CLONING AND CHARACTERISATION OF THE HUMAN UROPLAKIN 1B GENE

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by

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Summary

The uroplakin 1B gene has been cloned and characterised in only two species; mink (designated TI1) and cow. The cDNA sequences of mink TI1 and bovine uroplakin Ib have been isolated and putative protein sequences predicted. The mink TI1 gene is preferentially expressed during growth arrest mediated by transforming growth factor beta. The bovine uroplakin Ib gene is expressed as a terminal differentiation product of the asymmetric unit membrane of the bovine bladder and has urothelial-specific expression. The uroplakin 1B protein belongs to the tetraspan family of proteins, all of which have a similar protein structure with four highly conserved transmembrane domains interspersed with two more diverse extracellular domains. The tetraspan proteins are involved in a variety of cell functions including motility, activation and development. Recent studies have found that some of the tetraspan genes have altered patterns of expression in cancer and may act as metastasis suppressors.

This thesis describes the first cloning and characterisation of the human uroplakin 1B (UPK1B) gene, thereby confirming the existence of a human homologue of the mink TI1 and bovine uroplakin Ib genes. The cloning, by PCR techniques of the cDNA coding for the open reading frame of the human uroplakin 1B gene revealed homologies to both mink TI1 and bovine uroplakin Ib cDNA of greater than 90%. The cloning of 2.5 kb of contiguous human uroplakin 1B genomic sequence is described, along with the discovery of a *Taq*I restriction fragment length polymorphism. Chromosome mapping using two independent human uroplakin 1B genomic probes located the gene to human chromosome 3q13.3-21, a region with synteny to the location of the bovine UPKIb

gene to bovine chromosome 1. Mapping to mouse chromosomes revealed the location of mouse Upk1b to chromosome 16B5-C2, a region syntenic with human chromosome 3q.

Expression of the human uroplakin 1B gene in normal human urothelium was determined by Northern and RT-PCR analysis. A loss or marked reduction of expression of UPK1B mRNA was observed in approximately 70% of bladder carcinomas. All five bladder cancer cell lines analysed have no expression of uroplakin 1B mRNA detectable by Northern analysis. A search for a possible molecular mechanism for the observed frequent down-regulation of human uroplakin 1B mRNA expression involved both allelic loss studies and detection of UPK1B gene rearrangements. Using polymorphic markers located either side of the uroplakin 1B gene on chromosome 3q13.3-21, allelic loss was not detected in this chromosome region. Southern analysis using human UPK1B genomic probes did not detect gross rearrangements of the UPK1B gene, suggesting UPK1B gene rearrangements are not responsible for the down-regulation of UPK1B expression in bladder cancer.

To examine the biological function of UPK1B, the highly homologous mink TI1 cDNA, under the control of a constitutive cytomegalovirus (CMV) promoter, was transfected into the mink CCL64 cell line and two bladder cancer cell lines, T24 and 5637. The failure to propagate any stable clones expressing exogenous TI1 in any of the three cell lines suggested expression of TI1 was antiproliferative. There was an eight-fold reduction in the number of colonies propagated from

T24 cells transfected with the TI1/CMV plasmid when compared to vector-transfected cells, supporting this hypothesis.

In summary, this thesis reports the partial cloning and characterisation of the human uroplakin 1B gene. Cloning of partial human uroplakin 1B genomic sequences has allowed analysis and characterisation of the gene with regard to its structure, chromosomal localisation and integrity. Sequence comparisons of human UPK1B to mink TI1, bovine UPKIb and other tetraspan proteins were made possible by the cloning of the open reading frame of the human uroplakin 1B cDNA. The cloning of human uroplakin 1B cDNA has also enabled expression studies of UPK1B mRNA in normal urothelial tissue, bladder carcinomas and bladder cancer cell lines. Absent or greatly-reduced expression of UPK1B mRNA in a high proportion of bladder cancers and functional studies suggesting that the UPK1B gene is antiproliferative, all point to a potential role for UPK1B in the pathogenesis of bladder cancer.