

to develop immunological reagents and a sensitive and specific immunoassay for hK5, to measure this protein in human tissues and biological fluids and to study its clinical utility as a biomarker for endocrine-related malignancies. Recombinant hK5 was produced in a *P. pastoris* yeast expression system. The protein was used to generate mouse and rabbit monoclonal and polyclonal antibodies, for the purpose of developing an enzyme-linked immunosorbent assay (ELISA). The levels of hK5 were determined in 263 serum samples obtained from normal individuals and patients diagnosed with various malignancies including prostate, breast, ovarian, thyroid, colon, pancreatic and hepatocellular carcinomas, using the developed ELISA. We also prepared cytosolic extracts from 10 normal ovarian tissues, 10 tissues from benign ovarian disease patients and 20 ovarian cancer tissues and quantified their hK5 protein levels with the developed ELISA. Results were corrected according to the total protein content of each sample. Ascites fluids were obtained from patients with ovarian cancer stage FIGO II (n=9), III (n=18) and IV (n=4). While the levels of hK5 are almost undetectable in the serum of normal individuals (male and female) and patients with diverse malignancies, higher concentrations were found in a proportion of patients with ovarian (69%) and breast (49%) cancer. In many of these patients, the serum levels were more than 20 times higher than the cut-off value. Tissue levels of hK5 were higher in about 55-60% of patients with either benign ovarian disease or ovarian cancer. The levels of hK5 in ovarian cancer tissue extracts are significantly higher than levels in either normal or benign disease tissue extracts. All ascites samples were positive for hK5, with values ranging from 1 µg/L to 300 µg/L, with a mean of 32 µg/L and a median of 6.8 µg/L. Statistical analysis indicated that there were no significant correlations between ascites fluid hK5 concentration and either patient age, CA125 concentration or FIGO stage ($p > 0.05$ by the Fisher's exact test). We report the development of the first ELISA for hK5 and describe the distribution of hK5 in biological fluids and tissue extracts. Our preliminary data indicate that hK5 is a potential biomarker in patients with ovarian and breast cancer.

#6337 Human kallikrein 5 (hK5): a novel serum biomarker for breast and ovarian cancer. George M. Yousef, Linda Grass, Mary-Ellen Polymeris, Antoninus Soosaipillai, Pak-Cheung Chan, Andreas Scorilas, Carla A. Borgono, Nadia Harbeck, Barbara Schmafeldt, Julia Dorn, Manfred Schmitt, and Eleftherios P. Diamandis. *Dept. of Laboratory Medicine, Memorial University, St. John's, Newfoundland, Canada, Dept. of Pathology and Laboratory Medicine, Mount Sinai Hospital & Dept. of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada, Department of Clinical Pathology, Sunnybrook and Women's College Health Sciences Centre, Toronto, Ontario, Canada, National Center of Scientific Research Demokritos, IPC, Athens, Greece, and Clinical Research Unit, Dept. of Obstetrics and Gynecology, Technical University of Munich, Munich, Germany.*

The kallikrein family is a group of 15 serine protease genes clustered on chromosome 19q13.4. In addition to prostate specific antigen (PSA/hK3), which is an established prostate cancer tumor marker, recent evidence suggests that other members of the human kallikrein gene family are differentially regulated in endocrine-related malignancies. Human kallikrein gene 5 (*KLK5*) is differentially expressed at the mRNA level in breast and ovarian cancer. Until now, detection of its protein product, hK5, in either biological fluids or tissues has not been feasible due to lack of suitable reagents and methods. The aim of this study was