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ORIGINAL ARTICLE

Expression profile of undifferentiated cell transcription factor 1 in normal and cancerous human epithelia

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SUMMARY

Undifferentiated cell Transcription Factor 1 (UTF1) is a chromatin-bound protein involved in stem cell differentiation. It was initially reported to be restricted to stem cells or germinal tissues. However, recent work suggests that UTF1 is also expressed in somatic cells and that its expression may increase during carcinogenesis. To further clarify the expression profile of UTF1, we evaluated UTF1 expression levels immunohistochemically in eight normal human epithelia (from breast, prostate, endometrium, bladder, colon, oesophagus, lung and kidney) and their corresponding tumours as well as in several epithelial cell lines. We showed UTF1 staining in normal and tumour epithelial tissues, but with varying intensities according to the tissue location. *In vitro* analyses also revealed that UTF1 is expressed in somatic epithelial cell lines even in the absence of Oct4A and Sox2, its two main known regulators. The comparison of UTF1 levels in normal and tumoral tissues revealed significant overexpression in endometrial and prostatic adenocarcinomas, whereas lower intensity of the staining was observed in renal and colic tumours, suggesting a potential tissue-specific function of UTF1. Altogether, these results highlight a potential dual role for UTF1, acting either as an oncogene or as a tumour suppressor depending on the tissue. These findings also question its role as a specific marker for stem cells.

Keywords
cancer, epithelium, immunohistochemistry, screening, stem cell, undifferentiated cell transcription factor 1

Undifferentiated cell Transcription Factor 1 (UTF1) has been described as a transcriptional co-activator which was first identified in mouse embryonic stem cells (ESC) (Okuda *et al.* 1998). Several studies on ESC have described UTF1 as a transcriptional repressor, and it has been proposed as an important sensitive pluripotency marker (Fukushima *et al.* 1999; van den Boom *et al.* 2007; Tan *et al.* 2007). Available data indicate that *UTF1* expression is regulated by a dimer composed of Oct4A and Sox2 proteins acting on a specific fixation sequence located in the 3' flanking region (Nishimoto *et al.* 1999). To date, one isoform of this protein and no pseudogenes have been identified in humans. Investigations on human embryonic carcinoma and ESC demonstrated that UTF1 is a nuclear protein that is excluded from the nucleoli and strongly associated with chromatin during all phases of cell division (Kooistra *et al.* 2009). In addition, UTF1 was proposed to be expressed in the early phases of embryonic development and to be quickly downregulated during this process (Okuda *et al.* 1998).

In adults, UTF1 expression was reported to be restricted to germinal tissues (van Bragt *et al.* 2008; Kristensen *et al.* 2008). The expression pattern of UTF1 in somatic tissues has not been widely studied. Nevertheless, recent investigations revealed its presence in normal epithelium from prostate, uterine cervix and skin (Kristensen *et al.* 2010; Reinisch *et al.* 2011; Guenin *et al.* 2012).

Like many SC and pluripotency markers, such as Oct4 and Sox2, UTF1 has been assessed for its potential implication in tumorigenesis, and it was shown to be strongly expressed in germ cell tumours (Kristensen *et al.* 2008;

Wang *et al.* 2010). However, few data are available about its expression in the somatic tumour context. In brain tumours, UTF1 mRNA levels could help to discriminate between grade I–III and grade IV neuroblastoma tumours, whereas it was shown to be expressed at the same level in healthy epidermis and in skin squamous cell carcinoma (SCC) (Melone *et al.* 2009; Reinisch *et al.* 2011). In addition, we have previously shown that UTF1 overexpression was associated with cervical carcinogenesis (Guenin *et al.* 2012). Nevertheless, no further data are available about its expression profile in other human normal epithelia and their corresponding tumours.

Here, using immunohistochemistry, we screened the expression pattern of UTF1 in a panel of normal and cancerous epithelial tissues including breast, prostate, endometrium, bladder, colon, oesophagus, lung and kidney. We found that UTF1 was widely expressed in all epithelia or cell lines, although differentially, and was mainly localized in the nucleus. Also, by Western blotting, we showed UTF1 expression in several epithelial cell lines, even in the absence of its well-described regulators Oct4A and Sox2. Interestingly, the comparison of UTF1 expression levels in normal and cancer tissues revealed significant overexpression in endometrial and prostatic adenocarcinomas and sharp downregulation in colon and kidney carcinomas, suggesting a potential tissue-specific role of this protein.

Material and methods

Tissue samples

Normal and cancer formalin-fixed paraffin-embedded epithelial sections were obtained from the Tissue Bank of the University Hospital of Liege. Normal human epithelia included 94 cases distributed as follows: 20 specimens from endometrium, 13 from prostate, 14 from breast, 11 from colon, 10 from bladder and lung, 9 from oesophagus and 7 from kidney. We also evaluated 104 cases of corresponding tumours from endometrium ($n = 29$), colon and bladder ($n = 12$), oesophagus ($n = 14$), kidney and prostate ($n = 10$), breast ($n = 9$) and lung ($n = 8$). The study was approved by the ethics committee of the University Hospital of Liege (reference: B707201111603).

Tumour cell lines and cell culture

The teratocarcinoma cell line NCCIT (kind gift from Prof. Luc Grobet, Laboratory of Animal Histology and Embryology, University of Liege, Belgium) was cultivated in RPMI-1640 (Gibco, Carlsbad, CA, USA). DU146 cells (prostate) (kind gift from Dr Akeila Bellahcene, Laboratory of Research on Metastasis, University of Liege, Belgium) were cultivated in DMEM (Gibco). Ishikawa cells (endometrium) were purchased from Sigma-Aldrich (Sigma, St Louis, MO, USA) and cultivated in EMEM (Gibco). EO33 cell lines (oesophagus) were purchased from the European Collection of Cell Cultures and were cultivated in RPMI-1640. HCT116 (colon) and Detroit 562 (pharynx) cell lines were purchased from the American Type Culture Collections and were cultivated in McCoy's (Gibco) and MEM respectively. All cell culture media were supplemented with 10% foetal bovine serum (Gibco), 2 mM L-glutamine (Gibco), 1% sodium pyruvate (Gibco), 1% non-essential amino acids (Gibco), 100 U/ml penicillin G and 100 mg/ml streptomycin (Gibco). NCCIT treated or not with retinoic acid (all-trans retinoic acid, 10 mM, 11 days, Sigma) were used as negative and positive controls of UTF1 expression respectively.

Immunohistochemistry (IHC)

Tissue sections were deparaffinized in xylene and rehydrated through graded ethanol baths. For UTF1 expression, heat-induced antigen retrieval was performed in 10 mM citrate buffer (pH 6.0) using a microwave oven (5 min at 750 W followed by 15 min at 350 W). After treatment with endogenous peroxidase inhibitor (Dako, Glostrup, Danemark) for 5 min and blocking of endogenous biotin activity using Avidin (Vector, Burlingam, CA, USA) for 30 min, non-specific binding sites were blocked for 10 min in protein block serum-free solution (Dako). Sections were then incubated overnight at 4 °C with a mouse monoclonal anti-human UTF1 antibody (1:500, clone 5G10.2, MAB4337, Millipore, Darmstadt, Germany). The immunogen used to produce this antibody was a recombinant GST Fusion protein (human UTF1). We previously assessed the specificity of this antibody by Western blot using cells overexpressing the human protein (Guenin *et al.* 2012). This specificity was also validated by the fact that, up to now, no related protein or isoform was reported in human tissue, contrary to murine UTF1 which has two isoforms (Okuda *et al.* 1998). Negative controls included replacement of the primary antibody with either PBS or normal mouse IgG1. A secondary staining system (LSAB2, Dako) was used according to the manufacturer's instructions. The reaction was then visualized using diaminobenzidine (DAB, Dako), and sections were counterstained with Mayer haematoxylin.

Scoring of immunohistochemical

To assess IHC results, UTF1 staining was scored based on the intensity and percentage (*or extent*) of cells that stained positively according to an arbitrary scale. For staining intensity, 0 represented samples in which the immunoreactivity was undetectable, whereas 1, 2 and 3 denoted samples with low, moderate and strong staining respectively. For staining extent, 0, 1, 2 and 3 represented samples in which the immunoreactivity was, respectively, detectable in <6%, in 6% to 25%, in 26% to 75% and in more than 75% of the tumour cells. To provide a global score for each case, the results obtained using the two scales were multiplied, yielding a single scale of 0, +1, +2, +3, +4, +6 and +9 (Guenin *et al.* 2012). For scoring only the nuclear staining was taken into consideration.

Protein isolation and Western blotting

Cultured cells were harvested at 60–80% confluence and lysed in RIPA buffer (Pierce, Rockford, IL, USA) supplemented with 1 mM phenylmethyl sulphonyl fluoride (Sigma) and protease inhibitors (Roche, Vilvoorde, Belgium). Extracted proteins were quantified using BCA protein assay (Pierce) and separated by electrophoresis through 4–12% NuPAGE polyacrylamide gel (Invitrogen, Carlsbad, CA, USA) and transferred onto a PVDF membrane. The membrane was then blocked for 25 min in blocking buffer (5% skimmed milk in TBS-Tween-20, 0.1%) and incubated overnight at 4 °C with anti-human UTF1 (1:500, mouse monoclonal clone 5G10.2, Millipore), anti-Oct4A (1:500, mouse monoclonal sc-5279, Santa Cruz Biotechnology) or anti-Sox2 (1:1000 rabbit monoclonal clone D6D9, Cell Signaling) or for 2 h at room temperature with anti-Actin (1:2000, Sigma) antibodies. The membrane was then incubated with the appropriate secondary antibodies (Amersham Biosciences, Piscataway, NJ, USA). After washing, proteins were detected using an enhanced chemiluminescence system ECL Plus (Pierce).

Data analysis

All results are given as mean values ± SE. Statistical analyses were conducted and graphs drawn using GRAPHPAD PRISM 5 software (San Diego, CA, USA). The statistical tests used are indicated in the figure legends. All results were considered significant at the 5% level ($P < 0.05$).

Results

Expression and distribution of UTF1 protein in normal human epithelia

In this study, we characterized UTF1 expression in normal human tissues using a previously described monoclonal anti-human UTF1 (Kristensen *et al.* 2008; Wang *et al.* 2010; Reinisch *et al.* 2011; Guenin *et al.* 2012). Figure 1 shows representative images of UTF1 staining in each tissue type, with their corresponding negative controls (capital and lower case letters respectively). These results showed that UTF1 is expressed in all tested tissues and that staining is almost exclusively located in the epithelial components. UTF1 was rarely observed in cells within the stroma or in infiltrating lymphocytes, and staining was almost always confined to the nuclei.

Semi-quantitative analysis of the staining showed that UTF1 is differentially expressed according to tissue type. In endometrial epithelium (Figure 1A), UTF1 staining was remarkably low and stromal cell staining was seen in some cases (3/14). In breast epithelium (Figure 1B), UTF1 showed a similar pattern of expression in both ducts and lobules. Nevertheless, it should be noted that staining was mainly detected in luminal epithelial cells, whereas weak or no staining was detected in the basal myoepithelial layer. In the bladder (Figure 1C), the transitional epithelium showed low to moderate UTF1 staining in almost all epithelial cells. However, in many section areas, luminal epithelial cells were stained more intensely than underlying cells. Within the prostate (Figure 1D), epithelial cells were positively stained for UTF1. Importantly, as in breast and bladder epithelium, prostatic basal cells exhibited absent or weak UTF1 expression in comparison with luminal epithelial cells (Supplemental Figure 1). In colon epithelium (Figure 1E), UTF1 was mostly detected in all cells within crypts. In the oesophagus (Figure 1F), both squamous and glandular epithelial cells were positively stained. In the lungs (Figure 1G), staining was detected in all epithelial cells within the bronchioles and alveoli. In the kidneys (Figure 1H), epithelial cells showed strong nuclear UTF1 staining in both the distal and proximal tubules. Testicular tissue was used as a positive control as previously described (Kristensen *et al.* 2008) (Figure 1I).

To assess the UTF1 expression level within the epithelia, staining was evaluated using a semi-quantitative method. The results are shown in Figure 2 and Table 1. On the basis of the mean IHC score, these tissues could be classified into three categories (Figure 2): (a) tissues with weak UTF1 expression (score <3): breast prostate and endometrium; (b) tissues with weak to moderate UTF1 expression: bladder, colon, oesophagus and lung epithelium (score >3 and <6); and (c) tissues with strong UTF1 expression (score >6): kidney. Table 1 presents the statistical analysis of the staining intensity.

Expression of UTF1 with Sox2 and Oct4A in somatic epithelial cell lines

We investigated UTF1 expression in somatic epithelial cancer cell lines originating from various tissues. Positive and negative controls were, respectively, undifferentiated and differentiated (using all-trans-retinoic acid) human teratocarcinoma NCCIT cells. As shown in Figure 3, UTF1 was expressed in most of the tested cell lines although at different levels. Indeed, Ishikawa (endometrium), HCT116 (colon) and Detroit 562 (pharynx) cell lines showed high levels of UTF1 expression compared to NCCIT differentiated with retinoic acid. On the contrary, weak UTF1 expression was detected in DU146 (prostate) and EO33 (oesophagus). We also investigated the expression of Sox2 and Oct4A, which were reported to regulate UTF1 expression in stem cells (Nishimoto *et al.* 1999). As expected, with the exception of undifferentiated NCCIT, Oct4A was not detected in any of the analysed cell lines. However, in addition to NCCIT, Sox2 was also seen in Detroit 562, but not in the other cell lines.

Expression profile of UTF1 in human somatic epithelial cancers

We previously found that UTF1 overexpression is associated with cervical carcinogenesis (Guenin *et al.* 2012), and we therefore investigated whether UTF1 exhibited a specific pattern in the human epithelial tumours in comparison with

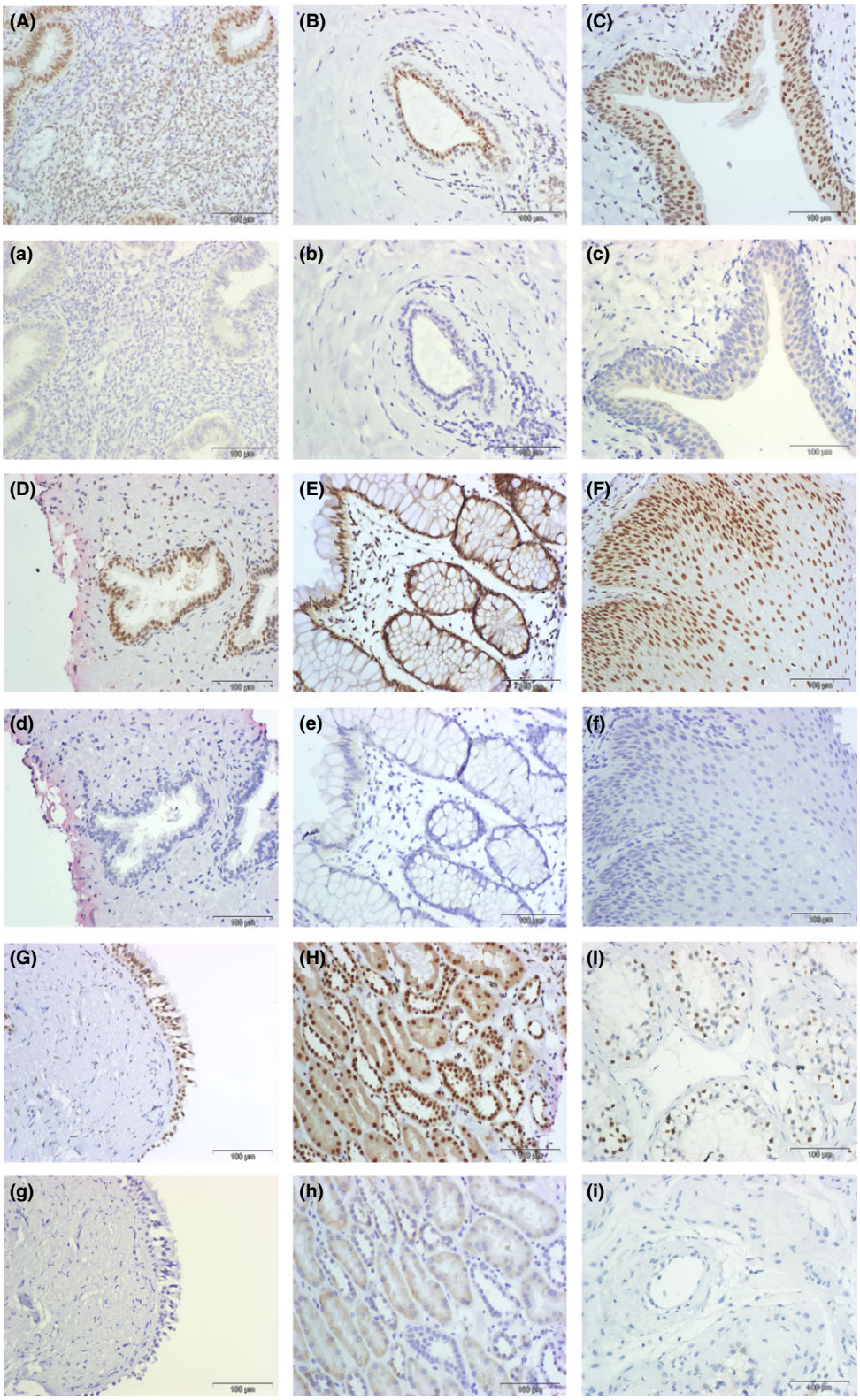

Figure 1 Immunohistochemical staining of undifferentiated cell transcription factor (UTF)1 in various normal human epithelia. UTF1 expression was detected in all tested tissues as shown here in representative images. (A) Endometrium, (B) Breast, (C) Bladder, (D) Prostate, (E) Colon, (F) Oesophagus, (G) Lung and (H) Kidney. Positive control (testis) is displayed in (I). Corresponding negative controls are shown in images indicated by lower case letters (a–i). Magnification ×200. Scale bar 100 μm.

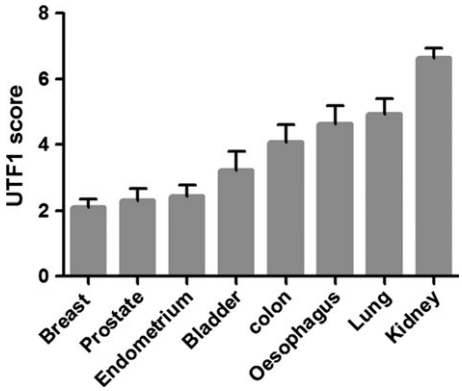

Figure 2 Undifferentiated cell transcription factor (UTF)1 expression profile in normal epithelia. UTF1 expression level was evaluated according to the semi-quantitative analysis described in the Materials and Methods section. Statistical analysis of staining is displayed in Table 1.

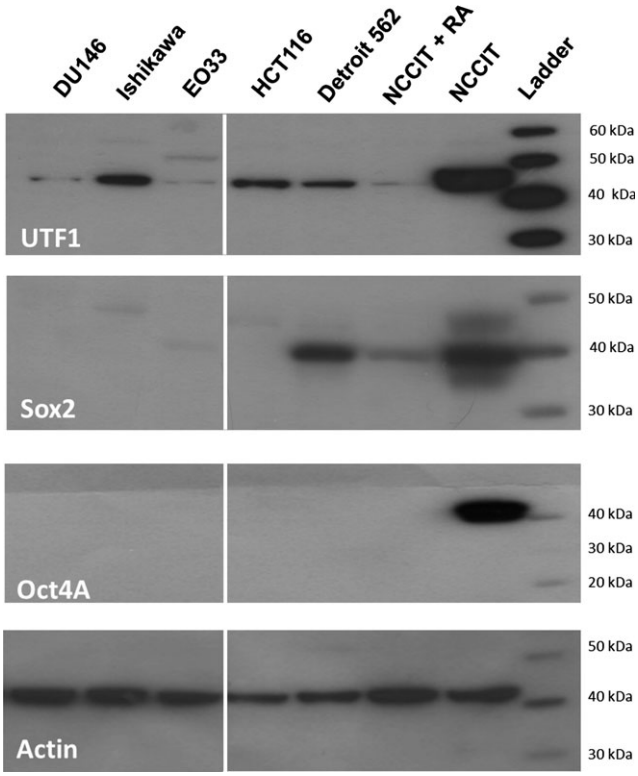

Figure 3 Western blot analysis of undifferentiated cell transcription factor 1, Sox2 and Oct4A expression in somatic epithelial cell lines. Actin was used as the loading control. White bars: line showing the removal of a lane from the original image of the gels that was not used for the purpose of this work.

their healthy counterparts. Thus, UTF1 expression was immunohistochemically analysed in epithelial tumour samples including 29 endometrioid adenocarcinomas, 9 breast carcinomas, 12 bladder transitional carcinomas, 10 prostatic adenocarcinomas, 12 primary colon adenocarcinomas, 14 oesophagus SCC, 8 lung SCC and 10 renal clear cell carcinomas. As shown in Figure 4, UTF1 immunoreactivity was seen in all tumour cases with the exception of one bladder and three colon samples. Representative images are displayed in Figure 4.

The semi-quantitative analysis of the staining in the tumour samples (Figure 5) revealed a significant increase in UTF1 expression in endometrial and prostatic adenocarcinomas ($P < 0.01$ and $P < 0.05$, respectively), whereas a significant decrease in UTF1 staining intensity was observed in colorectal adenocarcinomas ($P < 0.001$) and renal clear cell carcinomas ($P < 0.001$) in comparison with control tissues. The expression of UTF1 was similar in breast, bladder, lung and oesophageal tumours and controls.

Discussion

The specificity of some SC markers is currently under considerable debate. Indeed, numerous studies have reported the expression of some of them within normal somatic tissues, especially in epithelia. For example, Sox2 was found to be widely expressed in normal oesophageal (Gen *et al.* 2010), gastric (Otsubo *et al.* 2011) and lung epithelia (Wilbertz *et al.* 2011). A recent study also reported Oct4A expression by the epithelial cells within the human urogenital tract (Kristensen *et al.* 2010). Similarly, it was discovered that CD133 was not restricted to stem cells, as it was ubiquitously detected in differentiated epithelia in both murine and human colon mucosa (Shmelkov *et al.* 2008).

For many years, UTF1 was considered to be expressed exclusively in SC and gonadal cells in adults. Recently,

Table 1 Statistical analysis of undifferentiated cell transcription factor 1 staining in normal human epithelial tissue. Comparison between tissues was performed using one-way ANOVA followed by Tukey's multiple comparison test

P	Prostate	Endometrium	Bladder	Colon	Oesophagus	Lung	Kidney
Breast	ns	ns	ns	<0.05	<0.01	<0.001	<0.0001
Prostate		ns	ns	ns	<0.01	<0.001	<0.0001
Endometrium			ns	ns	<0.01	<0.001	<0.0001
Bladder				ns	ns	ns	<0.001
Colon					ns	ns	<0.05
Oesophagus						ns	ns
Lung							ns

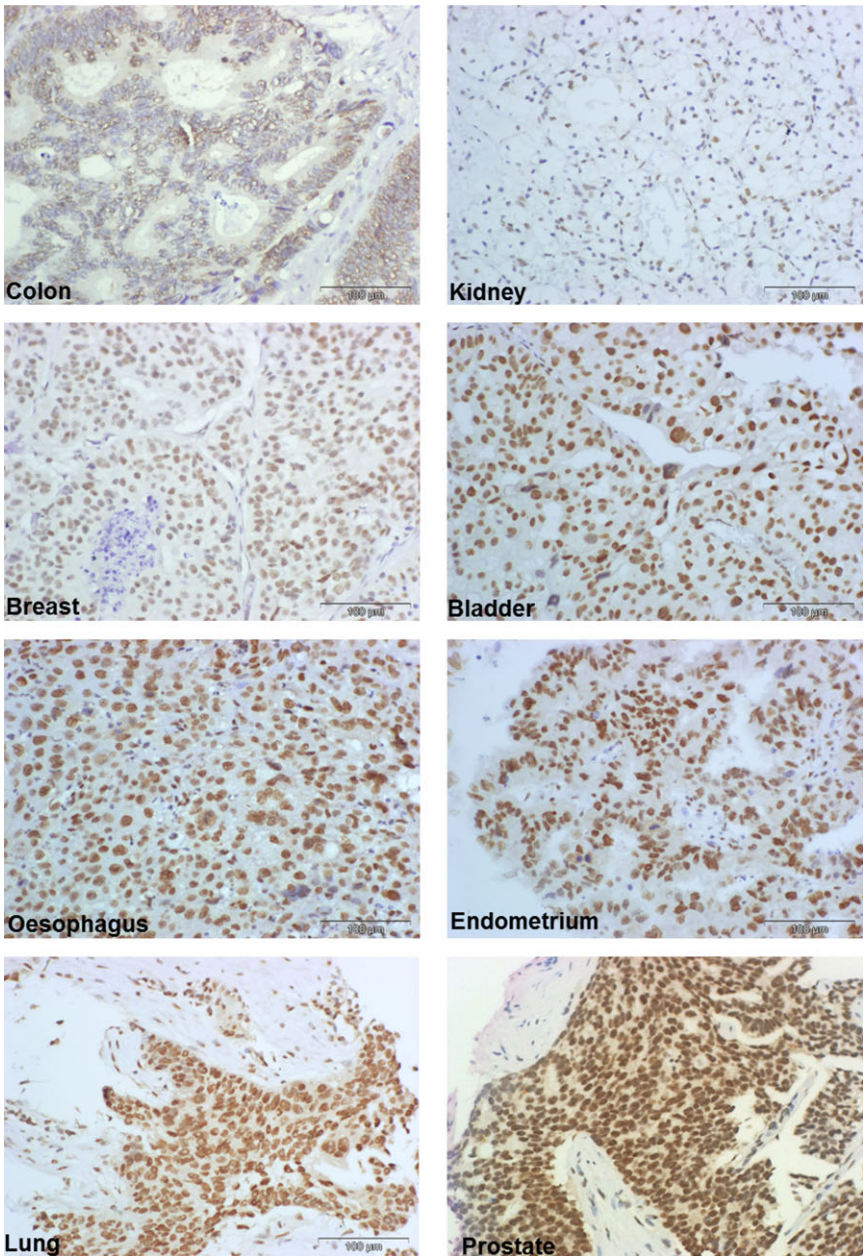

Figure 4 Undifferentiated cell transcription factor (UTF)1 expression in human epithelial tumours. Representative UTF1 immunostaining in tumour samples is shown in the main pictures, magnification ×200.

Kristensen and collaborators demonstrated the presence of UTF1 in normal epithelia from the human genital tract including the prostate, epididymis and seminal vesicle (Kristensen *et al.* 2010). Nevertheless, the authors did not discuss the fact that UTF1 protein could not be restricted to stem cells. More recently, Reinisch and collaborators demonstrated widespread UTF1 expression in normal skin epithelium, which was not restricted to skin SC. In that study, the authors questioned whether UTF1 is a skin SC marker and highlighted its role, not in SC fate determination, but rather in keratinocyte differentiation (Reinisch *et al.* 2011).

Recently, we showed that UTF1 was expressed in the normal cervical epithelium as well as in some cervical cell lines (Guenin *et al.* 2012). However, its expression and distribution patterns in other human tissues were not characterized. In the present study, we showed that UTF1 is widely expressed in normal human epithelial tissues, but, very rarely, in the stroma underlying the epithelial cells. Remarkably, UTF1 was differentially expressed according to the tissue type, at a markedly high level in the kidneys and at the lowest levels in breast, endometrium and prostate. In agreement with previously published data on ESC (van den Boom *et al.*

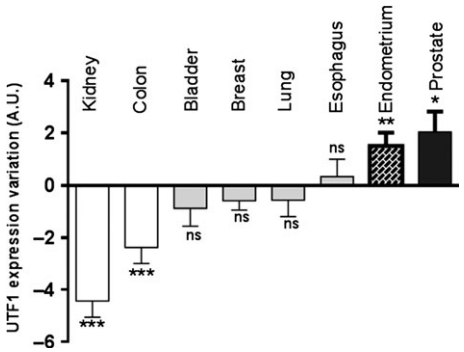

Figure 5 Variation in undifferentiated cell transcription factor (UTF)1 expression between tumours and normal epithelia. Semi-quantitative analyses of UTF1 expression in tumour samples were performed as described in the Materials and Methods section. The expression level of UTF1 in cancer was compared to that of normal tissues. Asterisks indicate statistically significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Student *t*-test).

2007), the immunostaining was almost always seen within the nucleus. In addition and consistent with the results found in skin epithelium (Reinisch *et al.* 2011), we noticed that UTF1 was more highly expressed in cells within the suprabasal layers. Such a pattern was clearly seen in the bladder, prostate and breast epithelia (Figure 1C and Supplementary Figure 1). These results suggest that UTF1 could be required at least for the initiation and progress of epithelial cell differentiation process. This hypothesis is supported by the fact that UTF1 depletion in ESC and ECC resulted in delayed or blocked differentiation (van den Boom *et al.* 2007).

Molecular analysis of UTF1 in epithelial cell lines demonstrated its expression at different levels. No differences in expression were found between EO33 or DU146 cells when compared to NCCIT differentiated cells. On the contrary, high UTF1 levels were seen in Ishikawa, HCT116 and Detroit 562 cells, which is consistent with results revealing the expression of UTF1 mRNA and protein in human somatic cell lines such as skin and cervical keratinocytes (Reinisch *et al.* 2011; Guenin *et al.* 2012). Interestingly, we found that UTF1 expression was not associated with that of Sox2 and Oct4A in any of the epithelial cell lines. Although the Detroit 562 cell line expressed Sox2, UTF1 expression did not seem to be related to that of Sox2. These observations strongly support the notion that UTF1 is likely to be regulated by factors other than Oct4A and Sox2. Indeed, UTF1 was detected in human spermatogonia in foetuses, children and postpuberty, but its expression was not associated with that of Sox2 and Oct4A (Wang *et al.* 2010). In addition, we previously suggested that methylation of the UTF1 promoter might play an important role in its expression (Guenin *et al.* 2012). However, the mechanisms underlying its regulation remain unknown. Thus, further molecular analyses are necessary to address this issue.

Many of the SC or pluripotency markers have been found to be involved in tumour development and/or progression. For example, it was shown that Sox2 was overexpressed in cervical, skin and penis tumours (Maier *et al.* 2011). Klf4, another stem cell marker, was reported to be upregulated in breast cancers (Foster *et al.* 2000). Furthermore, it has recently been shown that CD133 and CD44 were universally expressed in gastrointestinal stromal tumours (Chen *et al.* 2012).

In a pathological context, the analyses performed on ESC showed that UTF1 expression stimulated cell proliferation and increased teratoma-forming capability in nude mice (Nishimoto *et al.* 2005; Jia *et al.* 2012). Recent work investigated the expression level of UTF1 in some human cancers. Interestingly, it has been reported that UTF1 could be used as a diagnostic marker for seminoma and embryonal carcinoma of the testis (Wang *et al.* 2010). In somatic tumours, it was shown that UTF1 mRNA levels could serve to discriminate between stage I–III and IV neuroblastoma (Melone *et al.* 2009), and we have recently shown that UTF1 is upregulated during cervical carcinogenesis (Guenin *et al.* 2012).

Consistent with our results in cervical cancer, we found that UTF1 was significantly upregulated in endometrial and prostatic cancers, suggesting a potential role for this epigenetic regulator during genital tract carcinogenesis. It should be noted that UTF1 was also found to be significantly overexpressed in endometriosis (Forte *et al.* 2009). This pathology shares many cellular and molecular similarities with neoplastic development (Forte *et al.* 2009; Kokcu 2011). However, in a tumoral context, this is the first time that UTF1 has been linked to endometrial cancer. This also raised the possibility of putative hormonal regulation of UTF1 expression. This should be considered in further studies. In contrast, significant downregulation of UTF1 was found in colon and kidney tumours, suggesting a possible role in colon and renal carcinogenesis.

Altogether, these results suggest that UTF1 could play a dual role during carcinogenesis. Bivalent function was previously reported for some proteins that could act either as oncogenes or as tumour suppressors in a tissue-dependent manner. For example, although Sox2 was reported to be upregulated in several tumours, it was found to be downregulated in gastric cancer (Otsubo *et al.* 2008). This dual behaviour was seen for other markers such as Klf4, which is overexpressed in 70% of breast cancers, but inhibited in colorectal neoplasia (Foster *et al.* 2000; Patel *et al.* 2010). Such distinct expression patterns may be due to a histotype-specific function of the marker. Therefore, further cellular and molecular studies investigating, on the one hand, the role of UTF1 overexpression in genital carcinogenesis and, on the other hand, the role of its downregulation in renal and colon cancers could be of considerable interest.

In conclusion, we have shown, for the first time, that UTF1 is widely expressed in human epithelia with a nuclear localization. This distribution does not fit with the idea of its exclusive expression in stem cells and pinpoints a role of UTF1 in epithelial cell physiology. Moreover, we have shown that UTF1 is significantly upregulated in endometrial

and prostate cancers, but downregulated in kidney and colon cancers. These patterns indicate that UTF1 might play an important role in carcinogenesis but with relative tissue specificity. These findings open new perspectives in investigating the role of UTF1 in cell physiology and cancer.

Conflicts of interest

The authors declare no conflicts of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Decreased UTF1 expression in myoepithelial layers from prostate and breast.