

4461 In silico analysis of kallikrein gene expression in ovarian cancer.

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The human kallikrein gene family is formed of 15 serine proteases clustered on chromosome 19q13.4; an area that is non-randomly rearranged in some solid tumors, including ovarian cancer. In addition to prostate specific antigen (PSA), which is a known prostate cancer tumor marker, recent evidence suggests that other kallikreins are differentially regulated in ovarian cancer. The aim of this study was to examine kallikrein gene expression, at both the mRNA and protein levels, in normal and ovarian cancer tissues, and to assess the potential diagnostic/prognostic value of kallikreins in ovarian cancer. We utilized the Serial Analysis of Gene Expression (SAGE) and Expressed Sequence Tag (EST) databases of the Cancer Genome Anatomy Project to perform an in silico analysis of the 15 human kallikrein genes in normal and cancerous ovarian tissues and cell lines. Different analytical tools were used, including Virtual Northern blotting, Digital Differential Display and X-profiler. Human tissue extracts were prepared from histologically confirmed normal, benign and cancerous ovarian tissues. Kallikrein protein levels were measured by specific time-resolved immunofluorometric assays. Our results indicate that 7 kallikrein genes (KLK5-8, KLK10-11 and KLK14) are up-regulated in ovarian cancer. Probing two normal and 10 ovarian cancer SAGE libraries with gene-specific tags for each kallikrein indicated that while no expression was detected in any of the normal libraries, they were found to be expressed with moderate density (103-408 "tags per million", tpm) in 40-60 % of the ovarian cancer libraries analyzed. These data were verified by screening the independent EST databases, where all mRNA clones isolated for these genes were found to be from ovarian cancer libraries, except one normal KLK7 clone. Three to sixteen clones were isolated for each gene from cancer tissues. X-profiler comparison of the pools of normal and cancerous ovaries further verified the presence of significant difference in expression levels of 6 of these 7 kallikreins. To verify the in-silico mRNA overexpression results and to examine whether these mRNA up-regulations reflect changes at the protein level, we compared the protein levels of these kallikreins in normal, benign and cancerous ovarian tissues. A step-wise increase was found between normal, benign and cancer. These differences were statistically significant for hK11 ($p < 0.001$), hK5, 6 and 10 (p values of 0.048, 0.044 and 0.027, respectively) but not for hK7 and hK8. Moderate to strong correlations were observed between kallikreins with high statistical significance (r_s 0.45 – 0.74 and $p < 0.001$). We conclude that seven kallikrein genes are overexpressed at both the mRNA and protein levels in ovarian cancer and have the potential of being used as diagnostic/prognostic markers. Further large scale studies are needed to establish the clinical utility of kallikreins.