Cellular Response to Immobilized vs. Soluble Growth Factors

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Statement of Purpose

The delivery of biomolecules in biomaterials and tissue engineering applications is often complicated by inability of the biomolecule to reach its target due to premature degradation or diffusion away from the target site. Moreover, many biomolecules such as growth factors and cytokines require prolonged exposure to cells in order to elicit a response. To compensate for these challenges encountered in delivering biomolecules, many research groups have investigated achieving biomolecule delivery via covalent immobilization of these molecules on substrates or scaffolds. Our own group has demonstrated that immobilized epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) can induce accelerated and directed keratinocyte migration in vitro (Stefonek TJ. et al., Wound Rep Regen, 2007; Stefonek TJ. et al, Ann Biomed Eng, 2008, In Press).

However, even though many biomolecules are presented in a 'bound' form in vivo, and despite the increasing use of growth factor (GF) tethering in biomaterials applications, very little is known about the nature of cellular interactions with immobilized biomolecules. Thus, we have investigated the cellular response to immobilized vs. soluble (i.e., untethered or 'free') growth factors. Characterization of these events is sorely needed in order to: 1) engineer environments that control cell function, 2) better understand how cells may recognize bound GFs in vivo, and 3) enable comparisons between the use of soluble vs. immobilized GFs.

Methods

EGF and IGF-1 were rendered photoactive and patterned onto 2-D substrates at two concentrations (1, 10 ng/cm^2) as previously described (Stefonek TJ. et al, Ann Biomed Eng, 2008, In Press). These concentrations were selected based upon their ability to stimulate keratinocyte migration in the aforementioned study. For soluble EGF and IGF-1 conditions, two concentrations (10, 100 ng/ml) were added to medium just prior to cell seeding. Negative controls consisted of DMEM (low serum) alone. Keratinocytes were seeded at 62,500 cells per cm^2, 6 wells per condition, per assay. Quantitative ELISAs for total and phosphorylated EGF receptor (EGFR; Tyr1173), IGF-1 receptor (IGF-1R), Akt (Thr308), and Erk 1/2 (Thr202/Tyr204,Thr185/Tyr187) were used to assay cultured cells at 30 minutes, 1 hour, and 24 hours following exposure to either soluble or immobilized EGF or IGF-1. In order to perform a functional analysis of how immobilized vs. soluble growth factors impact cell behavior, the migration of keratinocytes seeded on: a) a constant concentration of immobilized GF, b) a concentration gradient of immobilized GF, or c) unpatterned substrates treated with GF in solution, was also examined.

Results/Discussion

As shown in Figure 1, significant differences were found in the keratinocyte response to immobilized vs. soluble EGF. Phospho-EGFR levels increased in a dose-dependent manner for both immobilized and soluble EGF, but immobilized EGF stimulated a much stronger phospho-EGFR response than soluble EGF ($p<0.0001$). This hyperstimulation of EGFR by immobilized EGF was accompanied by a large downregulation in Erk phosphorylation ($p<0.002$ vs. soluble EGF). These results suggest the presence of significant differences between immobilized vs. soluble EGF with respect to GF internalization and receptor metabolism. These trends remained at the 1 hour time point, but, by 1 day, the EGFR and Erk levels were similar across all conditions. Data for immobilized vs. soluble IGF-1 and cell migration upon EGF or IGF-1 treatment also indicated differences between immobilized vs. soluble conditions.

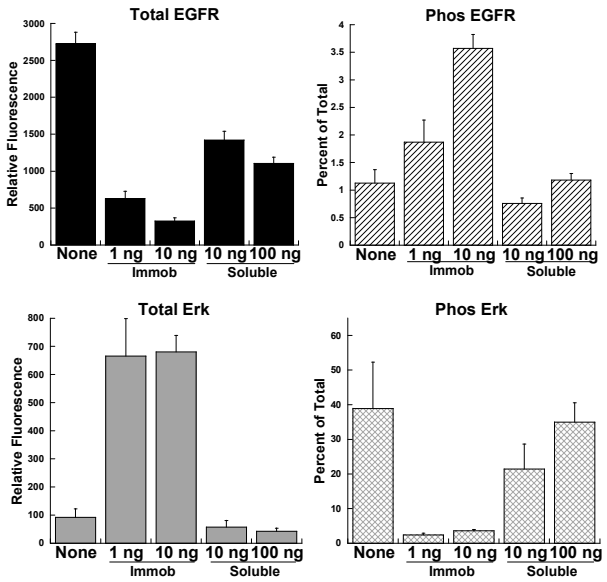

Figure 1. Results shown are 30 minutes after cell seeding. Top: Total EGFR (left) and phosphorylated (right) EGFR as percent of total. Bottom: Total Erk 1/2 (left) and phosphorylated (right) Erk 1/2 as percent of total.

Conclusions

The goals of engineering controlled environments that regulate cell function or translating our extensive body of knowledge on soluble growth factors to create immobilized GF systems necessitates characterization of the mechanisms by which cells interact with tethered GFs. Our results suggest that cells interact quite differently with immobilized GFs than they do soluble GFs, which may result in different functional outcomes for the cells. Revealing such differences between immobilized vs. soluble GF recognition and signaling may be useful in both designing bioactive materials and understanding natural physiological phenomena.