Tropical Biomedicine 25(1): 46–57 (2008)

Overexpression of *WNT2* and *TSG101* genes in colorectal carcinoma

Ma Xiang Ru[1], Edmund Sim Ui Hang[1], Pauline Balraj[2], Patricia Lim[3] and Rahman Jamal[4]
[1] Immunology-Human Molecular Genetics Laboratory, Department of Molecular Biology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.
[2] Institute for Medical Research (IMR), Jalan Pahang, 50588 Kuala Lumpur, Malaysia.
[3] Malaysian Bio-Diagnostics Research Sdn. Bhd. (MBDR), Block Intron-Ekson, UKM-MTDC Smart Technology Centre, 43650 Bangi, Selangor, Malaysia.
[4] Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Kuala Lumpur, Malaysia.
Corresponding author email: uhsim@frst.unimas.my
Received 30 January 2008; received in revised form 28 February 2008; accepted 3rd March 2008.

Abstract. Colorectal carcinoma (CRC) arises as a result of mutational activation of oncogenes coupled with inactivation of tumour suppressor genes. Mutations in *APC*, *K-ras* and *p53* have been commonly reported. In a previous study by our group, the tumour susceptibility gene 101 (*TSG101*) were found to be persistently upregulated in CRC cases. *TSG101* was reported to be closely related to cancers of the breast, brain and colon, and its overexpression in human papillary thyroid carcinomas and ovarian carcinomas had previously been reported. The wingless-type MMTV integration site family member 2 (*WNT2*) is potentially important in the Wnt/β-catenin pathway and upregulation of *WNT2* is not uncommon in human cancers. In this study, we report the investigation for mutation(s) and expression pattern(s) of *WNT2* and *TSG101*, in an effort to further understand their role(s) in CRC tumourigenesis. Our results revealed no mutation in these genes, despite their persistent upregulation in CRC cases studied.

INTRODUCTION

The predisposition of colorectal carcinoma (CRC) has been linked to a series of genetic alterations which includes mutations that activate oncogenes and inactivate tumour suppressor genes. Fearon & Vogelstein (1990) proposed a model for the genetic basis of colorectal tumourigenesis, linking genetic alterations such as mutations in adenomatous polyposis coli (*APC*), Kirsten-ras (*K-ras*) and *p53* to different stages of tumour development. Following the establishment of this genetic model, genetic alterations such as loss of heterozygosity (LOH), Deleted in Colon Cancer (*DCC*) gene, mutations in mismatch repair genes (MMR) and others have been reported (Fearon *et al.*, 1990; Kinzler & Vogelstein, 1996; Ogunbiyi *et al.*, 1998; Lanza *et al.*, 1998; Renkonen *et al.*, 2003).

A recent study by our group (employing microarray approach) had detected the upregulation of *WNT2* (unpublished data) and *TSG101* in CRC cases relative to normals (Sim *et al.*, 2006), suggesting the involvements of these genes in CRC progression. Wnt signaling has been identified as one of the key signaling pathways in cancer, targeting genes that regulate cell proliferation, developmental processes and tumour progression (Zang, 2000; You *et al.*, 2002). *WNT2*, being a member of the Wnt family, has been reported to be associated with human neoplasms especially colorectal cancer (Vider *et al.*, 1996; Katoh, 2001; Holcombe *et al.*, 2002). Vider *et al.* (1996) reported its low level expression in normal colon but over-expression in tumour tissue samples irrespective of their cancer stages. In the case of *TSG101*, its overexpression in

association with tumourigenesis has been reported by Liu *et al.* (2002) and Young *et al.* (2007). Liu *et al.* (2002) in their study on 20 human papillary thyroid carcinomas reported overexpression of *TSG101* at both transcriptional and protein levels using in-situ hybridization and immuno-histochemistry techniques. This provided the first evidence that linked overexpression of *TSG101* to carcinogenesis. The presence of truncated transcripts of *TSG101* in tumour samples has also been linked to tumourigenesis (Turpin *et al.*, 1999). Yet, this remains a contention since there were reports that the truncated transcripts can be PCR artifacts (Hampl *et al.*, 1998). To date, the question of whether *TSG101* plays a role in tumourigenesis as a tumour suppressor gene or tumour-enhancing gene remains debatable (Li *et al.*, 1997; Carney *et al.*, 1998; Turpin *et al*, 1999; Zhu *et al.*, 2004).

Following the discovery on associations of *WNT2* and *TSG101* with tumourigenesis, the classical CRC genetic model proposed by Fearon & Vogelstein might be further expanded since the involvements of these genes in the *APC-Wnt-β catenin* and also *p53* pathways have previously been reported. However, the role(s) of these genes in progression of CRC remains to be elucidated. Therefore, we aimed to study the existence of mutations (if any) and the expression pattern(s) of these genes in local CRC biopsy samples in order to understand the role(s) of these genes in CRC progression.

In this study, we screened 11 human CRCs in comparison to their normals by semi-quantitative RT-PCR to explore the role of *WNT2* and *TSG101* in colorectal tumourigenesis.

MATERIALS AND METHODS

Total RNA

Commercially available human normal colon (cat. no. 64065-1) and colon tumour (cat. no. 64014-1) were purchased from BD BioSciences (Clontech Laboratories, USA) and designated as C01 pair in this study. Ten clinical specimens of CRCs were collected from local patients during surgery for colectomy whereas the paired normal tissues were obtained with informed consent from these patients. The normal and tumour biopsies were then snap-frozen in liquid nitrogen prior to RNA extraction using the Trizol method. Basically, the frozen CRC and paired normal tissues were cut into smaller pieces with sterile surgical blade and then homogenized in 1 ml Trizol reagent (Invitrogen) using a polytron homogenizer. Total RNA extraction was later performed according to manufacturer's instructions. The total RNAs from the CRC tissues and their paired normals were designated as C02, C03 up to C11, respectively. The concentration and purity of the total RNA obtained were then determined using a spectrophotometer.

Oligonucleotides

The oligonucleotides used in this study for PCR amplification of each specific product were synthesized by MWG Biotech (German) and Research Biolabs (Singapore). All primers were designed using Primer3 Output free software available online (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi), and their specific sequences are as listed (Table 2).

RT-PCR

First-strand cDNA was synthesized from 1 microgram of total RNA using oligo-dT primers, catalysed by MML-V reverse transcriptase in a reaction volume of 20 µl. Then, 2.5 µl of the first strand cDNA was used as template for subsequent PCR amplification. PCR was carried out in a mixture volume of 25 µl with final reaction concentrations of 1X (1.5 mM $MgCl_2$)[2] GoTaq™ Reaction Buffer, 0.2 mM dNTPs, 1 pmole/µl of each forward and reverse primers, and 1.25 U GoTaq™ DNA polymerase. In the case where GoTaq® Flexi DNA Polymerase was used, a final concentration of 1.5 mM $MgCl_2$ was added to the final volume. PCR amplifications were performed using PTC-2000 Peltier Thermal Cycler (MJ Research). The mixture was first incubated at 96°C for 10 min prior to addition of polymerase. Then, the PCR reaction was performed with denaturation at 95°C for 50

Table 1. *WNT2* and *TSG101* expression and cDNA sequencing analysis

CRC Sample	Overexpression[a]		Mutation[b]	
	WNT2	*TSG101*	*WNT2*	*TSG101*
C01	$+^{N}$	+	–	$–^{P}$
C02	$+^{N}$	+	–	V
C03	NE	$+^{N}$	–	–
C04	NE	NE	ND	ND
C05	$+^{N}$	NE	–	V
C06	$+^{N}$	+	–	–
C07	$+^{N}$	+	–	–
C08	$+^{N}$	+	–	–
C09	$+^{N}$	+	–	–
C10	$+^{N}$	+	–	V
C11	+	$–^{D}$	–	–

[a] Overexpression was determined by semi-quantitative RT-PCR by comparing the relative amount of transcripts expressed in tumour to matched normal CRC. + indicated overexpression in tumour sample relative to its normal counterpart. $+^{N}$ indicated overexpression in which transcripts are detected in tumour but not in normal sample. $–^{D}$ indicated downregulation of transcripts in tumour counterpart. NE: no expression detected.

[b] cDNA encompassing the entire coding region was amplified by PCR, and the sequence status was determined by automatic sequencing. –: no mutation, $–^{P}$: no mutation in partial sequence amplified, ND: not determined, V: nucleotide variant at nucleotide position 834 (C→T).

s; annealing at temperature as stated in Table 2 for 2 min; and extension at 72°C for 2 min. The complete amplification procedure was carried out for 40 cycles followed by further incubation at 72°C for 10 min. In order to control for errors in input of cDNA used in PCR reactions, amplification of house keeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was performed using specific primers (forward: 5'-TGCACCACCAACTGCTTAGC-3'; reverse: 5'-GGCATGGACTGTGGTCATGAG-3'). Aliquots of PCR product were later size-fractionated by agarose gel electrophoresis.

Acquisition of Gel Images and Quantitative Analysis

Images of the RT-PCR ethidium bromide-incorporated agarose gels were acquired with a gel documentation system (Quantity One® 4.2.3, The Discovery Series™, Bio-Rad Laboratory, CA). The gel images were then exported as TIFF images. The expression of transcripts was determined quantitatively using BioNumerics version 4.50 software (Applied Biomaths, USA). Band intensity was expressed as relative concentration units by comparing the intensity of desired PCR product bands to intensity of reference bands (with known concentrations).

Sequence Analysis

Desired PCR products were purified using the gel extraction spin method. It was done

Table 2. Oligonucleotide primers

Primer	Nucleotide sequence 5' – 3'	Amplified fragment size/bp	Annealing temperature/ °C
TSG101F	GTGCCGACTTCCTGTTGTTT	1315	64
TSG101R	CCTCCAGCTGGTATCAGAGAA		
Wnt2F	AGCTGAGCGCTTCTGCTCT	1204	64
Wnt2B	GGAGTCCTTGTAGAAGGGAAGG		
P53-F	GACACGCTTCCCTGGATT	1320	62
P53-R	CAAGCAAGGGTTCAAAGAC		
APC-F3	GAAGCATTATGGGACATGGG	1103	61
APC-R3	TTCCTTGATTGTCTTTGCTCAC		

by using the Gel Extraction System extraction kit (Viogene) according to manufacturer's instructions. Purified samples were processed commercially by 1st Base Laboratory Sdn Bhd (Selangor, Malaysia) and Solgent Company Limited (Daejeon, South Korea). All purifed DNA samples were sequenced in both forward and reverse directions. All sequences obtained were verified by comparative analysis with the sequences in GenBank database (*WNT2*, Acc. No. NM_003391; *TSG101*, Acc. No. NM_006292). The sequence corresponding to the amplified gene or region of interest was searched for using the Blastn program (http://www.ncbi.nlm.nih.gov) with the nucleotide sequence obtained as a query sequence. Verifications of forward and reverse sequences were performed using blast2q alignment tool available at the NCBI website.

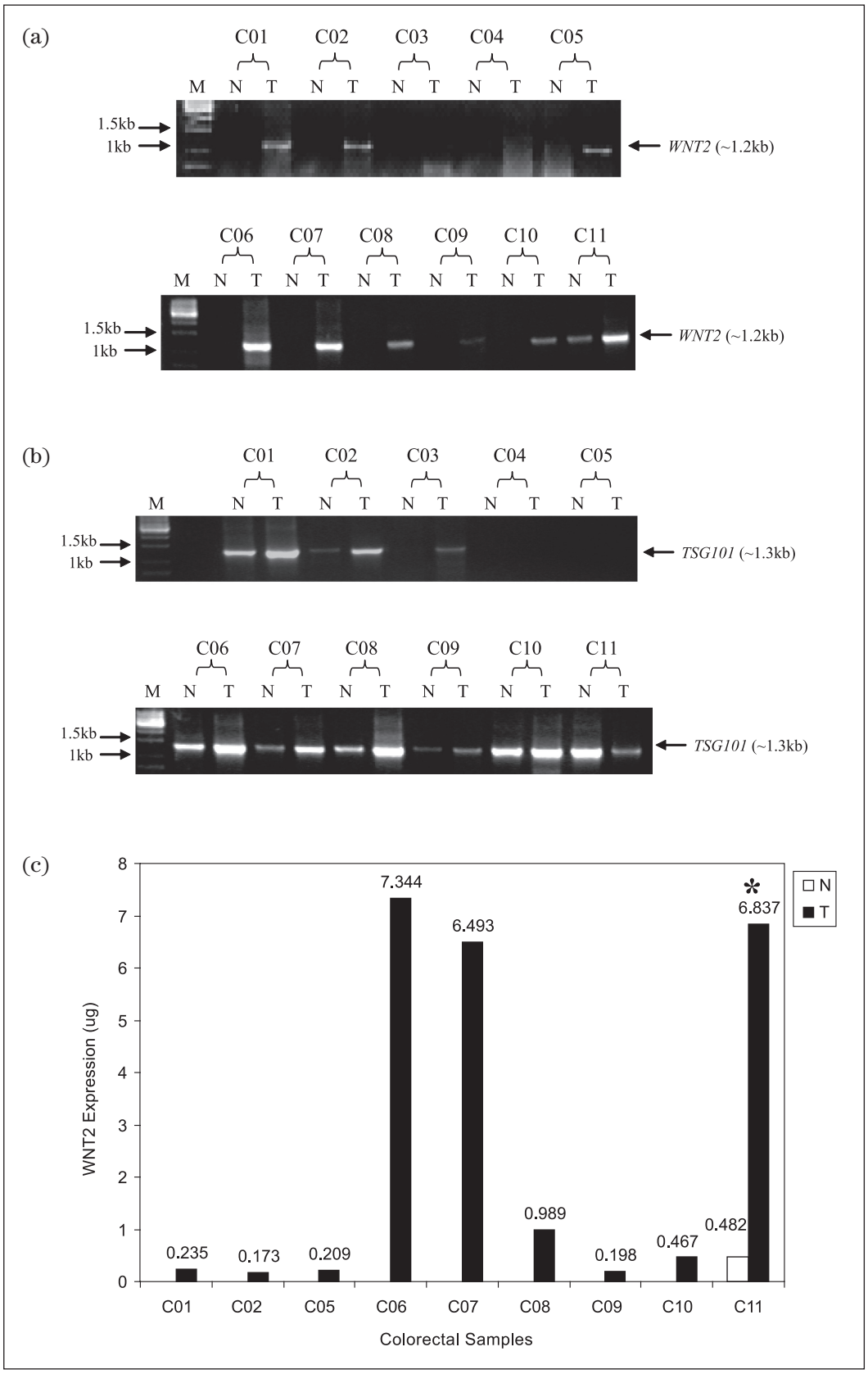
RESULTS

Overexpression of *WNT2* and *TSG101* are detected in CRC specimens by semi-quantitative RT-PCR

To investigate the expression and subsequently to determine the relative amount of *WNT2* and *TSG101* transcripts in tumour and normal colorectal tissues, semi-quantitative RT-PCR was performed on 10 pairs of biopsy sample and a pair of commercial colorectal total RNA. Figures 1(a) and (b) show the result of PCR amplification of *WNT2* and *TSG101* in these 11 CRC samples and their paired normal. *WNT2* and *TSG101* were differentially expressed in 9 out of 11 paired samples studied. For sample pairs that exhibited differential expression, *WNT2* was detected to be overexpressed in all tumour cases (9 of 9) whereas *TSG101* was consistently upregulated (8 of 9). A control experiment was carried out and it showed that varying the annealing temperature had no significant effect on the results obtained (data not shown). To compare the amounts of the amplified PCR products, we performed a density-based quantification by detecting the DNA band intensity as observed under UV irradiation (Figures 1(c) and (d)) using gel documentation system (Quantity One® 4.2.3, The Discovery Series™, Bio-Rad Laboratories, CA, USA). The value was determined using analysis software (BioNumerics trial version 4.50, Applied Biomaths, USA). As indicated in Figure 1(c), the amount of *WNT2* expressed was relatively higher in all tumour samples. Its expression was not detected in all normal samples except for C11. In C11, *WNT2* was upregulated by 14 fold in the tumour relative to its matched normal. As for *TSG101*, the relative amount of its expression in 5 of the tumour samples was more than twofold higher than that of their normal counterparts (Figure 1(d)). The *GAPDH* control affirmed the equimolar concentration of starting total RNA used in this study (Figure 1(e)). In short, our result from semi-quantitative RT-PCR revealed persistent overexpression of *WNT2* and *TSG101* in CRC specimens studied.

Upregulation of *WNT2* and *TSG101* are not caused by the mutation in the coding region

To detect the presence of genetic alterations that causes the upregulation, full length cDNA encompassing the entire coding region of *WNT2* and *TSG101* were amplified and sequenced. PCR amplifications performed on *WNT2* cDNA (using primers Wnt2F and Wnt2B) and *TSG101* (using primers TSG101F and TSG101R) gave rise to product fragments of about 1.2 kb and 1.3 kb, respectively. As summarized in Table 1, no mutation in the entire coding region of *WNT2* and *TSG101* could be detected in the CRCs analysed. However, for *TSG101*, a nucleotide variant at nucleotide position 834 was observed in specimens C02, C05, C10 (Figure 2). This transitional substitution of C by T is also observed in the paired normal samples. A GAC to GAT change was detected in codon 236 but it still encodes for the same amino acid, Asparagine (Asp), indicating a silent mutation or inconsequential variation. All the sequences were verified in both forward and reverse directions. Sequence analysis showed that upregulation of *WNT2* and *TSG101* were not due to mutation in the coding region of these genes.



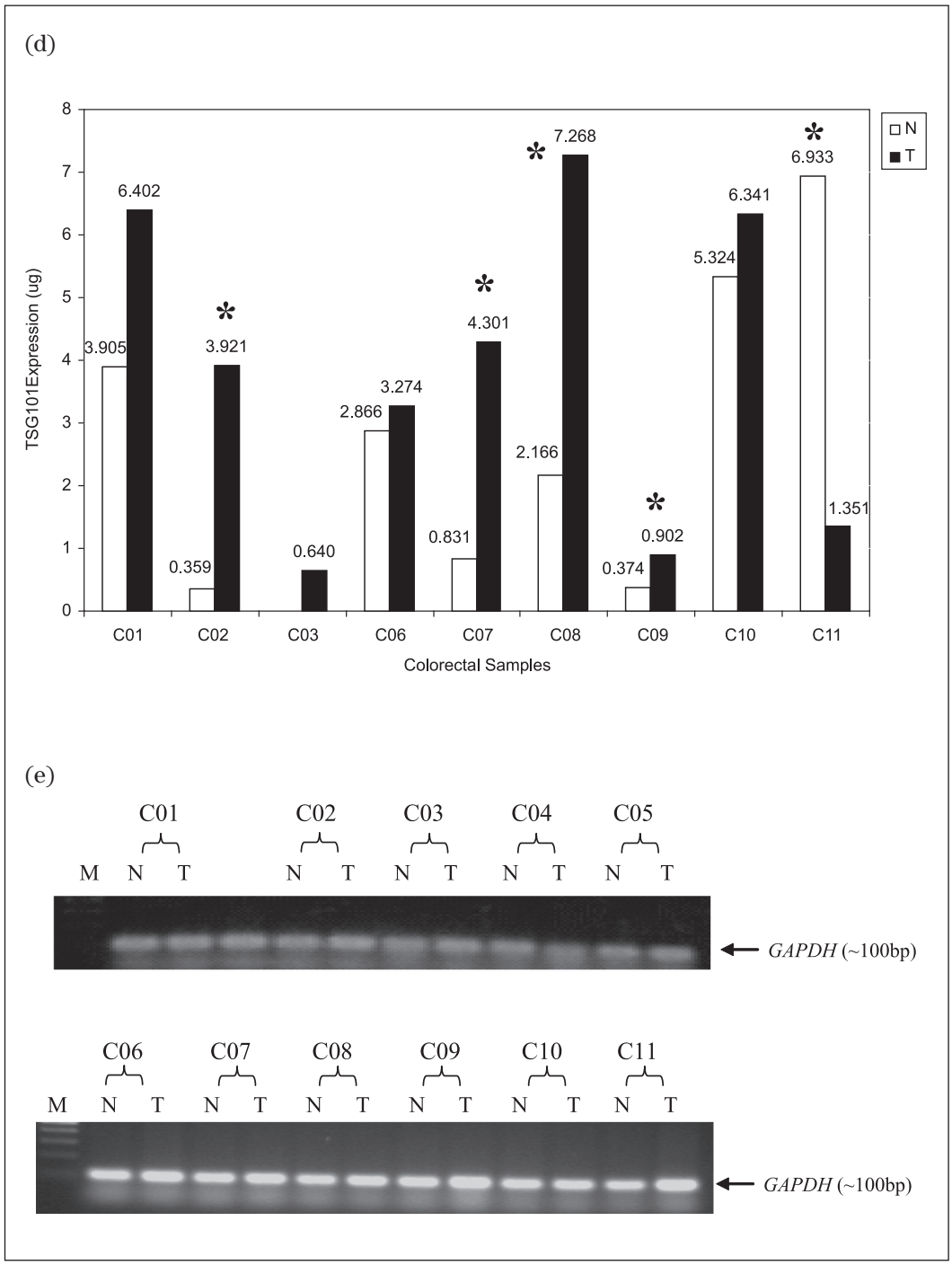


Figure 1. Expression of *WNT2* and *TSG101* in CRCs. M: Marker; N: Normal; T: Tumour. (a) Expression of *WNT2* in 11 paired CRCs. In each case, 1 µg of RNA was used as template for RT-PCR. Differential expression of *WNT2* was observed in 9 of 11 pairs studied. (b) Differential expression of *TSG101* as observed in 9 of 11 pairs studied. (c) & (d) Bar graphs demonstrating the relative levels of expression of *WNT2* and *TSG101* detected using semi-quantitative RT-PCR. Asterisks indicate sample pairs in which the differential in expression between the normal and tumour tissues exceeds two fold. (e) *GAPDH* cDNA was almost equally detected.

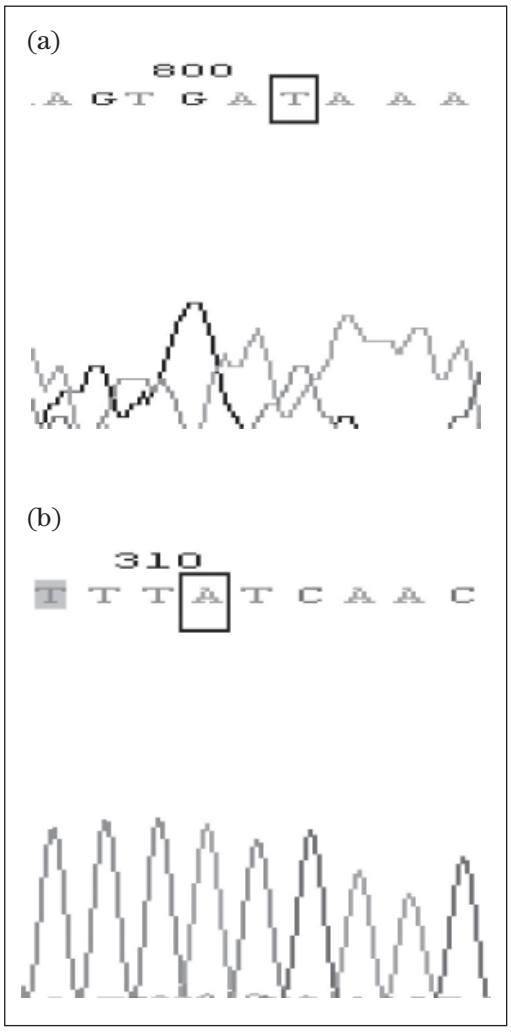


Figure 2. Sequence analysis of *TSG101* showing nucleotide variation at nucleotide 834 of sample C02. (a) Forward sequence. (b) Reverse sequence. Black box indicates nucleotide that was altered. The C to T change was observed in forward sequence whereas G to A change was observed in reverse sequence.

***APC* is expressed relatively higher than *WNT2* in normal colorectal tissues**

APC is reported to be an important regulator in Wnt signaling pathway (Bienz & Clevers, 2000), thus we tried to investigate its relation with *WNT2* by comparing the expression of both genes in 7 paired CRC specimens. The expression of *APC* was determined by semi-quantitative RT-PCR and the value was compared with that of *WNT2* (Figures 3(a) and (b)). The result from the study within normal tissues showed consistent expression pattern in which *APC* expression level was relatively higher in all samples except C09 (Figure 3(a)). In C09, no comparison was performed because expressions of *APC* and *WNT2* were not detected in the normal specimen. As for the case of tumour samples, a less uniform pattern was observed, in which C03, C07, C08, C11 displayed higher expression of *WNT2* while the rest were not (Figure 3(b)).

***TSG101* expression is higher than *p53* in most of the normal and tumour CRCs**

TSG101 was reported to serve as both a regulator and also a target of the *MDM2/p53* circuitry (Li *et al.*, 2001). Its relation with *p53* was examined in our study by comparing the expression of both genes in CRCs and also their normal counterparts. Data obtained (via semi-quantitative approach) for both *TSG101* and *p53* was represented in Figures 4(a) and (b). All normal (except C06 and C09) and tumour samples (except C09, C11) exhibited higher expression of *TSG101*. The expression patterns of *p53/TSG101* in C06 and C11 in normal samples were inconsistent with that of the tumour tissues. In C06 normal tissue, *p53* expression was relatively higher than *TSG101*, but in tumour, *p53* expression was relatively lower than *TSG101*. As for C11, *TSG101* was expressed relatively higher in normal but not in tumour tissue.

DISCUSSION

The frequent upregulation of *WNT2* in human cancers is not uncommon and has been reported previously (Katoh, 2001). In this study, we demonstrated overexpression of *WNT2* in all 9 CRC cases examined using RT-PCR technique. Our finding tallies with work done by Holcombe *et al.* (2002) in which they reported absence of *WNT2* expression in colonic crypts but expression in colon cancer and cell lines using in situ hybridization technique. Out of the 9 CRC samples screened by us, one was diagnosed with Dukes' D, one with Dukes' C with lymph node metastasis, one with Dukes' B2

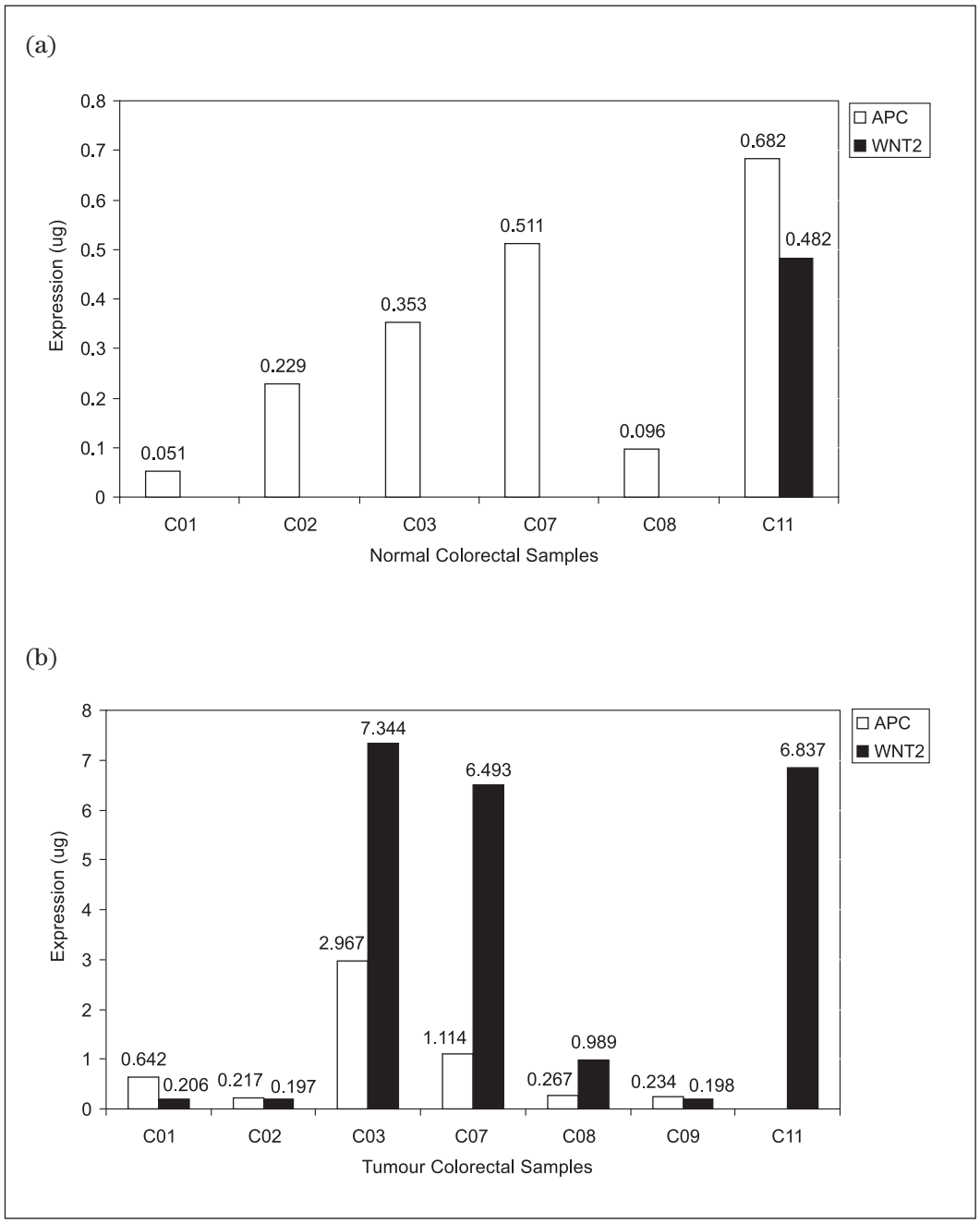


Figure 3. (a) & (b) Bar graphs demonstrating the relative levels of expression of *APC* and *WNT2* in normal and tumour colorectal samples, respectively.

whereas the rest were Dukes' B. The expression of *WNT2* in all CRC cases regardless of their stages has also been previously reported by Vider *et al.* (1996). Their study demonstrated low expression in normal colon and overexpression in all tumour tissue samples at different Dukes' stages of CRC progression which included premalignant polyps and liver metastases. The involvement of *WNT2* in progression of CRC is probably via the Wnt signaling pathway as *WNT2* is a member of the Wnt gene family. The Wnt genes encode secreted signaling proteins that during development

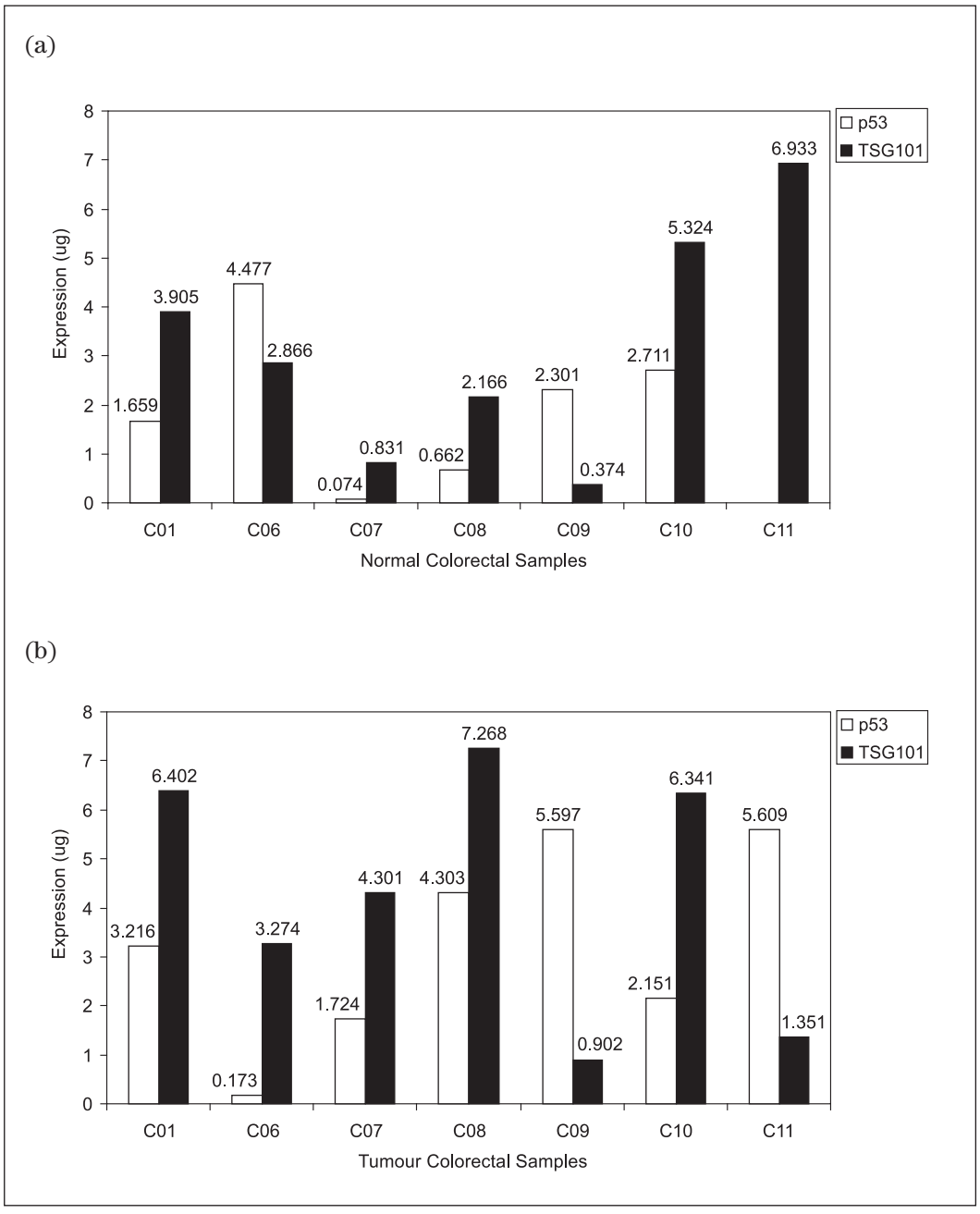


Figure 4. (a) & (b) Bar graphs demonstrating the relative levels of expression of *p53* and *TSG101* in normal and tumour colorectal samples, respectively.

play diverse roles in governing cell fate, proliferation, migration, polarity and death (Miller, 2001). Wnts have also been implicated in tumourigenesis through the inappropriate activation of the canonical Wnt/ *β-catenin* pathway (Bienz & Clevers, 2000). Although the results of others and ours strongly suggest the involvement of *WNT2* in colorectal cancer, there is no evidence to show that the expression level of *WNT2* correlates with the different stages of CRC progression. This has to be examined further.

A previous study by our group revealed upregulation of *TSG101* in 2 CRC cases using

microarray approach (Sim *et al.*, 2006). Here, we reported overexpression of *TSG101* in 8 out of 9 CRC cases studied. Our data from RT-PCR confirmed our previous finding with an increased sample size. Our findings provided new evidence that overexpression of *TSG101* could be associated with CRC tumourigenesis. Association of *TSG101* overexpression with human tumours was first reported by Liu *et al.* (2002). In their study using 20 human papillary thyroid carcinomas (PTC), the upregulation of *TSG101* was reported at the RNA transcript and protein levels. The reduction of *TSG101* protein has also been demonstrated to have negative impact on tumour cell growths (Zhu *et al.*, 2004). More recently, immunoanalysis using ovarian cancer samples and microtissue array also revealed elevated *TSG101* levels in human ovarian carcinomas (Young *et al.*, 2007). The association of *TSG101* with tumourigenesis is not unanticipated as *TSG101* has been reported to be essential for cell growth, proliferation, cell survival, and normal function of embryonic and adult tissues (Ruland *et al.*, 2001; Wagner *et al.*, 2003). On the other hand, presence of TSG101 aberrant transcripts had been suggested to have a role in breast and ovarian cancer (Carney *et al.*, 1998; Turpin *et al.*, 1999). However, it has not been linked to colorectal cancer. In a study employing RT-PCR assay to investigate the expression of *TSG101* in cancerous and normal breast tissues, 21% of primary breast cancer examined was reported to have at least three truncated transcripts but none of the normal tissues had more than two aberrant transcripts, and there was no report on any differential expression of *TSG101* in the tissues studied (Carney *et al.*, 1998). Although presence of truncated transcripts was observed in some of the samples examined in our study, we could not substantiate its correlation with tumourigenesis of CRC. In fact, by performing gradient PCR using total RNA from the commercial colorectal pair, we revealed that the presence of such truncated transcripts as somehow influenced by the annealing temperature used, of which the temperature tested was between the range of 52.2°C-66.6°C (data not shown). We suspected that these truncated fragments are PCR artifacts- a phenomenon of *TSG101* that has also been observed by Hampl and co-workers (1998).

Previous studies rarely focused on sequence analysis of *WNT2* (Vider *et al.*, 1996; Katoh, 2001; Holcombe *et al.*, 2002). Here, our sequence analysis data revealed that the overexpression of *WNT2* at transcript level is not due to the mutation of the gene itself. Its overexpression in CRC might be due to some other indirect influence. Liu *et al.* (2002), in their study using human PTCs, performed sequence analysis of *TSG101* cDNA but limited it to the region that encodes the steadiness box (a conserved sequence responsible for autoregulation of *TSG101* steady-state level, located near *TSG101*'s COOH-terminal end). They did not detect any mutation within that region. Although no mutation was identified in our study, a nucleotide variant occurring in samples C02, C05, C10 and their paired normals was detected. This C to T change was located at nucleotide 834, and resulted in a change in codon 236 from GAC to GAT. We suspect that this variant has no significant role in terms of CRC progression because the change did not result in amino acid substitution (a polymorphism). No deletion was detected in our case. This is in contradiction to a study by Balz *et al.* (2002) which reported a deletion in breast cancer that suggests germline mutation.

We tried to correlate expressions of *APC* with *WNT2* because of their close association with one another but could not gather a consistent pattern. However, in all normal colorectal samples studied, expression of APC was observed to be relatively higher than *WNT2*. We suspect that the expression pattern in which *APC* was expressed at a relatively higher level than *WNT2* is essential in maintaining the normal state of the cells. This is in agreement with the role of *APC* as a regulator, and *WNT2* as an activator in the Wnt signaling pathway (Bienz & Clevers, 2000).

It has been reported that in *TSG101* knockout mice, accumulation of *p53* protein was observed but its level of transcripts

was not affected (Ruland *et al.*, 2000). Other evidence suggesting the close interaction of *TSG101* with *p53* lies within the *p53/ MDM2* feedback control loop that upon deregulation results in tumourigenesis. In a study by Li *et al.* (2001), the Ubc domain of *TSG101* was reported to interfere with ubiquitination of *MDM2*, in which *TSG101* inhibited *MDM2* decay and elevated its own steady-state level, and these were associated with the downregulation of *p53* protein. On the other hand, elevation of *p53* upregulated *MDM2* and subsequently accelerated decaying of *TSG101*, suggesting that *TSG101* acts as both regulator of, and target of, *MDM2/ p53* circuitry. Yet, in our study we could not identify a consistent pattern that correlates expressions of *TSG101* with *p53*, at least at the transcript level, which could be associated with CRC progression.

On overall, our results on *WNT2* tally that of others. Our findings support the role of *WNT2* in cancer progression and strongly suggest its involvement in CRC tumourigenesis, perhaps via the Wnt signaling pathway. In terms of *TSG101*, our results strengthened the findings of others that its overexpression might play a role in mediating tumourigenesis. Our results also confirm previous findings that overexpression of *TSG101* is associated with CRC. We suggest that instead of being a tumour suppressor, *TSG101* might act in a manner that enhances tumour progression.

Acknowledgements. This work was supported by the research grant from Malaysian National Biotechnology Directorate- Medical Biotechnology Cooperative Centre research initiatives (Project No. 06-05-01-003 BTK/ER/018).

REFERENCES

Balz, V., Prisack, H.B., Bier, H. & Bojar, H. (2002). Analysis of BRCA1,TP53, and TSG101 germline mutations in German breast and/or ovarian cancer families. *Cancer Genetics and Cytogenetics* **138**: 120-127.

Bienz, M. & Clevers, H. (2000). Linking colorectal cancer to Wnt signaling. *Cell* **103**: 311-320.

Carney, M.E., Maxwell, G.L., Lancaster, J. M., Gumbs, C., Marks, J., Berchuck, A. & Futreal, P.A. (1998). Aberrant splicing of the TSG101 tumor suppressor gene in human breast and ovarian cancers. *Journal of Society for Gynecologic Investigation* **5**: 281-285.

Fearon, E.R. & Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. *Cell* **61**: 759-767.

Hampl, M., Hampl, J., Plaschke, J., Fitze, G., Schackert, G., Saeger, H.D. & Schackert, H.K. (1998). Evidence that TSG101 aberrant transcripts are PCR artifacts. *Biochemical and Biophysical Research Communications* **248**: 753-760.

Holcombe, R.F., Marsh, J.L., Waterman, M.L., Lin, F., Milovanovic, T. & Truong, T. (2002). Expression of Wnt ligands and frizzled receptors in colonic mucosa and in colon carcinoma. *Journal of Clinical Pathology* **55**: 220-226.

Katoh, M. (2001). Frequent up-regulation of WNT2 in primary gastric cancer and colorectal cancer. *International Journal of Oncology* **19**: 1003-1007.

Kinzler, K.W. & Vogelstein, B. (1996). Lessons from hereditary colorectal cancer. *Cell* **87**: 159-170.

Lanza, G., Matteuzzi, M., Gafa, R., Orvieto, E., Maestri, I., Santini, A. & del Senno, L. (1998). Chromosome 18q allelic loss and prognosis in stage II and III colon cancer. *International Journal of Cancer* **79**: 390-395.

Li, L., Li, X., Francke, U. & Cohen, S.N. (1997). The TSG101 tumor susceptibility gene is located in chromosome 11 band p15 and is mutated in human breast cancer. *Cell* **88**: 143-154.

Li, L., Liao, J., Ruland, J., Mak, T.W. & Cohen, S.N. (2001). A TSG101/MDM2 regulatory loop modulates MDM2 degradation and MDM2/p53 feedback control. *PNAS* **98**: 1619-1624.

Liu, R.T., Huang, C.C., You, H.L., Chou, F.F., Hu, C.C.A., Chao, F.P., Chen, C.M. & Cheng, J.T. (2002). Overexpression of

tumor susceptibility gene TSG101 in human papillary thyroid carcinomas. *Oncogene* **21**: 4830-4837.

Miller, J.R. (2001). The Wnts. *Genome Biology* **3**: 3001.1-3001.15.

Ogunbiyi, O.A., Goodfellow, P.J., Herfarth, K., Gagliardi, G., Swanson, P.E., Birnbaum, E.H., Read, T.E., Fleshman, J.W., Kodner, I.J. & Moley, J.F. (1998). Confirmation that chromosome 18q allelic loss in colon cancer is a prognostic indicator. *Journal of Clinical Oncology* **16**: 427-433.

Renkonen, E., Zhang, Y., Lohi, H., Salovaara, R., Abdel-Rahman, W.M., Nilbert, M., Aittomaki, K., Jarvinen, H.J., Mecklin, J.P., Lindblom, A. & Peltomaki, P. (2003). Altered expression of MLH1, MSH2, and MSH6 in predisposition to hereditary nonpolyposis colorectal cancer. *Journal of Clinical Oncology* **21**: 3629-3637.

Ruland, J., Sirard, C., Elia, A., MacPherson, D., Wakeham, A., Li. L., Pompa, J.L.D.L., Cohen, S.N. & Mak, T.W. (2000). P53 early accumulation, defective cell proliferation, and early embryonic lethality in mice lacking tsg101. *PNAS* **98**: 1859-1864.

Sim, E.U.H., Bong, I.P.N., Balraj, P., Tan, S.K., Jamal, R., Sagap, I., Nadeson, S., Rose, I.M. & Lim, P.K.M. (2006). A preliminary study of differentially expressed genes in Malaysian Colorectal Carcinoma Cases. *Journal of Bioscience* **17**(1): 27-45.

Turpin, E., Dalle, B. de Roquancourt, A., Plassa, L.F., Marty, M. Janin, A., Beuzard, Y., de The, H. (1999). Stress-induced aberrant splicing of TSG101: association to high tumor grade and p53 status in breast cancers. *Oncogene* **18**: 7834-7837.

Vider, B.Z., Zimber, A., Chastre, E., Prevot, S., Gespach, C., Estlein, D., Wolloch, Y., Tronick, S.R., Gazit, A. & Yaniv, A. (1996). Evidence for the involvement of the Wnt 2 gene in human colorectal cancer. *Oncogene* **12**: 153-158.

Wagner, K.U., Krempler, A., Qi, Y., Park, K.R., Henry, M.L.D., Triplett, A.A., Riedlinger, G., Rucker III, E.B. & Hennighausen, L. (2003). Tsg101 is essential for cell growth, proliferation, and cell survival of embryonic and adult tissues. *Molecular and Cellular Biology* **23**: 150-162.

Xie, W., Li, L. & Cohen, S.N. (1998). Cell cycle-dependent subcellular localization of the TSG101 protein and mitotic and nuclear abnormalities associated with TSG101 deficiency. *PNAS* **95**: 1595-1600.

You, Z., Saims, D., Chen, S., Zhang, Z., Guttridge, D.C., Guan, K., MacDougald, O.A., Brown, A.M.C., Evan, G., Kitajewski, J. & Wang, C.Y. (2002). Wnt signaling promotes oncogenic transformation by inhibiting c-Myc-induced apoptosis. *The Journal of Cell Biology* **157**: 429-440.

Young, T.W., Mei, F.C., Rosen, D.G., Yang, G., Li, N., Liu, J. & Cheng, X. (2007). Up regulation of tumour susceptibility gene 101 protein in ovarian carcinomas revealed by proteomics analyses. *Molecular & Cellular Proteomics* **6**: 294-304.

Zang, T., Zhuang, L., Zhang, Z., Xin, D. & Guo, Y. (2000). Aberrant activity of Wnt/Frizzled signaling pathway in renal cancer cell lines. *Chinese Science Bulletin* **45**: 1703-1707.

Zhu, G., Gilchrist, R., Borley, N., Chng, H.W., Morgan, M., Marshall, J.F., Camplejohn, R.S., Muir, G.H. & Hart, I.R. (2004). Reduction of TSG101 protein has a negative impact on tumor cell growth. *International Journal of Cancer* **109**: 541-547.