Human Mesenchymal Stem Cell Response to Tethered Epidermal Growth Factor as a Surface Modification on Bone Implants

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Localized controlled delivery of growth factors is a promising approach to effect in vivo tissue regeneration. In bone wound healing mesenchymal stem cells (MSC) originated from marrow adhere to the surface of the scaffold, hence it may be possible to stimulate growth factor receptors on MSC by tethering the factor to the surface. Further, it is believed that migration and proliferation of MSC's involved in the bone healing process can be regulated by the epidermal growth factor (EGF) receptor. This project investigates the involvement of EGF receptor signalling pathway in modulating MSC behavior in response to tethered EGF (tEGF) as an implant surface modification.

Methodology

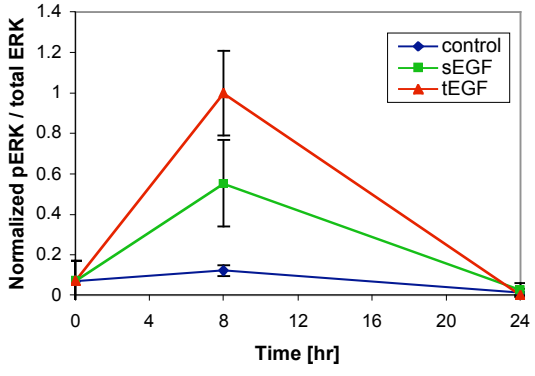
Surface Preparation. Murine EGF (mEGF) was coupled to a comb copolymer thin film activated by tresyl-choride or 4-nitrophenyl chloroformate. Activated copolymer at 20μg/mL in methyl ethyl ketone was spin-coated onto 12-mm round glass coverslips. Phosphate buffered EGF solution was applied to each coverslip to allow covalent bonding. Unreacted active sites were blocked with tris buffer.

Cell Culture. hTERT immortalized human MSC's (hMSCs) were a gift from Dr. Junya Toguchida and were maintained in DMEM 10% FBS, 1% pyruvate, 1% L-glutamine, 1% non-essential amino acids, and 1% penicillin-streptomycin (Invitrogen), at 37°C in a humidified atmosphere of 5% CO2/95% air.

Tethered EGF Stimulation. Cultured pMSCs were serum-starved with Advanced DMEM ~16 hours prior to growth factor stimulation. Cells were detached with EDTA and seeded onto the tEGF surfaces at approximately $20x10^3$cells/cm^2. Cells were lysed at 0, 8, and 24 hours. Cell lysate were collected and stored at -80°C.

ERK Signalling Measurement. ERK phosphorylation was monitored by Western Blot and normalized to the total amount of ERK to reflect the percentage of ERK phosphorylation.

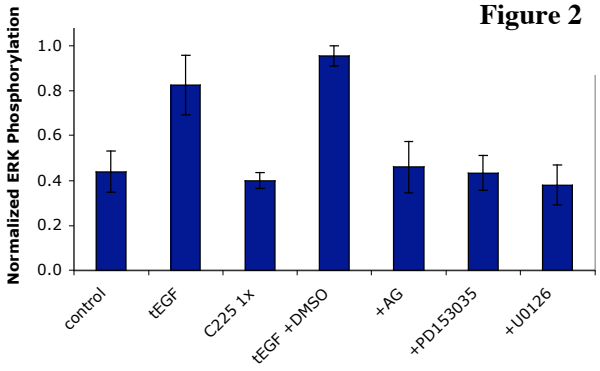
Figure 1



Results

Figure 1 shows that tEGF induced a greater ERK phosphorylation, compared to the control surface with or without 100 ng/mL soluble EGF (control and sEGF, respectively).

Figure 2



We introduced inhibition of individual points of the EGF receptor signaling cascade with anti-EGF blocking antibody (C225, 1 μg/mL), EGFR tyrosine kinase inhibitors (AG1478, 1 μM; PD153035: 1 μM), or MEK inhibitor (U0126;1 μM). The inhibitors abrogated ERK activation at 8 hours (Figure 2) and continued to do so at 24 hours (data not shown). DMSO alone was included as a vehicle control. Next, we investigated the dependence of tEGF-induced morphology on EGF-binding and EGF receptor signaling. Cell images taken at 8 hours showed the morphological difference expressed by the human MSC's on tethered EGF surfaces— noticeably less rounded cells, and more prominent and extended lamellapodia compared to the control group. Corresponding to the reduced ERK activation, cell spreading was reverted by the inhibitors at 8 hours. We could see that the majority of the cells resumed the round morphology with few lamellapodia. At 24 hours, C225 and PD153035 continued to inhibit cell spreading, but some degree of cell recovery was observed in the AG1478 and U0126 groups, where the cells expressed more extensive lamellapodia indicating increased spreading. A closer look revealed that the more potent inhibitors (i.e., PD153035) worked better in blocking cell spreading and as did the further upstream inhibitors (i.e., C225). This suggested that ERK was involved in controlling the tethered-EGF-induced cell spreading.

Conclusions

We have shown that ERK phosphorylation in hMSC's was greater on tEGF than control surfaces. Additionally, cell spreading on tethered EGF is prevented by inhibitors of EGFR signaling pathways.

These data suggest that that tEGF-modified surfaces activate progrowth and prosurvival signals, which may enhance bone regeneration in tEGF-modified implants.