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Immunolabeling of TGF-β during Muscle Regeneration

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Abstract : Muscle regeneration after injury (as irradiation) is of great importance. However, the molecular and cellular mechanisms are still unclear. Cytokines are believed to play fundamental role in the different stages of muscle regeneration. They are secreted by many cell populations, but the predominant producers are macrophages and helper T cells. On the other hand, it has been shown that adipose tissue derived stromal/stem cell (ASC) injection could improve muscle regeneration. Stem cells probably induce the coordinated modulations of gene expression in different macrophage cells. Therefore, we investigated the patterns and timing of changes in gene expression of different cytokines occurring upon stem cells loading. Muscle regeneration was studied in an irradiated muscle of minipig animal model in presence or absence of ASC treatment (irradiated and treated with ASCs, IRR+ASC; irradiated not-treated with ASCs, IRR; and non-irradiated no-IRR). We characterized macrophage populations by immunolabeling in the different conditions. In our study, we found mostly M2 and a few M1 macrophages in the IRR+ASC samples. However, only few M2b macrophages were noticed in the IRR muscles. In addition, we found intensive fibrosis in the IRR samples. With in situ hybridization and immunolabeling, we analyzed the cytokine expression of the different macrophages and we showed that M2d macrophage are the most abundant in the IRR+ASC samples. By in situ hybridization, strong expression of the transforming growth factor β (TGF-β) was observed in the IRR+ASC but very week in the IRR samples. But when we analyzed TGF-β level with immunolabeling the expression was very different: many M2 macrophages showed week expression in IRR+ASC and few cells expressing stronger level in IRR muscles. Therefore, we investigated the MMP expressions in the different muscles. Our data showed that the M2 macrophages of the IRR+ASC muscle expressed MMP2 proteins. Our working hypothesis is that MMP2 expression of the M2 macrophages can decrease fibrosis in the IRR+ASC muscle by capturing TGF-β.

Keywords : adipose tissue derived stromal/stem cell, cytokine, macrophage, muscle regeneration
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