**Matrix Rigidity Regulates Osteolytic Gene Expression in Oral Squamous Cell Carcinomas**

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**Statement of Purpose:** Oral squamous cell carcinomas (OSCC) account for the majority of oral cancers observed in patients and are a significant problem worldwide, where OSCC is the 8th most common cancer with some countries displaying higher incidence rates[1]. Approximately 50% of patients with OSCC develop invasion of the tumor into the lower jaw or mandible[2], with the majority of the tumors growing within the marrow space similar to other solid tumors that metastasize to bone (e.g., breast, lung, and prostate)[3]. OSCC patients require bone resection, which significantly impacts their quality of life, since the mandible is important for normal speech, eating, and appearance. Despite the significance of the clinical problem, little is known about the molecular mechanisms involved in OSCC invasion into the mandible. Previous studies suggest that parathyroid hormone-related protein (PTHrP) is over-expressed in OSCCs, resulting in an increase in Receptor Activator of NF-kB Ligand (RANKL) expression and bone destruction, but the mechanisms that regulate PTHrP in this system are not clear. Similar to OSCCs, solid tumors that metastasize to bone also express PTHrP[4]. In these tumors, there is a well-described vicious cycle of bone destruction in which the tumor cells stimulate bone destruction and release of growth factors that further stimulate tumor growth and the expression of proteins that stimulate bone destruction[5]. We have shown that in solid tumors that metastasize to bone, PTHrP is regulated by transforming growth factor beta (TGF-β) and the transcription factor Gli2[6]. Furthermore, we have demonstrated that the rigid mineralized bone extracellular matrix regulates PTHrP expression through mechanically transduced signaling pathways[7].

**Methods:** In order to mimic the tissue rigidities involved in OSCC cancers, liquid reactive polyurethanes (PUR) were utilized. PUR films were cast into a 60 mm polystyrene culture dish and allowed to cure overnight at 60° C. The liquid PUR precursors are hexamethylene diisocyanate trimer (HDIt) and a polyester triol (polyol). The mechanical properties of the cured PUR films are a function of polyol molecular weight. Compliant films representing soft tissue (elastic modulus 30-70 MPa) were produced with a 3000 g/mol polyol while rigid films representing bone (elastic modulus 0.5-1 GPa) were produced with 300 g/mol polyol. To identify candidate genes that may play a role in local soft tissue and bone invasion, we compared differential gene expression profiles of two commercially available OSCCs, the highly invasive Cal27 and the less invasive SCC4 cells, using microarray analysis. The effects of matrix rigidity, TGF-β, and Gli inhibitors on gene expression in OSCCs were also investigated. 2D PUR films of varying rigidity were seeded with SCC4 cells and cultured for 24 hours with treatments ±TGF-β or +TGF-β and Gli inhibitors. The expression of PTHrP was analyzed via RT-qPCR. Exogenous TGF-β and a Gli inhibitor (GANT 58) were added to identify changes in PTHrP expression seen in solid tumors that metastasize to bone.

**Results:** From the microarray analysis, PTHrP was in the top ten of 33,000 genes analyzed with significantly increased levels (27 fold) of expression in the invasive Cal27 cells. These results were confirmed by qPCR, demonstrating that PTHrP was expressed in the Cal27 cells at high levels but at very low levels in the SCC4 cells indicating a link to PTHrP expression and invasiveness. Additionally, we investigated the expression of Hh transcription factors in these cells (which were not on the micro-array), which demonstrated that Gli proteins were expressed in both cell types. The effects of culturing the less invasive SCC4 cells on compliant and rigid PUR films showed that PTHrP expression was significantly increased with changes in rigidity and exogenous TGF-β. Furthermore, this increase in PTHrP can be completely negated by targeting the Gli2 transcription factor with GANT58.

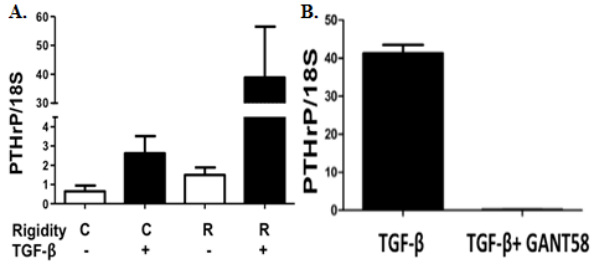


Figure 1. (A.) Effets of 2D substrate rigidity (compliant, C or rigid, R) and TGF-β dose (0 or 5 ng/ml) on PTHrP. R+ TGF-β was significantly ($p<0.05$) higher than other groups; (B.) GANT58 (50 uM) significantly ($p<0.05$) inhibits PTHrP expression induced by TGF-β (5 ng/ml) in SCC4 cells.

**Conclusions:** OSCCs present challenging clinical issues, but there are similarities to metastatic tumors of common cancers (e.g. breast, lung, and prostate) that can potentially be exploited for therapeutic targets. 2D PUR films and 3D PUR scaffolds with porosity and pore size comparable to trabecular bone can be tuned to mimic both soft and hard tissue. These biomaterials offer unique opportunities to investigate the effects of matrix rigidity on tumor cells that have been shown to metastasize to many different tissues in the body. Specifically, this model will allow for molecular signaling studies and testing of potential inhibitors of the mechanotransduction pathway that can be clinically translated to tumor-targeting therapies.

**References:** [1]Moore, S. R., et al. Oral diseases 2000;**6**: 65-74. [2]Rao, L. P., et al. Int J Oral Maxillofac Surg 2004;**33**:454-457. [3]Pandey, M., et al. World J Surg Oncol 2007;**5:**12. [4]Powell, G. J., et al. Cancer Res 1991;**51:** 3059-3061. [5]Guise, T. A., et al. J Clin Invest 1996;**98**: 1544-1549. [6]Biswas, S., et al. PLoS ONE 2011;**6:**e27090. [7]Ruppender, N. S., et al. A. PLoS ONE 2011;**5**:e15451.