are suggested to favor inflammation through the production of proinflammatory chemokines and factors enhancing macrophages activity. iASC also produces angiogenic factors, matrix remodeling proteases, chemokines, and keratinocytes/fibroblast growth inductors to help for later steps of healing. This pilot evaluation confirms the feasibility of harvesting, amplifying autologous ASC from superficial fat of ischemic patients ex vivo, as well as regenerative properties compatible with cell therapy of ischemic ulcers, including for the diabetic foot.

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