

propensity for bladder wall invasion and metastatic spread. Immunohistochemistry may be useful in the differential diagnostic work-up of a carcinoma with micropapillary features, as MPC of different organs exhibit almost identical histologic appearances. Positive immunostaining for CK7, CK19 and CK20, and negative staining for TTF1 and ER/PR in an MPC strongly suggests a urinary bladder primary.

70 Kallikreins are potential new breast cancer biomarkers

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Recent evidence suggests that many kallikrein genes are differentially regulated in different malignancies. In this study, we utilized the Serial Analysis of Gene Expression (SAGE) and Expressed Sequence Tag (EST) databases of the Cancer Genome Anatomy Project to perform *in-silico* analyses of the expression of the 15 human kallikreins in normal and cancerous breast tissues using different analytical tools including Virtual Northern blotting (VNB), Digital Differential Display (DDD) and X-profiler analysis. We also experimentally verified our findings. Our results indicate that 5 kallikreins (*KLK5*, 6, 8, 10) are down-regulated in breast cancer. Analyzing 8 normal and 24 breast cancer SAGE libraries indicated moderate to high kallikrein expression densities in normal breast (27–319 tags per million; tpm, in 2–5 out of 8 libraries), compared to no or low expression (0–34 tpm in 0–2 libraries out of 24) in cancerous tissues. Screening the EST databases also showed that all mRNA clones isolated for these genes, except for one, were from normal breast, with no clones detected from breast cancer (with the exception of *KLK8*). X-profiler comparison of two pools of normal and breast cancer libraries further verified this significant down-regulation. We experimentally verified our findings by RT-PCR analysis. While *KLK5* was expressed at high levels in all normal breast tissues, it was only detectable in 3 out of 14 cancerous breast tissues examined. *KLK6* was strongly positive in normal breast tissue, but was not expressed in 9 out of 14 tumor tissues, lower than normal in 3. *KLK8* also showed strong bands in normal tissues, compared to undetectable expression in 5 tumor tissues, lower than normal in 7 tumors.

KLK10 showed no expression in 8 tumors, lower than normal in 5 and compared to normal in one tumor. Our results indicate that kallikreins are potential diagnostic/prognostic biomarkers of breast cancer.

71 The human Kallikrein protein 5 (hK5) is enzymatically active, glycosylated and forms complexes with two protease inhibitors

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Kallikreins are a group of 15 serine proteases. Binding of kallikreins to protease inhibitors is an important mechanism for regulating their activity and, as is the case with PSA (hK3) has potential clinical applications. Human kallikrein protein 5 (hK5) is newly discovered kallikrein that was shown to be differentially expressed in different malignancies. Incomplete recovery of hK5 was observed in serum, suggesting its binding to inhibitors. In this study, recombinant hK5 protein was produced in yeast and mammalian systems. HPLC fractionation, followed by hK5-specific and hybrid assays, immunoblotting, and radiolabeling experiments were performed to study the interactions of hK5 and proteinase inhibitors. Deglycosylation analysis was also done and enzymatic activity of hK5 was tested using different synthetic trypsin and chymotrypsin fluorogenic substrates. Our results show that in addition to the free form, hK5 forms complexes with α_1 -antitrypsin and α_2 -macroglobulin in serum and ascites fluid. These complexes were detected by hybrid assays using anti-hK5 monoclonal antibody for capture and different inhibitor antibodies for detection. The ability of hK5 to bind to these inhibitors was also verified in-vitro. Serum samples spiked with I¹²⁵-labeled hK5 showed the distribution of the protein in two higher molecular mass (bound) forms, in addition to the unbound form. In addition, mixing α_2 -macroglobulin with hK5 in a BSA medium resulted in a significant decrease in detectable hK5. The hK5 mature enzyme is enzymatically active and shows trypsin-, but not chymotrypsin-like, activity. Glycosylation/Deglycosylation analysis showed that hK5 has a higher than predicted molecular mass due to glycosylation, which returns to normal after deglycosylation.