American Association for Cancer Research
95th AACR Annual Meeting
March 27-31, 2004
Orange County Convention Center
Orlando, Florida

3454 Cloning and characterization of novel isoforms of the human kallikrein 6 gene.

Georgios Pampalakis, Eleftherios P. Diamandis, Georgia Sotiropoulou. *Department of Pharmacy, University of Patras, Rion, Patras, Greece and Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada.*

The gene encoding human kallikrein 6 (protease M/zyme/neurosin) (KLK6) was originally identified by differential display based on its absent expression in a metastatic breast tumor as compared to the primary tumor derived from the same patient. The KLK6 gene was found highly overexpressed in a subset of breast and ovarian primary tumor tissues and cell lines but downregulated or inactivated in corresponding metastatic tumors. Human kallikrein 6 is a potential circulating biomarker for diagnosis and monitoring of ovarian cancer but may also be involved in pathologies of the CNS, such as Alzheimer's disease and multiple sclerosis. KLK6 is a member of the kallikrein multigene family of 15 genes tandemly arranged on human chromosome 19q13.3-13.4. Multiple isoforms have been described for most kallikrein genes. Specific isoforms may be important in clinical diagnosis, since they may crossreact with antibodies in established immunoassays as is the case for prostate specific antigen (PSA/KLK3) or may represent potential biomarkers for specific tumor types as, for example, a KLK13 isoform that is specifically produced by testicular tumors. Here, we report the cloning and characterization of three novel transcript variants of the human KLK6 gene that encode for wild-type kallikrein 6 protein. Transcript variants were identified by RLM-RACE, contain one untranslated exon, and are likely products of an alternative promoter that utilizes multiple transcriptional start sites located inside intron 1. Both KLK6 gene promoters lack a TATA-box in the immediate 5' upstream region. The longest and the most abundant transcript variants were deposited in GenBank™ with accession numbers: AY318867 and AY318869, respectively. The KLK6 transcript variants showed higher expression in most human tissues except for brain and spinal cord. In addition, three novel KLK6 splice variants were cloned that are produced by splicing out coding exons. Splice variant 2 (AY318870) lacks exon 3 and the translational start site ($A^{246}TG$) of the classical KLK6 gene, while splice variant 3 (AY318868) lacks exon 4 and encodes a predicted truncated protein of 40 amino acids with partial homology to kallikrein 6. Finally, splice variant 4 (AY457039) corresponds to a transcript of 1.1 kb that lacks exons 5 and 6 and codes for a truncated protein of 120 amino acids with partial homology to kallikrein 6. Given the potential diagnostic applications of the KLK6 gene at both the mRNA and protein levels, we have developed a multiplex RT-PCR assay, in order to differentially detect and quantitate mRNA species that correspond to the identified splice variants. We show that splice variants account for approximately 10-20% of all KLK6 mRNA species both in normal mammary epithelial cells and mammary tumor cell lines that overexpress the KLK6 gene.

Supported by PENED2001 (01EΔ557) grant funded by the Greek Secretariat of Research and Technology.

Citation information: Proceedings of the AACR, Volume 45, March 2004