

Abstract

Integrins are a transmembrane receptor family of key importance in fundamental cell mechanisms such as cell adhesion, spreading, migration, differentiation and proliferation. This project focuses on specific $\alpha 5\beta 1$ integrin ($\alpha 5\beta 1$) activation, because its crucial roles in differentiation and in adhesion have already been studied. The project is articulated in two related parts. The first and main one is dedicated to specific $\alpha 5\beta 1$ activation with the goal of controlling cell differentiation. The second and minor part focuses on the engineering of a fusion protein capable of activating both receptors simultaneously if close enough. These two subprojects converge towards a better understanding and control of $\alpha 5\beta 1$ activation and subsequent adhesion and differentiation activities, which is of crucial interest in the perspective of tissue-repair.

$\alpha 5\beta 1$ and differentiation:

Previous work on cell signaling has revealed three findings that provide interesting insights in terms of tissue repair. First, studies have shown that $\alpha 5\beta 1$ is a key trigger much more involved than $\alpha \nu \beta 3$ integrin ($\alpha \nu \beta 3$) in this process (Moursi et al., 1997, Gronthos et al., 2001, Stephansson et al., 2002). Second, $\alpha 5\beta 1$ binding, has been demonstrated, contrarily to $\alpha \nu \beta 3$, to be catalyzed by a synergy site on FNIII9 fibronectin module (Danen et al., 1995). Third, a point-mutated FN fragment (Leu1408 to Pro) comprising the FNIII9 and FNIII10 modules (FNIII9*-10) has been shown to bind $\alpha 5\beta 1$ with much more affinity than $\alpha \nu \beta 3$ (Altroff et al., 2004).

In a tissue-engineering perspective, these results suggest that one can use FN fragments (point-mutated FNIII9*-10 or wild-type FNIII9-10) so as to specifically activate $\alpha 5\beta 1$ rather than $\alpha \nu \beta 3$. Such control would allow instructing cells (e.g. MSC) to differentiate towards a specific cell-type in a bio-compatible environment.

Our previous 2D assays have shown that MSC activity is higher in the presence of FNIII9*-10 than in the presence of wild-type full-length FN. Therefore, we chose to use a 3D fibrin matrix, since it provides one step more towards tissue-repair. Our hypothesis was that cells would express more osteoblast specific proteins (OsBP) when cultured with FNIII9*-10 than with full-length FN or without fibronectin, due to specific activation of $\alpha 5\beta 1$. MSC OD was therefore quantified in 3D fibrin matrix, as a variable of FN molecules type. To measure that, MSC were seeded into fibrin gels containing either wild-type full-length FN or mutant fragment FNIII9*-10, or fibrin gels without any fibronectin molecules respectively referred to as "FN", "FN*" and "only cells" conditions). Presence of three OsBP (i.e. osteopontin (OPN), bone sialo-protein (BSP) and collagen I (COL I)) in the gel was quantified with specific fluoro-labeled antibodies after a 14-day incubation in OD medium.

Results do not show significant differences in terms of average OsBP signal because of technical problems to be improved, like cell viability, but reveal a more focal cell-shaped BSP signal in the FN* condition than in the FN and only cells conditions, thus partly corroborating our hypothesis. We explain negative results by unspecific binding of labeled antibodies used to measure signal and excessive background.

Integrin-syndecan collaboration:

Previous studies have highlighted the existence of a cross-talk between the two transmembrane receptors integrin and syndecan (Mostafavi-Pour Z. et al., 2003). The goal of this subproject was then to design and produce a fusion protein (FP) containing binding sequences of laminin alpha-3 chain (LAMA3) and FN, the ligands that are known to activate SDC-4 and $\alpha 5\beta 1$ respectively. Such an FP would allow simultaneous activation of both receptors if located close enough from each other and would therefore permit better understanding and control of the requirements in terms of simultaneity and proximity for an optimal synergy.