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Original Paper

Significant Increase of a Specific Variant *TSG101* Transcript during the Progression of Cervical Neoplasia

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The human tumour susceptibility gene *TSG101* has recently been identified on chromosomal locus 11p15.1-15.2 which is frequently affected by genetic alterations in neoplastic lesions of the uterine cervix. Aberrant transcripts of the *TSG101* gene have been reported in various tumour entities, including breast, ovarian and prostate cancers, but also in several non-neoplastic tissues. We analysed *TSG101* transcription by reverse transcription–polymerase chain reaction (RT–PCR) in a total of 139 clinical samples of cervical tissues and in cervical carcinoma cell lines. Variant transcripts were observed in all cell lines, in 69 of 122 (57%) cervical dysplasia and carcinoma samples and in five of 17 (29%) normal cervical tissues. One specific variant *TSG101* transcript (*Δ154-1054*) was detected with a significantly increased frequency in advanced preneoplastic cervical lesions. However, the relative abundance of variant *TSG101* transcripts appeared to be generally low, as only wild-type, but no variant transcripts were detectable in Northern blot analyses of cervical carcinoma cell lines. These data point to a progressive loss of stringent splice control functions or to extended alternative splicing in advanced dysplasia and neoplasia. The relative amounts of variant transcripts do not support a major functional role of *TSG101* variants in cervical carcinogenesis. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: ***TSG101* transcription, cervical dysplasia, neoplasia**
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INTRODUCTION

CANCER OF the uterine cervix emerges through progression of preneoplastic dysplastic lesions of the cervical epithelium (referred to as CIN I, II, III) which are induced by persistent infections with specific high-risk human papillomavirus (HPV) types [1, 2]. Besides the aetiological role of specific HPV types, additional genetic alterations seem to be required for the development and progression of cervical lesions. Alterations at chromosomal loci 3p, 6p, 11q, 11p and 18q are frequently detected in cervical carcinoma cells, pointing to the inactivation of putative tumour suppressor genes in these genomic regions [3–8]. The transfer of intact chromosome 11 by microcell fusion has resulted in the suppression of the oncogenic phenotype of two cell lines derived from cervical carcinomas [9, 10]. Detailed analyses of these hybrid cells have provided further evidence for the existence of cellular genes on chromosome 11p11-15 which might regulate the HPV16 early enhancer promotor [11]. In summary, these observations suggest the possible location of a tumour suppressor gene on chromosome 11p11-15.

Recently, the human *TSG101* gene has been mapped to chromosome 11p15.1-p15.2 [12]. Detailed analyses of the amino acid sequences of both the human TSG101 protein and its murine homologue have revealed that the amino terminal part of TSG101 resembles a conserved domain with homology to a class of apparently inactive homologues of ubiquitin-conjugating enzymes [13, 14]. Homozygous inactivation of the *tsg101* gene in mouse 3T3 fibroblasts has resulted in cellular transformation and in the ability to form

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metastatic tumours in nude mice [15]. Further analyses have indicated that deficiency of the murine *tsg101* gene might result in genomic instability [16].

Several reports have shown that the human *TSG101* gene is aberrantly transcribed in various tumour entities [12, 17–20]. Large intragenic deletions have been reported for breast cancer samples which might explain the generation of truncated *TSG101* transcripts [12]. However, recently this initial finding of major genomic deletions as analysed by long-distance genomic polymerase chain reaction (PCR) has been shown to be unreproducible [21]. Southern blot analyses of large sets of primary breast carcinoma and other clinical tumour samples did not confirm the presence of major genomic alterations at the *TSG101* locus [17, 18, 22]. Moreover, variant *TSG101* transcripts were also detected in various normal tissues, although with a lower frequency [17, 19, 22]. These observations suggest that either alternative splicing or relaxation of the splicing control might be responsible for the occurrence of truncated *TSG101* transcripts. Most recently, a detailed sequence analysis of the genomic structure of the mouse *tsg101* gene revealed the presence of additional introns within the N-terminal part of the murine gene [23]. By comparing the genomic structure of the mouse *tsg101* gene with its human homologue, Wagner and colleagues [23] predicted the existence of four additional introns in a region of the human *TSG101* gene which was considered to represent the first exon [12]. This finding might further explain the molecular mechanisms leading to the generation of the variant *TSG101* transcripts and, thus, corroborates their authenticity.

In this study, we investigated the transcript pattern of the human *TSG101* gene in clinical samples comprising normal cervical tissues, low grade and high grade preneoplastic lesions (CIN I–III) and primary cervical carcinomas, as well as various cervical cancer cell lines. Furthermore, we analysed the relative abundance of variant *TSG101* transcripts in cervical cancer cell lines by Northern blot experiments.

MATERIALS AND METHODS

Tissue samples and tumour cell lines

Tissue samples obtained from surgical specimens or punch biopsies were divided into two. One part was kept for histological evaluation the other was immediately snap frozen in liquid nitrogen and stored at − 80°C. Slices of approximately 30 μm thickness were used for microdissection of dysplastic and neoplastic tissues. Four human cervical tumour cell lines, containing DNA from HPV type 16 (SiHa), 18 (HeLa, SW756) or 68 (Me180), one HPV negative cervical tumour cell line (C33a) and one human keratinocyte cell line derived from sun-exposed skin (HaCaT) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum.

RNA extraction and reverse transcription–PCR (RT–PCR)

Total RNA was extracted using a commercial RNA isolation system (GlassMax, Life Technologies GibcoBRL, Karlsruhe, Germany). RT of 1 μg of total RNA was performed with 200 U of M-MLV reverse transcriptase (Superscript II, Life Technologies GibcoBRL) and 25 μM of the *TSG101*-specific RT primer [12] in a 20 μl reaction. After initial denaturation of the RNA samples for 10 min at 70°C, the RT reaction was performed at 42°C for 60 min. The reaction was stopped by inactivating the enzyme at 90°C for 5 min. PCR amplification of *TSG101* transcripts was performed as described, using primers P1 to P4 [12]. All PCR reactions were carried out at least twice using independent RT reactions.

Southern and Northern blot hybridisation

A cDNA probe comprising nucleotides 141–1214 of the cDNA sequence of *TSG101* (GenBank entry U82130) was used for the hybridisation analysis of the PCR fragments after Southern blot transfer to Nylon membranes (HybondN+, Amersham Life Science, Buckinghamshire, U.K.). A chemiluminescence-based labelling and detection system (ECL, Amersham Life Science) was used according to the recommendations of the manufacturer. For Northern blot analysis, 10 μg of total RNA from the carcinoma cell lines was hybridised with [^{32}P] labelled *TSG101*-specific cDNA according to the protocol described by Church and Gilbert [24].

DNA sequencing

PCR fragments were isolated from agarose gels, purified (Qiaquick Gel Extraction Kit, Qiagen, Hilden, Germany) and subsequently ligated into vector pCR2.1 (Invitrogen, San Diego, California, U.S.A.). DNA sequencing was performed using a Cy5′-AutoRead sequencing kit (Pharmacia Biotech, Freiburg, Germany) and an ALFexpress DNA sequencing device (Pharmacia Biotech).

Statistical analysis

Fisher's exact test was performed using the software package S-PLUS (version 3.4, MathSoft Inc.). The results were defined as statistically significant when the P value was less or equal to 0.05.

RESULTS

TSG101 transcription was analysed by nested RT–PCR [12] in a total of 139 samples of normal cervical epithelium, preneoplastic lesions and cervical carcinoma specimens as well as in cell lines derived from cervical cancers (C33a, HeLa, Me180, SiHa, SW756) and from sun-exposed skin (keratinocyte cell line HaCaT). To control for the authenticity of the truncated *TSG101* transcripts, all RT–PCR reactions were performed in duplicate. By comparing the results from these independent RT–PCR amplifications, no differences in the *TSG101* transcription patterns were observed. The specificity of the individual truncated amplicons was demonstrated by Southern blot hybridisation using an internal *TSG101* cDNA probe (Figure 1).

In addition to wild-type transcript which was observed in all analysed samples, truncated *TSG101* RT–PCR products were detected with comparable frequencies in 49 of 89 (55%) preneoplastic cervical lesions (CIN I–III) and in 20 of 33 (61%) primary cervical cancer biopsies (Table 1). All of the investigated cell lines revealed at least one variant transcript. However, the generation of aberrant *TSG101* transcripts was also observed in five of 17 (29%) normal cervical tissue samples. Furthermore, the occurrence of variant transcripts appears to be independent of the HPV status of the respective biopsy samples, which is further confirmed by the existence of variant *TSG101* transcripts both in HPV positive (SW756, SiHa, HeLa, Me180) and HPV negative (HaCaT, C33a) cell lines (Figure 2).

The precise structure of all six types of variant *TSG101* transcripts obtained in this study was determined by DNA sequencing. These analyses revealed that only a single specific transcript, showing a deletion of nucleotides 154–1054

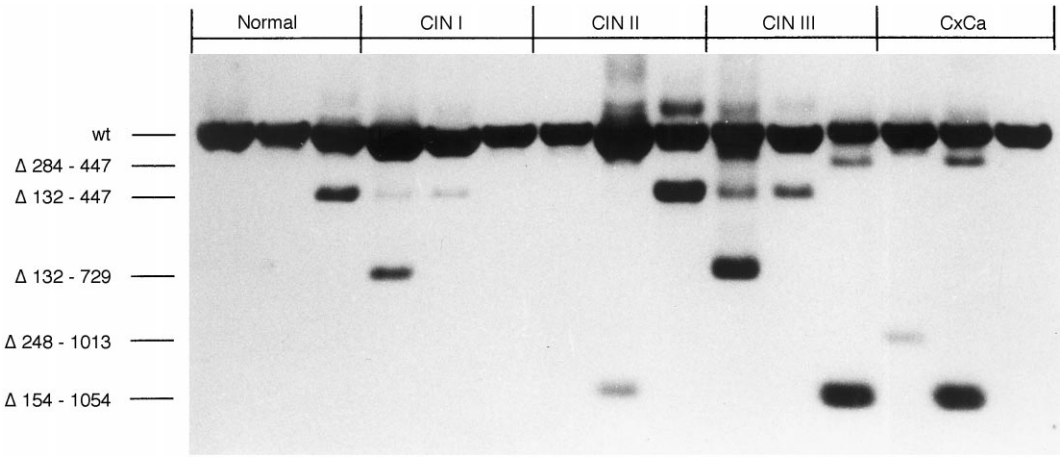

Figure 1. Variant *TSG101* transcripts in normal cervical epithelia, in preneoplastic lesions (CIN I, II, III) and in primary cervical carcinomas (CxCa). Southern blot analysis of the reverse transcription–polymerase chain reaction (RT–PCR) products from primary clinical specimens revealed the authenticity of the variant patterns of *TSG101* transcripts.

(transcript *Δ154-1054*), contained canonical splice donor and acceptor sites at the junctions of the deleted region of wild-type *TSG101* transcript. All other truncated RT–PCR products lacked conserved splice donor and/or acceptor sites. Furthermore, the majority of the truncated transcripts (transcripts *Δ284-447*, *Δ132-729*, *Δ248-1013* and *Δ278-1069*) were generally detected only with low frequencies (0–16%) in the samples analysed here, whereas transcript *Δ132-447* was observed in 29–60% of the clinical specimens (Table 1). The obtained frequencies of these five variant transcripts showed no significant differences with respect to the dysplastic or neoplastic progression of the clinical samples. In contrast, transcript *Δ154-1054* was not detectable in any of the analysed normal cervical tissues (0/17) or low grade preneoplastic lesions (CIN I, 0/20). However, with progression of the dysplastic lesions, the frequencies of transcript *Δ154-1054* increased from 6% (2/32) in CIN II lesions and 16% (6/37) in CIN III lesions to 42% (14/33) in primary cervical carcinoma samples (Table 1, Figure 3). Transcript *Δ154-1054* showed a statistically significant increase ($P < 0.001$, Fisher's exact test) in clinical cervical samples during the progression from preneoplastic lesions to malignant carcinomas. This transcript was additionally demonstrated to be present in five of six (83%) analysed cervical carcinoma and keratinocyte cell lines.

Although a nested RT–PCR protocol was used, the abundance of several of the obtained variant transcripts appeared to be relatively low when compared with the wild-type transcript. In particular, truncated transcripts *Δ284-447* and *Δ132-729*, resulting in RT–PCR products of 984 and 551 bp, respectively, were obtained as relatively weak PCR signals. In contrast, especially transcript *Δ154-1054* was observed in most cases as a major RT–PCR product. As this may be partially due to the nature of PCR reactions preferentially favouring the exponential amplification of smaller PCR fragments, we performed Northern blot analyses in order to evaluate the relative abundance of the variant *TSG101* transcripts. Total RNA preparations from cervical carcinoma cell lines C33a, HeLa, Me180, SiHa and SW756, as well as from the keratinocyte cell line HaCaT were subjected to Northern hybridisation using a radiolabelled *TSG101* cDNA as the probe. Although all of these cell lines were shown to harbour at least one truncated transcript in the RT–PCR analysis (Figure 2a), only hybridisation signals corresponding to wild-type *TSG101* transcripts were detectable in Northern blot hybridisation (Figure 2b).

DISCUSSION

We have shown that variant *TSG101* transcripts can be detected by RT–PCR both in preneoplastic and in malignant cervical tissue samples, as well as in a variety of cell lines derived from cervical carcinomas and from ultraviolet-irradiated skin keratinocytes. However, identical aberrant transcripts were also found in normal cervical tissue samples, although the overall frequency (29%) was lower than the frequencies observed in preneoplastic dysplasia (50–60%) and

Table 1. *Frequencies of variant* TSG101 *transcripts in preneoplastic and malignant cervical tissue samples and in carcinoma cell lines*

Variant *TSG101* transcript	Junction sites* (nucleotide sequences)	PCR fragment	Normal (%)	CIN I (%)	CIN II (%)	CIN III (%)	Cervical cancer (%)	Cell lines (%)
Δ284-447	GAG*gtaata…cac*CCA	984 bp	2/17 (12)	1/20 (5)	4/32 (13)	6/37 (16)	5/33 (15)	5/6 (83)
Δ132-447	CAA*gtacaa…cac*CCA	833 bp	5/17 (29)	12/20 (60)	13/32 (41)	15/37 (41)	15/33 (45)	5/6 (83)
Δ132-729	CAA*gtacaa…gtt*GGT	551 bp	1/17 (6)	2/20 (10)	3/32 (9)	6/37 (16)	3/33 (9)	3/6 (50)
Δ248-1013	TAA*tgaacc…tca*GTC	381 bp	0/17 (0)	1/20 (5)	2/32 (6)	3/37 (8)	4/33 (12)	0/6 (0)
Δ278-1069	CTT*atagag…aac*AGA	356 bp	0/17 (0)	1/20 (5)	0/32 (0)	1/37 (3)	1/33 (3)	0/6 (0)
Δ154-1054	ACT*gtacgt…cag*CTC	247 bp	0/17 (0)	0/20 (0)	2/32 (6)	6/37 (16)	14/33 (42)	5/6 (83)
Frequencies of variant *TSG101* transcripts			5/17 (29)	12/20 (60)	16/32 (50)	21/37 (57)	20/33 (61)	6/6 (100)

PCR, polymerase chain reaction; CIN, cervical intraepithelial neoplasia. *Nucleotides retained in the variant transcripts are indicated in capital letters; adjacent sequences not present in the respective variant transcripts are shown in italics.

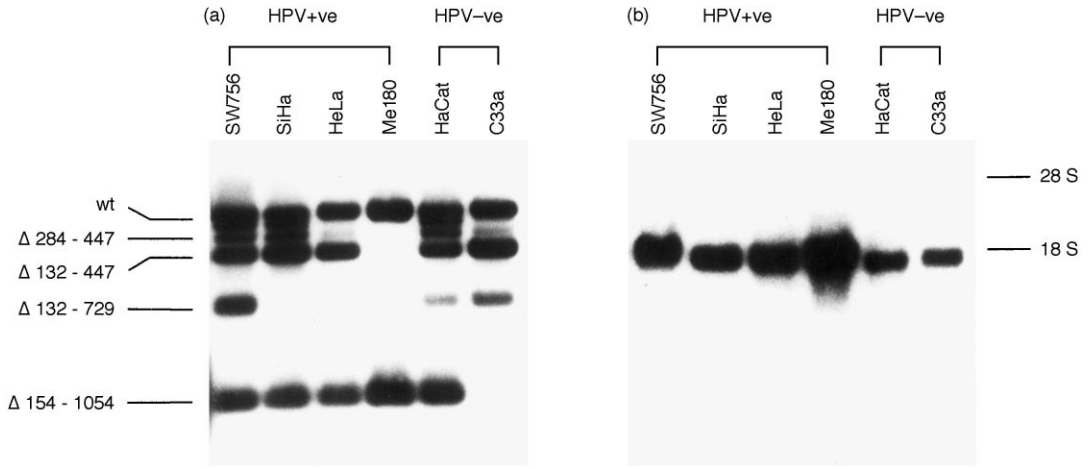

Figure 2. Analyses of *TSG101* transcription in cervical carcinoma and keratinocyte cell lines. (a) *TSG101*-specific reverse transcription–polymerase chain reaction (RT–PCR) of cervical carcinoma cell lines (SW756, SiHa, HeLa, ME180, C33a) and of a keratinocyte cell line (HaCaT) revealed variant transcripts in all cell lines. Southern blot analysis of the RT–PCR products using a chemiluminescence detection system confirmed the authenticity of the variant *TSG101* transcripts. (b) Northern blot hybridisation of total RNA from the identical cell lines using a radiolabelled *TSG101* cDNA probe.

malignant lesions (61%) (Table 1). These findings support the recent observations that the generation of variant *TSG101* transcripts is not restricted to carcinomas, but may also be observed in normal tissues [17, 19, 22].

Detailed analyses of the six variant transcripts obtained in this study revealed that three of them (transcripts *Δ132-447*, *Δ132-729* and *Δ154-1054*) have already been described in previous reports [12, 17, 19, 20]. Only one of the variant transcripts found in cervical tissues (transcript *Δ154-1054*) showed genuine splice donor and acceptor sequences at the junctions of the deleted part of the *TSG101* transcript. Interestingly, the frequency of this variant transcript type increased significantly ($P < 0.001$) during the progression from low grade to high grade dysplastic lesions and cervical carcinomas, whereas all other truncated *TSG101* RT–PCR products did not show major differences in their respective frequencies with increasing dysplastic progression. These observations may indicate that transcript *Δ154-1054* might be specifically generated by alternative or aberrant splicing of the *TSG101* gene in tissues with increased proliferation rates. The potential correlation of the occurrence of transcript *Δ154-1054* with the proliferative capacity of the individual tissue sample is further substantiated by the detection of this transcript in various fetal tissue samples (heart, gut, lung, tongue, skin and kidney) [17]. Furthermore, this transcript has been shown to be present at high frequencies (approximately 50%) in primary carcinoma samples of different histologies (mainly breast, ovarian, lung and prostate cancers [12, 17, 19, 20]). In contrast, it has been either not detected in various normal tissue samples [17, 20] or it has been shown to occur only with a low frequency (16%) in a set of 44 normal tissue samples which, however, also comprised 22 Epstein–Barr virus (EBV)-immortalised B cell lines [19]. The lack of this transcript in all normal (0/17) and low grade (CIN I, 0/20) preneoplastic cervical lesions and the consistent increase of the frequencies of this transcript during the progression of CIN II and CIN III lesions to cervical carcinomas support the hypothesis that variant transcript *Δ154-1054* might represent an oncodevelopmental marker reflecting the proliferative capacity of the respective tissues.

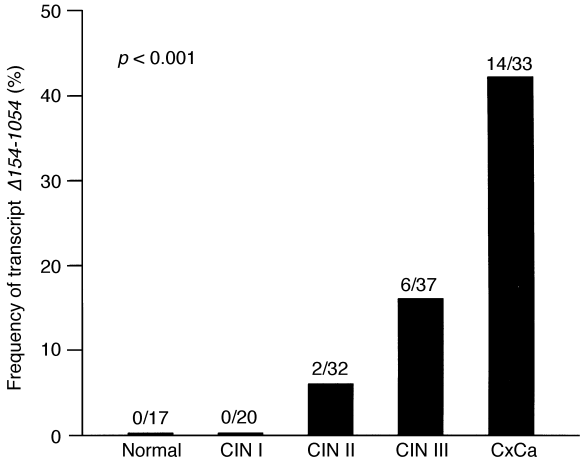

Figure 3. Frequency of variant *TSG101* transcript *Δ154-1054* in normal and dysplastic cervical epithelia (CIN I, II, III) and in cervical carcinomas (CxCa) as determined by reverse transcription–polymerase chain reaction (RT–PCR). CxCa, primary cervical carcinoma.

Recently, there has been a report questioning the authenticity of truncated *TSG101* transcripts as detected by nested RT–PCR [25]. These authors observed non-reproducible and highly variable results which might suggest that the aberrant transcripts represent PCR artifacts. Here, we controlled for the authenticity of the aberrant *TSG101* transcripts by performing all PCR reactions at least twice using independent RT reactions. Furthermore, we used oligonucleotide primers P3 and P4 for the nested PCR reactions [12], which were demonstrated by Hampl and co-workers [25] not to result in illegitimate, artificial PCR fragments. In addition, we performed control PCR reactions using 3′-oligonucleotide primers specific for each individual *TSG101* exon in combination with a single 5′-oligonucleotide primer, thus verifying the presence of the aberrant transcripts in the respective sample (data not shown).

Most recently, Wagner and colleagues [23] demonstrated the existence of at least four additional introns within the N-terminal part of the human *TSG101* gene, which may explain the generation of the variant, truncated transcripts by

mechanisms of alternative or aberrant splicing. The detailed analysis of the complete genomic sequence of the human *TSG101* gene remains necessary to clarify the exact mechanisms involved in the generation of its variant transcripts. It may be that their occurrence simply reflects a relaxed splicing control which might be observed in similar analyses of unrelated genes by applying sensitive RT–PCR-based detection protocols [17, 19]. The low abundance of the variant *TSG101* transcripts, as analysed by Northern blot hybridisation (Figure 2b), and the detection of identical aberrant transcripts in various normal tissues support this view. The observation that most of the aberrant *TSG101* transcripts are only detectable by sensitive nested RT–PCR amplification protocols [25, 26], further underlines the low abundance of variant *TSG101* transcripts.

As most of the intragenic deletions of the variant transcripts result in disruption of the *TSG101* reading frame, it seems unlikely that they encode proteins of functional relevance. This is also particularly true for variant transcript *Δ154-1054*, which codes only for the 18 N-terminal amino acid residues of the native TSG101 protein, linked to a new peptide sequence of seven amino acids due to a frameshift at the junction site and an adjacent translational stop codon. Thus, it remains to be clarified whether this or other variant *TSG101* transcripts have any physiologically relevant role in the pathogenesis of cervical cancers.

1. Ponten J, Adami H-O, Bergström R, *et al*. Strategies for global control of cervical cancer. *Int J Cancer* 1995, **60**, 1–26.
2. zur Hausen H. Human papillomaviruses in the pathogenesis of anogenital cancer. *Virology* 1991, **184**, 9–13.
3. Mullokandov MR, Kholodilov NG, Atkin NB, Burk RD, Johnson AB, Klinger HP. Genomic alterations in cervical carcinomas: losses of chromosome heterozygosity and human papilloma virus tumor status. *Cancer Res* 1996, **56**, 197–205.
4. Mitra AB, Murty VS, Li RG, Pratap M, Luthra UK, Chaganti RSK. Allelotype analysis of cervical carcinoma. *Cancer Res* 1994, **54**, 4481–4487.
5. Wistuba II, Montanello FD, Milchgrub S, *et al*. Deletions of chromosome 3p are frequent and early events in the pathogenesis of uterine cervical carcinoma. *Cancer Res* 1997, **57**, 3154–3158.
6. Rader JS, Kamarasova T, Huettner PC, Li L, Li Y, Gerhard DS. Allelotyping of all chromosomal arms in invasive cervical cancer. *Oncogene* 1996, **13**, 2737–2741.
7. Riou G, Barrois M, Sheng ZM, Duvillard P, Lhomme C. Somatic deletions and mutations of c-Ha-ras gene in human cervical cancers. *Oncogene* 1988, **3**, 329–333.
8. Atkin NB, Baker MC, Fox MF. Chromosome changes in 43 carcinomas of the cervix uteri. *Cancer Genet Cytogenet* 1990, **44**, 229–241.
9. Saxon PJ, Srivatsan ES, Stanbridge EJ. Introduction of human chromosome 11 via microcell transfer controls tumorigenic expression of HeLa cells. *EMBO J* 1986, **5**, 3461–3466.
10. Koi M, Morita H, Yamada H, Satoh H, Barrett JC, Oshimura M. Normal human chromosome 11 suppresses tumorigenicity of human cervical tumor cell line SiHa. *Mol Carcinog* 1989, **2**, 12–21.
11. Smits PH, Smits HL, Jebbink MF, ter Schegget J. The short arm of chromosome 11 likely is involved in the regulation of the human papillomavirus type 16 early enhancer-promoter and in the suppression of the transforming activity of the viral DNA. *Virology* 1990, **176**, 158–165.
12. Li L, Li X, Francke U, Cohen SN. The *TSG101* tumor susceptibility gene is located in chromosome 11 band p15 and is mutated in human breast cancer. *Cell* 1997, **88**, 143–154.
13. Koonin EV, Abagyan RA. *TSG101* may be the prototype of a class of dominant negative ubiquitin regulators. *Nature Genet* 1997, **16**, 330–331.
14. Ponting CP, Cai Y-D, Bork P. The breast cancer gene product *TSG101*: a regulator of ubiquitination? *J Mol Med* 1997, **75**, 467–469.
15. Li L, Cohen SN. *TSG101*: a novel tumor susceptibility gene isolated by controlled homozygous functional knockout of allelic loci in mammalian cells. *Cell* 1996, **85**, 319–329.
16. Xie W, Li L, Cohen SN. Cell cycle-dependent subcellular localization of the *TSG101* protein and mitotic and nuclear abnormalities associated with *TSG101* deficiency. *Proc Natl Acad Sci USA* 1998, **95**, 1595–1600.
17. Lee MP, Feinberg AP. Aberrant splicing but not mutations of *TSG101* in human breast cancer. *Cancer Res* 1997, **57**, 3131–3134.
18. Steiner P, Barnes DM, Harris WH, Weinberg RA. Absence of rearrangements in the tumour susceptibility gene *TSG101* in human breast cancer. *Nature Genet* 1997, **16**, 332–333.
19. Gayther SA, Barski P, Batley SJ, *et al*. Aberrant splicing of the *TSG101* and FHIT genes occurs frequently in multiple malignancies and in normal tissues and mimics alterations previously described in tumours. *Oncogene* 1997, **15**, 2119–2126.
20. Sun Z, Pan J, Bubley G, Balk SP. Frequent abnormalities of *TSG101* transcripts in human prostate cancer. *Oncogene* 1997, **15**, 3121–3125.
21. Li L, Francke U, Cohen SN. Retraction for Li *et al.* [12]. *Cell* 1998, **93**, 0.
22. Willeke F, Ridder R, Bork P, *et al*. Identical variant *TSG101* transcripts in soft tissue sarcomas and various non-neoplastic tissues. *Mol Carcinog* 1998, **23**, 195–200.
23. Wagner K-U, Dierisseau P, Rucker III EB, Robinson GW, Hennighausen L. Genomic architecture and transcriptional activation of the mouse and human tumor susceptibility gene *TSG101*: common types of shorter transcripts are true alternative splice variants. *Oncogene* 1998, **17**, 2761–2770.
24. Church GM, Gilbert W. Genomic sequencing. *Proc Natl Acad Sci USA* 1984, **81**, 1991–1995.
25. Hampl M, Hampl J, Plaschke J, *et al*. Evidence that *TSG101* aberrant transcripts are PCR artifacts. *Biochem Biophys Res Commun* 1998, **248**, 753–760.
26. Trink B, Pai SI, Spunt SL, *et al*. Absence of *TSG101* transcript abnormalities in human cancers. *Oncogene* 1998, **16**, 2815–2818.

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