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1819 Human kallikrein 13: Evaluation of its role in the degradation of extracellular matrix proteins and characterization of its substrate specificity.

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The human kallikrein family is a group of 15 serine protease genes clustered on chromosome 19q13.4 and share a high degree of homology. These proteolytic enzymes have diverse physiological functions in many different tissues. Growing evidence suggests that many kallikreins are differentially expressed in cancer and may play a role in metastasis. Human kallikrein gene 13 (KLK13) is a member of this family and codes for a trypsin-like, secreted serine protease (hK13) that is over expressed in ovarian cancer patients. The aim of this study was to determine if hK13 plays a role in tumor metastasis and to characterize its substrate specificity.Recombinant hK13 was produced in yeast and purified using cation exchange and reversed-phase chromatography. Its enzymatic activity was verified by synthetic tri-peptide fluorogenic substrates. This active hK13 was then incubated with various extracellular matrix proteins. These mixtures were then evaluated by Western Blot to see if any of the ECM proteins are cleaved. hK13 secreting cancer cell lines were also utilized in the chemotaxis invasion chamber that was coated with the various extracellular matrix proteins in order to determine the role of hK13 in tumor invasion. Soluble fluorogenic protease substrate libraries possessing diversity at the P1-P2-P3-P4 positions were constructed in order to evaluate hK13 substrate specificity.The Western Blot results showed that hK13 was able to cleave most of the major components of the extracellular matrix. It also cleaved gelatin when run on a zymogram. In the chemotaxis invasion chamber experiment it was found that tumor cells treated with an hK13 neutralizing antibody migrated less than the control. hK13 substrate profiling using combinatorial fluorogenic substrate libraries showed that it was highly selective for the P1 (Arg) and P3 (Arg) positions. These experiments suggest that hK13 may play a role in tissue remodeling and/or tumor invasion and metastasis.

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