Role of platelets in the phenotypic plasticity of fibroblasts

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After an injury, the wound repair process commonly leads to a non-functioning mass of fibrotic tissue known as a scar, the consequence of which may be defects in functionality (Goldberg et al., 2007). In these processes, fibroblasts/myofibroblats play a crucial role. Activated platelets may participate in the early events of chronic inflammation associated with fibrosis (Huang et al., 2007) through the direct adhesion to stromal cells (fibroblasts/myofibroblasts) and the release of several soluble mediators. To address this hypothesis, we performed co-culture experiments with human intestinal myofibroblasts and isolated human platelets to assess: i)the biosynthesis of the five prostanoids, thromboxane(TX)B_2 (the hydrolysis product of TXA_2), prostaglandin(PG)E_2, PGF_{2a}, 6-keto-PGF_{1a} (the hydrolysis product of PGI_2) and PGD_2; ii) the release of platelet a-granule proteins (TGF-b1 and PDGF-BB); iii)the expression of different markers of inflammation, myofibroblast phenotype, proliferation and cellular movement. Finally, we verified whether the selective inhibition of platelet function by aspirin prevented the molecular changes induced in myofibroblasts. Human intestinal myofibroblasts ($9x10^4$cells) were cultured for 24h alone or with isolated human platelets($0.5x10^8$). In culture media, prostanoids levels, PDGF-BB and TGFb1 were evaluated by specific immunoassays (Dovizio et al., 2013). In intestinal myofibroblast lysates, we assessed the protein levels of cyclooxygenase(COX)-2, a-smooth muscle actin (a-SMA) (the most widely used myofibroblast marker) and proliferating cell nuclear antigen (PCNA) (a S phase related proliferating cell nuclear antigen) by Western blot. Moreover, the mRNA levels of COX-2, a-SMA, RhoA (a member of the Rho Family GTPases involved in dynamic cellular processes) and two mesenchymal markers, i.e. vimentin and fibronectin, were assessed using q-PCR. In some experiments, platelets were pretreated with aspirin (300 mM to completely suppress COX-1 activity) and then washed before their incubation with myofibroblasts. Myofibroblasts and platelets cultured alone, produced low levels of the five prostanoids (<1 ng). In myofibroblast-platelet co-cultures, a remarkable increase of prostanoids was detected. TXB_2, PGE_2 and PGF_{2a} were the most abundant products (250±10 ng, 66±10 ng and 16±1 ng, respectively; mean ±SEM, $P<0.01$ versus platelets or myfibroblasts cultured alone). The use of aspirin-treated platelets showed that >90% of released prostanoids were from platelets. In myofibroblast-platelet co-cultures, released PDGF and TGF-b1 levels (4.5±0.2 and 0.44±0.11 ng, respectively) were substantially enhanced versus the cells cultured alone ($P<0.01$) and their levels were reduced (50%) by aspirin pre-treatments of platelets. These results suggest that the interaction with myofibroblasts caused the activation of platelets through an aspirin-sensitive mechanism. However, the interaction of platelets with myofibroblasts led to several molecular changes in myofibroblasts. COX-2 and PCNA were induced while a-SMA was reduced. Moreover, the mRNA levels of RhoA, vimentin and fibronectin were increased. All the changes were prevented by the incubation of myofibroblasts with aspirin-treated platelets. In summary, our results show that platelets may induce changes in the myofibroblast expression profile of proteins involved in inflammation, proliferation, migration and contraction. Interestingly, selective inhibition of platelet function by aspirin prevented the phenotypic changes induced by platelet/myofibroblast interaction, thus suggesting the central role of COX-1-dependent products in altered wound healing.

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