ABSTRACT INTRODUCTION:
Giant Cell Tumor of bone (GCT) is an aggressively osteolytic primary bone tumor that is characterized by the presence of abundant multinucleated osteoclast-like giant cells, hematopoietic monocytes and a distinct mononuclear mesenchymal stromal cell component. Currently, the treatment of GCT is limited to surgery accompanied by a variety of surgical adjuvant therapies and reconstructive techniques that collectively permit limb-sparing excision. Developing an understanding of the biology of the tumour is important to subsequent creation of more effective therapeutic options. Preliminary data has shown that parathyroid-hormone-related protein (PTHrP), which is expressed by cancer cells in osteolytic bone disease and is highly expressed by the stromal cells of GCT, serves to increase osteoclastogenesis in GCT. However an intriguing serendipitous finding turned our attention to another more important role for PTHrP in GCT: the stromal cells exposed to a monoclonal antibody to PTHrP ceased to replicate and quickly died in vitro. The purpose of this study was to examine the effects of PTHrP neutralization on cell proliferation, cell cycle regulation and apoptosis using both transcriptomic & proteomic microarray analyses.

METHODS:
GCT sample collection & Primary cell lines and cultures
The use of all patient-derived material was approved by our institution’s Research Ethics Board, and patient informed consent was obtained individually according to the laws and regulations of Canada. Primary cell cultures of homogeneous GCT stromal tumor cells from fresh GCT tissue was established by subculturing over 5 successive passages until the multinucleated giant cells and monocytes were eliminated from the culture. A human fetal osteoblast cell line (hFOB) was used as a control.

PTHrP treatments on GCT cell cultures
GCT stromal cells were treated with either 10 mg/ml anti-PTHrP neutralizing antisera (T-4512, Bachem Inc., CA), or IgG vehicle, or 10 nM recombinant PTHrP peptide (a.a.1–34, Bachem Inc.) in serum-free medium for 24h. After 24h of PTHrP exposure, cells were then lysed and total RNA and protein were extracted for assays separately.

Microarray Analysis
Global gene expression analysis using the Affymetrix GeneChip Human Gene 1.0 ST Array was performed for the hFOB control cell line and 3 individual GCT cell lines treated with anti-PTHrP antibodies (Pab), recombinant PTHrP peptide (Pp) and IgG vehicle as a control. Enrichments of gene ontology (GO) categories were computed using the hypergeometric probability distribution.

Real-time PCR for cell-cycle regulation and apoptosis
The expression levels of various genes in cell-cycle regulation and apoptosis in GCT mesenchymal stromal cells exposed to anti-PTHrP antibodies and recombinant PTHrP peptide were validated using real-time PCR.

Proteomics using antibody microarray on cell-cycle and apoptosis
The Antibody Microarray (BD Clontech, Palo Alto, CA) contains 512 highly specific and sensitive monoclonal antibodies against human proteins, including signal transduction, cell-cycle regulation, gene transcription, and apoptosis. Total protein from GCT cells was extracted, labeled with Cy3/5 and incubated with the array slides as per manufacture’s instruction. Slides were scanned using a fluorescent microarray scanner with the excitation wavelength and the emission filter wavelength for Cy3/5 at 570 nm/670 nm respectively.

Statistical analysis
All data are presented as mean ± standard error of the mean. Using unsupervised clustering analysis, changes that are 2-fold or higher compared with the control were identified. All statistical analyses were performed using analysis of variance (ANOVA). A P value <0.05 was considered as significant.

RESULTS:
Microarray data identified genes that were differentially expressed in GCT stromal cells under various PTHrP interventions. The dendrogram produced by unsupervised hierarchical clustering identified a set of 1200 genes shared by IgG control, Pab and Pp. The hierarchical clustering separated hFOB and all GCT IgG and Pp samples from the Pab treated GCT samples (Figure 1). Two selected lists of the differentially expressed genes with a false discovery rate (FDR) of less than 5% and with a focus of apoptosis and cell cycle are shown in Figures 2A and 2B of GCT stromal cells with anti-PTHrP antibodies showed a strong correlation in apoptotic gene expression in 41 genes (Figure 2A) and cell cycle related gene expression for 37 genes (Figure 2B) relative to the IgG control. Triplicate real-time PCR experiments on all specimens confirmed microarray expression data (Figure 3). Proteomics data confirmed microarray expression patterns (data not shown).

DISCUSSION:
In this study, we demonstrated that PTHrP stimulates cell proliferation in GCT by regulating apoptosis and cell cycle progression. Microarray data followed by GO investigation displayed that anti-PTHrP antibodies affect expression of genes mainly involved in cell cycle and apoptosis. Multiple genes involved in the G1/S cell cycle checkpoint were identified in our experiments. The frequent deregulation of genes involved in cell cycle control suggests that cellular growth pathways are a common downstream target for PTHrP. The direct corollary dictates that PTHrP, which is highly secreted by GCT stromal cells, serves to protect these cells from apoptosis and cell cycle regulation, and therefore contributes a neoplastic phenotype. This data constitutes a starting point for clinical evaluation of anti-PTHrP strategies against primary GCT of bone.

SIGNIFICANCE:
The concept that PTHrP serves to propagate proliferation in a paracrine/autocrine manner in GCT stromal cells is an intriguing model for further investigation into this neoplastic phenomenon. To further investigate this possibility, future studies exploring an in vivo intrasosseous model for GCT using anti-PTHrP treatment will be essential to promote ‘bench to bedside’ research in the management of this clinical entity.

ACKNOWLEDGEMENT:
CHR, Hamilton Health Sciences New Investigator Fund, McMaster University Department of Surgery, Juravinski Cancer Centre Foundation.

Poster No. 1478 • ORS 2012 Annual Meeting