World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i24.2909

World J Gastroenterol 2011 June 28; 17(24): 2909-2923
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Genomic imbalances in esophageal carcinoma cell lines involve Wnt pathway genes

Jacqueline Brown, Hannelie Bothma, Robin Veale, Pascale Willem

Jacqueline Brown, Hannelie Bothma, Pascale Willem, National Health Laboratory Services and University of the Witwatersrand, York Rd, Parktown Johannesburg 2193, South Africa
Robin Veale, School of Molecular and Cell Biology, University of the Witwatersrand, Yale Rd, Johannesburg 2193, South Africa
Author contributions: Brown J performed most experiments, did the analysis and wrote this manuscript; Bothma H performed analysis of conventional cytogenetics; Veale R provided and cultured the cell lines; Willem P conceptualized the study, assisted with results analysis and wrote this manuscript.
Supported by The Cancer Association of South Africa, the National Health Laboratory Services Research Trust and the Medical Research council of South Africa
Correspondence to: Jacqueline Brown, MSc, National Health Laboratory Services and University of the Witwatersrand, York Rd, Parktown Johannesburg 2193,
South Africa. jacqueline.brown@nhls.ac.za
Telephone: +27-11-4898575 Fax: +27-11-4898480
Received: October 7, 2010 Revised: October 30, 2010
Accepted: November 6, 2010
Published online: June 28, 2011

Abstract

AIM: To identify molecular markers shared across South African esophageal squamous cell carcinoma (ESCC) cell lines using cytogenetics, fluorescence *in situ* hybridization (FISH) and single nucleotide polymorphism (SNP) array copy number analysis.

METHODS: We used conventional cytogenetics, FISH, and multicolor FISH to characterize the chromosomal rearrangements of five ESCC cell lines established in South Africa. The whole genome copy number profile was established from 250K SNP arrays, and data was analyzed with the CNAT 4.0 and GISTIC software.

RESULTS: We detected common translocation breakpoints involving chromosomes 1p11-12 and 3p11.2, the latter correlated with the deletion, or interruption of the *EPHA3* gene. The most significant amplifications involved the following chromosomal regions and genes: 11q13.3 (*CCND1*, *FGF3*, *FGF4*, *FGF19*, *MYEOV*), 8q24.21(*C-MYC*, *FAM84B*), 11q22.1-q22.3 (*BIRC2*, *BIRC3*), 5p15.2 (*CTNND2*), 3q11.2-q12.2 (*MINA*) and 18p11.32 (*TYMS*, *YES1*). The significant deletions included 1p31.2-p31.1 (*CTH*, *GADD45α*, *DIRAS3*), 2q22.1 (*LRP1B*), 3p12.1-p14.2 (*FHIT*), 4q22.1-q32.1 (*CASP6*, *SMAD1*), 8p23.2-q11.1 (*BNIP3L*) and 18q21.1-q21.2 (*SMAD4*, *DCC*). The 3p11.2 translocation breakpoint was shared across four cell lines, supporting a role for genes involved at this site, in particular, the *EPHA3* gene which has previously been reported to be deleted in ESCC.

CONCLUSION: The finding that a significant number of genes that were amplified (*FGF3*, *FGF4*, *FGF19*, *CCND1* and *C-MYC*) or deleted (*SFRP2* gene) are involved in the Wnt and fibroblast growth factor signaling pathways, suggests that these pathways may be activated in these cell lines.

Key words: Esophagus; Cancer; Single nucleotide polymorphism arrays; Fluorescent *in situ* hybridization

Peer reviewer: Julian Swierczynski, MD, PhD, Professor, Department of Biochemistry, Medical University of Gdansk, 80-211 Gdansk, Poland

Brown J, Bothma H, Veale R, Willem P. Genomic imbalances in esophageal carcinoma cell lines involve Wnt pathway genes. *World J Gastroenterol* 2011; 17(24): 2909-2923 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i24/2909.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i24.2909

INTRODUCTION

Esophageal squamous cell carcinoma (ESCC) is a major cause of cancer-related death in the world and it is char-

acterized by a peculiar epidemiology with worldwide geographic pockets of high incidence. In South Africa, the region of the Eastern Cape shows the highest incidence and ESCC represents the leading cancer affecting men and the second most common cancer in woman with a prevalence of 31.3 and 18 per 100 000 individuals, respectively[1]. The Gauteng (Johannesburg) and the Western Cape regions are also affected but to a lesser extent[2,3]. The tumors are diagnosed at advanced stages of the disease and patients have a poor survival rate. The etiology of this cancer is unresolved and while the most common risk factors associated with ESCC include smoking and alcohol consumption, these factors are surprisingly lacking in some parts of the world that have a high incidence such as in Iran and northern parts of China[4]. Additional risk factors have been proposed to play a role in some regions. In particular, exposure to fumonisin, a Fusarium fungi toxin that grows on maize, was reported in South Africa and China[5,6] as well as human papillomavirus (HPV) infection[7]. Poor nutrition is associated with ESCC in most parts of the world[4], and chronic inflammation was described in endemic parts of South Africa[8]. The respective part played by environmental risk factors and potential genetic susceptibility remains unclear and it is possible that different combinations of factors may be at play in different parts of the world.

It is widely accepted that recurrent chromosomal breakpoints in malignancies often pinpoint genes involved in the initiation or progression of cancer[9]. A major limitation in assessment of the chromosome complement in ESCC specimens is the difficulty in obtaining metaphases from fresh tumors, and established cell lines provide a unique resource for such investigations. The number of ESCC cell lines that have been reported to date remains limited and were all established in China[10-12] and Japan[13]. These cell lines have been investigated with one or several low resolution molecular cytogenetic techniques including cytogenetics, fluorescence *in situ* hybridization (FISH), multicolor FISH (M-FISH) or SKY and conventional comparative genomic hybridization (CGH). Various clonal aberrations have been identified and the most common abnormalities across studies involved over representation of chromosomes 1q, 3q, 11q and 8q as well as breakpoints in the centromeric or near centromeric regions of chromosomes 1, 3 and 8[10-12,14].

Five cell lines have previously been established from South African ESCC patients[15,16] but apart from cell line SNO, which was karyotyped, these cell lines were never characterized for their genetic constitution. We used conventional cytogenetics, FISH, and M-FISH to identify common chromosome structural abnormalities across these cell lines. Affymetrix 250K single nucleotide polymorphism (SNP) arrays were performed to investigate DNA copy number changes and the common aberrations detected by M-FISH and conventional cytogenetics. Here we describe clonal aberrations shared by these cell lines and highlight preferential targets for chromosomal rearrangements and copy number changes. These are the first ESCC cell lines from Africa to be genetically characterized to our knowledge.

MATERIALS AND METHODS

Cell lines

The five esophageal carcinoma cell lines used in this study were previously isolated from male patients with moderately differentiated ESCCs[16]. These cell lines were previously described and are designated as WHCO1, WHCO3, WHCO5, WHCO6 and SNO cell lines[15,16].

Cytogenetics

Cell lines were cultured at 37℃, 5% CO_2 in Dulbecco's Modified Eagles medium: HAMS F12 (3:1) (GIBCO®, Invitrogen Corporation, USA) containing 10% fetal calf serum, 100 μg/mL streptomycin (ICN, Costa Mesa, CA, USA) and 100 IU/mL of penicillin (ICN, Costa Mesa, CA, USA). When the cultures were half confluent, the cells were incubated with a final concentration of 0.44 μg/mL of Karyomax® Colcemid® (Invitrogen Corporation, USA) for 4 h. Cells were then harvested by standard cytogenetic procedure. Slides were either prepared for metaphase analysis using GTG banding by standard procedures or for FISH preparations.

M-FISH

M-FISH was performed using the SpectraVysion™ Assay (Vysis®, Abbott Molecular Inc, IL, USA) according to the manufacturer's protocol. The slides were analyzed on an Olympus BX41 fluorescent microscope with six single band pass filters for visualization of the six fluorophores. Genus™ CytoVision 3.0 software (Applied Imaging Corporation, San Jose, California, USA) was used for image acquisition and analysis. Ten metaphases were analyzed per cell line.

FISH

FISH was performed on metaphase chromosomes from all cell lines in order to confirm or refine translocation derivative breakpoints and clarify their composition. Probes specific for the short and long arms of chromosome 3, the short arm of chromosome 1 and the long arm of chromosome 22 (Qbiogene, Strasbourg, France) as well as the Vysis® Cep 3 Alpha and Cep 1 Alpha SpectrumOrange probes (Abbott Molecular Inc., IL, USA) were hybridized to further map the breakpoints. The Vysis® LSI IGH and Vysis® LSI RARA, both dual color break apart rearrangement probes (Abbott Molecular Inc., IL, USA) were used to confirm the involvement of chromosomes 14 and 15 in translocation derivatives seen in cell lines WHCO1 and WHCO3 respectively. In order to establish if the *EGFR* (epidermal growth factor receptor) gene was involved in a marker chromosome 7 the Vysis® LSI EGFR SO/CEP 7 SG probe (Abbott Molecular Inc., IL, USA) was hybridized to metaphase chromosomes.

FISH was also performed on interphase nuclei from all cell lines using the Vysis® LSI C-MYC SpectrumOrange

probe and the Vysis® LSI t(11;14) dual color probe (Abbott Molecular Inc., IL, USA). These probes target the *C-MYC* gene (Spectrum Orange) on 8q24 and the *CCND1* gene (Spectrum Orange) on chromosome 11q respectively and were used to confirm SNP array copy number results. A hundred interphase nuclei were analyzed in each cell line. All FISH experiments were performed according to the manufacturer's instructions and analyzed on an Olympus BX41 fluorescent microscope equipped with appropriate fluorescence filters. The Genus™ CytoVision 3.0 software was used for image acquisition and analysis.

DNA isolation

DNA was extracted from the cell lines and from six blood specimens obtained from healthy and population-matched volunteers using standard phenol-chloroform extraction methods. DNA was quantified on the ND-1000 Spectrophotometer (Nanodrop® Technologies, Rockland, DE) and quality assessed by gel electrophoresis.

SNP arrays and data analysis

Cell lines and control DNA were hybridized to the Affymetrix® GeneChip® Human Mapping 250 K Nsp Arrays (Santa Clara, CA). The GeneChip® mapping 500 K protocols were used. The control group used as a reference for analysis was DNA samples extracted from whole blood from black male volunteers.

Data analysis was performed using the Affymetrix®, Genotyping Console™ 2.0 and the copy number analysis tool (CNAT 4.0) software (Affymetrix®, Santa Clara, USA). Subsequently data were analyzed with third party software, Genepattern[17]. The signal intensities from the CEL files were normalized by the PM-MM (perfect match minus mismatch) probe intensity and quantile normalization against the median intensity of the controls. The raw copy number was then estimated as a ratio against the signal intensities of the normal reference samples. The log2ratios were smoothed using GLAD (Gain and Loss analysis of DNA)[18].

To determine the significant common regions of amplification and deletion (i.e. driver aberrations as opposed to random passenger aberrations) across the five cell lines, the GISTIC (Genomic Identification of Significant Targets in Cancer) algorithm[19] was applied to the smoothed data. The algorithm first scores the regions of copy number change according to their frequency and amplitude, which indicates the likelihood of these regions to represent a driver aberration (G-score). The statistical significance of each G-score is calculated by comparison of these scores against a null model of random aberrations. This significance is represented as a q-value (False discovery rate), which is the likelihood that the data was generated by chance. The most probable locations for oncogenes or tumor suppressor genes are identified by calculating the minimal common regions of aberration, which are most significantly altered i.e. high amplitude change. The regions of aberrations with high G-scores and minimal q-values (less than 0.25) are therefore more likely to contain target genes that have a significant role in carcinogenesis. The chromosomal regions are then classified as high or low level where low level amplification is considered for a log2ratio greater than 0.1 but smaller than 0.9 and high level amplification is considered for a log2ratio > 0.9 (0.9 corresponds to at least 3.7 copies per diploid cell). Similarly low level deletions (hemizygous) were considered for a log2ratio of -0.1 and homozygous deletions were considered for a log2ratio < -1. The GISTIC algorithm has been used for copy number analysis in previous studies[20,21].

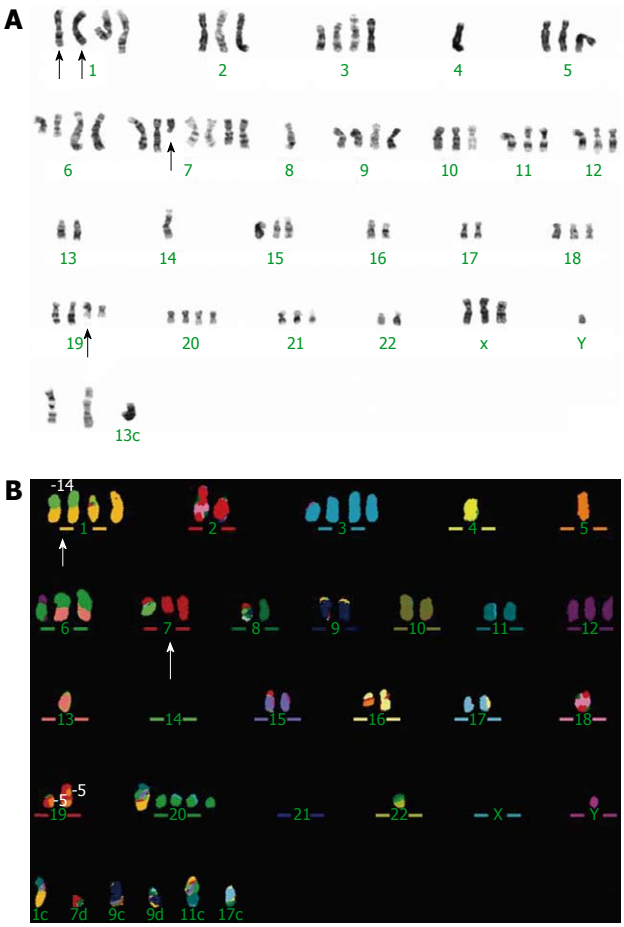

Figure 1 G-banded and multicolor fluorescence *in situ* hybridization representative karyotypes of cell line WHCO1. A: G-banded karyotype; B: Multicolor fluorescence *in situ* hybridization (M-FISH) karyotype, the arrows indicate the marker chromosomes, der(1) t(1;14)(p11;q11) and del(7)(q21).

RESULTS

Cytogenetics and M-FISH

Cytogenetic analysis revealed complex numerical and structural chromosome aberrations in all cell lines with a high variability observed between cells from the same cell line (Figures 1 and 2). The G-banded karyotype of cell line WHCO1 and a corresponding M-FISH karyotype that illustrates the degree of variation from one metaphase to another are shown in Figure 1. The M-FISH data confirmed the complexity of the karyotypes and revealed

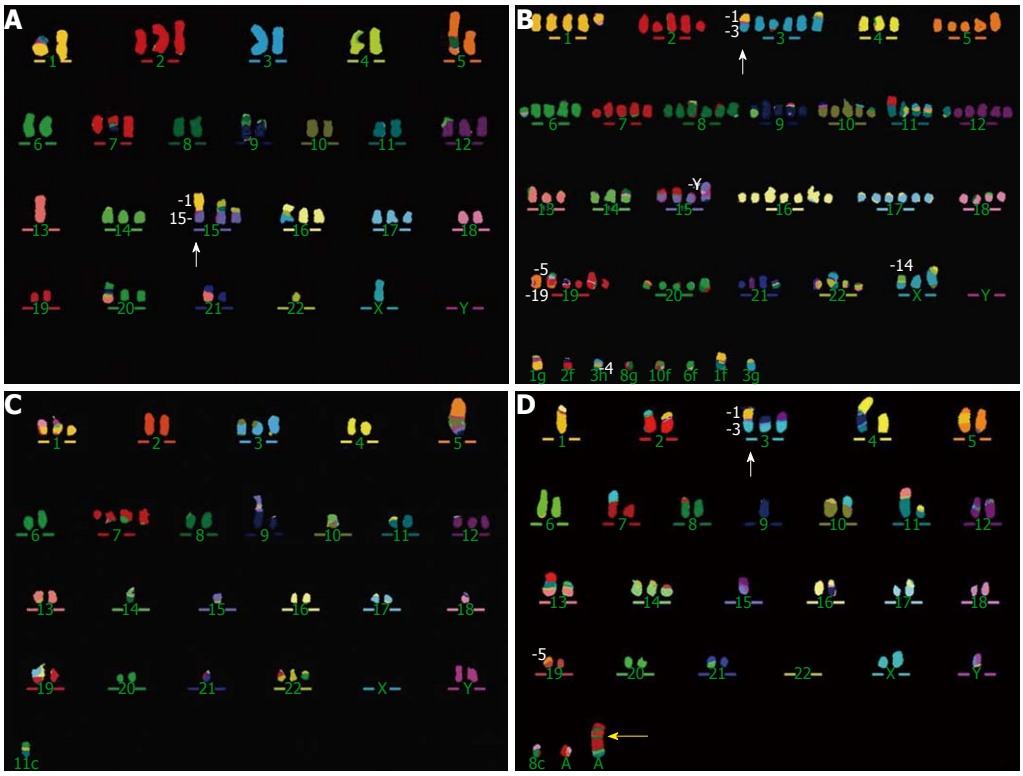

Figure 2 Multicolor fluorescence *in situ* hybridization representative karyotypes showing complex rearrangements in four cell lines. A: Cell line WHCO3, the white arrow indicates the der(15)t(1;15)(q11;p11); B: Cell line WHCO5, the white arrow points to the der(3)t(1;3)(p11-12;q11); C: Cell line WHCO6; D: Cell line SNO, the white arrow points to the der(3)t(1;3)(p11-12;p11) and the yellow arrow indicates the marker chromosome 7, mar(7), which involves the *EGFR* (epidermal growth factor receptor) locus (Figure 6).

the genetic composition of recurrent chromosome markers that could not be identified on G-banded metaphases (Figure 2); some of these markers are further discussed below. The detailed composite karyotypes obtained from twenty metaphases in each cell line are summarized in Table 1. All cell lines were near diploid except for cell line WHCO5 which was near tetraploid (Figure 2). Across the five cell lines a total of 97 translocations, 19 trisomies and 11 monosomies were detected. The breakpoints amounted to 203, with 78 of these clustering around the centromeric regions of chromosomes. The chromosomes involving the highest number of abnormalities were chromosomes 1, 3, 5, 7, 8, 9, 10, 11, 13, 14, 15, 18, 19, 20 and 22. G-banded metaphases also revealed the presence of isochromosomes involving the D group acrocentric chromosomes in all the cell lines. In particular isochromosome 13q, i(13)(p10) was common to cell lines WHCO3, WHCO5 and WHCO6, isochromosome 14q, i(14)(p10) was common to cell lines WHCO1, WHCO5 and WHCO6, and isochromosome 15q, i(15)(p10) was seen in cell lines WHCO3 and WHCO5.

The combined results of cytogenetics, FISH and M-FISH revealed a common translocation derivative, der (3)t(1;3)(p11;q11) that combined chromosome 3 and chromosome 1 short arms in cell lines WHCO5 and SNO (Figures 2-4). The SNP array copy number data did not bring further information on these breakpoints, possibly due to a lack of SNP probes in this region.

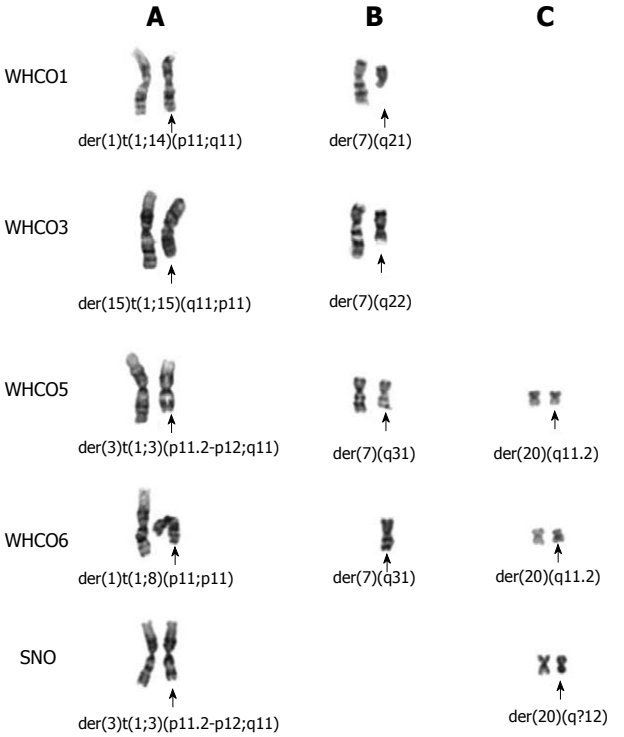

Figure 3 Partial G-banded karyotypes of the five cell lines showing shared chromosomal aberrations across cell lines. Chromosome derivatives from unbalanced translocations involving (A) chromosome 1p11-12 breakpoints in four cell lines, 1q11 in cell line WHCO3, (B) deletions of chromosome 7q in four cell lines, and (C) deletions of chromosome 20q in three cell lines.

Table 1 Composite karyotypes

Cell line	Composite combined cytogenetics and M-FISH karyotype
WHCO1	42~73, XY, +X, +X [4], +1 [10], del 1(?) [4], + der(1)t(1;14)(p11;q11) x2 [18], t(1;14;22)(?;?;q?) [4], t(1;22)(?;q?) [6], + 2 [8], t(2;8)(?;?) [4], +3 [8], +del 3(p21p13) x2 [4], der(3;22)(q10;q10) [4], -4 [8], t(5;21)(?;?) [8], + der(5)t(5;?)(q23;?) [4], + del(6)(q?21) x2 [4], t(6;12)(?q;?p) [16], t(6;13)(?q25;?q14) x2 [18], +7 [4], + del(7)(q21) [4], + der(7;14)(p10;q10) [20], der(7)t(7;18)(p21;q23) [16], der(7)t(7;19)(p22;p13) [4], der(8)t(8;?19)(q?24;q?11) [4], t(8;22)(?;?) [18], +9 [10], + der(10)t(9;10)(q13;q?11.2) [4], +11 [8], t(11;17;20)(?;?;?) [4], +12 [10], t(13;22)(?;?) [4], add(14)(q?) [4], i(14)(q10) [6], der(15;19)(q10;q10) [20], der(16)t(5;16)(?;?) [8], +18 [4], der(19)t(5;19)(p?12;q11) [12], der(19) t(5,19)(q33;q13.4) [18], + der(19)t(9;19)(q?13;q?13) x2 [4], t(19;21)(?;?) [14], +20, +20 [20], + der (20)t(1;11;20)(?;?;?) [4], +1~7mar [20] [cp20]
WHCO3	46~50, X, -Y, +2 [12], der(5)t(5;8,18)(q?;?;?) [20], + del 7(q22) [4], der(7)t(7;9)(?;?) [6], t(7;9;16;18)(?;?;?;?) [14], t(7;15)(?;?) [4], +12 [6], der(12)t(6;12)(?;?) [16], i(13)(q10) [6], t(13;14)(?;?) [6], t(13;14;20)(?;?;?) [6], +14 [12], + der(15)t(1;15)(q11;p11) [6], + der(15)t(1;15;11)(?;?;?) [10], der(15;22)(q10;q10) [20], i(15)(q10) [4], der(16)t(3;16;22)(p?11.2;?;q?10) [20], +17 [10], der(?20)t(9;13;20)(?;?;?) [10], der(21)t(13;21)(?;?) [12] [cp20]
WHCO5[1]	99~108, XY, t(X;4;10.22)(?;?;?;q?) [14], t(1;19)(?;?) [8], t(1;18)(?;?) [6], del 2 [2], der(2)t(2;9)(q12;q13) [10], t(2;9)(?q31;?q34) [8], +der(3)t(1;3)(p11-12;q11) [12], t(3;11;13;22)(?;?;?;?) [8], t(3;11;22)(?;?;?) [16], t(3;22)(p11;q11) [12], der(5;20)(p10;p10) [4], t(6;13)(?;?) [4], del(7)(q31) [4]; t(8;14;18)(?;?;?) [16], t(8;18)(?;?) [10], der(9)t(9;14)(?;?) [16], t(9;15)(q?;q?) [10], t(9;19)(?;?) [6], t(12;19)(?;?) [4], i(13)(q10) [6], i(14)(q10) [8], der(15)t(Y;15)(?;?)[12], der(15)t(7;15)(?;?) x2 [14], i(15)(q10) [4], der(19)del(19)(q13.?2)t(5;19)(p?12;p11) [12], del(20)(q11.2) [12] [cp20]
WHCO6	44~54, Y, -X, der(1)t(1;8)(p11;q11) [14], t(3;10)(?;?) [4], t(3;10)(?q13.3;p10) [6], t(4;10)(?;?) [4], t(5;10)(?;?) [10], t(5;22)(?;q?) [4], +6 [2], del(6)(q?21) [4]; t(6;11)(p12;q13) [6], der(22)t(6;22)(?;?) [6], +7[7], del(7)(q31) [8]; +8 [8], t(9;15)(?;?) [10], t(10;14)(?;?) [6], -11 [6], t(11;22)(p?;q?) [6], +12 [10], i(13)(q10) [6]; i(14)(q10) [6], +16 [8], t(17;19)(?;?) [10], +18 [4], del(20)(q?11.2) [6]; -21 [12], t(21;22)(?;?) [6], der(22)t(6;22)(?;?) [6], [cp14]
SNO	29~43, XY, +X, del X(?) [14], der(Y;15)(q10;q10) [12], t(1;16)(?;?) [14], der(2)t(X;2)(?;?) [16], der(2)t(1;2)(?;?) [18], der(3)t(1;3)(p11-12;q11) [20], t(3;9)(?;?) [4], t(3;10)(?;?) [14], t(3;12)(?;?) [20], t(3;20)(?;?) [8], t(4;9;11)(?;?;?) [20], t(4;11)(?;?) [12], der(5)t(1;5)(?;?) [20], -6 [18], del(6)(q?23) [20], der(7)t(3;7)(?q25;p22) [18], t(7;20;11;8;2)(?;?;?;?;?) [18], der(8)t(2;8)(?;?) [14], t(8;18)(?;?) [8], -9 [10], t(9;18)(?;?) [4], t(10;22)(q?;q?) [12], t(11;13)(?;?)[8], t(12;15)(?;?)[8], t(12;21)(?;?)[20], t(13;11;20)(?;?;?) [14], der(14;22)(p10;q10) x2 [20], t(14;19)(?;?) [20], -16 [14], der(16)t(9;16)(q?22;q?13) [20], der(17)t(6;17)(?;?) [12], -18 [16], -19 [16], der(19)del(19)(q13.?2)t(5;19)(q?12;q10) [16], +20 [10], del(20)(q?12) [16]; der(20;21)(q10;p10) [6], -21 [18], -22 [20] [cp20]

[1]Only structural rearrangements are listed due to the complexity of this cell line.

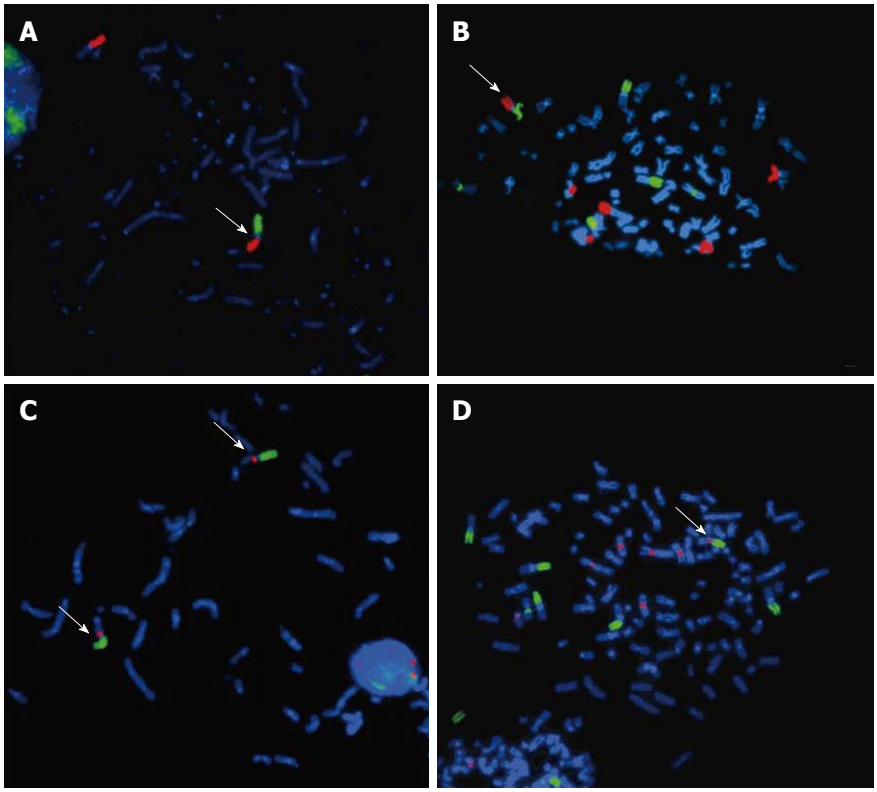

Figure 4 DAPI stained metaphasic chromosomes showing the der(3)t(1;3)(p11-12;q11) in cell lines SNO and WHCO5. A, B: Arm-specific paint for chromosomes 1p (green) and 3p (red) in cell lines SNO and WHCO5 respectively showing the derivatives der(3)t(1;3)(p11-12;q11) arrowed; C, D: Arm specific paint for chromosome 1p (green) and Cep 3 α probe (red) in cell lines SNO and WHCO5 respectively showing that the centromere of chromosome 3 is retained on the derivatives der(3)t(1;3)(p11-12;q11).

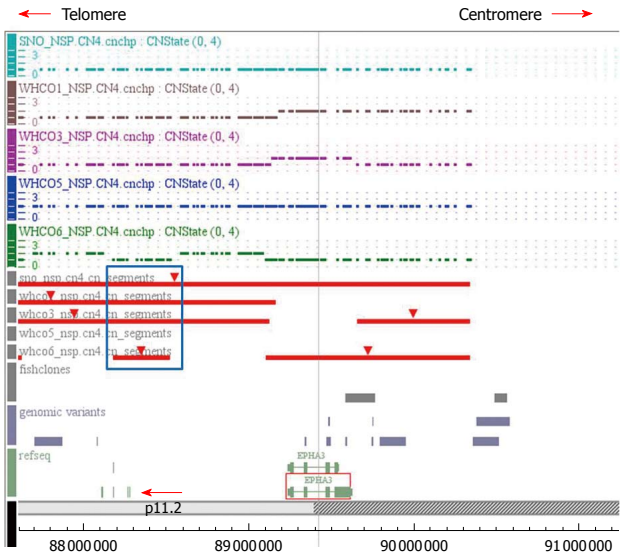

Figure 5 CNAT plot of single nucleotide polymorphism hybridization results showing the map of the region of deletion on chromosome 3p11.2-12.1 in cell lines WHCO1, WHCO3, WHCO5, WHCO6 and SNO. The red bars indicate the segment of deletion as determined by CNAT 4.0. The dotted lines indicate the copy number status for each single nucleotide polymorphism probe for each cell line. The blue block indicates the minimal common region of deletion and the red arrow indicates the two genes in this region, *c3orf38* and *CGGBP1*.

Interestingly, the 1p11 breakpoint was also involved in cell lines WHCO1 and WHCO6 in differing unbalanced translocations (Figure 3). The M-FISH and FISH results with probes for the respective partner chromosomes and centromeric Cep1, confirmed the interpretation of these derivatives as der(1)t(1;14)(p11;q11) in cell line WHCO1 and der(1)t(1;8)(p11;p11) in cell line WHCO6 (results not shown). In contrast, a translocation derivative also involving the chromosome 1 pericentromeric region in cell line WHCO3 (Figure 3) was shown not to involve 1p11 but 1q11, and was interpreted as der(15)t(1;15)(q11-12;p11) (result not shown).

Translocation derivatives involving chromosomes 3 and 22 were seen in cell lines WHCO3 and WHCO5 and were interpreted as der(16)t(3;16;22)(p11;?;q?11) and t(3;22)(p11;q11) respectively (Table 1). The array data showed corresponding hemizygous deletions at 3p11.2, in cell line WHCO3. Interestingly deletions at 3p11.2 were also observed in cell lines WHCO1, SNO and WHCO6. The minimal region of overlap was 343 kb (88 184 220-88 527 215 bp) in size and involved *c3orf38* and CGG triplet repeat binding protein (*CGGBP1)* genes. In addition the *EPHA3* gene was deleted in cell lines WHCO6 and SNO (Figure 5). The *EPHA3* gene encodes a tyrosine kinase, which is mutated in lung and breast cancers[22-24].

Cell lines WHCO1, WHCO3, WHCO5 and WHCO6 all showed a deletion of chromosome 7 long arm with varying breakpoints, q21 to q31, on G-banded metaphases (Figure 3). However GISTIC analysis of the SNP array data identified a significant common focal deletion (q-value of 0.09) of approximately 5.16 Mb at 7q33-q34 (133 721 542-138 880 555 bp) in only three of these cell lines. This region contained 26 genes including the potential target gene homeodomain-interacting protein kinase-2 (*HIPK2)* the product of which activates p53 expression and is pro apoptotic[25].

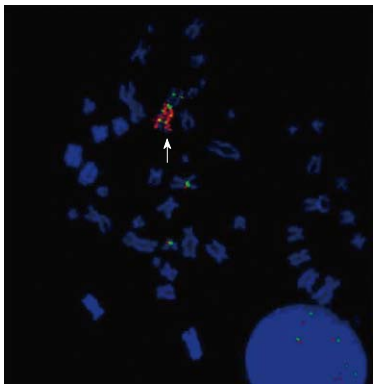

Figure 6 DAPI stained metaphase from cell line SNO showing fluorescence *in situ* hybridization results with the locus specific EGFR probe (red) and Cep7 α (green). The arrow indicates the chromosome 7 marker with a homogeneously stained region that contains the *EGFR* locus.

Although deletions at 20q11.2 were detected in cell lines WHCO5, WHCO6 and at 20q12 in cell line SNO on G-banded metaphases (Figure 3), copy number analysis (CNAT) revealed that there was in fact amplification of 20q sequences in all cell lines implying that complex rearrangement occurred for these sequences to be relocated elsewhere in the genome. Two minimal regions of amplification were identified, the 20q11.21-q11.22, of approximately 1.12 Mb in size (31 799 867-32 906 584 bp), which contained 14 genes and a smaller region at 20q13.12 of 149.48 kb which contained five genes. Both these regions were amplified in all cell lines, yet these amplifications were not found to be significant on GISTIC analysis.

Cell line SNO showed a large marker chromosome 7 on metaphases analyzed by M-FISH. This marker was interpreted as a possible inverted duplication of chromosome 7p sequences (Figure 2D). FISH with an *EGFR* probe, revealed a high amplification of the *EGFR* gene in 14% of the cells (Figure 6). A high level amplification at 7p13-7p11.2 (genomic location of *EGFR*) was confirmed by GISTIC (q-value 0.14) on array analysis in this cell line, while low level amplification was observed in the remaining four cell lines in agreement with the presence of 4 to 7 copies on FISH analysis.

Copy number changes

In view of the high cell to cell heterogeneity in each cell line, and in order to separate the potential driver aberrations out of the background of aberrations that may have occurred by chance, we used the software GISTIC specifically designed for this purpose. Target genes, defined as genes whose alteration confers a cell growth advantage, are likely to reside in the regions amplified or deleted to the highest degree in a majority of cells and within a common region of overlap amongst all cell lines. Fourteen common regions of amplification and 20 regions of deletion were

Table 2 Amplification peaks as detected by the GISTIC algorithm

Cytoband	Location (kb)	Approx size (Mb)	Frequency (*n*/5)	Mean log2ratio[1]	q-value	Genes[2]
1p34.2	Chr1:39027237-40780163	1.75	3	1.10	0.18	*MYCL1*
3q11.2-q12.2	Chr3:95917505-101945216	6.02	4	1.40	0.17	*MINA*
5p15.2	Chr5:10051329-11800765	1.75	2	1.36	0.09	*CTNND2*
7p11.2-p13	Chr7:45294289-57299457	12.01	1	1.24	0.13	*EGFR*
8q24.21	Chr8:127445828-129661846	2.22	5	2.10	0.0007	*MYC*
9q31.1	Chr9:100905143-102152148	1.25	1	2.57	0.15	
10q12.33-q21.3	Chr10:17811791-65388337	47.58	1	3.05	0.13	
11q13.3	Chr11:68753086-69985447	1.23	4	1.81	5.76E^{-05}	*CCND1, CTTN, FGF3, FGF4, FGF19, MYEOV*
11q22.1-q22.3	Chr11:100815801-103042620	2.23	2	2.35	0.03	*BIRC2, BIRC3, YAP1*
18p11.32	Chr18:1-1118244	1.12	2	1.04	0.13	*TYMS, YES1*
20p11.1-p11.22	Chr20:22140447-26145930	4.01	2	1.24	0.13	*PYGB*
22q11.21	Chr22:16558724-17937900	1.38	2	1.20	0.21	*BID, CLDN5*
22q11.21-q11.22	Chr22:18577713-20667607	2.10	2	1.20	0.13	*CRKL, MAPK1*
22q12.3	Chr22:31889314-32003182	0.11	2	1.15	0.13	*LARGE*

[1]The mean log2ratio for the samples with a log2ratio > 0.9 (equivalent to 3.7 copies per diploid cell); [2]The selected genes from the peak region.

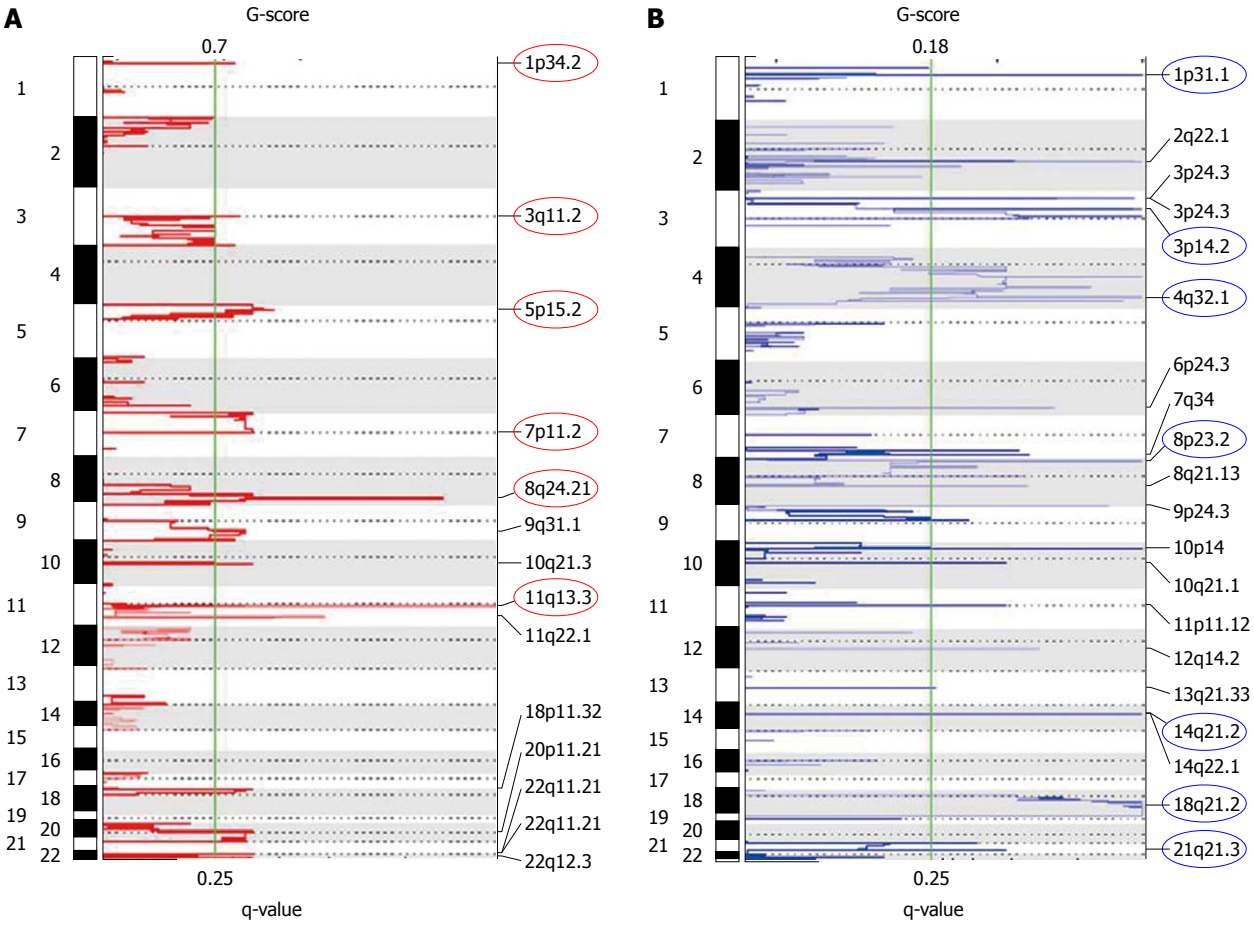

Figure 7 Plots of recurrent genomic amplifications (A) and deletions (B) detected in the esophageal squamous cell carcinoma cell lines from GISTIC analysis of single nucleotide polymorphism array data. The x-axis shows the G-score (top) and false discovery rate (q value; bottom). The green line indicates the false discovery rate cut off of 0.25. The circles indicate the peaks of the most significantly aberrant chromosomal regions.

identified (Figure 7, Tables 2 and 3). The most significant chromosomal regions of amplification were, in descending order of significance: 11q13.3 and 8q24.21, in four and five cell lines respectively, 11q22.1-q22.3 and 5p15.2 both detected in two cell lines. Chromosomal regions of less significant amplification were 10p12.33-q21.3, 18p11.32, 20p11.1-p11.22, 22q11.21-q11.22, 22q12.3, 9q31.1, 22q11.21, 3q11.2-q12.2 and 1p34.2 (Figure 7). Similarly, chromosomal regions of deletion were seen in the following order of significance: 1p31.1-p31.2, 2q22.1,

Table 3 Deletion peaks as detected by the GISTIC algorithm

Cytoband	Location (kb)	Size (Mb)	Frequency (*n*/5)		Mean log2ratio[1]	q-value	Genes[2]
			Hemi	Homo			
1p31.1-p31.2	Chr1:66691991-71187083	4.50	3	1	-3.03	0.02	*CTH*
2q22.1	Chr2:141590067-141951947	0.36	3	2	-1.66	0.19	*LRP1B*
3p26.3-q29	Chr3:1-199344050	199.34	2	1	-2.90	0.022	
3p12.1-p14.2	Chr7:60424050-85108679	24.70	4	1	-2.99	0.02	*FHIT, ADAMTS9*
4q22.1-q32.1	Chr4:91972774-162358674	70.40	3	2	-2.19	0.02	*CASP6, SMAD1*
6q24.3-q27	Chr6:147967444-170914576	22.95	2	1	-2.22	0.07	
7q33-q34	Chr7:133721542-138880555	5.16	2	1	-1.72	0.09	*HIPK2*
8p23.2-q11.1	Chr8:4078057-47043375	43.00	4	1	-2.47	0.02	*BNIP3L, INDO*
8p23.3-q21.13	Chr8:1-146308819	146.31	1	1	-2.27	0.09	
9p24.2-p24.3	Chr9:1151516-2459741	1.31	2	1	-2.53	0.03	
10p12.31-p14	Chr10:10309026-19155158	408.85	2	1	-2.74	0.02	
10q11.23-q22.1	Chr10:50393324-70694787	20.30	1	1	-2.35	0.12	
11p11.12-q12.2	Chr11:50256798-61426521	11.20	1	1	-1.90	0.12	
12p13.33-q24.33	Chr12:1-132078379	132.10		1	-2.80	0.08	
13q21.33-q34	Chr13:68772537-113042980	44.30	1	1	-1.70	0.24	
14q21.2	Chr14:42828345-44176016	1.35	3	1	-4.50	0.02	
14p13-q32.33	Chr14:1-105311216	105.30		1	-5.10	0.02	
18q21.1-q21.2	Chr18:46081464-51919972	5.80	5		-1.03	0.02	*DCC, SMAD4*
21p13-q21.3	Chr21:1-29932926	29.90	3			0.12	*BAGE*

[1]The mean of the log2ratio of those samples with log2ratios < -1.3 (< 0.9 copies per diploid cell); [2]The selected genes within the deletion peaks.

Table 4 Fluorescence *in situ* hybridization for detection of *CCND1* and *C-MYC* amplification

Cell line	FISH signals	Amplitude[1]	Log2Ratio
CCND1			
WHCO1	5-15	2	1.56
WHCO3	> 20	2	2.57
WHCO5	10-20	1	0.78
WHCO6	4-8	1	0.55
SNO	15-20	1	0.79
C-MYC			
WHCO1	4-6	1	0.12
WHCO3	15-> 20	2	1.83
WHCO5	10-> 20	2	1.83
WHCO6	10-> 20	2	2.06
SNO	4-> 20	2	1.71

[1]Amplitude threshold where log2ratio < 0.1 = 0, log2ratio > 0.1 < 0.9 = 1 and log2ratio > 0.9 = 2 as determined by SNP array copy number analysis. FISH: Fluorescence *in situ* hybridization.

3p12.1-p14.2, 4q22.1-q32.1, 8q11.1-p23.2, 14q21.2, 18q21.1-q21.2 and 21p13-q21.3 detected in all or four cell lines. Other regions of less significant deletion, and only seen in one or two cell lines, included: 10p12.31-p14, 14p13-q32.33, 3p26.3-q29, 9p24.2-p24.3, 6q24.3-q27, 12p13.33-q24.33, 7q33-q34, 8p23.3-q24.3, 10q11.23-q22.1, 11p11.12-q12.2 and 13q21.33-q34 (Figure 7). Together the regions of amplification and deletion encompassed a total of 4595 genes.

Significant gains

We selectively describe below the five regions of amplification that were the most significant on GISTIC analysis (q-value < 0.25) (Figure 7). Chromosomes 11q13.3 (68 753 086-69 985 447 bp) and 8q24.21 (127 445 828-129 661 846 bp) were the two most amplified and most significant regions with a q-value of 5.76E^{-05} and 0.0007 respectively. The 11q13.3 region was 1.23 Mb in size and was highly amplified in cell lines WHCO3, WHCO5, WHCO6 and SNO while amplified to a lesser degree in cell line WHCO1. This region harbors seven candidate genes including the cyclin D1 (*CCND1*), the cortactin (*CTTN*), the protein tyrosine phosphatase, receptor type, polypeptide, interacting protein α 1 (*PPFIA1*), *FGF3*, *FGF4* and *FGF19* and the myeloma overexpressed (*MYEOV*) genes, which could all play a role in ESCC oncogenesis. FISH validated these findings and confirmed the amplification of *CCND1* (Table 4, Figure 8). The common amplicon of 2.22 Mb at 8q24.21 was highly amplified in cell lines WHCO1 and WHCO3 and moderately amplified in cell lines WHCO5, WHCO6 and SNO. This amplicon involved the oncogene v-myc myelocytomatosis viral oncogene homolog (*C-MYC*) and the family with sequence similarity 84, member B (*FAM84B*) gene. Locus specific FISH confirmed the amplification of *C-MYC* in cell lines WHCO1, WHCO3, WHCO5, WHCO6 and SNO (Table 4, Figure 8).

A second focal region of high amplification on chromosome 11 was observed at 11q22.1-q22.3 (2.23 Mb in size) in cell lines WHCO5 and SNO. This region included the regulators of apoptosis *BIRC2* (*cIAP1*) and *BIRC3* (*cIAP2*), the matrix metalloproteinases (*MMP*) and the Yes associated protein (*YAP-1*) genes all potential target genes. The *BIRC2* gene was previously described as a target of amplification /increased expression in cervical cancers[26] and the *YAP-1* gene product is a cellular adaptor protein, which can induce *BIRC2* expression. *YAP-1* was reported to be over expressed in hepatic and mammary cancers[27]. In turn, the MMP genes, which include

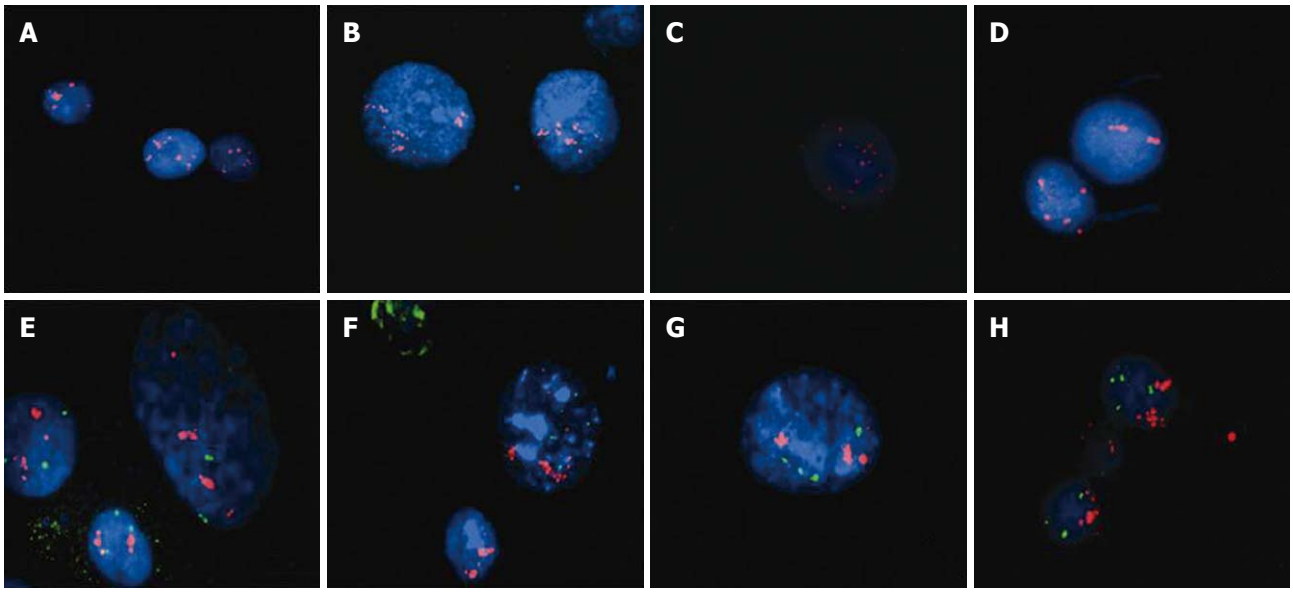

Figure 8 DAPI stained interphase nuclei hybridized with locus specific probes for C-MYC Spectrum Orange (red) (A-D) and *CCND1* (red)/*IGH* (green) (E-H). C-*MYC* amplification can be seen in cell lines WHCO1 (A), WHCO3 (B), WHCO5 (C) and SNO (D). *CCND1* amplification was detected in cell lines WHCO3 (E), WHCO5 (F), WHCO6 (G) and SNO (H).

MMP1, *MMP7*, and *MMP13*, have been shown to be co-expressed in early stage ESCC correlating with a poorer prognosis[28].

A 1.75 Mb region on chromosome 5p15.2 (10 051 329-11 800 765 bp) was highly amplified in cell lines WHCO6 and WHCO5 and moderately amplified in cell lines WHCO1 and SNO. This region hosts the potential target gene, delta catenin (*CTNND2*), overexpressed in prostate cancer[29].

Four cell lines, WHCO6, WHCO3, WHCO5 and SNO, had focal gain on chromosome 3q. The minimal common region of amplification mapped at 3q11.2-12.2, and was 6.02 Mb in size (95 917 505-101 945 216 bp) (q-value = 0.17). This region is commonly amplified in a variety of cancers[20,30] including esophageal squamous carcinoma[31] and it involved the potential oncogene, MYC induced nuclear antigen (*MINA*).

The 18p11.32 sub-band was highly amplified in cell lines WHCO3 and WHCO6 and moderately amplified in the remaining 3 cell lines. This region of 1.12 Mb in size (1-1 118 244 bp) has previously been described in ESCC[32] and involves the potential oncogenes *TYMS* and *YES-1*. Both genes have been implicated in gastro intestinal cancer. The *TYMS* gene codes for a thymidylate synthase involved in DNA synthesis and targeted by the chemotherapy agent fluorouracil (5FU). *TYMS* overexpression leads to 5FU treatment resistance[32] and affects colorectal cancer treatment[33]. *YES-1* is a homologue of the Yamaguchi sarcoma virus v-*yes* amplified and overexpressed in gastric cancers and ESCC[32].

Significant losses

The most significantly deleted chromosomal regions were 1p31.1-p31.2, 2q22.1, 3p12.1-p14.2, 4q22.1-q32.1, 8p23.2-q11.1, 14q21.2 and 18q21.1-q21.2 (in descending order of significance) in all or four of the cell lines (Figure 7). Less significant regions detected in less than four cell lines are depicted in Table 3. Three small regions of deletions involved chromosomes 1p, 2q and 18q. The 4.5 Mb deletion on chromosome 1 short arm, 1p31.2-p31.1 (66 691 991-71 187 083 bp) was seen in four cell lines (homozygous deletion in cell line SNO and hemizygous in cell lines WHCO1, WHCO5, WHCO6) with a high significant q-value of 0.02. Three genes with a reported tumor suppressor activity were involved in this deletion, the cystathionine γ-lyase (*CTH*), the growth arrest and DNA damage-45 α (*GADD45α*) and the DIRAS family, GTP-binding RAS-like 3 (*DIRAS3*) genes. The *DIRAS3* gene was shown to be down regulated in hepatocellular carcinoma and breast cancers[34,35] and is postulated to have a tumor suppressive activity. Both the *CTH* and *GADD45* genes were shown to negatively control cell growth[36,37].

Chromosome 2, sub-band q22.1 (141 590 067-141 951 947 bp) was lost in all cell lines (homozygous deletion in cell lines WHCO3 and WHCO5 and hemizygous in the other 3 cell lines) (q-value = 0.019). The low density lipoprotein 1B (*LRP1B*) tumor suppressor gene, deleted in lung cancer[38,39] maps in this region.

A chromosome 18q sub-band, q21.1-q21.2 (46 081 464-51 919 972 bp) (5.8 Mb) was hemizygously deleted in all five cell lines, involving both the *SMAD4* and deleted in colorectal carcinoma (*DCC*) genes.

Three large regions of deletion involved chromosomes 3p, 4q and 8p. First, the 24.7 Mb region of deletion at 3p12.1-p14.2 (60 424 050-85 108 679 bp) was significant (q-value of 0.02) in all cell lines (homozygous deletion in cell line SNO). This region houses the FRA3B associated gene, Fragile Histidine triad (*FHIT*), whose deletions were previously detected by MLPA analysis in these cell lines[40]. The potential tumor suppressor *ADAMTS9* gene, a me-

talloproteinase family member involved in inhibition of angiogenesis[41] was also involved in this deletion.

Second, the 4q22.1-q32.1 (91 972 774-162 358 674 bp) (75 Mb) region was homozygously deleted in cell lines WHCO3 and WHCO5 and hemizygously deleted in the three other cell lines with a q-value of 0.02. This region encompasses many genes but of interest are the Bone morphogenetic protein receptor 1B (*BMPR1B*), the caspase 6 (*CASP6*), the secreted frizzled-related protein 2 (*SFRP2*) and the SMAD protein 1 (*SMAD1*) genes, all potential tumor suppressor genes.

Lastly, a chromosome 8p23.2-q11.1 (4 078 057-47 043 375 bp) (43 Mb) deletion was seen in all cell lines (homozygous in cell line WHCO3 and hemizygous in the other 4 cell lines) (q value of 0.02), including five potential target genes, the BCL2/adenovirus E1B 19kDa interacting protein 3-like (*BNIP3L*), the leucine zipper tumor suppressor 1 (*LZTS1*) and the three tumor necrosis factor related superfamily genes *TNFRSF10A, TNFRSF10B* and *TNFRSF10C*.

DISCUSSION

We have characterized the karyotype and genomic constitution of five ESCC cell lines established in SA using a combination of traditional cytogenetics, M-FISH, FISH and SNP arrays. The number of ESCC cell lines genetically described worldwide is limited. Only eight ESCC cell lines have been investigated previously with traditional cytogenetics to our knowledge[10,12,42] and 10 with M-FISH[11,43,44], these are the only two techniques that can detect recurrent translocation breakpoints. In this study the five cell lines had complex karyotypes and were hyperploid with WHCO5 being near tetraploid. There was a high level of intra cell-line heterogeneity. The chromosomes most frequently involved in translocations were chromosomes 1, 3, 5, 7, 8, 9, 10, 11, 13, 14, 15, 18, 19, 20 and 22. These features were comparable to ESCC cell lines previously characterized[10-12,42,43] and across all studies that involved karyotyping, including the karyotyping of fresh ESCC tumor samples[14], chromosomes 1, 3 and 8 were the most commonly affected by translocation breakpoints[10-12,43].

Forty percent of translocation breakpoints occurred in near centromeric regions (Table 1). These were represented by unbalanced whole arm chromosome translocations, frequently involving chromosomes 1 and 3, and by isochromosomes for the D group acrocentric chromosomes 13, 14 and 15. Indeed, frequent centromeric breakpoints have been described in squamous carcinoma including ESCC[10,12] with up to 60% of all breakpoints being in centromeric regions[43] supporting the idea that centromeric disruption is a frequent event in epithelial cancers. It has been suggested that environmental factors may preferentially interact with centromeric sequences[45], and clastogenic compounds, such as mitomycin C, induce breaks in centromeric of chromosomes 1, 9 and 16[46]. Smoking is a major risk factor associated with ESCC and nicotine is known to induce single strand DNA breaks[47]. Although active smokers exhibit an increased number of breaks at fragile sites[48], it is not known if centromeric regions are also targeted.

Two chromosomal breakpoints were shared across cell lines. First, chromosome 1p11 was involved in a translocation in four cell lines, translocation t(1;3)(p11.2-12; q11) in cell lines SNO and WHCO5 and translocations with differing partners in cell lines WHCO1 and WHCO6. Second, chromosome 3p11-12 was involved in translocations in two cell lines and deletions in, or near the *EPHA3* locus was seen in four cell lines.

This gene codes for a receptor tyrosine kinase, with a tumor suppressor activity[49]. It was found to be mutated in lung and breast cancers[22-24] and interestingly, found to be deleted in 18.2% of ESCC patients in a previous study[31].

Breakpoints in, or near the centromeric regions of chromosomes 1 and 3 have previously been reported in several ESCC cell lines[10-12], as well as in fresh ESCC tumor samples that were karyotyped[14], and in ESCC cell lines obtained by *in vitro* transformation with HPV[50]. This strongly points to 1p11 and 3p11 translocation hotspots in ESCC that may affect genes and/or regulatory sequences not identified by SNP array. Deletions affecting or near the *EPHA3* gene may point to a role for this gene which was previously found to be deleted in 18.2% of ESCC patients[31].

We knew from previous studies that these ESCC cell lines, all overexpress the *EGFR* gene[16]. *EGFR* DNA amplification observed in cell line SNO is likely to contribute to *EGFR* overexpression whilst other factors are likely to be involved in the other four cell lines where low levels of *EGFR* amplification were observed.

In view of the high clonal heterogeneity observed in each cell line, we used the GISTIC software in addition to CNAT to analyze SNP array data and evidence the significant targets of amplification and deletions. The most stable genetic rearrangements are thought to reflect a proliferative cell growth advantage. In this context, the GISTIC algorithm allowed us to prioritize amplicons and regions of deletions in term of their likelihood to host driver genes. The five most interesting significant regions of amplification included chromosomal regions: 11q13.3, 8q24.21, 11q22.1-q22.3, 5p15.2 and 3q11.2-q12.2in decreasing order of significance.

The 8q24.21, 11q13.3 and 3q11.2-q12.2 regions have all previously been reported in a variety of carcinomas[51-53] and they often co-exist with one another. Genomic amplification at 8q24 occurs in a large variety of cancers[51,53-56], and most amplicons described in the literature involve both the *C-MYC* and *FAM84B* genes, as was observed in this study in four ESCC cell lines. In previous reports the target of amplification has been attributed to either both genes[54], or to one or the other[55,56] based on their respective increased transcription.

The 11q13.3 amplicon covered a large region containing a number of potential target genes. The *CCND1* and *MYEOV* genes (11q13.3) were co-amplified in four cell

lines. Co-amplification of these genes has been reported in multiple myeloma, breast cancer and ESCC[14]. *CCND1* is a downstream effector in the Wnt2/β-catenin pathway and the most frequent target of amplification in several ESCC studies[14,53,57]. While the *MYEOV* gene has been associated with cell proliferation in colon cancer[58], its amplification in ESCC is not always matched by increased transcription due to its silencing by epigenetic mechanisms[59]. The cortactin gene, *CTTN,* involved in cell motility[60], was previously shown to be over expressed in ESCC pre-cancerous lesions, as well as in carcinogen induced murine ESCC supporting a role for this gene in ESCC carcinogenesis[61]. The three fibroblast growth factor (FGF) genes, *FGF3*, *FGF4* and *FGF19* were part of the 11q13.3 amplicon. FGF and Wnt signaling pathways cross talk in a number of carcinogenesis scenarios[62]. Activated FGF receptors activate the FRS-GR2-GAB1-PI3K-AKT signaling cascade, and downregulate GSK-3β protein activity, thus hampering β-catenin phosphorylation and degradation[62]. In particular, FGF19 ligand downregulates GSK-3β activity, which results in the release and nuclear accumulation of β-catenin. Nuclear β-catenin activates the transcription of downstream genes including *C-MYC* and *CCND1*[63].

In addition to the amplification of Wnt pathway activators, the *SFRP2* tumor suppressor gene locus was deleted at chromosome 4q22.1-q32.1 (Figure 7). The *SFRP2* gene encodes a frizzled-related protein and is part of the SFRP family of Wnt inhibitors. Loss of *SFRP2* is detected in medulloblastoma and is suggested to contribute to carcinogenesis through loss of inhibition of the Wnt pathway[64].

The copy number data therefore suggests that the Wnt signaling pathway may be at work in these ESCC cell lines through one or the combined effects of genes activating the β-catenin transcriptional activity and/or the FGF signaling pathways as well as deletions of genes, at 4q22.1-q32.1, inhibiting this pathway. Amplicons at both 8q24 and 11q13.3-13.4 have been described in a variety of squamous cell carcinoma[54,65-67] suggesting that the activation of pathways through the combined effects of genes at 8q24 and 11q13.3-13.4 contributes to the development and aggressiveness of SCC.

In addition to the *SFRP2* gene, the three tumor suppressor genes *BMR1B, SMAD1* and *CASP6* were also targets of deletion at 4q22.1-q32.1. Both *BMR1B* and *SMAD1* genes have previously been reported to have decreased expression in gliomas correlating with poor survival[68], and *BMPR1B* decreased expression in breast cancer is associated with increased cell proliferation and poor prognosis[69]. The *CASP6* gene encodes the pro-apoptotic caspase-6 protein[70].

Although large 3q amplicons are commonly observed in squamous carcinoma[71,72] in this study the 6 Mb, 3q11.2-12.2 amplicon was focal and involved the *MINA* gene. This gene has previously been reported to be over-expressed in 83% of ESCC in one study and its inhibition was shown to suppress ESCC cell proliferation[73].

A 43 Mb region of deletion at 8p23.2-q11.1 (4 078 057-47 043 375 bp) was observed in the five cell lines and involved five potential target genes. These included the *BNIP3L* gene deleted or downregulated in prostate cancer and malignant melanomas, respectively[65,74], the *LZTS1* gene, deleted in oral squamous cell carcinomas and downregulated in breast carcinomas[75,76]; and the three *TNFR* genes, *TNFRSF10A*, *TNFRSF10B* and *TNFRSF10C,* whose epigenetic inactivation was reported in gastric cancers[77]. An 8p loss, was previously detected by conventional CGH in a study performed on 29 South African black and colored ESCC patients[78]. Chromosome 8p22 loss has also been reported in prostate, breast, lymphoma, hepatocellular and colorectal cancers[79-83].

Active smoking is linked to increased fragile site expression[84] and is also one of the primary risk factors associated with ESCC in South Africa[85]. We previously hypothesized that deletions affecting anti-oncogenes located at fragile sites may contribute to the etiology of ESCC in South Africa and reported *FHIT* intragenic deletions in these cell lines and a small cohort of patients[40]. *FHIT* gene deletions were confirmed here supporting its role in ESCC carcinogenesis.

Deletion at 18q23 involved the *SMAD4* and *DCC* genes in all cell lines. Both genes have previously been reported to be down regulated in ESCC either by deletion, mutation or methylation[86]. Decreased expression of the *SMAD4* gene, a tumor suppressor of the transforming growth factor β family signaling pathway, has been associated with ESCC tumor invasion[87]. The *DCC* gene was shown to be frequently methylated in ESCC tumor specimens[88].

In summary, breakpoints at 1p11 and 3p11 were recurrent in the five ESCC cell lines and may point to genes such as *EPHA3* that may be involved in ESCC carcinogenesis. Copy number alterations involved both amplicons previously reported in squamous cell carcinoma (8q24, 11q13 and 3q11) as well as novel regions of significant amplification (11q22.1-q22.3, 5p15.2 and 18p11.32). The finding that a significant number of genes that were amplified (*FGF3, FGF4, FGF19, CCND* and *C-MYC)* or deleted (*SFRP2* gene) are involved in the Wnt and FGF signaling pathways suggest that these pathways may be activated in these ESCC cell lines. These results warrant expression studies of these genes in both cell lines and patients' specimens. Of interest, should *FGF* gene expression be increased, ESCC patients may benefit from the respective FGF targeted therapies recently developed[89,90].

ACKNOWLEDGMENTS

We thank Elsabe Scott for culturing the cell lines.

COMMENTS

Background

Esophageal squamous cell carcinoma (ESCC) is a major cause of cancer death in the world and has a peculiar epidemiology with worldwide geographic

pockets of high incidence particularly in Asia and Africa. In South Africa, in the Eastern Cape region, ESCC represents the leading cancer affecting men and the second most common cancer in woman. There is a need to identify biomarkers to better understand the pathophysiology of this cancer and inform future diagnostic and therapeutic strategies for these patients. Established cell lines provide a unique resource to investigate both the presence of chromosomes translocations and copy number imbalances.

Research frontiers

A limited number of ESCC cell lines, all established in Asia, were reported to date. These have been investigated with molecular cytogenetic techniques of limited resolution and no key pathways have been reported previously.

Innovations and breakthroughs

This is the first comprehensive molecular cytogenetic study of five ESCC cell lines established in South Africa. The authors combined high-resolution whole genome array copy number analysis with conventional cytogenetics and multicolor fluorescence *in situ* hybridization to assess common chromosomal imbalances. Interestingly, a significant number of genes that were amplified (*FGF3*, *FGF4*, *FGF19*, *CCND1* and *C-MYC*) or deleted (*SFRP2* gene) are involved in the Wnt and FGF signaling pathways. In addition, a deletion within or near the *EPHA3* gene was present in 4 of these cell lines, corresponding to a translocation breakpoint at 3p11.2 shared in some cell lines.

Applications

These results suggest that the Wnt and FGF pathways may be involved in the initiation or progression of ESCC. They also point to the *EPHA3* gene as an added potential key gene. Further study on patients' specimens and functional studies will determine the significance of these genes in ESCC pathogenesis.

Terminology

High-resolution 250K single nucleotide polymorphism (SNP) arrays cover at least one SNP per 100 kb of DNA, using an average of 24 probes per SNP. The copy number data are derived from the summary of non-polymorphic SNPs and examined as a ratio to a reference genome. Changes in intensity ratios are indicative of amplification/deletions. The Wnt and fibroblast growth factor (FGF) signaling pathways are involved in tissue homeostasis as well as in cell proliferation and differentiation. Although their mechanisms differ, these two pathways cross talk through GSK3β inhibition.

Peer review

The manuscript by Brown and coworkers demonstrates that a significant number of genes that were amplified or deleted are involved in the Wnt and FGF signaling pathways in five cell lines established from South African ESCC patients. They suggest that these pathways are activated in these cell lines. The overall goal of the paper is relevant. The data presented are solid and credible. The results are interesting and clinically important.

REFERENCES

1 **Somdyala NI**, Bradshaw D, Gelderblom WC, Parkin DM. Cancer incidence in a rural population of South Africa, 1998-2002. *Int J Cancer* 2010; **127**: 2420-2429
2 **Hendricks D**, Parker MI. Oesophageal cancer in Africa. *IUBMB Life* 2002; **53**: 263-268
3 **van Rensburg SJ**. Oesophageal cancer risk factors common to endemic regions. *S Afr Med J* 1987; **Suppl**: 9-11
4 **Kamangar F**, Malekzadeh R, Dawsey SM, Saidi F. Esophageal cancer in Northeastern Iran: a review. *Arch Iran Med* 2007; **10**: 70-82
5 **Marasas WF**, van Rensburg SJ, Mirocha CJ. Incidence of Fusarium species and the mycotoxins, deoxynivalenol and zearalenone, in corn produced in esophageal cancer areas in Transkei. *J Agric Food Chem* 1979; **27**: 1108-1112
6 **Sun G**, Wang S, Hu X, Su J, Huang T, Yu J, Tang L, Gao W, Wang JS. Fumonisin B1 contamination of home-grown corn in high-risk areas for esophageal and liver cancer in China. *Food Addit Contam* 2007; **24**: 181-185
7 **Yao PF**, Li GC, Li J, Xia HS, Yang XL, Huang HY, Fu YG, Wang RQ, Wang XY, Sha JW. Evidence of human papilloma virus infection and its epidemiology in esophageal squamous cell carcinoma. *World J Gastroenterol* 2006; **12**: 1352-1355
8 **Matsha T**, Stepien A, Blanco-Blanco E, Brink LT, Lombard CJ, Van Rensburg S, Erasmus RT. Self-induced vomiting -- risk for oesophageal cancer? *S Afr Med J* 2006; **96**: 209-212
9 **Mitelman F**, Johansson B, Mertens F. The impact of translocations and gene fusions on cancer causation. *Nat Rev Cancer* 2007; **7**: 233-245
10 **Hu YC**, Lam KY, Law SY, Wan TS, Ma ES, Kwong YL, Chan LC, Wong J, Srivastava G. Establishment, characterization, karyotyping, and comparative genomic hybridization analysis of HKESC-2 and HKESC-3: two newly established human esophageal squamous cell carcinoma cell lines. *Cancer Genet Cytogenet* 2002; **135**: 120-127
11 **Wu YP**, Yang YL, Yang GZ, Wang XY, Luo ML, Zhang Y, Feng YB, Xu X, Han YL, Cai Y, Zhan QM, Wu M, Dong JT, Wang MR. Identification of chromosome aberrations in esophageal cancer cell line KYSE180 by multicolor fluorescence in situ hybridization. *Cancer Genet Cytogenet* 2006; **170**: 102-107
12 **Xiao S**, Feng XL, Geng JS, Yan FC, Liu QZ, Li P. Cytogenetic studies of five primary esophageal cancers. *Cancer Genet Cytogenet* 1991; **55**: 197-205
13 **Tada K**, Oka M, Hayashi H, Tangoku A, Oga A, Sasaki K. Cytogenetic analysis of esophageal squamous cell carcinoma cell lines by comparative genomic hybridization: relationship of cytogenetic aberrations to in vitro cell growth. *Cancer Genet Cytogenet* 2000; **117**: 108-112
14 **Jin Y**, Jin C, Law S, Chu KM, Zhang H, Strombeck B, Yuen AP, Kwong YL. Cytogenetic and fluorescence in situ hybridization characterization of clonal chromosomal aberrations and CCND1 amplification in esophageal carcinomas. *Cancer Genet Cytogenet* 2004; **148**: 21-28
15 **Bey E**, Alexander J, Whitcutt JM, Hunt JA, Gear JH. Carcinoma of the esophagus in Africans: establishment of a continuously growing cell line from a tumor specimen. *In Vitro* 1976; **12**: 107-114
16 **Veale RB**, Thornley AL. Increased single class low-affinity EGF receptors expressed by human oesophageal squamous carcinoma cell lines. *S Afr J Sci* 1989; **85**: 375-379
17 **Reich M**, Liefeld T, Gould J, Lerner J, Tamayo P, Mesirov JP. GenePattern 2.0. *Nat Genet* 2006; **38**: 500-501
18 **Hupé P**, Stransky N, Thiery JP, Radvanyi F, Barillot E. Analysis of array CGH data: from signal ratio to gain and loss of DNA regions. *Bioinformatics* 2004; **20**: 3413-3422
19 **Beroukhim R**, Getz G, Nghiemphu L, Barretina J, Hsueh T, Linhart D, Vivanco I, Lee JC, Huang JH, Alexander S, Du J, Kau T, Thomas RK, Shah K, Soto H, Perner S, Prensner J, Debiasi RM, Demichelis F, Hatton C, Rubin MA, Garraway LA, Nelson SF, Liau L, Mischel PS, Cloughesy TF, Meyerson M, Golub TA, Lander ES, Mellinghoff IK, Sellers WR. Assessing the significance of chromosomal aberrations in cancer: methodology and application to glioma. *Proc Natl Acad Sci USA* 2007; **104**: 20007-20012
20 **Haverty PM**, Fridlyand J, Li L, Getz G, Beroukhim R, Lohr S, Wu TD, Cavet G, Zhang Z, Chant J. High-resolution genomic and expression analyses of copy number alterations in breast tumors. *Genes Chromosomes Cancer* 2008; **47**: 530-542
21 **Weir BA**, Woo MS, Getz G, Perner S, Ding L, Beroukhim R, Lin WM, Province MA, Kraja A, Johnson LA, Shah K, Sato M, Thomas RK, Barletta JA, Borecki IB, Broderick S, Chang AC, Chiang DY, Chirieac LR, Cho J, Fujii Y, Gazdar AF, Giordano T, Greulich H, Hanna M, Johnson BE, Kris MG, Lash A, Lin L, Lindeman N, Mardis ER, McPherson JD, Minna JD, Morgan MB, Nadel M, Orringer MB, Osborne JR, Ozenberger B, Ramos AH, Robinson J, Roth JA, Rusch V, Sasaki H, Shepherd F, Sougnez C, Spitz MR, Tsao MS, Twomey D, Verhaak RG, Weinstock GM, Wheeler DA, Winckler W, Yoshizawa A, Yu S, Zakowski MF, Zhang Q, Beer DG, Wistuba II, Watson MA, Garraway LA, Ladanyi M, Travis WD, Pao W, Rubin MA, Gabriel SB, Gibbs RA, Varmus HE, Wilson RK, Lander ES, Meyerson M. Characterizing the cancer genome in lung adenocarcinoma. *Nature* 2007; **450**: 893-898

22 **Davies H**, Hunter C, Smith R, Stephens P, Greenman C, Bignell G, Teague J, Butler A, Edkins S, Stevens C, Parker A, O'Meara S, Avis T, Barthorpe S, Brackenbury L, Buck G, Clements J, Cole J, Dicks E, Edwards K, Forbes S, Gorton M, Gray K, Halliday K, Harrison R, Hills K, Hinton J, Jones D, Kosmidou V, Laman R, Lugg R, Menzies A, Perry J, Petty R, Raine K, Shepherd R, Small A, Solomon H, Stephens Y, Tofts C, Varian J, Webb A, West S, Widaa S, Yates A, Brasseur F, Cooper CS, Flanagan AM, Green A, Knowles M, Leung SY, Looijenga LH, Malkowicz B, Pierotti MA, Teh BT, Yuen ST, Lakhani SR, Easton DF, Weber BL, Goldstraw P, Nicholson AG, Wooster R, Stratton MR, Futreal PA. Somatic mutations of the protein kinase gene family in human lung cancer. *Cancer Res* 2005; **65**: 7591-7595

23 **Stephens P**, Edkins S, Davies H, Greenman C, Cox C, Hunter C, Bignell G, Teague J, Smith R, Stevens C, O'Meara S, Parker A, Tarpey P, Avis T, Barthorpe A, Brackenbury L, Buck G, Butler A, Clements J, Cole J, Dicks E, Edwards K, Forbes S, Gorton M, Gray K, Halliday K, Harrison R, Hills K, Hinton J, Jones D, Kosmidou V, Laman R, Lugg R, Menzies A, Perry J, Petty R, Raine K, Shepherd R, Small A, Solomon H, Stephens Y, Tofts C, Varian J, Webb A, West S, Widaa S, Yates A, Brasseur F, Cooper CS, Flanagan AM, Green A, Knowles M, Leung SY, Looijenga LH, Malkowicz B, Pierotti MA, Teh B, Yuen ST, Nicholson AG, Lakhani S, Easton DF, Weber BL, Stratton MR, Futreal PA, Wooster R. A screen of the complete protein kinase gene family identifies diverse patterns of somatic mutations in human breast cancer. *Nat Genet* 2005; **37**: 590-592

24 **Wood LD**, Calhoun ES, Silliman N, Ptak J, Szabo S, Powell SM, Riggins GJ, Wang TL, Yan H, Gazdar A, Kern SE, Pennacchio L, Kinzler KW, Vogelstein B, Velculescu VE. Somatic mutations of GUCY2F, EPHA3, and NTRK3 in human cancers. *Hum Mutat* 2006; **27**: 1060-1061

25 **Puca R**, Nardinocchi L, Sacchi A, Rechavi G, Givol D, D'Orazi G. HIPK2 modulates p53 activity towards pro-apoptotic transcription. *Mol Cancer* 2009; **8**: 85

26 **Imoto I**, Tsuda H, Hirasawa A, Miura M, Sakamoto M, Hirohashi S, Inazawa J. Expression of cIAP1, a target for 11q22 amplification, correlates with resistance of cervical cancers to radiotherapy. *Cancer Res* 2002; **62**: 4860-4866

27 **Da CL**, Xin Y, Zhao J, Luo XD. Significance and relationship between Yes-associated protein and survivin expression in gastric carcinoma and precancerous lesions. *World J Gastroenterol* 2009; **15**: 4055-4061

28 **Gu ZD**, Li JY, Li M, Gu J, Shi XT, Ke Y, Chen KN. Matrix metalloproteinases expression correlates with survival in patients with esophageal squamous cell carcinoma. *Am J Gastroenterol* 2005; **100**: 1835-1843

29 **Burger MJ**, Tebay MA, Keith PA, Samaratunga HM, Clements J, Lavin MF, Gardiner RA. Expression analysis of delta-catenin and prostate-specific membrane antigen: their potential as diagnostic markers for prostate cancer. *Int J Cancer* 2002; **100**: 228-237

30 **Or YY**, Hui AB, Tam KY, Huang DP, Lo KW. Characterization of chromosome 3q and 12q amplicons in nasopharyngeal carcinoma cell lines. *Int J Oncol* 2005; **26**: 49-56

31 **Chen J**, Guo L, Peiffer DA, Zhou L, Chan OT, Bibikova M, Wickham-Garcia E, Lu SH, Zhan Q, Wang-Rodriguez J, Jiang W, Fan JB. Genomic profiling of 766 cancer-related genes in archived esophageal normal and carcinoma tissues. *Int J Cancer* 2008; **122**: 2249-2254

32 **Nakakuki K**, Imoto I, Pimkhaokham A, Fukuda Y, Shimada Y, Imamura M, Amagasa T, Inazawa J. Novel targets for the 18p11.3 amplification frequently observed in esophageal squamous cell carcinomas. *Carcinogenesis* 2002; **23**: 19-24

33 **Jensen SA**, Vainer B, Witton CJ, Jørgensen JT, Sørensen JB. Prognostic significance of numeric aberrations of genes for thymidylate synthase, thymidine phosphorylase and dihydrofolate reductase in colorectal cancer. *Acta Oncol* 2008; **47**: 1054-1061

34 **Huang J**, Lin Y, Li L, Qing D, Teng XM, Zhang YL, Hu X, Hu Y, Yang P, Han ZG. ARHI, as a novel suppressor of cell growth and downregulated in human hepatocellular carcinoma, could contribute to hepatocarcinogenesis. *Mol Carcinog* 2009; **48**: 130-140

35 **Shi Z**, Zhou X, Xu L, Zhang T, Hou Y, Zhu W, Zhang T. [NOEY2 gene mRNA expression in breast cancer tissue and its relation to clinicopathological parameters]. *Zhonghua Zhongliu Zazhi* 2002; **24**: 475-478

36 **Rosemary Siafakas A**, Richardson DR. Growth arrest and DNA damage-45 alpha (GADD45alpha). *Int J Biochem Cell Biol* 2009; **41**: 986-989

37 **Yang G**, Cao K, Wu L, Wang R. Cystathionine gamma-lyase overexpression inhibits cell proliferation via a H2S-dependent modulation of ERK1/2 phosphorylation and p21Cip/WAK-1. *J Biol Chem* 2004; **279**: 49199-49205

38 **Liu CX**, Ranganathan S, Robinson S, Strickland DK. gamma-Secretase-mediated release of the low density lipoprotein receptor-related protein 1B intracellular domain suppresses anchorage-independent growth of neuroglioma cells. *J Biol Chem* 2007; **282**: 7504-7511

39 **Nagayama K**, Kohno T, Sato M, Arai Y, Minna JD, Yokota J. Homozygous deletion scanning of the lung cancer genome at a 100-kb resolution. *Genes Chromosomes Cancer* 2007; **46**: 1000-1010

40 **Willem P**, Brown J, Schouten J. A novel approach to simultaneously scan genes at fragile sites. *BMC Cancer* 2006; **6**: 205

41 **Lo PH**, Lung HL, Cheung AK, Apte SS, Chan KW, Kwong FM, Ko JM, Cheng Y, Law S, Srivastava G, Zabarovsky ER, Tsao SW, Tang JC, Stanbridge EJ, Lung ML. Extracellular protease ADAMTS9 suppresses esophageal and nasopharyngeal carcinoma tumor formation by inhibiting angiogenesis. *Cancer Res* 2010; **70**: 5567-5576

42 **Cheung LC**, Tang JC, Lee PY, Hu L, Guan XY, Tang WK, Srivastava G, Wong J, Luk JM, Law S. Establishment and characterization of a new xenograft-derived human esophageal squamous cell carcinoma cell line HKESC-4 of Chinese origin. *Cancer Genet Cytogenet* 2007; **178**: 17-25

43 **Yang Y**, Chu J, Wu Y, Luo M, Xu X, Han Y, Cai Y, Zhan Q, Wang M. Chromosome analysis of esophageal squamous cell carcinoma cell line KYSE 410-4 by repetitive multicolor fluorescence in situ hybridization. *J Genet Genomics* 2008; **35**: 11-16

44 **Yen CC**, Chen YJ, Chen JT, Hsia JY, Chen PM, Liu JH, Fan FS, Chiou TJ, Wang WS, Lin CH. Comparative genomic hybridization of esophageal squamous cell carcinoma: correlations between chromosomal aberrations and disease progression/prognosis. *Cancer* 2001; **92**: 2769-2777

45 **Jin Y**, Jin C, Salemark L, Martins C, Wennerberg J, Mertens F. Centromere cleavage is a mechanism underlying isochromosome formation in skin and head and neck carcinomas. *Chromosoma* 2000; **109**: 476-481

46 **Johansson B**, Mertens F. Frequency and distribution of mitomycin C-induced structural chromosome aberrations in lymphocytes from non-Hodgkin lymphoma patients. *Cytogenet Cell Genet* 1988; **48**: 79-83

47 **Kleinsasser NH**, Sassen AW, Semmler MP, Staudenmaier R, Harréus UA, Richter E. [Does nicotine add to the carcinogenic strain of tobacco smoke?]. *HNO* 2006; **54**: 369-372, 374-375

48 **Stein CK**, Glover TW, Palmer JL, Glisson BS. Direct correlation between FRA3B expression and cigarette smoking. *Genes Chromosomes Cancer* 2002; **34**: 333-340

49 **Clifford N**, Smith LM, Powell J, Gattenlöhner S, Marx A, O'Connor R. The EphA3 receptor is expressed in a subset of rhabdomyosarcoma cell lines and suppresses cell adhesion and migration. *J Cell Biochem* 2008; **105**: 1250-1259

50 **Zhang H**, Jin Y, Chen X, Jin C, Law S, Tsao SW, Kwong YL. Cytogenetic aberrations in immortalization of esophageal epithelial cells. *Cancer Genet Cytogenet* 2006; **165**: 25-35

51 **Burkhardt L**, Grob TJ, Hermann I, Burandt E, Choschzick M,

Jänicke F, Müller V, Bokemeyer C, Simon R, Sauter G, Wilczak W, Lebeau A. Gene amplification in ductal carcinoma in situ of the breast. *Breast Cancer Res Treat* 2010; **123**: 757-765
52 **Freier K**, Joos S, Flechtenmacher C, Devens F, Benner A, Bosch FX, Lichter P, Hofele C. Tissue microarray analysis reveals site-specific prevalence of oncogene amplifications in head and neck squamous cell carcinoma. *Cancer Res* 2003; **63**: 1179-1182
53 **Bass AJ**, Watanabe H, Mermel CH, Yu S, Perner S, Verhaak RG, Kim SY, Wardwell L, Tamayo P, Gat-Viks I, Ramos AH, Woo MS, Weir BA, Getz G, Beroukhim R, O'Kelly M, Dutt A, Rozenblatt-Rosen O, Dziunycz P, Komisarof J, Chirieac LR, Lafargue CJ, Scheble V, Wilbertz T, Ma C, Rao S, Nakagawa H, Stairs DB, Lin L, Giordano TJ, Wagner P, Minna JD, Gazdar AF, Zhu CQ, Brose MS, Cecconello I, Jr UR, Marie SK, Dahl O, Shivdasani RA, Tsao MS, Rubin MA, Wong KK, Regev A, Hahn WC, Beer DG, Rustgi AK, Meyerson M. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. *Nat Genet* 2009; **41**: 1238-1242
54 **Camps J**, Nguyen QT, Padilla-Nash HM, Knutsen T, McNeil NE, Wangsa D, Hummon AB, Grade M, Ried T, Difilippantonio MJ. Integrative genomics reveals mechanisms of copy number alterations responsible for transcriptional deregulation in colorectal cancer. *Genes Chromosomes Cancer* 2009; **48**: 1002-1017
55 **Huang XP**, Rong TH, Wang JY, Tang YQ, Li BJ, Xu DR, Zhao MQ, Zhang LJ, Fang Y, Su XD, Liang QW. Negative implication of C-MYC as an amplification target in esophageal cancer. *Cancer Genet Cytogenet* 2006; **165**: 20-24
56 **van Duin M**, van Marion R, Vissers KJ, Hop WC, Dinjens WN, Tilanus HW, Siersema PD, van Dekken H. High-resolution array comparative genomic hybridization of chromosome 8q: evaluation of putative progression markers for gastroesophageal junction adenocarcinomas. *Cytogenet Genome Res* 2007; **118**: 130-137
57 **Janssen JW**, Cuny M, Orsetti B, Rodriguez C, Vallés H, Bartram CR, Schuuring E, Theillet C. MYEOV: a candidate gene for DNA amplification events occurring centromeric to CCND1 in breast cancer. *Int J Cancer* 2002; **102**: 608-614
58 **Moss AC**, Lawlor G, Murray D, Tighe D, Madden SF, Mulligan AM, Keane CO, Brady HR, Doran PP, MacMathuna P. ETV4 and Myeov knockdown impairs colon cancer cell line proliferation and invasion. *Biochem Biophys Res Commun* 2006; **345**: 216-221
59 **Janssen JW**, Imoto I, Inoue J, Shimada Y, Ueda M, Imamura M, Bartram CR, Inazawa J. MYEOV, a gene at 11q13, is coamplified with CCND1, but epigenetically inactivated in a subset of esophageal squamous cell carcinomas. *J Hum Genet* 2002; **47**: 460-464
60 **Hofman P**, Butori C, Havet K, Hofman V, Selva E, Guevara N, Santini J, Van Obberghen-Schilling E. Prognostic significance of cortactin levels in head and neck squamous cell carcinoma: comparison with epidermal growth factor receptor status. *Br J Cancer* 2008; **98**: 956-964
61 **Hsu NY**, Yeh KT, Chiang IP, Pai LY, Chen CY, Ho HC. Cortactin overexpression in the esophageal squamous cell carcinoma and its involvement in the carcinogenesis. *Dis Esophagus* 2008; **21**: 402-408
62 **Katoh M**. Cross-talk of WNT and FGF signaling pathways at GSK3beta to regulate beta-catenin and SNAIL signaling cascades. *Cancer Biol Ther* 2006; **5**: 1059-1064
63 **Novak A**, Dedhar S. Signaling through beta-catenin and Lef/Tcf. *Cell Mol Life Sci* 1999; **56**: 523-537
64 **Kongkham PN**, Northcott PA, Croul SE, Smith CA, Taylor MD, Rutka JT. The SFRP family of WNT inhibitors function as novel tumor suppressor genes epigenetically silenced in medulloblastoma. *Oncogene* 2010; **29**: 3017-3024
65 **Liu W**, Xie CC, Zhu Y, Li T, Sun J, Cheng Y, Ewing CM, Dalrymple S, Turner AR, Sun J, Isaacs JT, Chang BL, Zheng SL, Isaacs WB, Xu J. Homozygous deletions and recurrent amplifications implicate new genes involved in prostate cancer. *Neoplasia* 2008; **10**: 897-907
66 **Janssen JW**, Vaandrager JW, Heuser T, Jauch A, Kluin PM, Geelen E, Bergsagel PL, Kuehl WM, Drexler HG, Otsuki T, Bartram CR, Schuuring E. Concurrent activation of a novel putative transforming gene, myeov, and cyclin D1 in a subset of multiple myeloma cell lines with t(11; 14)(q13; q32). *Blood* 2000; **95**: 2691-2698
67 **Fantozzi I**, Grall D, Cagnol S, Stanchi F, Sudaka A, Brunstein MC, Bozec A, Fischel JL, Milano G, Van Obberghen-Schilling E. Overexpression of cortactin in head and neck squamous cell carcinomas can be uncoupled from augmented EGF receptor expression. *Acta Oncol* 2008; **47**: 1502-1512
68 **Liu S**, Tian Z, Yin F, Zhang P, W Y, Ding X, Wu H, Wu Y, Peng X, Yuan J, Qiang B, Fan W, Fan M. Expression and functional roles of Smad1 and BMPR-IB in glioma development. *Cancer Invest* 2009; **27**: 734-740
69 **Bokobza SM**, Ye L, Kynaston HE, Mansel RE, Jiang WG. Reduced expression of BMPR-IB correlates with poor prognosis and increased proliferation of breast cancer cells. *Cancer Genomics Proteomics* 2009; **6**: 101-108
70 **Chan JY**, Phoo MS, Clement MV, Pervaiz S, Lee SC. Resveratrol displays converse dose-related effects on 5-fluorouracil-evoked colon cancer cell apoptosis: the roles of caspase-6 and p53. *Cancer Biol Ther* 2008; **7**: 1305-1312
71 **Haverty PM**, Hon LS, Kaminker JS, Chant J, Zhang Z. High-resolution analysis of copy number alterations and associated expression changes in ovarian tumors. *BMC Med Genomics* 2009; **2**: 21
72 **Kanao H**, Enomoto T, Kimura T, Fujita M, Nakashima R, Ueda Y, Ueno Y, Miyatake T, Yoshizaki T, Buzard GS, Tanigami A, Yoshino K, Murata Y. Overexpression of LAMP3/TSC403/DC-LAMP promotes metastasis in uterine cervical cancer. *Cancer Res* 2005; **65**: 8640-8645
73 **Tsuneoka M**, Fujita H, Arima N, Teye K, Okamura T, Inutsuka H, Koda Y, Shirouzu K, Kimura H. Mina53 as a potential prognostic factor for esophageal squamous cell carcinoma. *Clin Cancer Res* 2004; **10**: 7347-7356
74 **Su DM**, Zhang Q, Wang X, He P, Zhu YJ, Zhao J, Rennert OM, Su YA. Two types of human malignant melanoma cell lines revealed by expression patterns of mitochondrial and survival-apoptosis genes: implications for malignant melanoma therapy. *Mol Cancer Ther* 2009; **8**: 1292-1304
75 **Chen L**, Zhu Z, Sun X, Dong XY, Wei J, Gu F, Sun YL, Zhou J, Dong JT, Fu L. Down-regulation of tumor suppressor gene FEZ1/LZTS1 in breast carcinoma involves promoter methylation and associates with metastasis. *Breast Cancer Res Treat* 2009; **116**: 471-478
76 **Ono K**, Uzawa K, Nakatsuru M, Shiiba M, Mochida Y, Tada A, Bukawa H, Miyakawa A, Yokoe H, Tanzawa H. Down-regulation of FEZ1/LZTS1 gene with frequent loss of heterozygosity in oral squamous cell carcinomas. *Int J Oncol* 2003; **23**: 297-302
77 **Lee KH**, Lim SW, Kim HG, Kim DY, Ryu SY, Joo JK, Kim JC, Lee JH. Lack of death receptor 4 (DR4) expression through gene promoter methylation in gastric carcinoma. *Langenbecks Arch Surg* 2009; **394**: 661-670
78 **Du Plessis L**, Dietzsch E, Van Gele M, Van Roy N, Van Helden P, Parker MI, Mugwanya DK, De Groot M, Marx MP, Kotze MJ, Speleman F. Mapping of novel regions of DNA gain and loss by comparative genomic hybridization in esophageal carcinoma in the Black and Colored populations of South Africa. *Cancer Res* 1999; **59**: 1877-1883
79 **Bova GS**, MacGrogan D, Levy A, Pin SS, Bookstein R, Isaacs WB. Physical mapping of chromosome 8p22 markers and their homozygous deletion in a metastatic prostate cancer. *Genomics* 1996; **35**: 46-54
80 **Di Benedetto M**, Pineau P, Nouet S, Berhouet S, Seitz I, Louis S, Dejean A, Couraud PO, Strosberg AD, Stoppa-Lyonnet D, Nahmias C. Mutation analysis of the 8p22 candidate tumor

suppressor gene ATIP/MTUS1 in hepatocellular carcinoma. *Mol Cell Endocrinol* 2006; **252**: 207-215
81 **Flanagan JM**, Healey S, Young J, Whitehall V, Trott DA, Newbold RF, Chenevix-Trench G. Mapping of a candidate colorectal cancer tumor-suppressor gene to a 900-kilobase region on the short arm of chromosome 8. *Genes Chromosomes Cancer* 2004; **40**: 247-260
82 **Thomassen M**, Jochumsen KM, Mogensen O, Tan Q, Kruse TA. Gene expression meta-analysis identifies chromosomal regions involved in ovarian cancer survival. *Genes Chromosomes Cancer* 2009; **48**: 711-724
83 **Ying J**, Li H, Murray P, Gao Z, Chen YW, Wang Y, Lee KY, Chan AT, Ambinder RF, Srivastava G, Tao Q. Tumor-specific methylation of the 8p22 tumor suppressor gene DLC1 is an epigenetic biomarker for Hodgkin, nasal NK/T-cell and other types of lymphomas. *Epigenetics* 2007; **2**: 15-21
84 **Sozzi G**, Sard L, De Gregorio L, Marchetti A, Musso K, Buttitta F, Tornielli S, Pellegrini S, Veronese ML, Manenti G, Incarbone M, Chella A, Angeletti CA, Pastorino U, Huebner K, Bevilaqua G, Pilotti S, Croce CM, Pierotti MA. Association between cigarette smoking and FHIT gene alterations in lung cancer. *Cancer Res* 1997; **57**: 2121-2123
85 **Pacella-Norman R**, Urban MI, Sitas F, Carrara H, Sur R, Hale M, Ruff P, Patel M, Newton R, Bull D, Beral V. Risk factors for oesophageal, lung, oral and laryngeal cancers in black South Africans. *Br J Cancer* 2002; **86**: 1751-1756
86 **Metzger R**, Schneider PM, Warnecke-Eberz U, Brabender J, Hölscher AH. Molecular biology of esophageal cancer. *Onkologie* 2004; **27**: 200-206
87 **Fukuchi M**, Masuda N, Miyazaki T, Nakajima M, Osawa H, Kato H, Kuwano H. Decreased Smad4 expression in the transforming growth factor-beta signaling pathway during progression of esophageal squamous cell carcinoma. *Cancer* 2002; **95**: 737-743
88 **Park HL**, Kim MS, Yamashita K, Westra W, Carvalho AL, Lee J, Jiang WW, Baek JH, Liu J, Osada M, Moon CS, Califano JA, Mori M, Sidransky D. DCC promoter hypermethylation in esophageal squamous cell carcinoma. *Int J Cancer* 2008; **122**: 2498-2502
89 **Pai R**, Dunlap D, Qing J, Mohtashemi I, Hotzel K, French DM. Inhibition of fibroblast growth factor 19 reduces tumor growth by modulating beta-catenin signaling. *Cancer Res* 2008; **68**: 5086-5095
90 **Stemke-Hale K**, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, Carey M, Hu Z, Guan Y, Sahin A, Symmans WF, Pusztai L, Nolden LK, Horlings H, Berns K, Hung MC, van de Vijver MJ, Valero V, Gray JW, Bernards R, Mills GB, Hennessy BT. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res* 2008; **68**: 6084-6091

S- Editor Tian L **L- Editor** Cant MR **E- Editor** Zheng XM