

Desmoid Cell Migration is Induced *in vitro* by rhEGF

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PURPOSE:

Desmoid tumors are locally invasive myofibroblastic lesions that arise predominantly in the abdominal wall or shoulder and are prone to aggressive local recurrences without metastases. Desmoids have been associated with trauma, hormonal activity, and genetic alterations and frequently develop in women during or after pregnancy. Epidermal growth factor (EGF) and transforming growth factor alpha (TGF α) are essential participants in the process of wound healing and their common receptor is typically upregulated in uteroplacental tissues during childbirth. We propose that EGF and/or TGF α are involved in the initiation and/or progression of Desmoid tumors and might be influential in regulating specific Desmoid attributes, such as tumor invasiveness. This study investigated the relationship between Desmoid cell migration *in vitro* and growth factor stimulation.

METHODS AND MATERIALS:

Tissue acquisition and processing: Eighteen Desmoid tumors were acquired from three institutions (University of Utah, SUNY Upstate Medical University, Sinai Hospital Cancer Institute) and processed according to IRB approved and HIPAA compliant protocols. Total RNA was extracted and purified from the homogenized tissues using RNeasy® kits supplied by Qiagen (Germantown, MD). RNA quality was evaluated using an Agilent Bioanalyzer (Santa Clara, CA).

Desmoid cell lines: Three Desmoid tumors resected at the University of Utah were enzymatically dissociated for 2 h at 37° C in Collagenase 1A (Sigma C9891;100 collagen digestion units/mL; St. Louis, MO). Desmoid cell cultures, maintained in DMEM culture medium containing 15% fetal bovine serum, were grown to near-confluency, and then subdivided for continued *in vitro* culture or cell freeze.

Real time RT-PCR: Tumor mRNA content was quantified by real time RT-PCR using TaqMan® Gene Expression Assays and the ABI PRISM® 7900 HT Sequence Detection System with related software (Applied Biosystems, Foster City, CA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the reference standard.

Boyden Chamber migration assays: Desmoid cell cultures derived from dissociated tumors were serum starved for 24 h, then treated under serum-free conditions with rhEGF (E9644; Sigma) or rhTGF α (T5403; Sigma), each at 100-300 ng/mL, for an additional 24 h. The cultures were then assayed for cell migration using Boyden Chambers (Cell Biolabs, Inc. San Diego, CA). Other Desmoid cultures were pre-treated for 24 h under serum-free conditions with the EGF receptor (EGFR) inhibitor AG1478 (tyrphostin; T4182; Sigma), alone or in combination with the TGF β 1 receptor inhibitor SB-431542 (Tocris Biosciences, Ellisville, MO), and then stimulated with rhEGF or rhTGF α for an additional 24 h prior to being assayed for cell migration.

Statistical analysis: The interrelationships between EGF/TGF α , and their common receptor, EGFR, were analyzed by multiple regression using the least squares method (Microsoft Office Excel statistical analysis tools), with EGF and TGF α as predictor variables, and EGFR as the response variable. Student's *t*-tests (Excel) were used to test for differences in EGF, TGF α , and EGFR mRNA content in the 18 Desmoid tumors.

RESULTS:

EGF/TGF α /EGFR mRNA content in tumors: All 18 Desmoid tumors tested positive for EGF, TGF α , and EGFR mRNA based on real time RT-PCR. The mRNA concentrations of TGF α exceeded EGF content in 13 of the 18 tumors (median fold-difference = 3.6-fold higher; range = 1-fold to 580-fold), while in four tumors, EGF content was higher than TGF α (median = 6.0-fold higher; range = 1.2-fold to 50-fold); one tumor showed equivalent concentrations of EGF and TGF α mRNA. The differences in tumor EGF and TGF α mRNA content were not statistically significant. EGFR mRNA content, on the other hand,

significantly exceeded the transcript content of both growth factors, averaging 70-fold higher than TGF α content ($t = 17.7$; $p = 4.14E-18$; 32 df), and 155-fold higher than EGF ($t = 8.6$; $p = 3.9E-08$; 20 df). Multiple regression analysis indicated that the variance in EGFR mRNA content of tumors was best predicted by TGF α transcript concentrations ($p = 0.002$); EGF showed no significant association with EGFR content. **Boyden Chamber migration assays:** We found a direct dose-dependent relationship between rhEGF stimulation and the number of Desmoid cells that migrated through the Boyden Chamber membrane (Fig. 1). rhTGF α , in contrast, was ineffective at inducing cell migration.

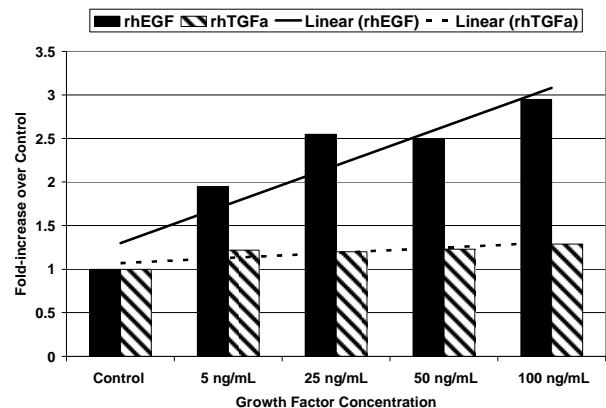


Figure 1. Desmoid cell migration is induced in a dose-dependent manner by rhEGF.

The extent of rhEGF-induced cell migration could be diminished, but not reduced to control levels, by inhibiting the EGFR with AG1478 (Fig. 2). When EGF and TGF β 1 receptors were inhibited simultaneously, the level of rhEGF-induced cell migration was reduced an additional 12% beyond the 17% reduction generated by AG1478.

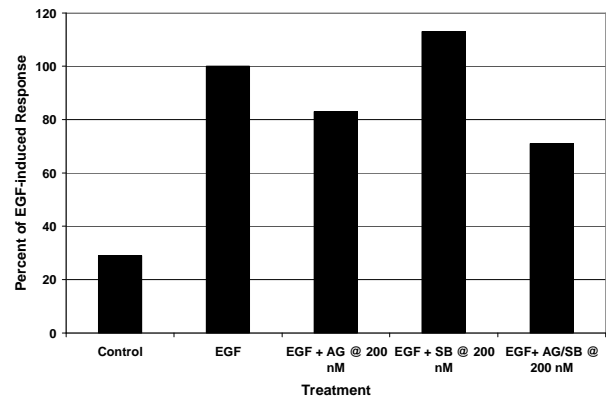


Figure 2. EGF-induced cell migration is diminished, but not eliminated, by inhibiting the EGFR.

CONCLUSIONS: Desmoid cell cultures displayed enhanced migration following rhEGF stimulation, but were not responsive to rhTGF α . Inhibition of the EGFR did not abrogate rhEGF-induced cell migration, suggesting that EGF may signal through receptors in addition to the recognized EGFR.

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