Mimicking cell-cell interactions at the biomaterial-cell interface for control of stem cell differentiation

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Statement of Purpose: The ability to regulate stem cell proliferation and differentiation has relevance in numerous medical applications, including medical devices, tissue engineering and regenerative medicine. To control cellular behavior at the biomaterial interface, many studies have employed surface modifications that mimic the extracellular matrix. Strikingly absent is the immobilization of cell-surface ligands to the biomaterial surface. One cell-to-cell signaling pathway that has been shown to regulate tissue development and stem cell fate is the Notch pathway. Utilizing this knowledge, we applied an affinity immobilization scheme designed to attach and orient the Notch ligand, Jagged-1, in an active conformation on a biomaterial surface and measured its ability to drive epithelial stem cell differentiation.

Methods:

Immobilization of Jagged-1 to polystyrene. The wells of a tissue-culture polystyrene (TCPS) plate (Corning Costar, Acton, MA) were incubated with recombinant Protein G (50μg/mL) and then with recombinant rat Jagged-1/Fc or human Fc (0.1-10nM precoat). The presence of Jagged-1/Fc on the surface was confirmed using an ELISA.

Immobilization of Jagged-1 to polyHEMA. Poly (2-hydroxyethyl methacrylate) (polyHEMA) was spin cast as previously described.[1] The polyHEMA surface was activated with 20mM 1,1'-carbonyldiimidazole (CDI) and rabbit anti-human Fc (15μg/ml) covalently linked to the activated surface. The surfaces were characterized using Electron Spectroscopy for Chemical Analysis (ESCA) with a Surface Science Instruments (SSI) M-Probe. Surfaces were then incubated with Jagged-1/Fc or Fc.

Confirmation of Notch Signaling. Rat esophageal epithelial stem cells (REEC) were enzymatically isolated and cultured as previously described.[2] To evaluate Notch signaling through CSL-regulated transcription, a CBF-1 luciferase assay was performed as previously described using a firefly luciferase construct with four adjacent copies of the CBF-1 binding sequence (gift of L. Liaw, Maine Medical Center Research Institute).[3]

Assessment of epithelial differentiation. REEC were treated for 72hrs with either TCPS-bound or soluble Jagged-1/Fc to evaluate the effect of Jagged-1 on differentiation. Western blots were employed to evaluate protein expression. In addition, Jagged-1/Fc or human Fc (10nM precoat) was immobilized to the surface of a transwell insert (12 mm dia, 0.4μm pore size) as detailed above for TCPS. At 72hrs, inserts were fixed, processed for paraffin embedding, and stained with H&E.

Results / Discussion:

Surface-bound Jagged-1 signals the Notch/CSL pathway. Signaling of Notch through the CSL-dependent pathway by surface-bound Jagged-1 on polystyrene was confirmed using a CBF-1 luciferase assay and was dose-dependent on the Jagged-1/Fc precoat concentration. Whereas bound Jagged-1/Fc (10nM precoat) significantly signaled the Notch pathway (10-fold luciferase increase), soluble Jagged-1/Fc (10nM) did not activate Notch/CBF-1. To test alternative biomaterial surfaces, polyHEMA was functionalized with Jagged-1/Fc. Epithelial cells plated on the surface showed dose-dependent activation of the Notch pathway, with a 3-fold luciferase increase seen at a 10nM Jagged-1 precoat concentration.

Bound Jagged-1 drives both intermediate- and late-stage differentiation. We further investigated the effects of the bound ligand on epithelial stem cell differentiation. When REEC were cultured on bound Jagged-1 the result was a marked increase in both intermediate- (involucrin and CK10) and late- (filaggrin) stage epithelial differentiation markers. Soluble Jagged-1 showed only slight increases of involucrin and filaggrin expression compared to its Fc control. Bound Jagged-1 demonstrated a higher potency compared to soluble Jagged-1 in inducing Notch/CSL signaling and increasing expression of intermediate- and late-stage epithelial differentiation markers.

Bound Jagged-1 induces clustering and stratification of epithelial cells. REEC plated on human Fc proliferated to a monolayer and then began to pile and stratify (see figure). In contrast, REEC plated on bound Jagged-1 rapidly stratified (<72hrs), forming up to 3-5 layers of cells in tight clusters compared to 1-2 layers on the Fc controls (see figure 1).

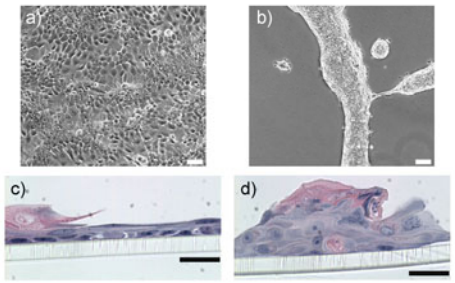

Figure 1. Phase image of REEC plated on surface-bound Fc (a) or Jagged-1/Fc (b) after 48hrs (bar 100μm). Cross-sectional views of REEC plated on bound human Fc (c) or bound Jagged-1/Fc (d) after 72hrs (bar 50μm).

Conclusions: In this study, we have shown that Jagged-1 can be immobilized to a surface, the immobilized Jagged-1 is active, and the presentation of this ligand results in enhanced differentiation and stratification of the epithelial stem cells. Harnessing cell-cell signaling pathways, such as Notch, for surface modification represents a powerful and previously untapped means to control cell behavior at the biomaterial interface.

References: 1. J Biomed Mater Res A 70A:10-9. **2.** Biomaterials 26:6217-28. **3.** J Biol Chem 276:32022-30.