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## ORIGINAL ARTICLE

# Increased expression of TRPV1 in squamous cell carcinoma of the human tongue

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**OBJECTIVES: Recent reports have unambiguously identified the presence and the growth-modulatory role of transient receptor potential vanilloid-1 (TRPV1), a central integrator of pain sensation, on numerous non-neuronal cell types and, of great importance, in certain malignancies. In this study, we have investigated the molecular expression of TRPV1 in the human tongue and its high-incidence malignant (squamous cell carcinoma, SCC) and premalignant (leukoplakia) conditions.**

**METHODS: Immunohistochemistry, Western blotting and quantitative 'real-time' Q-PCR were performed to define the expression of TRPV1.**

**RESULTS: A weak and sparse TRPV1-specific immunoreactivity was identified in the basal layers of the healthy human tongue epithelium. By contrast, we observed a dramatically elevated TRPV1-immunoreactivity in all layers of the epithelium both in precancerous and malignant samples. Furthermore, statistical analysis revealed that the marked overexpression of TRPV1 found in all grades of SCC showed no correlation with the degree of malignancy of the tumours. Finally, the molecular expression of TRPV1 was also identified in an SCC-derived cell line and was shown to be increased in parallel with the accelerated growth of the cells.**

**CONCLUSION: Collectively, our findings identify TRPV1 as a novel, promising target molecule in the supportive treatment and diagnosis of human tongue SCC.**
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**Keywords:** transient receptor potential vanilloid-1 (TRPV1); human tongue epithelium; human tongue squamous cell carcinoma; leukoplakia; overexpression

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## Introduction

Transient receptor potential vanilloid-1 (TRPV1), a member of the large TRP channel family, is a non-selective calcium-permeable cation channel which was originally described on nociceptive sensory afferents as a central integrator of pain sensation (Caterina *et al*, 1997; Tominaga *et al*, 1998). Indeed, TRPV1 can be activated by numerous exogenous and endogenous agents such as capsaicin (alkaloid of hot chilli peppers), resiniferatoxin (pungent compound isolated from *Euphorbia resinifera*), heat, low pH, inflammatory mediators, etc (Di Marzo *et al*, 2002; Ugawa *et al*, 2002). The activation of TRPV1 results in depolarization of the sensory afferents, firing of action potentials and hence the onset of pain sensation (Moran *et al*, 2004).

Recent reports, however, have unambiguously identified the presence of TRPV1 on numerous non-neuronal cell types as well. We and others have found that functional TRPV1 is expressed, for example, on various epithelial cells such as human skin keratinocytes (Denda *et al*, 2001; Inoue *et al*, 2002; Southall *et al*, 2003; Bodó *et al*, 2004, 2005), bronchial epithelium (Veronesi *et al*, 1999), urothelium (Birder *et al*, 2001; Lazzeri *et al*, 2004), cells of the gastrointestinal tract (Geppetti and Trevisani, 2004; Faussone-Pellegrini *et al*, 2005) as well as on mast cells (Bíró *et al*, 1998a), glial cells (Bíró *et al*, 1998b), etc. Moreover, it was also shown that the activation of TRPV1 on these cells may result in changes e.g. in proliferation, apoptosis, differentiation and/or cytokine release (Bíró *et al*, 1998a; Veronesi *et al*, 1999; Birder *et al*, 2001; Bodó *et al*, 2004; Lazzeri *et al*, 2004).

In relation to these mostly *in vitro* functional data on regulation of cell growth, it is also of great importance that TRPV1 is expressed at various levels in certain malignancies (Prevarskaya *et al*, 2007). For example, elevated TRPV1 expression was identified in carcinomas of the human prostate (Sanchez *et al*, 2005), colon (Domotor *et al*, 2005), pancreas (Hartel *et al*, 2006), or urinary bladder (Lazzeri *et al*, 2005). Moreover, certain

data also indicate that the level of TRPV1 may alter in relation to the degree of malignancy; e.g. positive (prostate cancer) (Sanchez *et al*, 2005) or negative [bladder carcinoma (Lazzeri *et al*, 2005), glioma (Amantini *et al*, 2007)] correlations were equally found with increasing grades of the respective tumours.

Although sparse reports indicate that dietary capsaicin may inhibit tongue carcinogenesis in rats, intriguingly, we lack data on the existence of the 'capsaicin receptor' TRPV1 on structures of the oral cavity which comprise the primary 'target' of the regularly consumed TRPV1 agonist capsaicin (Tanaka *et al*, 2002). Therefore, in this study, by focusing on the human tongue, we investigated the expression of the molecule in the epithelial cells of the organ. Moreover, we also defined the putative alterations in the level of TRPV1 in human squamous cell carcinoma (SCC) of the tongue which comprises one of the highest incidence malignancies of the oral cavity (indeed, it accounts for 20–50% of all oral cavity neoplasias with a 5-year mortality rate of approximately 50% and a poor survival index that has not changed significantly in the past half-century) (Byers *et al*, 1998; Nagler *et al*, 2007; Molina *et al*, 2008).

## Methods

*Human tissues*

The study was approved by the Institutional Research Ethics Committee and written consent was obtained from all patients (Table 1). Seven normal adult (healthy) tongue epithelial tissue samples were obtained for routine diagnosis. The control patients had no history of pre- or malignant oral mucosal lesions. Eight epithelial leukoplakia lesions and 18 tongue SCC samples were involved in the study and were verified by histopathological evaluations by expert pathologists. Neither the leukoplakia patients nor the SCC patients had the previous or contemporary oral malignancies.

*Human tissue sample preparation*

In general, the fresh tissue specimens were divided into two parts (Varga *et al*, 2004). One part of the samples was fixed in 4% paraformaldehyde, embedded in paraffin and processed for histopathology grading and for immunohistochemistry (see below). The second part was frozen in liquid nitrogen and to collect tissue parts containing epithelial tissue-enriched samples (and, therefore, free of non-epithelial tissues such as muscle or connective tissue, which may contain TRPV1) (Miyamoto *et al*, 2005; Cavuoto *et al*, 2007), the samples were serially cut using a cryomicrotome starting from the surface of the tumour until the lamina propria was reached (This was verified by serial haematoxylin–eosin stained sections as described in our earlier reports) (Varga *et al*, 2004). The sections were then collected on ice and were processed for either quantitative 'real-time' PCR (Q-PCR) or Western blot analysis (see below). Unfortunately, the volume of the leukoplakia specimens was so limited that we could perform only immunohistochemistry on these samples.

**Table 1** Histopathological and clinical information about studied samples

| *Histopathological diagnosis* | *Case ID no.* | *Age, year (mean, range)* | *Sex (men/women)* |
|---|---|---|---|
| Normal tissue | 1–7 | 56 (51–60) | 4/3 |
| Leukoplakia | 8–15 | 48 (42–59) | 5/3 |
| Well-differentiated SCC (grade 1) | 16–22 | 53 (42–60) | 4/3 |
| Moderately differentiated SCC (grade 2) | 23–31 | 61 (49–69) | 6/3 |
| Poorly differentiated SCC (grade 3) | 32–33 | 58 | 2/0 |

SCC, human tongue squamous cell carcinoma.

*Immunohistochemistry*

The expression of TRPV1 was determined using horseradish-peroxidase (HRP) based method using diaminobenzidine (DAB) as a chromogene. In brief, paraffin-embedded sections (5 $\mu$m), after antigen retrieval (in citrate-buffer, pH 6.0, at 750 W in microwave oven for 10 min), were first incubated with a primary rabbit anti-TRPV1 antibody which recognizes the C-terminus of TRPV1 (1:1000; Sigma-Aldrich, St Louis, MO, USA). Sections were then incubated with a goat anti-rabbit HRP-polymer-conjugated secondary antibody (EnVision kit; DAKO, Glostrup, Denmark). Immunoreactions were finally visualized using DAB-substrate (EnVision kit DAKO) and the sections were counterstained by haematoxylin (Sigma-Aldrich).

To assess specificity of the immunostaining, primary labelling was also performed using another set of antibodies (both from Santa Cruz, Santa Cruz, CA, USA); i.e. rabbit anti-TRPV1 *vs* the N-terminus of TRPV1 (H-150, sc-20813, 1:50 dilution) and goat C-terminus-specific anti-TRPV1 (D-20, sc-12502, 1:50 dilution). The application of these latter primary antibodies resulted in identical staining patterns (data not shown). In addition, for negative controls of the labelling procedure, antibodies were either omitted from the procedure or were preabsorbed by control blocking peptides provided (along with appropriate protocols) by the manufacturers. For positive controls, human skin (Bodó *et al*, 2004, 2005) and prostate tissues (Sanchez *et al*, 2005) were employed (data not shown).

*Image analysis*

Immunohistochemical images were captured and digitalized using an RT Spot Colour CCD camera (Diagnostic Instruments Inc., Sterling Heights, MI, USA) integrated on a Nikon Eclipse 600 fluorescence and light microscope (Nikon, Tokyo, Japan). Digitalized images were then analysed using Image Pro Plus 4.5 (Media Cybernetics, Bethesda, MD, USA) image analysis software as detailed in our previous reports (Bodó *et al*, 2004, 2005). The intensity of TRPV1-ir was measured at 10 randomly placed, equally areas of interest (AOI) and the average of immunopositive pixels of the 10 AOI (expressed as mean ± s.e.m.) was determined (Bodó *et al*, 2005).

*Cell culturing*
The CAL27 cell line (Gioanni *et al*, 1988), derived from human tongue SCC, was purchased from LGC Promochem (Wesel, Germany). Cells were cultured in Dulbecco's Modified Eagle's Medium (Sigma-Aldrich) supplemented with 10% foetal bovine serum (Invitrogen, Paisley, UK), 2 mM Glutamine (Sigma-Aldrich), 50 U $ml^{-1}$ penicillin and 50 $\mu g$ $ml^{-1}$ streptomycin (both from Biogal, Debrecen, Hungary). Medium was changed every other day and cells were subcultured at 80% confluence.

*Immunocytochemistry*
CAL27 cells growing on glass cover slips were washed with phosphate-buffered saline, fixed in acetone for 5 min at 4°C, air dried and blocked at room temperature for 30 min in a blocking solution containing 0.6% Triton X-100 and 1% bovine serum albumin (all from Sigma-Aldrich). Cells were first incubated with the appropriate rabbit anti-TRPV1 antibody for 1 h (1:1000; Sigma-Aldrich) and then with a fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (1:300; Vector, Burlingame, CA, USA) for 1 h. Cell nuclei were counterstained by 4,6-diamidino-2-phenylindole (DAPI) (Vector). Confocal microscopy images were acquired using a Zeiss LSM 510 microscope (Oberkochen, Germany) and images were stored for further analysis (Varga *et al*, 2004; Czifra *et al*, 2006).

*Western blotting*
Tissues and cells were homogenized in lysis buffer (20 mM Tris-Cl, pH 7.4, 5 mM EGTA, 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, 20 $\mu$M leupeptin, all from Sigma-Aldrich) and the protein content of samples was measured by a modified BCA protein assay (Pierce, Rockford, IL, USA). The samples were subjected to SDS-PAGE (8% gels were loaded with 120 $\mu g$ protein per lane), transferred to nitrocellulose membranes (BioRad, Vienna, Austria), and then probed with the above primary anti-TRPV1 antibody (1:1000, Sigma-Aldrich). HRP-conjugated secondary antibodies (EnVision; DAKO) were then employed and the immunoreactive bands were visualized by enhanced chemiluminescence (Pierce). To assess equal loading (and to obtain an endogenous control), membranes were stripped in 200 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 2% SDS and 0.1 $\beta$-mercaptoethanol (all from Sigma-Aldrich) at 65°C for 1 h and were re-probed with a $\beta$-actin antibody (1:100; Santa Cruz) followed by a similar visualization procedure as described above. To quantitatively assess the immunosignals, immunoblots were finally subjected to densitometric analysis using an Intelligent Dark Box (Fuji, Tokyo, Japan) and the Image Pro Plus 4.5.0 software (Media Cybernetics). The values of the densitometric analysis in several independent experiments were normalized to the average of the immunosignal of the controls and expressed as mean ± s.e.m. (Varga *et al*, 2004; Czifra *et al*, 2006).

*Quantitative 'real-time' Q-PCR*
The Q-PCR was carried out on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) by using the 5′ nuclease assay according to our previous reports (Bodó *et al*, 2005; Griger *et al*, 2007). Briefly, frozen tissues were pulverized under liquid $N_2$ and total RNA was isolated using TRIzol (Invitrogen). Three micrograms of total RNA were then reverse transcribed into cDNA by using 15 units of AMV reverse transcriptase (Promega, Madison, WI, USA) and 0.025 $\mu g$ $\mu l^{-1}$ random primers (Promega). PCR amplification was carried out by using the TaqMan primers and probes (Assay ID: Hs00218912_m1 for human TRPV1) using the TaqMan Universal PCR Master Mix Protocol (Applied Biosystems). As internal controls, transcripts of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were determined (Assay ID: Hs99999905_m1 for human GAPDH), and the amount of TRPV1 transcripts were normalized to those of GAPDH using the ΔΔCT method.

*Statistical analysis*
Statistical analysis was carried out using SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA). For statistical analysis, a two-tailed un-paired *t*-test was employed and $P < 0.05$ values were regarded as significant differences.

## Results

Using immunohistochemistry, a minor TRPV1-specific (see negative controls in Figure 1a⁄xi,a⁄xii) immunoreactivity (ir) was identified on epithelial cells of healthy (control) human tongue. On the ventral surface of the tongue, this faint immunosignal was exclusively localized to the most upper layers of the stratum (str.) superficiale (Figure 1a⁄i,a⁄ii). However, on the specialized epithelium of the dorsal surface of the tongue, mostly intracellular TRPV1-ir (with characteristic granular pattern) was found in the basal epithelial cells of the str. basale (Figure 1a⁄iii,a⁄iv). By contrast, neither the cells of the lamina propria nor of the submucosa showed immunoreaction.

Intriguingly, the epithelium of the premalignant leukoplakia samples exhibited an intense and characteristics TRPV1-ir when compared with the healthy tissues (Figure 1a⁄v,a⁄vi). On all cells of the str. basale and str. spinosum, TRPV1-ir was clearly localized to the cell membrane. Furthermore, this immunopositivity was also identified on degenerated cells of the str. superficiale as well as on the hyper-orthokeratotic surface.

On human SCC samples, on tumour epithelial cells infiltrating the submucosa, we observed a markedly increased TRPV1-ir when compared with the controls (Figure 1a⁄vii,a⁄viii). It was also evident that besides the weak surface membrane-localized immunosignals, the dominant TRPV1-specific staining pattern (i.e. intracellular⁄granular localization) highly resembled to those found on cells on the str. spinosum in the control tissues.

Investigation of the epithelium surrounding the tumour invasion revealed another intriguing phenomenon. Namely, on this thickened epithelium which still possesses the characteristic morphological appearance of that of the 'healthy' tissues, TRPV1-ir was also dramatically increased (when compared with the controls) on all layers

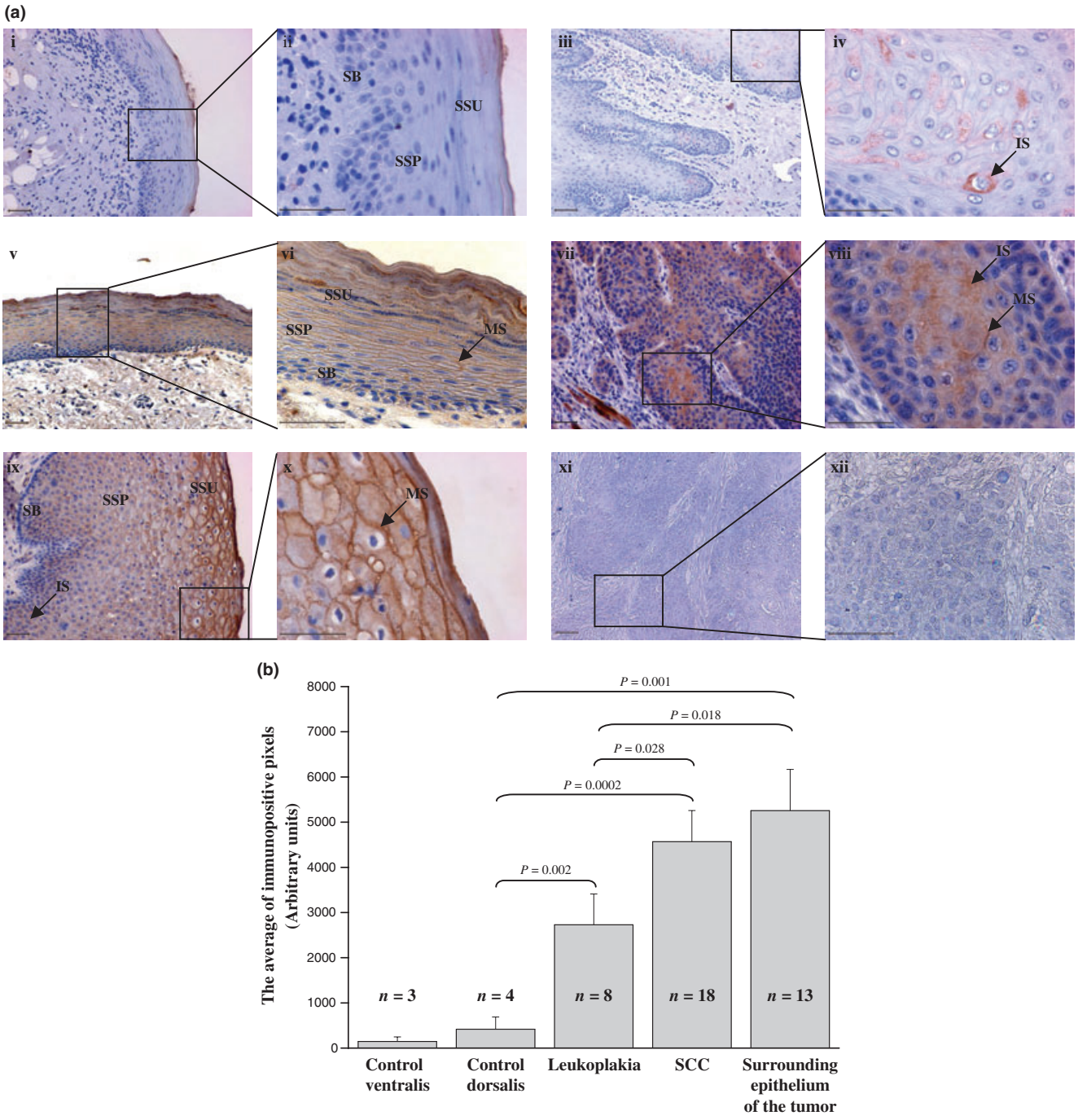


**Figure 1** *Localization of transient receptor potential vanilloid-1 (TRPV1) in healthy, premalignant (leukoplakia) and squamous carcinoma of the human tongue*. (**a**) TRPV1-specific immunoreactivity (ir) with diaminobenzidine as a chromogene (brown staining) on healthy and diseased human tongue samples. Nuclei were counterstained by haematoxylin. Ventral (i, ii) and dorsal (iii, iv) surfaces of the healthy tongue. (v, vi) Leukoplakia. (vii, viii) Squamous cell carcinoma; tumour epithelial cell invasion of the submucosa. (ix, x) Epithelium surrounding the tumour invasion. (xi, xii) Preabsorption negative control on tumour sections. Scale bars, 50 $\mu$m. MS, membrane staining, IS, intracellular staining, SB, str. basale, SSP, str. spinosum, SSU, str. superficiale. (**b**) Digitalized images obtained on numerous samples (*n* values) were analysed using Image Pro Plus 4.5 image analysis software (see Methods). The intensity of TRPV1-ir was measured at 10 randomly placed, equal areas of interest (AOI) and the average (expressed as mean ± s.e.m.) of immunopositive pixels of the 10 AOI was defined. *P* values were determined using two-tailed un-paired *t*-test

of the epithelium (Figure 1a/ix,a/x). Basal cells of the str. basale and str. spinosum, similar to described above, exhibited intracellular/granular staining pattern. However, TRPV1-ir was rather localized to the cell membrane on cells of the more apical (str. spinosum and superficiale) layers. Moreover, the intensity of TRPV1-ir gradually increased towards the surface and reached in maximal values in the upper layers of the str. superficiale.

The intensity of immunosignals was then quantitated by image analysis software (as detailed under Methods). Comparison of the average intensity values revealed that TRPV1-ir was markedly and significantly higher in

sections from leukoplakia ($P = 0.002$), at the site of SCC invasion ($P = 0.0002$) and on the superficial epithelium surrounding the tumours ($P = 0.001$) when compared with those of the dorsal epithelium of the control samples (which exhibited the strongest immunosignals on the healthy tongue, Figure 1a′iii,a′iv). In addition, mutual comparison of the diseased samples also defined significant differences between TRPV1-ir values of the precancerous leukoplakia and the SCC samples ($P = 0.028$) and of the epithelium surrounding the tumours ($P = 0.018$) (Figure 1b).

Although these results clearly indicated the overexpression of TRPV1 in human tongue SCC, because of the rather semi-quantitative nature of the above technique, we also investigated the level of TRPV1 in SCC samples using Western blot (followed by quantitative densitometry analysis) and Q-PCR techniques. These two complementary techniques concordantly revealed that the expression of the TRPV1-specific mRNA transcripts and protein, yet exhibiting marked inter-individual variations, was higher in all tumour samples investigated (Figure 2a,b). Statistical analysis of densitometry and Q-PCR values of all SCC samples indicated that this elevation was significantly different in the grade 1 ($P = 0.005$ for Western blot, $P = 0.0002$ for Q-PCR) and grade 2 ($P = 0.003$ for Western blot, $P = 0.001$ for Q-PCR) SCC groups, when compared with the controls (Figure 2c). Although (at least) a similar increase in TRPV1 expression was also identified in grade 3 tumour samples, the low number of the available tumour samples ($n = 2$) made it impossible to perform statistical analysis. Of further importance, we found statistically insignificant differences when respective values of the tumour samples with various grades were mutually compared with one another (Figure 2c).

Finally, we investigated the expression of TRPV1 at the cellular levels using the human SCC-derived cells line CAL27. As assessed by immunocytochemistry followed by confocal microscopy analysis, TRPV1-ir was localized both to the surface membrane and in the cytoplasm of the cells (Figure 3a), similar to tumour cells *in situ* (see Figure 1a′vii,a′viii). In addition, quantitative Western blot and Q-PCR analyses also revealed that the expression of TRPV1 (both at the protein and mRNA levels) significantly and, of importance, gradually increased in parallel to the accelerated growth rate (hence the confluence) of the cell cultures (Figure 3b,c).

## Discussion

Dietary capsaicin primarily acts on cells of the gastrointestinal tract. The previous studies have clearly identified the existence the 'capsaicin receptor' TRPV1 on parietal cells of the human stomach (Faussone-Pellegrini *et al*, 2005) and on certain epithelial cell types of this organ system (Ward *et al*, 2003; Geppetti and Trevisani, 2004; Domotor *et al*, 2005). However, to our best knowledge, our current study provides the first evidence that TRPV1 is expressed (both at the mRNA and protein levels) on the primary 'target' of capsaicin, i.e. on epithelial cells of the human tongue.

Mutually complementary immunohistochemical, Western blot and Q-PCR analyses have also shown that the relatively low level of TRPV1 expression

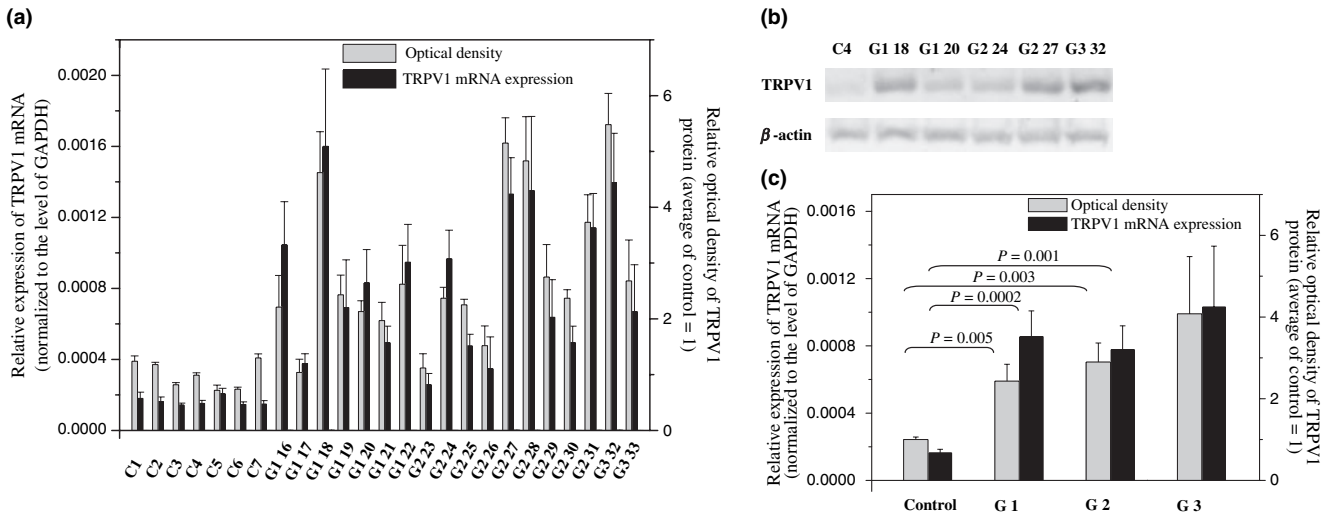


**Figure 2** *Expression of transient receptor potential vanilloid-1 (TRPV1) is increased in squamous carcinoma of the human tongue.* (**a**) Epithelial tissues of normal tongue (control, C) and squamous carcinomas of the human tongue with various tumour grades (G1–G3) were collected, processed as described under Methods and subjected to Western blot (followed by densitometry analysis) and Q-PCR to determine TRPV1 expression. During Western blot, the amount of TRPV1 was quantitated using densitometry in triplicates and normalized to those of $\beta$-actin. Panels represent mean ± s.e.m. values compared with the average of the control samples (C1–C7) defined as 1. During Q-PCR, data of TRPV1 expression (obtained in three triplicate determinations of each sample) were normalized to the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) of the same sample and are expressed as mean ± s.e.m. (**b**) Representative Western blot data of several determinations yielding similar results of control (sample no. 4) and on squamous carcinomas with grade (G) 1 (samples no. 18, 20), G2 (samples no. 24, 27) and G3 (sample no. 32). Equal loading was assessed by determining the expression of $\beta$-actin. (**c**) Statistical analysis of Western blot and Q-PCR data presented in panel A. *P* values were determined using two-tailed un-paired *t*-test

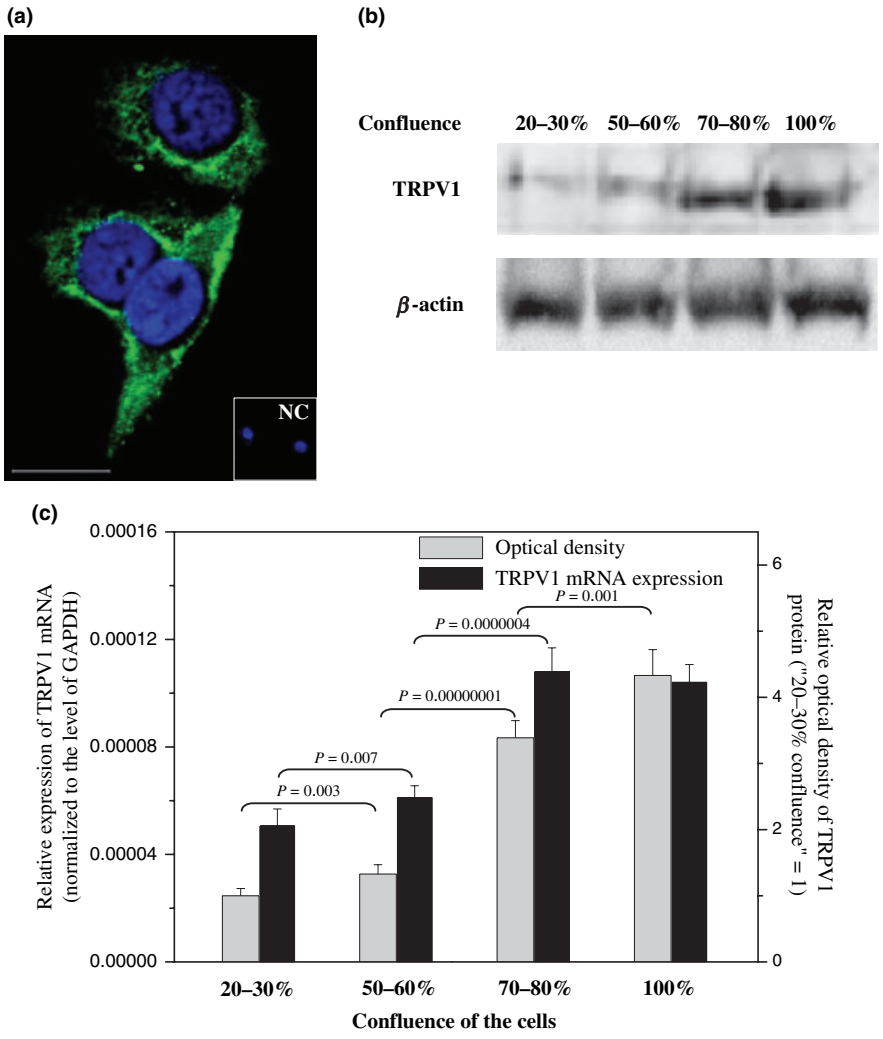


**Figure 3** *Transient receptor potential vanilloid-1 (TRPV1) is expressed on cultured human tongue squamous carcinoma-derived CAL27 cells and its level alters in parallel to the proliferation of the cells*. (**a**) TRPV1 immunoreactivity on CAL27 cells (green fluorescence), as visualized by confocal microscopy. Nuclei were counterstained by 4,6-diamidino-2-phenylindole (DAPI) (blue fluorescence). Inset, preabsorption negative control (NC). Scale bar, 10 $\mu$m. (**b**) Western blot analysis of TRPV1 expression on cell lysates of CAL27 cells harvested at various confluences. Equal loading was assessed by determining the expression of $\beta$-actin. (**c**) Statistical analysis of Western blot and Q-PCR analysis performed on CAL27 cells harvested at various confluences. During Western blot, in each sample, the amount of TRPV1 was quantitated by densitometry and normalized to those of $\beta$-actin; optical density value of the '20–30% confluence' sample was defined as 1. During Q-PCR, data of TRPV1 expression were normalized to the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) of the same sample and are expressed as mean ± s.e.m. of three independent determinations. Three additional experiments yielded similar results. *P* values were determined using two-tailed un-paired *t*-test

(localized mostly to the basal layers of the epithelium in healthy tongue tissues) was markedly increased in all grades of human tongue SCC samples. These intriguing data suggested that TRPV1 – similarly to findings on other epithelial cells such as human skin keratinocytes (Denda *et al*, 2001; Inoue *et al*, 2002; Southall *et al*, 2003; Bodó *et al*, 2004, 2005), bronchial epithelium (Veronesi *et al*, 1999) and urothelium (Birder *et al*, 2001; Lazzeri *et al*, 2004) – may participate in the growth control of the cells. This idea was further supported by showing that the expression of TRPV1 gradually increased with the accelerated growth rate of the human tongue SCC-derived cell line CAL27. Although further (both *in vitro* and *in vivo*) studies are invited to clarify the growth-modulatory role of TRPV1 (similar to the work of Tanaka *et al* (2002) suggesting that dietary capsaicin may inhibit tongue carcinogenesis in rats), our findings identify TRPV1 as a novel, promising target molecule in the putative supportive treatment of human tongue SCC.

Intriguingly, the above evaluations have also revealed that the elevated TRPV1 expression in SCC tissues of all grades did not correlate with the degree of malignancy of the tumours. It appears therefore, that in contrast to findings on prostate (Sanchez *et al*, 2005) carcinomas as well as on gliomas (Amantini *et al*, 2007) where definite correlations were described, TRPV1 may not serve as a prognostic factor in the clinics of human tongue SCC.

Nonetheless, several lines of evidence demonstrate that TRPV1 may rather act as a novel diagnostic molecule in human tongue transformation. For example, in the current study, we also present that TRPV1 is highly overexpressed already in the grade 1 (low malignancy) SCC group. Moreover, of further importance, markedly elevated levels of TRPV1 were identified in the precancerous leukoplakia samples and also in the 'healthy' epithelium surrounding the tumour invasion. These data suggest that the overexpression of the molecule may be a relatively early step in the process of tumour genesis; hence, determination of TRPV1 levels may hold out a promise for the benefits of early diagnosis.

Finally, we have also observed that – similar to other neuronal and non-neuronal cell types such as e.g. sensory neurons (Eun *et al*, 2001), mast cells (Turner *et al*, 2003), various skin cells (Bodó *et al*, 2004, 2005), hepatoblastoma cells (Vriens *et al*, 2004; Waning *et al*, 2007), – the specific TRPV1-ir was not restricted to the

plasma membrane of the cells but intracytoplasmic staining patterns were also found. Moreover, here we also show that the subcellular localization pattern of the receptor is markedly different in the various diseased samples; e.g. mostly intracytoplasmic staining in the healthy epithelium and in the submucosal SCC islets whereas prominent surface membrane TRPV1-ir in the leukoplakia samples, in the more superficial layers of the SCCs and in the epithelium surrounding the SCC (see Figure 1). Although further studies are invited to define the exact functional role of the intracellular TRPV1 in SCC-derived cells, these data demonstrate that TRPV1 may have a central role in the transformation of the epithelium of the human tongue leading to unwanted growth. This hypothesis is supported by the previous findings showing that the intracellularly localized TRPV1 indeed functions as Ca-release channel and hence may act as a key regulator of cell morphology, viability and migration (Vriens *et al*, 2004; Han *et al*, 2007; Nilius *et al*, 2007; Waning *et al*, 2007).

Collectively, our current findings identify TRPV1 as a novel, promising target molecule in the supportive treatment and diagnosis human tongue SCC.

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## Conflict of interest

The authors declare no competing financial interests.

## Author contributions

Rita Marincsák and Tamás Bíró contributed to the research design. Rita Marincsák, Balázs I. Tóth , Gabriella Czifra contributed to the acquisition and analysis of data. Rita Marincsák and Tamás Bíró contributed to the interpretation of data. Pál Rédl and Ildikó Tar contributed to the collection of the human samples and László Tóth contributed to the hystopathological analysis of these samples. Rita Marincsák and Tamás Bíró contributed in drafting the paper. Ildikó Márton and László Kovács worked on the critical revision of the paper.

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