

β -Catenin Splice Variants and Downstream Targets as Markers for Neoplastic Progression of Esophageal Cancer

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This study characterizes the frequency of exon 3 *CTNNB1* mutations and compares the expression of *CTNNB1* transcript variants and downstream targets *MYC* and *WAF1* (p21) across the neoplastic progression of esophageal squamous cell carcinomas (ESCCs). Mutational analysis was performed on 56 tumors and corresponding germline DNA, using primers to exon 3 of *CTNNB1* and SSCP DNA sequencing gels. Quantitative Real Time RT-PCR was performed on 45 foci representing the histological spectrum from normal to invasive cancer, using specific primer sets for alternative splice variants that differ by the presence (16A) or absence (16B) of a 159-bp noncoding segment of exon 16 of *CTNNB1*, in conjunction with downstream targets *MYC* and *WAF1*. Two unique mutations were identified, S37F in the SxxxS repeat region, and a germline polymorphism, T59A. Thus, mutation of *CTNNB1* exon 3 is a rare event in this population. RT-PCR analysis successfully confirmed the presence of both β -catenin splice variants in histologically normal and preneoplastic squamous epithelium, and invasive tumors of the esophagus, and identified a significant reduction in the 16A/16B ratio ($P = 0.014$) and an accompanying significant increase in the *MYC/WAF1* expression ratio ($P = 0.001$) with progression from normal mucosa to dysplasia. This represents the first identification of two *CTNNB1* transcripts in histologically "normal" esophageal squamous cells, squamous dysplasia, and invasive ESCC. These results show an increase in the minor mRNA (16B) isoform and changes in the expression of downstream markers consistent with increased transcription during the histological progression from normal to squamous dysplasia. Published 2005 Wiley-Liss, Inc.†

INTRODUCTION

β -Catenin (*CTNNB1*) is located in a chromosomal region (3p) associated with both early and late genetic events and appears to be overexpressed in esophageal squamous cell carcinoma (ESCC) at the protein level (Roth et al., 2001, 2002). Other studies of esophageal cancer show overexpressed *CTNNB1* competing for binding partners such as APC (Kimura et al., 1999; Osterheld et al., 2002) and, subsequently, excess *CTNNB1* is stabilized and available for nuclear translocation (Bienz and Clevers, 2003). Despite the findings of generalized *CTNNB1* overexpression and genetic changes at 3p, it is still not clear whether alteration in expression of *CTNNB1*, downstream targets and/or nuclear or cytoplasmic localization of *CTNNB1* are associated with the development of ESCC. These potential associations are further complicated by alternative splice forms associated with *CTNNB1*, as initially described by Nollet et al.

Genomic cloning shows the *CTNNB1* locus to consist of 16 exons stretching over a region of 23 kb and carrying a splice variant (16B) for a truncated portion of the noncoding region of exon 16 (Nollet et al., 1996). In a small set of human colon cancer cell lines, both the wild type and variant have been identified at similar levels by RT-PCR. However, this variant has not been studied in the setting of ESCC neoplasia, in which it may be biologically significant given that 3' untranslated regions (UTRs) may possess translational control of regulatory elements that govern the spatial and temporal expression of mRNA (Mendez and Richter, 2001;

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Hu et al., 2002; Kuersten and Goodwin, 2003; Kalnina et al., 2005).

As an adherens junction or zonula adherens protein, CTNNB1 is associated with cell signaling through the APC or Wnt pathways, with complex binding of E-cadherin, APC, TCF, AXIN, GSK3 β , and α -catenin. Some of the functions of CTNNB1 include mediating adhesion between cells and subsequently regulating normal cell growth and behavior, including embryogenesis, wound healing, and tumor metastasis. The latter is, in part, related to the participation of CTNNB1 in transcription via interaction with the T-cell family (TCF) of transcription factors. Stabilized CTNNB1 interacts with TCF and activates transcription of downstream target genes such as *MYC* and *WAF1* (He et al., 1998; Bieche et al., 1999). Degradation of β -catenin requires a multi-protein complex that includes APC and the serine/threonine kinase GSK3 β . In tumors, this degradation can be blocked by mutations typically involving exon 3 of the *CTNNB1* gene (Behrens, 2000).

The goal of this study was to characterize the frequency of exon 3 *CTNNB1* mutations and to compare the expression of *CTNNB1* transcript variants and the expression of downstream targets such as *MYC* and *WAF1* across the neoplastic progression of ESCC from a high-risk region.

MATERIALS AND METHODS

Patients and Samples

All cases and samples were obtained from subjects residing in regions of north central China where rates (>100/100,000 year) of ESCC are extraordinarily high. This study was approved by the Institutional Review Boards of the collaborating institutions, Shanxi Cancer Hospital and Institute, Taiyuan, Shanxi; the Cancer Institute of the Chinese Academy of Medical Sciences (CICAMS), Beijing; and the US National Cancer Institute, Bethesda, MD.

Mutation Analysis

Patients presenting to the Shanxi Cancer Hospital in Taiyuan, Shanxi, PR China, who were diagnosed with ESCC and considered candidates for curative surgical resection, were identified and recruited to participate in this study. Ten milliliters of venous blood and a portion of tumor fixed in ethanol were obtained from 56 patients and analyzed for *CTNNB1* exon 3 mutations using SSCP. Genomic and somatic DNA were extracted and

purified from venous blood and tumor tissue, respectively, using methods previously described (Hu et al., 2001). Briefly, tumor cells were microdissected under light microscopic visualization, and mutations in exon 3 of *CTNNB1* were screened by PCR-SSCP using primers (5'-CTAATGCTAATACTGTTTTCGT-3' and 5'-TACTCTTACCAGCTACTTGTTCCT-3') producing a 228-bp PCR product, after adjusting the annealing temperature to 53°C. The DNA was subsequently sequenced and all mutations were confirmed by repeating the procedures outlined earlier.

Real Time RT-PCR Analysis

Resection specimens from six patients were fully submitted in histology cassettes and stored at -70°C until Real Time RT-PCR analysis of *CTNNB1*, *MYC*, and *WAF1*. Serial 5- μ m frozen sections were cut from each histology cassette and representative foci of normal ($N = 11$), low grade dysplasia (LGD, $N = 11$), high grade dysplasia (HGD, $N = 8$), carcinoma in situ ($N = 4$), and invasive squamous cell carcinoma ($N = 11$) from the six fully submitted esophageal resections were chosen based on histological review by a pathologist (MR) of corresponding H&E-stained slides, using accepted histological criteria (Dawsey et al., 1994). RNA was isolated from these laser capture microdissected tissue foci using a standard Trizol extraction protocol in the presence of yeast carrier tRNA. Approximately 25% of the resultant RNA was used from each laser capture microdissection sample for the synthesis of cDNA. Using the Thermoscript RT-PCR Synthesis System (Invitrogen, Carlsbad, CA), oligo dT and random hexamer primers were used to synthesize cDNA following the manufacturer's protocol. Primers specific for the 16A and 16B isoforms of *CTNNB1*, *MYC*, *WAF1* (*p21*), *GAPDH*, *18s rRNA*, and β -*actin* (Table 1) were used to amplify specific products either with SYBR green (50 cycles Real Time RT-PCR) or without SYBR green (35 cycles conventional gel-based RT-PCR). Unless specified, all reaction conditions were conducted in Platinum Supermix (Invitrogen) using standard PCR conditions, including a reannealing temperature of 55°C. For standardizing Real Time PCR product concentrations, purified inserts of the appropriate products were used in serial dilutions and then plotted on a log-scale to calculate starting quantity (threshold starting cycle). All samples were normalized to housekeeping controls (β -actin) prior to calculating the levels of gene expression.

TABLE 1. Specific Primers for Real Time RT-PCR Analysis

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
<i>CTNNB1</i> complete cDNA	AGC CAC AAG ATT ACA AGA AAC	AGG CTA GGG TTT GCT AAA TTC
<i>CTNNB1 16A</i> isotype (exons 11–16)	GTT ATC AAG AGG ACT AAA TAC CA	GAC AAT ACA GCT AAA TGA TGA T
<i>CTNNB1 16B</i> isotype (exons 11–16)	GTT ATC AAG AGG ACT AAA TAC CA	GTA TTG TTA CTC CTA AAG GAT GA
<i>MYC</i> (exons 2 and 3)	GCC CCT GGT GCT CCA TGA	ACC CTC TTG GCA GCA GGA TA
<i>WAF1</i> (exons 1 and 2)	ACA GCA GAG GAA GAC CAT GTG	GGG CTT CCT CTT GGA GAA GAT
<i>18s rRNA</i>	TCAAGAACGAAAGTCCGAGG	GGACATCTAAGGGCATCACA
<i>β-Actin</i>	CCA CAC TGT GCC CAT CTA CG	CAG CGG AAC CGC TCA TTG CCA ATG G

Statistical Analysis

Gene expression values were transformed using natural log. Because of small numbers, LGD and HGD were analyzed together as DYS, and CIS and invasive squamous cell carcinoma were analyzed together as CA. The percent change in expression from normal to DYS and CA was estimated using linear mixed models including a random intercept for participant. Grade was described with two indicator variables that were treated as fixed effects. Because of the range of samples per specimen additional models with a fixed effect for the number of samples per specimen were examined to test for effect modification between the number of samples and histology (results not shown). All tests of statistical significance were two-sided. Statistical analyses were performed using S-PLUS (S-PLUS version 6.1 for Windows; Insightful Corporation, Seattle, WA, 2002).

RESULTS

We find exon 3 of *CTNNB1* to be infrequently mutated in ESCC tumors (2 of 56 (4%) ESCC cases) and have identified a single somatic TCT-TTT change (S37F), resulting in a serine-to-phenylalanine substitution in the SxxxS repeat region, and a germline polymorphism ACC-GCC change (T59A) resulting in an threonine-to-alanine substitution (Fig. 1). This finding of a low frequency of mutations is consistent with that observed by others in ESCC (de Castro et al., 2000; Ninomiya et al., 2000) and histologically similar squamous cell cancers of the head and neck (Gonzalez et al., 1998). The absence of a corresponding somatic mutation in case 152 (Fig. 1) is also consistent with the LOH of 3p seen in preneoplastic lesions and invasive tumors and suggests that random (i.e., nonselective) chromosomal loss may frequently involve the *CTNNB1* locus (Roth et al., 2001). In this high-risk population, mutation of exon 3 does not seem to be responsible for the increase in protein expression identified in the majority of these esophageal tumors. Consequently, six fully blocked frozen esophagectomy specimens, with an average

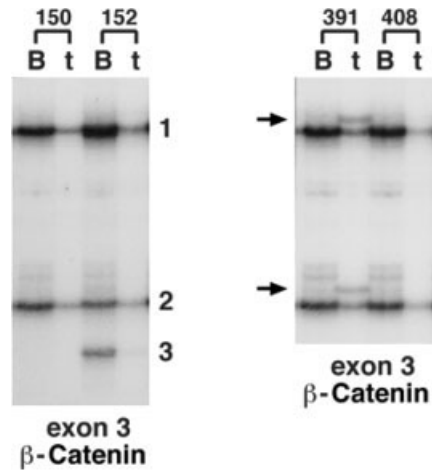


Figure 1. SSCP mutation analysis of exon 3 of *CTNNB1* was performed on 10 ml of venous blood (B) and matched tumor (t). A single germline polymorphism ACC-GCC change (T59A) resulting in a threonine-to-alanine substitution (Case 152, band 3) and a somatic TCT-TTT change (S37F), resulting in a serine-to-phenylalanine substitution in the SxxxS repeat region (case 391, arrowhead) were identified.

of 7 (range, 3–15) foci per specimen representing the histological spectrum of neoplastic progression from normal to invasive squamous cell carcinoma were selected for RT-PCR analysis. From these resections, a total of 11 foci of histologically normal (Nml) epithelium were found in 4 esophagectomies, 11 foci of low grade dysplasia (LGD) were found in 6 esophagectomies, 8 foci of high grade dysplasia (HGD) were found in 4 esophagectomies, 4 foci of carcinoma in situ (CIS) were found in 2 esophagectomies, and 11 foci of invasive cancer were found in 5 esophagectomies. Because of the small number of foci and the fact that CIS was adjacent, that is, in the same section, to the invasive cancer, the expression results from LGD and HGD were combined into a DYS category and those from CIS and invasive SCC were combined into a Cancer (CA) category. RT-PCR analysis identified *CTNNB1* splice variants, *16A* and *16B* (Fig. 2), and successfully amplified RT-PCR products for *MYC* and *WAF1* in every histological category. Mean expression values for all normal, dysplasia, and cancer foci are shown in Table 2.

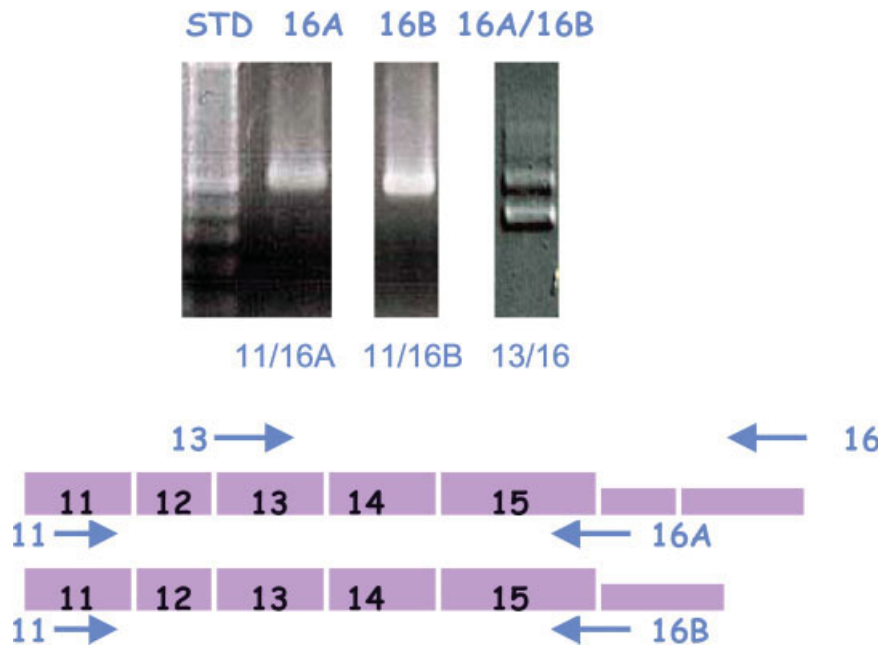


Figure 2. Gel electrophoretic RT-PCR results with primers extending from exon 11 to 16A, exon 11 to 16B, and exon 13 to exon 16 inclusive. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 2. Geometric Mean of mRNA Expression for Participants With Normal Histology and % Change (Δ) of Expression from Normal to Dysplasia and Cancer^a

	Normal, mean	Dysplasia, Δ^b	Cancer, Δ^b
CTNNB1 16A	2.2×10^{-8} (0.6–8.0) ^c	–69 (–91 to 9.4)	–22 (–79 to 197)
CTNNB1 16B	1.7×10^{-9} (0.4–6.9)	162 (–32 to 913)	94 (–55 to 735)
16A + 16B	3.4×10^{-8} (1.2–9.4)	–51 (–81 to 29)	–17 (–70 to 135)
16A/16B	13.1 (2.5–68)	–88 ^d (–97 to –40)	–61 (–93 to 108)
MYC	8.5×10^{-9} (4.6–16)	78 (–9.3 to 248)	45 (–29 to 196)
WAF1	9.6×10^{-7} (5.1–18.0)	–52 ^d (–74 to –11)	34 (–31 to 161)
MYC/WAF1	0.008 (0.003–0.02)	283 ^d (84 to 701)	31 (–42 to 194)

^aSix participants with a total of 43 observations for 16A + 16B and 16A/16B, 44 observations for 16A, 16B, WAF1, and MYC/WAF1 ratio; and 45 observations for MYC.

^bEstimates of percent change are from linear mixed models, including participant as a random effect and grade as a fixed effect treated as two indicator variables.

^cValues in parentheses indicates 95% CI.

^dCompared to normal tissue, the percent change of mRNA expression in dysplasia is significantly different for WAF1 ($P = 0.026$) and the 16A/16B ($P = 0.014$) and MYC/WAF1 ($P = 0.001$) ratios.

The geometric mean RNA expression of each *CTNNB1* product varies with histological severity between Nml, DYS, and CA (Table 2). As can be seen in the normal tissue, splice variant 16A was about ten times as abundant as 16B. The mean value for 16A was lower in DYS than in Nml, but higher in CA than in DYS. The mean value for 16B was higher in DYS than in Nml, but lower in CA than in DYS. In addition, compared to histologically normal epithelium, total *CTNNB1* (16A + 16B) mRNA expression in DYS was 51% lower. The reduction in total *CTNNB1* between Nml and DYS resulted from a nearly 70% decrease of the more abundant splice variant 16A and a 162% increase of the less abundant variant 16B. Consequently, there was a significant reduction in the

16A/16B mRNA expression ratio ($P = 0.014$) between Nml and DYS. Downstream markers *MYC* and *WAF1* also varied across the spectrum of histological lesions, with changes in *MYC* values paralleling those in 16B and *WAF1* values changing parallel to 16A. Quantification of downstream markers of transcription showed over a 78% higher *MYC* mRNA expression in DYS than in Nml epithelium, where as *WAF1* was 52% lower in DYS than in Nml ($P = 0.026$). As a consequence of these differences in directionality, the *MYC/WAF1* expression ratio was also reduced ($P = 0.001$).

DISCUSSION

This analysis represents the first identification of two *CTNNB1* transcripts, differing by the presence

(16A) or absence (16B) of a 159-bp noncoding segment of exon 16, in histologically normal esophageal epithelium, squamous dysplasia, and invasive ESCC. In contrast to prior immunohistochemical analysis, which showed an increase in protein, we found a reduction in the total *CTNNB1* mRNA expression in dysplastic epithelium. The current results also show that the ratio for the *CTNNB1* splice variants is significantly reduced with histological progression from normal to dysplastic epithelium. In the context of the protein findings and in the absence of exon 3 mutations, these results suggest that there may be preferential processing and an increase in the minor mRNA 16B isoform with progression from a normal histology to DYS. Although this study was not designed to determine the mechanism behind such a change, possible explanations include an increase in transcription efficiency, mRNA stability, or a decrease in nuclear export of the 16B isoform. Such mechanisms are consistent with studies showing that 3' UTRs can contain several regulatory elements governing the spatial and temporal expression of mRNA (Cok and Morrison, 2001; Hurlstone and Clevers, 2002; Kuersten and Goodwin, 2003; Kalnina et al., 2005).

The mean *CTNNB1* mRNA expression alterations are accompanied by increased mean *MYC* and decreased mean *WAF1* mRNA expression that synergistically favor an increase in cell transcription (Bitzer et al., 2003). This is consistent with the fact that *MYC* and *WAF* represent TCF target genes and are part of a potential malignant transformation cascade involving CTNNB1 in the gastrointestinal tract (Hurlstone and Clevers, 2002; van de Wetering et al., 2002). This is also consistent with the finding that the CTNNB1/TCF-4 complex controls proliferation versus differentiation in healthy and malignant intestinal epithelial cells by affecting *MYC* and *WAF1* activity, which control G1 arrest and differentiation (van de Wetering et al., 2002). These genes also seem to be involved in the neoplastic progression of esophageal tumors as well as sites outside of the gastrointestinal tract (Wang et al., 1998; Sarbia et al., 1999; Polakis, 2000; Tselepis et al., 2003).

We cannot yet explain why the changes in *CTNNB1*, *MYC*, and *WAF1* expression with progression from DYS to CA trend toward the levels found in histologically normal epithelium. However, this pattern suggests that these genes may play a more significant role in the earlier stages of neoplastic progression, from normal to DYS. These findings are limited by the large variation of expression between the foci with the same histol-

ogy. A larger sample size may help to reduce this variability.

A better understanding of these disease-specific changes will help in the development of more effective early detection and disease prevention strategies. Additional studies should be directed at localizing CTNNB1 protein and the message for its alternate splice forms to further elucidate possible mechanisms associated with the neoplastic progression of ESCC.

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REFERENCES

- Behrens J. 2000. Control of β-catenin signaling in tumor development. *Ann N Y Acad Sci* 910:21–33.
- Bieche I, Laurendeau I, Tozlu S, Olivi M, Vidaud D, Lidereau R, Vidaud M. 1999. Quantification of *MYC* gene expression in sporadic breast tumors with a real-time reverse transcription-PCR assay. *Cancer Res* 59:2759–2765.
- Bienz M, Clevers H. 2003. Armadillo/β-catenin signals in the nucleus—proof beyond a reasonable doubt. *Nat Cell Biol* 5:179–182.
- Bitzer M, Stahl M, Arjumand J, Rees M, Klump B, Heep H, Gabbert HE, Sarbia M. 2003. C-myc gene amplification in different stages of oesophageal squamous cell carcinoma: prognostic value in relation to treatment modality. *Anticancer Res* 23:1489–1493.
- Cok SJ, Morrison AR. 2001. The 3'-untranslated region of murine cyclooxygenase-2 contains multiple regulatory elements that alter message stability and translational efficiency. *J Biol Chem* 276:23179–23185.
- Dawsey SM, Lewin KJ, Liu SF, Wang GQ, Shen Q. 1994. Esophageal morphology from Linxian, China. Squamous histologic findings in 754 patients. *Cancer* 73:2027–2037.
- de Castro J, Gamallo C, Palacios J, Moreno-Bueno G, Rodriguez N, Feliu J, Gonzalez-Baron M. 2000. β-catenin expression pattern in primary oesophageal squamous cell carcinoma. Relationship with clinicopathologic features and clinical outcome. *Virchows Arch* 437:599–604.
- Gonzalez MV, Pello MF, Ablanado P, Suarez C, Alvarez V, Coto E. 1998. Chromosome 3p loss of heterozygosity and mutation analysis of the FHTT and β-cat genes in squamous cell carcinoma of the head and neck. *J Clin Pathol* 51:520–524.
- He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B, Kinzler KW. 1998. Identification of c-MYC as a target pathway of the APC pathway. *Science* 281:1509–1512.
- Hu N, Huang J, Emmert-Buck MR, Tang ZZ, Roth MJ, Wang C, Dawsey SM, Li G, Li W-J, Wang Q-H, Han X-Y, Ding T, Giffen C, Goldstein AM, Taylor PR. 2001. Frequent inactivation of the TP53 gene in esophageal squamous cell carcinoma from a high-risk population in China. *Clin Cancer Res* 7:883–891.
- Hu X, Bi J, Loh HH, Wei LN. 2002. Regulation of mouse κ Opioid receptor gene expression by different 3'-untranslated regions and the effect of retinoic acid. *Mol Pharmacol* 62:881–887.
- Hurlstone A, Clevers H. 2002. T-cell factors: turn-ons and turn-offs. *EMBO J* 21:2303–2311.
- Kalnina Z, Zayakin P, Silina K, Line A. 2005. Alterations of pre-mRNA splicing in cancer. *Genes Chromosomes Cancer* 42:342–357.
- Kimura Y, Shiozaki H, Doki Y, Yamamoto M, Utsunomiya T, Kawanishi K, Fukuchi N, Inoue M, Tsujinaka T, Monden M. 1999. Cytoplasmic β-catenin in esophageal cancers. *Int J Cancer* 84:174–178.
- Kuersten S, Goodwin EB. 2003. The power of the 3' UTR: translational control and development. *Nat Rev Genet* 4:626–637.
- Mendez R, Richter JD. 2001. Translational control by CPEB: a means to the end. *Nat Rev Mol Cell Biol* 2:521–529.

- Ninomiya I, Endo Y, Fushida S, Sasagawa T, Miyashita T, Fujimura T, Nishimura G, Tani T, Hashimoto T, Yagi M, Shimizu K, Ohta T, Yonemura Y, Inoue M, Sasaki T, Miwa K. 2000. Alteration of β -catenin expression in esophageal squamous-cell carcinoma. *Int J Cancer* 85:757–761.
- Nollet F, Bex G, Molemans F, van Roy F. 1996. Genomic organization of the human β -catenin gene (CTNNB1). *Genomics* 32:413–424.
- Osterheld MC, Bian YS, Bosman FT, Benhattar J, Fontollet C. 2002. β -catenin expression and its association with prognostic factors in adenocarcinoma developed in Barrett esophagus. *Am J Clin Pathol* 117:451–456.
- Polakis P. 2000. Wnt signaling and cancer. *Genes Dev* 14:1837–1851.
- Roth MJ, Hu N, Emmert-Buck MR, Wang Q-H, Dawsey SM, Guang L, Guo W-J, Zhang Y-Z, Taylor PR. 2001. Genetic progression and heterogeneity associated with the development of esophageal squamous cell carcinoma. *Cancer Res* 61:4098–4104.
- Roth MJ, Hu N, Paweletz CP, Iwamoto M, Qiao Y-L, Li W-J, Su H, Ahnen DJ, Dawsey SM, Taylor PR. 2002. Proteomic analysis of β -catenin during the neoplastic progression from precursor lesions to invasive squamous cell carcinoma of the esophagus. *Cancer Detect Prev* 26:4505.
- Sarbia M, Loberg C, Wolter M, Arjumand J, Heep H, Reifemberger G, Gabbert HE. 1999. Expression of Bcl-2 and amplification of *c-myc* are frequent in basaloid squamous cell carcinomas of the esophagus. *Am J Pathol* 155:1027–1032.
- Tselepis C, Morris CD, Wakelin D, Hardy R, Perry I, Luong QT, Harper E, Harrison R, Attwood SE, Jankowski JA. 2003. Upregulation of the oncogene *c-myc* in Barrett's adenocarcinoma: induction of *c-myc* by acidified bile acid in vitro. *Gut* 52:174–180.
- van de Wetering M, Sancho E, Verweij C, de Lau W, Oving I, Hurlstone A, van der HK, Battle E, Coudreuse D, Haramis AP, Tjon-Pon-Fong M, Moerer P, van den BM, Soete G, Pals S, Eilers M, Medema R, Clevers H. 2002. The β -catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cells. *Cell* 111:241–250.
- Wang LD, Zhou Q, Wei JP, Yang WC, Zhao X, Wang LX, Zou JX, Gao SS, Li YX, Yang C. 1998. Analysis of gene expression profile induced by EMP-1 in esophageal cancer cells using cDNA Microarray. *World J Gastroenterol* 4:287–293.