

Abstract Details

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Stem cell therapy induces a shift from an inflammatory environment towards an immune tolerant scenario promoting endometrial tissue regeneration

Biography

PhD student

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Study question:

Could bone marrow derived stem cell (BMDSC) transplantation induce an immunomodulatory milieu helping tissue recovery in human and murine models with endometrial pathologies?

Summary answer:

Differences in Neutrophil Elastase (NE) expression in treated women and a specific cytokine/chemokine profile in mouse models have evidenced a favourable ambient inducing endometrial regeneration.

What is known already:

Asherman Syndrome and Endometrial Atrophy (AS/EA), producing endometrial destruction, are two of the most frequent causes of uterine-type infertility. Cell therapy using CD133⁺BMDSCs has been described for treating these pathologies. It has been demonstrated that factors secreted by these cells stimulate tissue regeneration, for example in pathological situations where Neutrophil Elastase (NE) is overexpressed, creating a microenvironment based on pro-inflammatory and immunomodulatory processes. Our previous studies detected up- and down-regulated genes like SERPINE1/IL4/JUN and CXCL8/CCND1 respectively. Interestingly, NE can be inhibited by SERPINE1 and increases the release of CXCL8, a potent chemoattractant, thus potentiating the inflammatory response.

Study design, size, duration:

Our study design involves on one hand a retrospective study based on endometrial tissue analysis from 8 patients with endometrial pathologies (AS/EA) before and after autologous CD133⁺BMDSCs treatment (ClinicalTrials.gov-NCT02144987): gene and protein analysis were performed to elucidate how the regenerative process occurred. On the other hand, uterus from immunocompromised mice with damaged endometrium, where human CD133⁺BMDSCs were injected intrauterus (n = 5) or through the tail vein (n = 5), were analysed by Multiplex technologies.

Participants/materials, setting, methods:

We collected 2 endometrial biopsies from each of these 8 women: before/Control and after the Treatment. Genetic analysis from our previous study was assessed with bioinformatic analyses (*t*-test), and validated by individual PCR. All samples were analysed by immunohistochemistry against NE (M0752, Dako), the differential expression was analysed using Image-ProPlus-6.0 (Mann-Whitney test). These results were also supported from the animal model (intrauterus and tail vein) where Multiplex Immunoassays (ProcartaPlex Mouse-26plex Cytokine&Chemokine, ThermoFisher) were carried out.

Main results and the role of chance:

Bioinformatic analysis revealed 5 significant genes: JUN, SERPINE1 and IL-4, up-regulated, while CCND1

and CXCL8, down-regulated; when Treatment condition was compared to Control. These gene pattern correlates with the subsequent protein analysis we performed. Expression of NE protein resulted significantly lower in the Treatment group ($p=0.0286$) when compared to Control, which correlates with CXCL8 gene downregulation ($p=0.036$). The down-regulated expression of CXCL8 and NE (reliable markers of inflammation) in the human endometrium, caused by the transplantation of CD133⁺BMDSCs, results in a diminished inflammatory cascade in AS/EA patients promoting probably endometrial regeneration. All together correlates with the NE inhibitory capacity of SERPINE1 ($p=0.026$), which in absence of NE turned to be up-regulated. This change from an inflammatory environment toward an anti-inflammatory/tolerant phenotype allows proliferation, differentiation, anti-apoptosis and chemotaxis events. We observed evidences of these pro-regenerative processes, when analysing in detail our animal model by Multiplex immunoassays, we obtained a specific cytokine/chemokine pattern (IL-27, MCP-1, MIP-1 β and MIP2 among others) in the affected area after cell therapy. All these molecules seemed to be involved in tissue recovery after damage, pro-angiogenic events, wound healing and BMDSCs secretions, processes in that SERPINE1, IL-4 ($p=0.041$) and JUN ($p=0.037$) are also implicated.

Limitations, reasons for caution:

The limitation of our study is the low quantity of tissue collected due to human endometrial biopsies were coming from women suffering from AS/EA. And, in our animal model the use of Formaldehyde Fixed-Paraffin tissues to perform the Multiplex assays.

Wider implications of the findings:

These results support the regenerative effect of CD133⁺BMDSCs in endometrial pathologies like AS/EA via immune and inflammatory responses; what would bring us one step forward to understand the specific mechanisms of this cell therapy. The decrease of endometrial NE identified here may diminish the inflammatory response giving a proliferative scenario.

Trial registration number:

NCT02144987.

Keywords:

endometrial regeneration

immune system

cytokines

Bone marrow derived stem cells (BMDSCs)

neutrophil elastase