論文審査の結果の要旨

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The thesis paper consists of 9 chapters: abstract, introduction, hypothesis, material&methods, results, discussion, conclusion, references, annex and acknowledgements.

The abstract states that tissue type plasminogen activator can expand BM-derived mesenchymal stem cells. In the introduction, the fibrinolytic pathway and its role in growth factor and MMP activation/deactivation are described, followed by the role of the fibrinolytic factors within the bone marrow. Then follows a short overview on the definition and therapeutic potential of mesenchymal stem cells (MSC).

The hypothesis of the study is presented, namely that plasmin in part by modulating matrix metalloproteinases can influence MSC fate, i.e. proliferation or differentiation. The method part covers: the animal experimental protocol, incl. the use of gene deficient mice, *in vivo* treatment regiments including the use of recombinant tissue plasminogen activator (tPA), urokinase activator (uPA) or treatment with rec. kit ligand. Then blocking experiments both in *vitro* and *in vivo* describe the use of neutralizing antibodies against c-Kit (ACK2), and small drugs like Gleevec or FGF receptor inhibitor to block the PDGFR and FGFR and c-kit signaling. Then follows a detailed protocol how to isolate bone marrow mesenchymal stem cells: CD45-Ter119-Sca1+PDGFRa+ MSC follows and colony forming unit-fibroblast assay, and MSC differentiation assays. In the result part data are presented describing that tPA, but not uPA can expand bone marrow-derived MSC that show high proliferative capacity, increased clonogenic potential in the colony forming unit-fibroblast (CFU-F) assay and multilineage differentiation potential. Next, data are presented demonstrating that tPA-mediated MSC expansion requires plasminogen and MMP-9 by using plasminogen and MMP-9 deficient mice, and c-kit signaling in vitro and in vivo. She then demonstrates that tPA treatment upregulated the gene and protein expression of PDGF-BB and FGF-2, a process that required c-kit signaling. Next, data are presented showing that c-kit+ endothelial cells promote KitL- and tPA-induced MSC expansion and that the MSC-active growth factors PDGF-BB and FGF2 can upregulate PDGFRα on MSC expression. Then a MSC-endothelial cell coculture was set up to demonstrate the upregulation of the identified growth factors KitL, PDGF-BB and FGF2 expression on MSC (for KitL) and PDGF-BB and FGF2 on EC. To demonstrate the functional importance of PDGFRα and c-Kit signaling, blocking reagents were introduced both in vitro and in vivo to show that these pathways mediate tPA-initiated MSC expansion in the presence of EC. Finally data are presented demonstrating that endogenous tPA is required for MSC expansion after myelosuppression and it was proposed that upregulation of tPA modulate the BM niche cells *in vivo*. Finally, using a melanoma model, data are given showing that tumor-

derived tPA accelerates MSC expansion *in vitro* and that tPA knockdown in tumor cells impairs the number of MSC within the growing tumor. The discussion part includes references to studies showing that MSC are a source of KitL within the BM niche. Then evidence is given that PDGFRa signaling enhances MSC proliferation without altering MSC multipotency. Then studies are discussed in comparison with this study indicating that fibrinolytic factors driven neoangiogenesis might in part be driven by tPA-induced MSC expansion. The studies on PDGF and FGF2 signaling for MSC survival are presented indicating that these angiogenic factors actually might drive angiogenesis. It was then proposed that the MSC-endothelial crosstalk contributes to ischemic tissue regeneration and neoangiogenesis, wound healing, and cancer growth. It was discussed that other MSC niches, e.g. within adipose tissues can be modulated by tPA and the cancer niche. The conclusion part summarizes the results and shows a model on the mechanism how tPA expands MSC.

Douaa is the *main contributor* of this study. Under my supervision she designed the experiments, performed the experiments one her own. She analyzed the data, followed up on the literature associated with the described project and showed good team spirit. She established skills in doing in vivo experiments, incl. mouse handling, breeding, genotyping, mouse in vivo and in vitro treatment, and tissue collection.
She established the MSC – EC coculture system on her own, tried different cell lines, media etc. to use this model *in vitro* to demonstrate that the observed and suspected MSC –EC interaction really occurs the *in vitro* model. She searched for potential clinical applications of cell biological findings and we decided to investigate various tumors and studied the role of tPA for MSC recruitment.

I hereby guarantee that her work makes her eligible to receive a PhD (Medical Science) from the graduate school of Frontier Sciences (CBMS), Tokyo, Japan.

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