ImmunoTools *special* Award 2013

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Modification of mesenchymal stem cell migration and survival in a fracture hematoma model

Background

Fracture healing is often prolonged/impaired in patients with autoimmune diseases or with otherwise compromised immune functions (e.g. diabetes mellitus, cancer treatment) [1]. The elderly also exhibit prolonged/impaired fracture healing and limited immune function in advanced age does contribute to this phenomenon. Thus, the development of therapies of prolonged/impaired fracture healing is an important approach, especially in the context of increasing life expectancy.

Fracture healing can be divided in mainly three phases: (i) the initial inflammatory phase, (ii) the reparative phase, and (iii) the remodeling phase. The initial inflammatory phase is suggested to be among the crucial points to determine fracture healing [2, 3].

Preliminary work

We have previously shown that the early phase of human fracture healing is characterized by hypoxia [3]. Hypoxia is also known to promote proliferation, survival and migration of progenitor cells like mesenchymal stem cells (MSC). We have developed a fracture hematoma model mimicking the initial inflammatory phase of fracture healing incorporating the initial hypoxic conditions [4].

Project description

In this project we will analyse the ability of several cytokines and chemokines to increase the chemoattraction of MSC to the fracture gap. It is known that MSC positively influence bone healing. The goal is to identify cytokines and chemokines, which could be used in the therapy of prolonged/impaired fracture healing in patients at risk to develop non-unions. The aim is to identify a target for therapeutic intervention inducing chemoattraction of endogenous MSC, that would be a fast and cost effective alternative to a cell based therapy with cultured MSC.

In previous work we have defined in detail the cytokine/chemokine cocktail within the initial human fracture hematoma (Hoff et al., in preparation). In this project, we will test which cytokines or chemokines will (i) increase the migratory capacity and/or survival of MSC or (ii) negatively influence the MSC function (increased apoptosis,

decreased migration) in the context of a fracture hematoma (initial inflammatory phase of fracture healing). A therapeutic strategy could be the application of chemokines increasing the chemoattraction of MSC or the blockade of cytokines negatively influencing MSC function. The migratory capacity of MSC will be tested in transwell migration assays (MSC migration to the fracture hematoma model milieu). Apoptosis will be tested via Annexin V staining. We plan to test the following cytokines/chemokines from ImmunoTools: rh BMP-2, rh BMP-7, rh FGF-a / FGF-1, rh FGF-b / FGF-2, FGF19, rh IFNgamma, rh IL-1alpha / IL-1F1, rh IL-1beta /IL-1F2, rh IL-6, rh IL-8, rh IL-9, rh IL-10, rh IL-17A, rh IL-17F, rh IP-10 /CXCL10, rh MCP1 / CCL2, rh MCP2 / CCL8, rh MCP3 / CCL7, rh MIP-1α/ CCL3, rh PDGF-AA, rh PDGF-BB, rh PF4v1/CXCL4V1, rh RANTES / CCL5, rh TNFα.

The requested cytokines and chemokines will be titrated and added to the fracture hematoma model milieu. The special impact of this project is that we will not just test the influence of these reagents on MSC but we will test the effects within the fracture hematoma model allowing a complex interaction with inflammatory cells in this model mimicking the *in vivo* situation.

Outlook

This work will be used to define interesting targets with therapeutic potential. On the base of this data we will design a proof of concept study in an animal model and apply for a DFG grant.

References: Publications of Paula Hoff, née Kolar

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4. **Hoff P**, Maschmeyer P, Gaber T, Schutze T, Raue T, Schmidt-Bleek K, Dziurla R, Schellmann S, Lohanatha FL, Rohner E, Ode A, Burmester GR, Duda GN, Perka C, Buttgereit F (**2013**) Human immune cells' behavior and survival under bioenergetically restricted conditions in an in vitro fracture hematoma model. Cell Mol Immunol 10:151-158