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# Transcriptional control of the human urothelial-specific gene, uroplakin Ia

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**Abstract**

The transcriptional control elements of tissue-specific genes may be exploited in the design of therapeutic constructs for use in human gene therapy. The uroplakins are a family of four proteins which form the asymmetric unit membrane of the urothelium. We have cloned the human uroplakin Ia gene and defined its genomic structure and transcriptional start site. Using quantitative RT-PCR in an extended panel of normal tissues, we have demonstrated highly urothelial-specific expression of this gene. A Dual-Luciferase assay was used to assess the transcriptional activity of a variety of promoter fragments of the human uroplakin Ia gene. A highly specific promoter fragment (consisting of 2147 bp of 5′-flanking sequence, intron 1 and the 5′ UTR) was identified which regulated urothelial-specific expression in vitro. The human uroplakin Ia promoter identified has potential use in future gene therapy strategies to restrict transgene expression to the urothelium.


*Keywords:* Uroplakin 1a; Promoter; Tissue-specific; Urothelial; Gene therapy

## 1. Introduction

The transitional urothelium which lines the urinary tract is a specialised epithelium whose unique structure reflects its barrier function. Although transitional metaplasia is seen within other embryologically related tissues, normal transitional differentiation is only seen within the urinary tract, reflecting a pattern of urothelial-specific gene expression. This pattern of gene expression, the cis-regulatory transcriptional control elements which regulate it and the specific transcription factors which affect them are poorly defined.

The defining morphological feature of urothelial differentiation is the asymmetric unit membrane (AUM) seen on the apical membrane of superficial cells [1]. Four major transmembrane proteins, which comprise the AUM, the uroplakins, have been isolated and their genes cloned. Uroplakins Ia, II and III are specific to urothelium at both protein and RNA level [2–8]. Uroplakin Ib, although originally reported to be urothelial-specific, is probably more widely expressed, with human ESTs identified in cDNA libraries derived from a wide range of normal and/or malignant tissues in addition to those derived from the genitourinary tract. Following neoplastic transformation, with the associated loss of the terminally differentiated phenotype, uroplakin expression is variable [9–12]. However, many primary tumours and their associated lymph nodes may retain uroplakin expression [13,14]. Uroplakin expression has therefore been proposed as a marker for metastatic transitional cell carcinoma [15–20].

Despite the enthusiasm for gene therapy approaches in cancer, there has been little real clinical progress, in part because of the problems of generating selective gene expression in a given tissue or tumour site. Promoter elements which target-specific tissues are therefore a valuable tool for gene therapists. Human uroplakin Ia (hUP1a) is highly restricted in expression and retained in many human transitional cell carcinoma specimens. The present study was undertaken to examine the molecular basis for hUP1a expression. The specific aims were to determine the genomic structure of hUP1a gene, to identify the transcription start site, clone and sequence its promoter and to evaluate the ability of promoter elements to regulate urothelial-specific transcription.

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## 2. Materials and methods

### 2.1. Materials

Surgical specimens of normal/non-neoplastic human ureter were obtained from patients with no history of urothelial dysplasia or malignancy. Normal human urothelial cells (NHUs) were isolated from underlying stroma as previously described [10]. Non-urothelial cell lines (HepG2 (liver), DLD-1 and HT-29 (colon) and SK-BR-3 (breast)) were obtained from the American Type Culture Collection. The urothelial cell lines (VM-CUB-1, VM-CUB-3, RT-112, RT4) form part of a panel of cell lines previously described [21]. pGEM-T Easy, pGL-3-Basic, pGL-3-Promoter, pGL-3-Control and pRL-TK were obtained from Promega. A human urothelial cDNA library was prepared from normal human urothelium pooled from five independent donors as described previously [10].

### 2.2. Cell culture

NHU cells were maintained in culture in keratinocyte serum-free medium (KSFM) containing bovine pituitary extract and epidermal growth factor at the manufacturer's recommended concentrations (Gibco BRL) and 30 ng/ml cholera toxin (Sigma). Human transitional cell carcinoma (TCC) cell lines and non-urothelial cell lines were maintained in a 1:1 mixture of RPMI 1640 and Dulbecco's Minimal Essential Medium with 5% fetal bovine serum, as described previously [22]. All cultures were maintained at 37 °C in a humidified atmosphere of 5% $CO_2$ in air and passaged at near-confluence by incubation in 0.1% (w/v) EDTA in phosphate-buffered saline for 5 min followed by detachment using 0.25% (w/v) trypsin in 0.02% (w/v) EDTA.

### 2.3. RNA extraction

Total RNA was obtained from NHUs and cell lines using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. RNA was quantified using the optical density/absorbance at 260 nm, and quality assessed on 1% agarose formaldehyde gels. Poly(A)+ mRNA was isolated using oligo dT magnetic beads (Dynal).

### 2.4. Ribonuclease protection assay

Radio-labelled riboprobes were generated using an in vitro transcription kit (Promega) incorporating [$^{32}$P]CTP (Amersham International). The DNA template was destroyed by a 15-min incubation in the presence of 1.5 U of RNAse-free DNase (Promega). Probes were purified on Chromaspin 30-DEPC columns (Clontech). The 5′ end of hUP1a transcripts were mapped using the RPAII RPA kit (Ambion). A total of 2 fmol of labelled probe was mixed with 5 μg of total RNA (sample or yeast control). Hybridization and digestion were performed according to the manufacturer's instructions. After precipitation and electrophoresis on 5% denaturing polyacrylamide gels (Sequagel; Flowgen Instruments), protected probe fragments were visualized by autoradiography.

### 2.5. Cloning of human UP1a mRNA by RT-PCR

First strand cDNA synthesis was performed using oligo dT as primer. PCR of the hUP1a sequence was performed using a forward primer (5′-GCTCATCGTCTACATCTT-CGA-3′), reverse primer (5′-CTGACGTGAAGTTCACC-CAG-3′) and AmpliTaq DNA polymerase (Applied Biosystems, Warrington, UK). The PCR product was TA-cloned into pGEM-3Z (Promega) and sequenced using automated fluorescent sequencing using ABI prism BigDye terminators (Perkin-Elmer Applied Biosystems).

### 2.6. CapSelect RT-PCR

This was performed according to the CapSelect protocol [23] using an oligo dT primer for first strand cDNA synthesis with a hUP1a-specific primer (5′-CGTCAT-CCTTGCCTGAGACTC-3′) and a nested hUP1a-specific primer (5′-CACGGTACTGGTCGGCTGTC-3′) for subsequent PCR amplification. PCR products were cloned into pGEM-T Easy and sequenced, as above.

### 2.7. Quantitative RT-PCR (TaqMan)

Gene-specific reagents for hUP1a were: forward primer 5′-TGGTCCTCACGTACCTGGTG-3′, reverse primer 5′-CGGTGGGTGTAGGACGTGAT-3′, TaqMan probe 5′-ATGCTCATCGTCTACATCTTCGAGTGC-3′. Gene-specific reagents for β2-microglobulin (β2M) control were: forward primer 5′-GAGTATGCCTGCCGTGTG-3′, reverse primer 5′-AATCCACCTGCGGCATCT-3′, TaqMan probe 5′-CCTCCATGATGCTGCTTACATGTCTC-3′. First-strand RNA synthesis was performed using the TaqMan reverse transcription reagent kit (Perkin-Elmer) using random hexamers and MultiScribe reverse transcriptase according to the manufacturer's protocol. Quantitative PCR was performed to measure either hUP1a or β2M expression in independent 20 μl reaction volumes in capped 96 well plates (Perkin-Elmer) containing 1 × TaqMan buffer, 5.5 mM $MgCl_2$, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 400 μM dUTP, 100 nM gene-specific TaqMan probe, 200 nM gene-specific forward primer, 200 nM gene-specific reverse primer, AmpErase UNG (0.01 U/μl) and AmpliTaq Gold (0.05 U/μl) (all reagents, Perkin Elmer). The PCR reaction was performed in an ABI 7700 Sequence Detector (PE Biosystems) (PCR parameters: 50 °C for 2 min, 95 °C for 10 min and 40 cycles of 94 °C for 15 s, 60 °C for 1 min). Expression levels of hUP1a, relative to β2M, were calculated using the comparative $C_T$ method. Results were normalised to the level of expression in the HepG2 cell line and expressed as a percentage of that seen in normal human urothelium.