Expression and copy number analysis of TRPS1, EIF3S3 and MYC genes in breast and prostate cancer

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Molecular cytogenetic analyses, especially comparative genomic hybridisation (CGH), have indicated that gain of the long arm of chromosome 8 is one of the most common regions of amplification in cancers of several organs, especially carcinomas of the breast and prostate. TRPS1, MYC and EIF3S3 genes are located in one of the minimal regions of amplification, 8q23–q24, and have been suggested to be the target genes of the amplification. Here, our goal was to study copy number and expression of the three genes in order to investigate the significance of the genes in breast and prostate cancer. By using fluorescence in situ hybridisation (FISH), we first found that TRPS1 and EIF3S3 were amplified together in about one-third of hormone-refractory prostate carcinomas. Next, we analysed the mRNA expression of the three genes by real-time quantitative RT–PCR and the gene copy number by FISH in six breast and five prostate cancer cell lines. Breast cancer cell line, SK-BR-3, which contained the highest copy number of all three genes, showed overexpression of only EIF3S3. Finally, the expression levels of TRPS1, EIF3S3 and MYC were measured in freshly frozen clinical samples of benign prostate hyperplasia (BPH), as well as untreated and hormone-refractory prostate carcinoma. The TRPS1 and MYC expression levels were similar in all prostate tumour groups, whereas EIF3S3 expression was higher ($P = 0.029$) in prostate carcinomas compared to BPH. The data suggest that the expression of EIF3S3 is increased in prostate cancer, and that one of the mechanisms underlying the overexpression is the amplification of the gene.

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In addition, a recent cDNA microarray study suggested that upregulation of several genes of translation apparatus is generally involved in metastasis of cancer (Ramaswamy et al, 2003).

TRPS1 was recently found to be more expressed in androgen-dependent than androgen-independent LNCaP prostate cancer cell lines by differential display analysis (Chang et al, 2000). TRPS1 encodes a zinc-finger GATA-type nuclear protein, which has been implicated in apoptosis. Castration leads to increased expression of TRPS1 in rat ventral prostate (Chang et al, 2000, 2002). Germ-line mutations in the TRPS1 gene, on the other hand, cause tricho-rhino-phalangeal syndrome (TRPS) types I and III (Momeni et al, 2000, Lüdecke et al, 2001). In addition, it was reported that xenopus TRPS1 acts as a repressor of the xenopus GATA4 transcription factor (Malik et al, 2001). This suggests that human TRPS1 could be involved in gene regulation of the family of human GATA transcription factors.

In order to evaluate the significance of EIF3S3, MYC, and TRPS1 in breast and prostate cancer, we have analysed the expression and copy number of the three genes in cell lines and tumours using quantitative real-time RT–PCR and fluorescence in situ hybridisation (FISH).

MATERIALS AND METHODS

Cell lines and tumour samples

Five prostate cancer cell lines (PC-3, LNCaP, DU145, 22rv1 and NCI-H660) and six breast cancer cell lines (SK-BR-3, ZR75-1, MCF-7, MDA436, EFM19 and T47D) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured under the recommended conditions. Freshly frozen specimens from nine benign prostate hyperplasias (BPH), 35 untreated (from the Tampere University Hospital, was constructed according to previously published guidelines (Kononen et al, 1998). The use of clinical tumour material has been approved by the Ethical Committee of the Tampere University Hospital.

RT–PCR

One to three 20 μm frozen sections were cut using a cryotome. The total RNAs were isolated from the sections using Qiagen RNeasy MiniKit (Qiagen Inc., Valencia, CA, USA), and used for the first-strand cDNA synthesis with Superscript™ II reverse transcriptase and oligo (dT)$_{12-18}$ primer according to the manufacturer’s protocol (Life Technologies, Gaithersburg, MD, USA). For preparing the standard curve, 5 μg of total RNA from the prostate cancer cell line LNCaP (ATCC, Manassas, VA, USA) was reverse transcribed as described above. After the first-strand cDNA synthesis, serial dilutions (1:5) were made to correspond to cDNA transcribed from 500, 100, 20, 4, 0.8 and 0.16 ng of total RNA. Primers for the TRPS1, EIF3S3 and MYC genes were designed with the Primer3 program (available at http://www-genome.wi.mit.edu/cgi-bin/primer3.www.cgi). To avoid amplification of any genomic DNA, the forward and reverse primers for each gene were chosen from different exons. The sizes of the PCR products were designed to be under 400bp to optimise the RT–PCR measurements. The primer and probe sequences for the genes are given in Table 1.

The PCR reactions were performed with a LightCycler™ instrument using the LightCycler – FastStart DNA Master Hybridization Probes Kit (EIF3S3 and MYC) or FastStart DNA Master SYBR Green I Kit (TRPS1) (Roche Diagnostics, Mannheim, Germany). Thermocycling for each reaction was carried out in a final volume of 20 μl containing 2 μl of cDNA sample (or standard), 4 μl MgCl$_2$, 0.5 μM of each primer, 0.2 μM of fluorescein and 0.4 μM LC Red640 labelled probes (or SYBR Green I stain in TRPS1 assay), as well as 1 × ready-to-use reaction mix including Taq DNA polymerase, reaction buffer and dNTP mix. After 10 min of initial denaturation at +95°C, the cycling conditions of 50 cycles consisted of denaturation at +95°C for 1 s (EIF3S3 and MYC) or 10 s (TRPS1), annealing at +58°C for 10 s (EIF3S3 and MYC) or at +60°C for 7 s (TRPS1) and elongation at +72°C for 10 s (EIF3S3) or 13 s (MYC) or 7 s (TRPS1). After the PCR reaction and fluorescence measurements, fit point method together with background adjustment was used to determine the cycle in which the log-linear signal was distinguished from the background, and that cycle number was used as the crossing-point value. The software produced the standard curve by measuring the crossing point of each standard and plotting them against the logarithmic values of concentrations (Figure 1). The expression levels of TRPS1, EIF3S3 and MYC were normalised by the expression level of the housekeeping gene TATA binding protein (TBP), measured as previously described (Linja et al, 2001). The relative expression was illustrated by dividing the EIF3S3, TRPS1 and MYC values with the TBP value, and multiplying by 10. TBP was chosen for the reference gene, because there are no known retro-

| Table 1 | Primer and probe sequences used in the real-time RT–PCR |
|---------|--------------------------------------------------------|
| Gene    | Primer sequences (5’–3’) | Hybridization probe sequences (5’–3’)* |
| EIF3S3  | GCC CAG GCT CCT CAA GAA TAC | GCTGAACTCTCGCGAGCGGCTTTT-Fluorescein |
|         | ATA GCC AAA ATC GGC AAT GA  | Red640-CCCTTGCCCTTCTCCGTCGCC |
| C-MYC   | CCT ACC CTC TCA ACG ACA GC | GCCTCCCTCTACCTGGGAGGGACT-Fluorescein |
|         | GCC CTC TGG ACA TCC TCC TC | Red640-TCCTGCTGCCAAAGGGGTCAGTT |
| TRPS1   | GTA TCC TGC ATC GGG AGA AA | *purchased from Tib Mol Biol, Berlin, Germany. |
|         | AGC TCC TGG TAG AGG CCA CA  | |

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two-fold higher copy number of the locus-specific probe signals vs centromeric signals or ≥5 copies of locus specific probe signals were considered to contain a high-level amplification of either gene. Tumours with three to four copies of the gene signals were considered to have a gain of the gene.

Statistical analyses

The associations of the gene copy numbers, tumour types, histological grades and clinical stages with expression levels were calculated with nonparametric Kruskal–Wallis and Mann–Whitney U-tests. Outliers were detected by using extreme studentised deviate method (ESD).

RESULTS

TRPS1, EIF3S3 and MYC gene amplification

Tissue microarrays and FISH were first used to study gene copy number of EIF3S3 and TRPS1 in hormone-refractory prostate carcinomas. High-level amplification (≥5 copies) of EIF3S3 and TRPS1 was found in 11 out of 40 (28%) and 10 out of 36 (28%) of the cases, respectively. The gain (three to four copies) of EIF3S3 and TRPS1 was found in 19 out of 40 (48%) and 18 out of 36 (50%) of the cases, respectively. In the cases of high-level amplification, the genes were always coamplified. The coamplification was also verified by hybridizing differentially-labelled gene specific probes (biontin labelled EIF3S3 probe and AlexaFluro 594 labelled TRPS1 probe) simultaneously to the TMA.

Next, breast and prostate cancer cell lines were analysed for copy numbers of the EIF3S3, MYC and TRPS1 genes. Of the cell lines, the highest copy number of all the genes was found in the breast cancer cell line SK-Br-3, which showed 47 copies of TRPS1 and 21 copies of EIF3S3 and MYC and only one copy of chromosome 8 centromere (Figures 2 and 3). In addition, a high-level amplification (locus/centromere ratio ≥2) of all three genes was found in MDA436 and PC-3 cancer cell lines. EIF3S3 and MYC were highly amplified also in EFM19 (Figure 3).

Expression of TRPS1, EIF3S3 and MYC

The standard curves in the real-time quantitative RT–PCR assay showed wide dynamic range and the linear relationship between cycle number and fluorescent threshold was strong (r² ~1). In addition to TBP, the expression of β-actin was measured and used alternatively for normalisation of most of the samples (data not shown). The results were similar with both control genes. Due to the potential problems with the β-actin retropseudogenes, TBP was chosen for normalisation of the whole material.

Figure 3 illustrates the relative expression of TRPS1, EIF3S3 and MYC in breast and prostate cancer cell lines. In the breast cancer cell line SK-Br-3, which contains the highest copy number of all the three genes investigated, EIF3S3 was the only gene that showed remarkably high-level (three-to 10-fold compared to the other cell lines) overexpression. The relative expression of TRPS1 was highest in ZR75-1, which contains a loss of the gene compared to centromere copy number. Interestingly, in most breast cancer cell lines, the expression of TRPS1 was high compared to the expression levels of EIF3S3 and MYC. The high expression level of TRPS1 in the breast cancer cell lines has also been observed by Northern and Western blot analyses (Chang et al, unpublished).

Figure 4 illustrates the relative expression of TRPS1, EIF3S3 and MYC in BPH (n = 9), untreated primary (n = 30 for TRPS1, n = 35 for EIF3S3 and MYC) and locally recurrent hormone-refractory (n = 12 for TRPS1, n = 11 for EIF3S3 and MYC) prostate carcinomas. The expression of EIF3S3 was, on average, three-fold higher (P = 0.029) in carcinomas than in BPH. There was no difference in the level of expression of EIF3S3 in hormone...
refractory and untreated prostate carcinomas. MYC and TRPS1 were expressed in equal levels in BPH, untreated and hormone-refractory tumours. There were no significant associations between histological grade or clinical stage (T3–T4 and/or N+ and/or M+ vs T1–2N0M0) and the expression of any of the three genes in the untreated tumours.

**DISCUSSION**

Several oncogenes are activated by overexpression of the gene and one mechanism of the overexpression is amplification of the gene (Brodeur and Hogarty, 1998). Gain or amplification of chromosome 8q is one of the most common chromosomal alterations in breast and prostate cancer (Forozan et al, 1997). However, the target gene of the amplification is still unknown. The genes studied here, EIF3S3, TRPS1 and MYC, have been suggested to be putative target genes in 8q23–q24 (Jenkins et al, 1997; Nuopponen et al, 1999; Chang et al, 2000). In order to evaluate the significance of these genes in breast and prostate cancer, we analysed both the gene copy numbers as well as the expressions of the three genes.

**Figure 2** (A) Dual colour FISH with PAC probes for EIF3S3 (green) and TRPS1 (red) in metaphase preparation of breast cancer cell line SK-Br-3. Multiple copies of both genes are seen in marker chromosomes. (B) Copy numbers of TRPS1, EIF3S3, MYC and anonymous sequence tag sites (STSs) in SK-Br-3 breast cancer cell line by FISH. The copy numbers of the STSs are from a previous publication (Nuopponen et al, 2000). At the bottom, the order and distances in megabases (Mb) of the genes (marked in red) and STSs (marked in black) from the p-telomere (pter) of the chromosome 8 are shown.

**Figure 3** Relative expression and gene copy number of (A) EIF3S3, (B) MYC and (C) TRPS1 in prostate and breast cancer cell lines by real-time quantitative RT–PCR and FISH. The relative expression of the genes was calculated by dividing the expression value of the gene of interest with the expression value of housekeeping gene TBP. The relative gene copy number was calculated by dividing the signal copy number of the gene of interest with the signal copy number of chromosome 8 centromere. Of the cell lines, SK-Br-3 showed clearly the highest copy number of all genes. However, only EIF3S3 was overexpressed in the cell line.
We have previously shown that EIF3S3 and MYC are coamplified in about one-third of the locally recurrent hormone-refractory prostate carcinomas (Saramäki et al., 2001). Now, it was found that also EIF3S3 and TRPS1 are coamplified in about 30% of the hormone-refractory prostate tumours. The finding that all three genes are commonly coamplified in the hormone-refractory tumours indicates that the size of the amplicon is large. The TRPS1 and EIF3S3 genes are located about 12 and 11 Mb centromeric from MYC, respectively. The large size and the relatively low copy number of the amplicons have previously been implicated also by CGH and FISH studies (Visakorpi et al., 1995; Cher et al., 1996; Nupponen et al., 1998, 2000).

The majority of cancer cell lines have been established from metastatic lesions of cancer. They typically contain more chromosomal alterations than primary tumours, and the aberrations are more confined. For example, amplicons are often smaller and the copy numbers higher making the cell lines more informative than the primary tumours for mapping the amplicons (Kallioniemi et al., 1994). Of the breast and prostate cancer cell lines, however, only SK-Br-3 shows a high-level amplification of two (8q21 and 8q23–24) independent subarm regions by CGH (Kallioniemi et al., 1994; Nupponen et al., 1999). Of the three genes analysed here, TRPS1 showed the highest copy number, about twice as high as for either EIF3S3 or MYC in SK-Br-3. Together with our previous mapping data (Nupponen et al., 2000), the results indicate that SK-Br-3 contains high copy numbers of all the three genes with low copy numbers of the flanking regions (Figure 1B). Thus, the amplicon from TRPS1 to MYC, covering a chromosomal region of about 12 Mb, contains, at least, three independent subamplicons in SK-Br-3.

We used real-time quantitative RT-PCR approach to measure the expression of TRPS1, EIF3S3 and MYC. In real-time PCR, the quantification of the template is based on detection of the cycle in which the reaction enters the exponential phase, instead of measuring the amount of end product. Thus, none of the reagents is rate limiting in the reaction at the time of measurement of the fluorescence. Several studies have already shown that real-time RT-PCR is a highly quantitative and reliable method (Bieche et al., 1999; Linja et al., 2001; Savinainen et al., 2002). It is also especially useful in analysis of small tumour samples.

We first analysed the expression of EIF3S3, MYC and TRPS1 in the cell lines. In SK-Br-3, which showed the highest copy number of the genes, the expression of only EIF3S3 was remarkably high. The high-level expression of EIF3S3, shown here by quantitative RT-PCR, confirms our earlier Northern blot data (Nupponen et al., 1999). Surprisingly, the expression of TRPS1, whose copy number was highest of the three genes in SK-Br-3, was lower in SK-Br-3 compared to ZR75-1. The expression of TRPS1 was the highest in ZR75-1, which actually contains a relative loss of the gene. The expression of MYC varied among the cell lines. SK-Br-3 containing the highest copy number of the gene did not show clearly increased expression compared to the other cell lines. The data suggest that of the three genes, EIF3S3 is the most likely target gene of amplification in SK-Br-3.

In the clinical prostate cancer specimens, the level of EIF3S3 expression was significantly (P = 0.029) higher in prostate than in BPH. The data are consistent with our previous analyses by semiquantitative mRNA in situ hybridisation, which suggested that EIF3S3 is expressed more in hormone-refractory prostate carcinomas than in BPH (Nupponen et al., 1999). Here, it was found that the expression of EIF3S3 is increased also in untreated prostate cancers. Somewhat surprising, the expression of EIF3S3 was not higher in the hormone-refractory compared to the untreated tumours, despite the fact that hormone-refractory tumours, in general, contain higher frequency of 8q gain (Visakorpi et al., 1995). The data suggest that EIF3S3 is commonly overexpressed in prostate cancer, and also other mechanisms than gene amplification may lead to the overexpression of the gene. There were no...
significant differences between the expression of either MYC or TRPS1 in BPH, untreated and hormone-refractory carcinomas, suggesting that alterations in the expression of these two genes are not generally involved in the progression of prostate cancer.

In conclusion, the results indicate that overexpression of MYC and TRPS1 are rare in prostate cancer in vivo. In contrast, the expression of EIF3S3 is increased in prostate cancer. One mechanism for the overexpression of EIF3S3 seems to be amplification of the gene as demonstrated by the cell line SK-BR-3. Analyses of the expression levels of EIF3S3 in large clinical materials are now warranted.

REFERENCES

Alers JC, Rochat J, Krijtenburg PJ, Hop WC, Kranse R, Rosenberg C, Tanke HJ, Schroder FH, van Dekken H (2000) Identification of genetic markers for prostatic cancer progression. *Lab Invest* 80: 931–942

Anthony B, Carter P, De Benedetti A (1996) Overexpression of the proto-oncogene/translation factor 4E in breast-carcinoma cell lines. *Int J Cancer* 65: 858–863

Asano K, Vornlocher HP, Richter-Cook NJ, Merrick WC, Hinnebusch AG, HERSHEY JW (1997) Structure of cDNAs encoding human eukaryotic initiation factor 3 subunits. Possible roles in RNA binding and macromolecular assembly. *J Biol Chem* 272: 27042–27052

Bieche I, Laurendeau I, Tozlu S, Olivi M, Vidaud D, Lidereau R, Vidaud M (2003) Quantitation of MYC gene expression in sporadic breast tumors with a real-time reverse transcription-PCR assay. *Cancer Res* 59: 2759–2765

Brodeur GM, Hogarty MD (1998) Gene amplification in human cancers: biological and clinical significance. In *The Genetic Basis of Human Cancer*, Vogelstein B, Kinzler KW (eds) pp 161–172. New York: McGraw-Hill Companies Inc

Chang GT, van den Bemd GJ, Jhamai M, Brinkmann AO (2002) Structure and function of GC79/TRPS1, a novel androgen-repressible apoptosis gene. *Apoptosis* 7: 13–21

Cher ML, Bova GS, Moore DH, Small EJ, Carroll PR, Pin SS, Epstein JJ, Isaacs WB, Jensen RH (1996) Genetic alterations in untreated metastases and androgen-independent prostate cancer detected by comparative genomic hybridization and allelotyping. *Cancer Res* 56: 3091–3102

De Benedetti A, Rhoads RE (1990) Overexpression of eukaryotic protein synthesis initiation factor 4E in HeLa cells results in aberrant growth and morphology. *Proc Natl Acad Sci USA* 87: 8212–8216

Forozan F, Karhu R, Kononen J, Kallioniemi A, Kallioniemi OP (2002) Improved technique for analysis of formalin-fixed, paraffin-embedded tumors by fluorescence *in situ* hybridization. *Cytometry* 50: 93–99

Fukuchi-Shimogori T, Ishii I, Kashiwagi K, Mashiba H, Ekimoto H, Igarashi K, GIACCIA AJ, DENKO NC (2000) Multiple roles for deregulated MYC oncogene. *Cancer Res* 60: 1735–1740

Giaccia AJ, Denko NC (1999) Genotypic and phenotypic spectrum in tricho-rhino-phalangeal syndrome types I and III. *Am J Hum Genet* 65: 81–91

Hogarty MD, Brodeur GM, LIPECKY RP, SIEGEL SR, TUSCHL T, VISAKORPI T, KALLIONIEMI A, KALLIONIEMI OP (2001) Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. *Cancer Res* 61: 3550–3555

Lundeck HJ, Schaper J, MEINECKE P, MOMENI P, GROSS S, Von HULTOM D, HIRCHE H, ABRAMOWICZ MJ, ALBRECHT B, APACIC C, CHRISTEN HJ, Claussen U, Devriendt K, Fastnacht E, FORDERER A, FRIEDRICH U, GOODSHIP TH, GREIWE M, HAMM H, HENNEKAM RC, HINKEL GK, HOELTzenbein M, KAYSERLI H, MAJEWSKI F, Mathieu M, MCLeod R, MIDRO AT, MOOG U, NAGAI T, NIKAWA N, ORSTAVIK KH, PLOGCH E, SEITZ C, SCHMIDKIE J, TRANEBJAERG L, TAKAHARA M, WITTWER B, Zabel B, GILLESSEN-KAESCH G, HORSHEMKE B (2001) Genotypic and phenotypic spectrum in tricho-rhino-phalangeal syndrome types I and III.

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Microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* 4: 844–847

Linja MJ, Savinainen KJ, Saramäki OR, Tammela TL, Vessella RL, Visakorpi T (2001) Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. *Cancer Res* 61: 3550–3555

Malik TH, Sahooth SA, Latham P, Kroll TG, Peters LL, Shivdasani RA (2001) Transcriptional repression and developmental functions of the atypical vertebrate GATA protein TRPS1. *EMBO J* 20: 1715–1725

Nupponen NN, Momoni P, GLOCKNER G, SCHMIDTKE J, TRANEBJAERG L, TAKAHARA M, WITTWER B, ZABEL B, GILLESSEN-KAESCH G, HORSHEMKE B, Lundeck HJ (2000) Mutations in a new gene, encoding a zinc-finger protein, cause tricho-rhino-phalangeal syndrome type I. *Nat Genet* 24: 71–74

Nupponen NN, Isola J, Visakorpi T (2000) Mapping the amplification of EIF3S3 in breast and prostate cancer. *Genes Chromosomes Cancer* 28: 203–210

Nupponen NN, Kakkola L, KOIVISTO P, Visakorpi T (1998) Genetic alterations in hormone-refractory recurrent prostate carcinomas. *Am J Pathol* 153: 141–148

Nupponen NN, PORKKA K, Kakkola L, TANNER M, PERSSON K, BORG Å, ISOLA J, Visakorpi T (1999) Amplification and overexpression of p40 subunit of eukaryotic translation initiation factor 3 in breast and prostate cancer. *Am J Pathol* 154: 1777–1783

Ramaswamy S, Ross KN, LANDER ES, Golub TR (2002) A molecular signature of metastasis in primary solid tumors. *Nat Genet* 33: 94–98

Reiter RR, GU Z, Watake T, THOMAS G, SZIGETI K, DAVIS E, WAIL M, NISITANI S, YAMASHIRO J, LE BEAU MM, LODA M, WITTE ON (1998) Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer. *Proc Natl Acad Sci USA* 95: 1735–1740

Rummukainen JK, SALMINEN T, LUNDIN J, JOENSSU H, ISOLA J (2001) Amplification of c-myc oncogene by chromogenic and fluorescence *in situ* hybridization in archival breast cancer tissue array samples. *Lab Invest* 81: 1543–1551

Saramäki O, Willi N, Bratt O, Gasser TC, KOIVISTO P, NUPPONEN NN, BUBENDORF L, VISAKORPI T (2001) Amplification of EIF3S3 gene is associated with advanced stage in prostate cancer. *Am J Pathol* 159: 2089–2094

Savinainen KJ, Saramäki OR, LINJA MJ, Bratt O, TAMMELA TL, Isola J, Visakorpi T (2002) Expression and gene copy number analysis of ERBB2 oncogene in prostate cancer. *Am J Pathol* 160: 339–345

Tirkkonen M, TANNER M, KARHU R, KALLIONIEMI A, ISOLA J, KALLIONIEMI OP (1998) Molecular cytogenetics of primary breast cancer by CGH. *Genes Chromosomes Cancer* 21: 177–184

Visakorpi T, KALLIONIEMI AH, SYWANE AC, HYTTINEN ER, Karhu R, TAMMELA TL, Isola J, KALLIONIEMI OP (1995) Genetic changes in primary and recurrent prostate cancer by comparative genomic hybridization. *Cancer Res* 55: 342–347