American Journal of Pathology, Vol. 154, No. 6, June 1999

Amplification and Overexpression of p40 Subunit of Eukaryotic Translation Initiation Factor 3 in Breast and Prostate Cancer

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Amplification at the long arm of chromosome 8 occurs in a large fraction of breast and prostate cancers. To clone the target genes for this amplification, we used suppression subtraction hybridization to identify overexpressed genes in the breast cancer cell line SK-Br-3, which harbors amplification at 8q (8q21 and 8q23-q24). A differentially expressed gene identified by SSH, the p40 subunit of eukaryotic translation initiation factor 3 (eIF3), was localized to 8q23 and found to be highly amplified and overexpressed in the breast and prostate cancer cell lines studied. High-level amplification of eIF3-p40 was found in 30% of hormone-refractory prostate tumors and in 18% of untreated primary breast tumors. In the vast majority of the cases, p40 and c-*myc* were amplified with equal copy numbers. Tumors with higher copy numbers of p40 than c-*myc* were also found. Expression of p40 mRNA was analyzed with *in situ* hybridization. The amplification of eIF3-p40 gene was associated with overexpression of its mRNA, as expected for a functional target gene of the amplification. These results imply that genomic aberrations of translation initiation factors, such as eIF3-p40, may contribute to the pathogenesis of breast and prostate cancer. *(Am J Pathol 1999, 154:1777–1783)*

The development and progression of cancer is caused by multiple genetic alterations, such as gene amplification. Several amplified oncogenes have been identified in cancer,[1] but studies by comparative genomic hybridization (CGH)[2] have indicated that known oncogenes explain only part of the amplifications found in different chromosomal regions.[3] The long arm of chromosome 8 (8q) is one of the most common regions of amplification in cancers of several organs, especially carcinomas of breast and prostate.[4–7] Almost 80% of hormone-refractory and distant metastases but only 5% of untreated prostate carcinomas show gain of 8q.[4,7,8] In breast cancer gain at 8q is the second most common copy number imbalance, present in 40–50% of tumors.[6] In both cancer types, the gain of 8q is associated with more advanced stage and aggressive phenotype, and in breast cancer also with poor prognosis.[9,10] Therefore, identification of target genes for the 8q amplification may reveal important pathogenic mechanisms for the development and progression of breast and prostate carcinomas.

Within the 8q arm, two independently amplified subregions, 8q21 and 8q23-q24, have been identified,[4,8] suggesting the presence of several target genes. The c-*myc* oncogene, cloned over 10 years ago, is considered the putative target gene at 8q24.1. However, according to several studies, c-*myc* amplification is not always associated with its overexpression.[11,12] Large amplifications at 8q occur also without c-*myc* amplification, suggesting that multiple target genes might be involved, as with other amplicons in breast cancer.[11–13]

The two minimal commonly amplified regions (8q21 and 8q23-q24) comprise approximately 60 Mb of DNA containing possibly up to 1000 genes. We used 8q suppression subtractive hybridization (SSH)[14] between breast cancer cell lines with and without the 8q-amplification[2] to identify overexpressed target genes.

Materials and Methods

Cell Lines and Tumors

Breast cancer cell lines SK-Br-3, ZR-75-1, MDA436, and MCF-7 and prostate cancer cell lines PC-3, DU145, and LNCaP were obtained from the American Type Culture Collection (Manassas, VA) and cultured in recommended conditions. Formalin-fixed, paraffin-embedded hormone-refractory prostate carcinomas (n = 44) were obtained from transurethral resections, which were done to relieve urethral obstruction. The average time from diagnosis

Supported by grants from the Pirkanmaa Cancer Society, the Irja Karvonen Cancer Foundation, the Cancer Society of Finland, the Reino Lahtikari Foundation, Academy of Finland, the Medical Research Fund of Tampere University Hospital and CaPCURE.

Accepted for publication March 12, 1999.

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(the beginning of hormonal therapy) to progression was 44 months (range, 8–113 months). The second set of tumors comprised 39 freshly frozen primary invasive breast carcinomas taken from patients before any treatment. In addition, 19 breast carcinoma imprint touch preparations were obtained from the Department of Oncology, University of Lund (Lund, Sweden). These tumors were selected because they were known to contain c-*myc* amplification according to Southern analysis.[15]

Suppression Subtractive Hybridization (SSH)

SSH was done with PCR-Select cDNA Subtraction Kit (Clontech, Palo Alto, CA) with minor modifications, as earlier described.[14] Total RNAs were isolated from SK-Br-3 and ZR-75-1 breast cancer cell lines by TRIzol Reagent (Gibco BRL, Grand Island, NY), from which mRNAs were isolated using Dynabeads (Dynal A.S., Oslo, Norway) and used for cDNA synthesis. cDNA from SK-Br-3 was used as tester and cDNA from ZR75-1 as driver in the subtraction hybridization. The resulting subtracted cDNAs were subcloned into pCR 2.1-TOPO vector (Invitrogen, Carlsbad, CA). The inserts were amplified by polymerase chain reaction (PCR) using adapter-specific primers (Clontech) from randomly picked clones and sequenced using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer, Foster City, CA) and ABI310 sequencer (Perkin-Elmer).

Fluorescence in Situ *Hybridization (FISH)*

Metaphase and interphase cell preparations from the cancer cell lines, normal blood lymphocytes, and nuclei isolated from prostate and breast carcinomas were used for the FISH analysis. Metaphase and interphase FISH were performed as described in detail elsewhere.[16] After identification of the p40 subunit of eukaryotic translation initiation factor 3 (eIF3-p40), a locus-specific genomic probe was obtained by screening human PAC library with PCR using primers specific to eIF-p40 (5′-GCCCAGGCTCTTCAAGAATAC-3′ and 5′ATAGCCAAAATCGGCAATGA-3′). A genomic P1-probe for c-*myc* was obtained from RMC (RMC08P001, Berkeley, CA). The probes were labeled with biotin-16-dUTP or digoxigenin-11-dUTP (Boehringer Mannheim, Mannheim, Germany) using nick-translation. Texas Red-labeled chromosome 8 α-satellite (centromere) probe was used as a reference probe (CEP8, Vysis, Inc., Downers Grove, IL). Before hybridization, prostate cancer samples were pretreated by heating in 59% glycerol/0.1× standard saline citrate (SSC, pH 7.5) solution at 90°C for 3 minutes to improve hybridization efficiency of the probes. Slides were denatured in a 70% formamide-2× SSC solution at 73°C for 3 minutes. After hybridization the probes were detected immunochemically with avidin-fluorescein isothiocyanate and anti-digoxigenin rhodamine. Slides were counterstained with 0.1 μmol/L 4,6-diaminido-2-phehylindole in an antifade solution. Signal copy numbers were counted from 100 randomly chosen nonoverlapping nuclei. Control hybridizations included normal lymphocytes and formalin-fixed, paraffin-embedded benign prostate hyperplasia (BPH) samples (n = 10). These experiments showed that the probes recognized a single copy target and that the hybridization efficiencies were similar. The mean averages ± SD of p40 and c-*myc* signals in the BPH samples were 2.2 ± 0.3 and 2.1 ± 0.2, respectively. Digital images were captured with a Zeiss Axioplan 2 epifluorescence microscope (Carl Zeiss Jena GmbH, Jena, Germany) equipped with a Hamamatsu C9585 camera (Hamamatsu Photonics, Hamamatsu, Japan) and ISIS software program (Metasystems GmbH, Altslusheim, Germany). Tumors that showed >20% of nuclei with increased copy number of either eIF3-p40 or c-*myc* were considered to have amplification. In these cases, the level of amplification was determined counting only nuclei with an increased number of signals. The tumors were classified into three groups: nonamplified (no increase in p40 or c-*myc* copy number), low-level amplification (3–5 copies per cell), and high-level amplification (≥5 copies of the genes per cell or gene/centromere ratio >2).

Southern Blot Analysis

Genomic DNAs were extracted according to standard protocols. Following restriction enzyme digestion with *Bcl*I (MBI Fermentas, Vilnius, Lithuania), DNAs were separated on a 0.7% agarose gel and blotted onto a nylon membrane (Hybond-N, Amersham, Arlington Heights, IL). α^{32}P-labeled probes (Random Primed DNA labeling kit, Boehringer Mannheim) for eIF3-p40 (670-bp fragment corresponding to eIF3-p40 sequences from exon 3 to exon 5) and hypoxantine-guanine phosphoribosyltransferase (insert of EST 270419; GenBank accession no. N33128) were hybridized sequentially using standard protocols. The hybridization signals were detected with Phosphoimager (Molecular Dynamics, Inc., Sunnyvale, CA).

Northern Blot Analysis

Total RNAs from cancer cell lines were isolated by TRIzol Reagent (Gibco BRL). Twenty micrograms of total RNA were electrophoresed and transferred to a nylon membrane. α^{32}P-labeled probes for eIF3-p40 (1.2-kb insert of EST 346021; GenBank accession no. W72146), c-*myc* (2.2-kb insert of EST 51699; GenBank accession no. H24033), and β-actin (Clontech) were hybridized sequentially using standard protocols. The hybridization signals were detected and quantitated with Phosphoimager and ImageQuaNT software program (Molecular Dynamics).

Expression of eIF3-p40 in Clinical Tumors Studied with mRNA in Situ *Hybridization*

*Eco*RI-*Hin*cII-fragment (780 bp) from EST-clone 595376 (GenBank accession no. AA173710) was subcloned into pBluescript SK vector (Stratagene, La Jolla, CA) and used for *in vitro* transcription of eIF3-p40 to obtain anti-

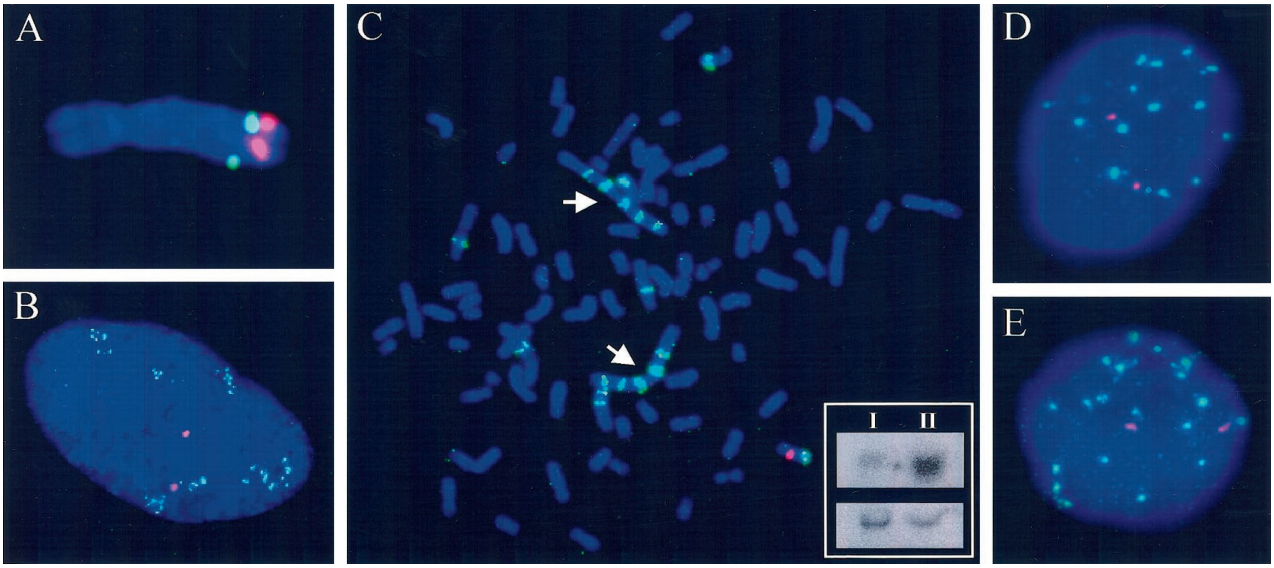

Figure 1. A: Two-color FISH analysis shows that eIF3-p40 (green signals) maps to chromosome 8q23, approximately 12 Mb centromeric from c-*myc* (red signals). FLpter-values, which were used to estimate the localization of eIF3-p40, were measured for both eIF3-p40 (mean FLpter-value 0.8096) and c-*myc* (mean FLpter-value 0.8894) using the Scilimage software program (TNO, Delft, The Netherlands). Examples of high-level amplification of the eIF3-p40 in the interphase nuclei of prostate cancer cell line PC-3 (B) and, in the metaphase preparation of breast cancer cell line SK-Br-3 (C). eIF3-p40 gene is present in several copies in two large marker chromosomes (arrows), as well as in several smaller chromosomes in SK-Br-3. There is only one chromosome 8 centromere signal (red signal). Inset Southern analysis shows amplification of eIF3-p40 in SK-Br-3. Hypoxantine-guanine phosphoribosyltransferase gene indicate equal loading of the DNAs. Examples of the interphase nuclei of hormone-refractory prostate tumor (D) and uncultured breast tumor (E) show multiple copies of eIF3-p40.

sense and sense cRNA probes. A cytokeratin antisense probe derived from a *Eco*RI-*Sma*I fragment (690 bp) of EST-clone 487868 (GenBank accession no. AA044589) was used to control the quality of RNA and hybridization of the samples. We hybridized 27 formalin-fixed, paraffin-embedded hormone-refractory prostate carcinomas, 34 primary breast carcinomas, 1 normal breast tissue, and 3 BPHs with ^{33}P-dUTP-labeled cRNA probes. Deparaffinized sections were rinsed in phosphate-buffered saline and digested with proteinase K (10 μg/ml) for 20 minutes at 37°C in phosphate-buffered saline. The sections were covered with hybridization solution (50% formamide, 2×SSC, 20 mmol/L Tris, 1 mmol/L EDTA, 1× Denhart's, 10% dextran sulfate, and 500 μg/ml yeast tRNA) containing the probe with the final activity of 10^7 cpm/ml and hybridized overnight at 55°C. After hybridization, the sections were washed and digested with RNase A (20 μg/ml) for 30 minutes at 37°C in 0.5 mol/L NaCl, 10 mmol/L Tris (pH 8.0), and 1 mmol/L EDTA.

For quantitation, the hybridized sections were exposed to Amersham β-max Hyperfilm for 3 days. After the film was developed, it was scanned using Personal Densitometer SI (Molecular Dynamics). The expression levels were quantitated with ImageQuaNT software using the volume quantitation option. First, representative objects of equal size were selected from each slide. The quantitation results were given as integrated intensity of all pixels in the objects excluding the background. For microscopic examination, the hybridized sections were immersed in autoradiographic emulsion NTB2 (Kodak) and exposed for 4 weeks at 4°C. After developing the autoradiographic signals (grains), the sections were counterstained with hematoxylin and examined in a Nikon Microphot-SA (Nikon Corp., Tokyo) microscope equipped with an epipolarization filter allowing simultaneous visualization of grains (using polarized epi-illumination light) and morphology by hematoxylin staining (using transmitted light).

Results

eIF3-p40 as a Candidate Target Gene of the Amplification

SSH was used to identify overexpressed transcripts in breast cancer cell line SK-Br-3. cDNAs from SK-Br-3 were subtracted against those from ZR-75-1. Database searches with BLASTN revealed that the first redundant clone, named A8, recognized an EST clone 595376 (accession no. AA173710), which, according to the Unigene database, was located in the region of interest between marker D8S276 (8q22.3) and D8S1799 (8q24). Next, we verified by Northern analysis that A8 was differentially expressed in SK-Br-3 and ZR-75-1.

Database search showed that the sequence of A8 was identical to the recently cloned gene, eukaryotic translation initiation factor 3 subunit p40 (eIF3-p40).[17] To map the gene precisely, we obtained genomic clone for eIF3-p40 by screening human PAC library. Using the PAC-probe and FISH, we localized the eIF3-p40 to 8q23, about 12 Mb centromeric from c-*myc* (Figure 1A).

eIF3-p40 Amplified in both Breast and Prostate Cancer

To study the p40 gene copy number status in breast and prostate cancer, we first analyzed three prostate (PC-3,

Table 1. Frequency of eIF3-p40 Gene Amplification in the Clinical Tumor Material Analyzed by FISH

Tissue type	No amplification	Low level amplification*	High level amplification*	Total number of tumors
Hormone-refractory prostate carcinoma	0	31 (70%)	13 (30%)	44 (100%)
Primary breast carcinoma	15 (38%)	17 (44%)	7 (18%)	39 (100%)
Selected breast carcinoma†	0	3 (16%)	16 (84%)	19 (100%)

*Low level amplification, 3–4 copies; high-level amplification, ≥5 copies.
†The selected tumors had been previously analyzed for c-*myc* amplification by Southern blot.

DU-145, LNCaP) and four breast (SK-Br-3, MDA-436, MCF-7, ZR-75-1) cancer cell lines by FISH. High-level amplification (5 or more copies of the gene or p40 and centromere ratio >2) of p40 was found in PC-3 (Figure 1B), SK-Br-3 (Figure 1C), MDA-436, and in MCF-7, in concordance with the gain of 8q found by CGH in these cell lines.[5,18] The amplification of eIF3-p40 gene in SK-Br-3 was also confirmed by Southern analysis (Figure 1C, inset). Figure 1, D and E, illustrates examples of high-level amplifications of eIF3-p40 in prostate and breast carcinomas determined by FISH. Table 1 summarizes the results in clinical tumor material obtained with FISH. Thirteen of 44 (30%) of the hormone-refractory prostate carcinomas showed high-level amplification of p40, and the remaining cases showed a low-level copy number gain (3 to 4 copies) of p40. In prostate tumors with amplification, the mean (± SD) copy number of p40 was 6.7 (± 1.5). Seven of 39 (18%) of the breast cancers showed high-level amplification of p40, 17 of 39 (43%) showed a low-level gain, and the remaining 15 tumors (39%) showed two copies of the p40. The mean copy number of p40 was 8.5 (± 2.9) in the breast tumors with amplification. Next, we analyzed 19 selected breast carcinomas with high-level c-*myc* amplification demonstrated by Southern blot.[15] Sixteen of these tumors showed high-level amplification of p40 with the mean copy number 21.8 (± 21.12).

*Coamplification of eIF3-p40 and c-*myc

To analyze coamplification of eIF3-p40 and c-*myc* oncogene, copy number of c-*myc* was also studied in the same samples by FISH. In breast and prostate cancer cell lines the copy numbers of c-*myc* and p40 were identical, except in PC-3, where p40 was present in 15 copies and c-*myc* in 9 copies per cell. All hormone-refractory prostate carcinomas showed similar copy number of p40 and c-*myc*. One of the unselected breast carcinomas showed high-level amplification of c-*myc*, but only a low-level amplification of p40. Three selected breast carcinomas showed ~5 times higher copy number of p40 than c-*myc*, whereas one case showed ~2 times more c-*myc* than p40 signals (Figure 2).

Amplification of eIF3-p40 Associated with Overexpression

The amplification of the target proto-oncogenes is thought to lead to their overexpression. We compared the expression levels of p40 and c-*myc* in cancer cell lines using Northern blot analysis. Whereas there was no clear association between the expression and amplification status of c-*myc*, the expression of p40 was related to its gene copy number (Figure 3A). The expression of p40 was examined in prostate and breast tumors with semiquantitative mRNA *in situ* hybridization (Figure 3, B-E). The hormone-refractory prostate carcinomas expressed over four times more p40 than benign prostate hyperplasia tissues (Figure 4) (Mann-Whitney *U* test; $P = 0.0021$). The level of p40 expression was higher in breast carcinomas with high-level amplification than low-level or no amplification (Kruskal-Wallis test; $P = 0.028$).

Discussion

We identified the eIF3-p40 gene as a candidate gene for the 8q amplification. The high-level amplification of the gene was found in one-third of the hormone-refractory prostate and in ~20% of the untreated breast carcinomas. The results indicate that the amplification of p40 belongs to the most commonly amplified genes in these tumor types.

The well-characterized proto-oncogene c-*myc* and eIF3-p40 were coamplified in a vast majority of tumors and cancer cell lines studied. The copy number for both genes was also similar in most tumors with coamplification. Thus, the amplicon either extends from eIF3-p40 to c-*myc* (approximately 12 Mb) or consists of several separate amplification peaks with nonamplified DNA in be-

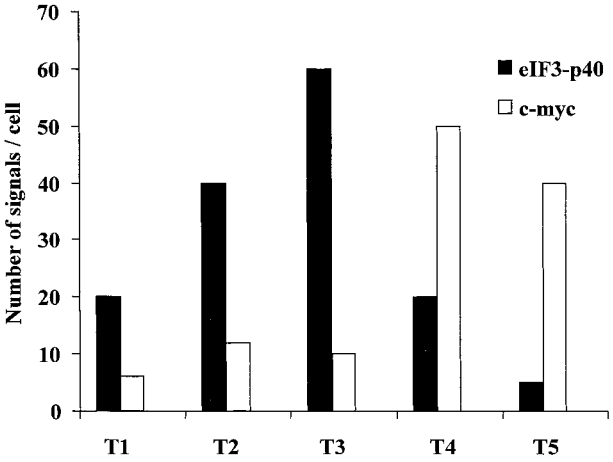

Figure 2. The mean copy numbers of eIF3-p40 and c-*myc* in five breast tumors. Tumors 1, 2, and 3 from the selected breast cancer material show clearly higher copy numbers of p40 than of c-*myc*, whereas one tumor (T4) from the selected breast cancer material and another (T5) from the unselected material display more c-*myc* than p40 signals.

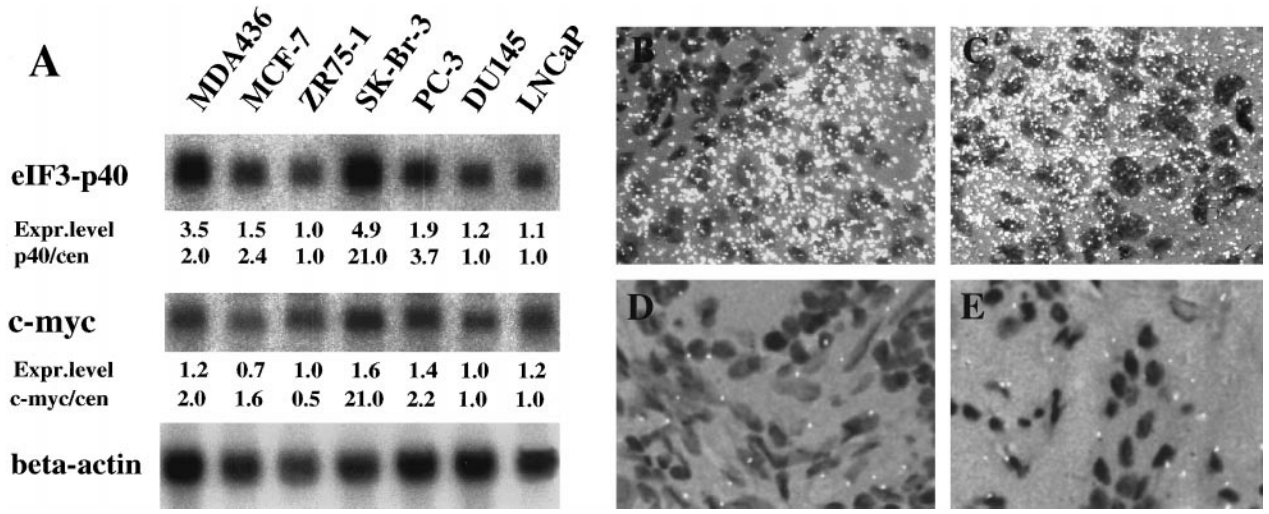

Figure 3. A: Increased expression of eIF3-p40 in the MDA436, MCF-7, SK-Br3, and PC-3 cell lines showing high-level amplification of eIF3-p40 by FISH, is found by Northern analysis, as compared to the expression level in the ZR75-1. Expression levels of c-*myc* quantitated using Phosphoimager clearly show less variation than eIF3-p40 expression levels. The relative level of expression of the genes is given in proportion to the expression in ZR75-1. The expression of β-actin was used to control the loading differences. The relative copy numbers (gene versus centromere copy number) of p40 and c-*myc* are also shown. eIF3-p40 mRNA *in situ* hybridization demonstrates overexpression in (B) hormone-refractory prostate carcinoma and in (C) primary breast carcinoma, and (D) low-level expression in benign prostate hyperplasia and (E) in primary breast carcinoma without eIF4-p40 amplification. B and C correspond to the FISH images in Figure 1D and 1E, respectively. Hybridization signals were visualized with epipolarization filter (magnification, ×400).

tween, as has been described in 20q12-q13 amplification in breast cancer.[19] Whatever the structure of the 8q23-q24 amplicon, it is clear based on the present study that the amplification contains several target genes. Thus, the amplification resembles those in 11q13, 17q23-q25, and 20q12-q13, where several target genes are often coamplified.[13,19–21]

Many genes may also be amplified simply because they are located near the target gene. The commonly used criterion for a putative target oncogene is that the amplification of the gene leads to its overexpression.[22–25] Therefore, we used SSH to identify transcripts that were overexpressed in the SK-Br-3 cancer cell line containing high-level amplification of 8q21 and 8q23-q24 regions. After identification of the eIF3-p40 by SSH, we confirmed differential expression pattern by Northern blot in the cancer cell lines. Subsequently, we screened more breast and prostate cancer cell lines for eIF3-p40 copy number and expression. The cancer cell lines containing high-level amplification expressed more of the gene than those with no amplification. Because the clinical material consisted of paraffin-embedded tumor blocks, we chose to use mRNA *in situ* hybridization to detect the gene expression in breast and prostate tumors. We have previously shown that mRNA *in situ* hybridization is a reliable technique for the quantitation of gene expression in formalin-fixed, paraffin-embedded tissue.[26] Breast carcinomas with the high-level amplification expressed significantly more p40 than tumors with low-level or no amplification, although there were individual cases with high-level expression without the gene amplification. The hormone-refractory prostate carcinomas expressed more p40 than BPHs. However, significant differences between the p40 expression levels in prostate tumors with high- and low-level amplification were not observed. Altogether, the results suggest that gene amplification is not the only mechanism that leads to overexpression of the eIF3-p40 gene. Alterations occurring in the regulation of eIF3-p40 expression could also be involved in the overexpression of the gene. Similarly, it has previously been shown that overexpression of ERBB2 oncogene, which is usually due to the high-level amplification of the gene, can also take place without the gene copy number changes.[27] Nevertheless, the expression studies demonstrated that all tumors containing eIF3-p40 amplification expressed the gene at high levels. There was also a trend between increased copy number of eIF3-p40 and enhanced expression of the gene, as would be expected for the true target gene of the amplification.

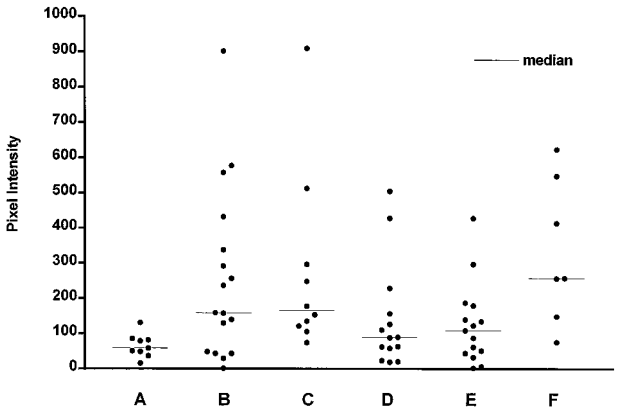

Figure 4. Expression of eIF3-p40 (A) in benign prostate hyperplasia (n = 9), in (B) hormone-refractory prostate tumors with low-level amplification (n = 17), and (C) with high-level amplification (n = 10), as well as in (D) unselected breast carcinomas with no amplification (n = 14), (E) with low level amplification (n = 15), and (F) with high-level amplification (n = 7) of eIF3-p40. The mRNA *in situ* hybridization signals were quantitated from autoradiograph film using Personal Densitometer SI (Molecular Dynamics, Inc). The expression of p40 was higher in hormone-refractory prostate carcinomas than in BPH (P = 0.002), as well as in breast carcinomas with high-level amplification than in breast carcinomas with low-level amplification (P = 0.029) of p40 gene.

In the prostate cancer and most of the breast cancers the gene copy number of p40 often remained relatively low (<10 copies) when compared with copy numbers reported, eg, in 20q13 amplification in breast cancer.[19] This may be due to the fact that, according to CGH studies, the 8q amplification usually comprises the whole chromosome arm. It may well be that even a few additional copies of p40 (and c-*myc*) are enough to cause overexpression and thereby contribute to the tumorigenesis. However, in the selected breast cancer material found to contain c-*myc* amplification by Southern analysis,[15] very high copy numbers of p40 (50–100 copies) were also found. Three tumors showed clearly higher copy number of eIF3-p40 than c-*myc*, suggesting that the genes may belong to separate subamplicons. However, the fact that the most frequent finding was coamplification with equal copy numbers suggests that both genes may contribute to and perhaps cooperate in the progression of breast and prostate cancer.

The finding that eIF3-p40 is amplified in breast and prostate cancer suggests a new functional category of amplified oncogenes in breast and prostate cancer. Most of the known oncogenes are involved in signal transduction (eg, EGFR, erbB-2, *ras*), regulation of transcription (eg, fos, jun, myc), or cell cycle regulation (cyclinD1). Several lines of recent evidence imply that aberrant regulation of translation could also be involved in the tumorigenesis. Overexpression of initiation factors eIF-4E and eIF-4G have been shown to transform NIH 3T3 cells as well as rat embryo fibroblasts in collaboration with v-*myc* or E1A.[28–30] It has also been suggested that the mitogenic and oncogenic activities of eIF-4E are mediated by *ras* oncogene.[31] On the other hand, the expression of eIF-4E and eIF-2α are regulated by c-*myc*.[32] Thus, amplified c-*myc* may increase cell growth by regulating the expression of these translation initiation factors. Translational apparatus also contains elongation factors. The constitutive expression of elongation factor 1α (EF-1α) causes cells to become susceptible to transformation by UV light or 3-methylcholantrene.[33] In addition, the PTI-1 gene, which, based on sequence analysis, is a mutated and truncated form of EF-1α, was recently cloned from LNCaP prostate cancer cell line using differential RNA display.[34] There is limited *in vivo* evidence on the role of translation initiation factors in the development and progression of cancer. Allelic imbalance of eIF3-p48 subunit, which is encoded by INT6, has been detected in breast carcinomas.[35,36] Amplification of eIF-4G has been found in a few squamous cell lung carcinomas.[37] In addition, overexpression of eIF-4E has been detected in breast cancer and it may be associated with the recurrence of the disease.[38]

The eIF3-p40 gene, which we found amplified and overexpressed in breast and prostate cancer, has not been implicated in the development or progression of cancer before. It is a subunit of the largest (~600 kd) eukaryotic translation initiation factor protein complex, which has a central role in the initiation of translation. eIF3 complex binds to 40S ribosomal subunits in the absence of other initiation factors and preserves the dissociated state of 40S and 60S ribosomal subunits. It also stabilizes eIF2-GTP-Met-tRNA binding to 40S subunits and mRNA binding to ribosomes.[39] Very little is known about the p40 subunit itself. Based on the sequence homology, it seems to be related to mouse protein Mov-34.[17] The gene product of human homologue of Mov-34 is a component of the 26S proteasome. However, due to the fragmentary nature of the current knowledge, it is not possible to draw conclusions about how eIF3-p40 could be involved in the tumorigenesis. Therefore, functional studies, in addition to studies aiming at defining the clinical significance, are required to elucidate the role of eIF3-p40 in the progression of breast and prostate cancer.

Acknowledgments

We thank Mrs. Sari Toivola, Mrs. Arja Alkula, and Mrs. Mariitta Vakkuri for technical assistance.

References

1. Alitalo K, Schwab M: Oncogene amplification in tumor cells. Adv Cancer Res 1986, 47:235–281
2. Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D: Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992, 258:818–821
3. Forozan F, Karhu R, Kononen J, Kallioniemi A, Kallioniemi OP: Genome screening by comparative genomic hybridization. Trends Genet 1997, 13:405–409
4. Cher ML, Bova GS, Moore DH, Small EJ, Carroll PR, Pin SS, Epstein JI, Isaacs WB, Jensen RH: Genetic alterations in untreated metastases and androgen-independent prostate cancer detected by comparative genomic hybridization and allelotyping. Cancer Res 1996, 56:3091–3102
5. Nupponen NN, Hyytinen ER, Kallioniemi AH, Visakorpi T: Genetic alterations in prostate cancer cell lines detected by comparative genomic hybridization. Cancer Genet Cytogenet 1998, 101:53–57
6. Tirkkonen M, Tanner M, Karhu R, Kallioniemi A, Isola J, Kallioniemi OP: Molecular cytogenetics of primary breast cancer by CGH. Genes Chromosomes Cancer 1998, 21:177–184
7. Visakorpi T, Kallioniemi AH, Syvanen AC, Hyytinen ER, Karhu R, Tammela T, Isola JJ, Kallioniemi OP: Genetic changes in primary and recurrent prostate cancer by comparative genomic hybridization. Cancer Res 1995, 55:342–347
8. Nupponen NN, Kakkola L, Koivisto P, Visakorpi T: Genetic alterations in hormone-refractory recurrent prostate carcinomas. Am J Pathol 1998, 153:141–148
9. Isola JJ, Kallioniemi OP, Chu LW, Fuqua SA, Hilsenbeck SG, Osborne CK, Waldman FM: Genetic aberrations detected by comparative genomic hybridization predict outcome in node-negative breast cancer. Am J Pathol 1995, 147:905–911
10. Van den Berg C, Guan X-Y, Von Hoff D, Jenkins R, Bittner M, Griffin C, Kallioniemi O, Visakorpi T, McGill J, Herath J, Epstein J, Saroscy M, Metzer P, Trent J: DNA sequence amplification in human prostate cancer identified by chromosome microdissection: potential prognostic implications. Clin Cancer Res 1995, 1:11–18
11. Escot C, Theillet C, Lidereau R, Spyratos F, Champeme MH, Gest J, Callahan R: Genetic alteration of the c-myc protooncogene (MYC) in human primary breast carcinomas. Proc Natl Acad Sci USA 1986, 83:4834–4838
12. Mariani-Costantini R, Escot C, Theillet C, Gentile A, Merlo G, Lidereau R, Callahan R: In situ c-myc expression, and genomic status of the c-myc locus in infiltrating ductal carcinomas of the breast. Cancer Res 1988, 48:199–205
13. van de Vijver M, van de Bersselaar R, Devilee P, Cornelisse C, Peterse J, Nusse R: Amplification of the neu (c-erbB-2) oncogene in human mammmary tumors is relatively frequent and is often accompanied by amplification of the linked c-erbA oncogene. Mol Cell Biol 1987, 7:2019–2023

14. Diatchenko L, Lau YF, Campbell AP, Chenchik A, Moqadam F, Huang B, Lukyanov S, Lukyanov K, Gurskaya N, Sverdlov ED, Siebert PD: Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. Proc Natl Acad Sci USA 1996, 93:6025–6030
15. Borg A, Baldetorp B, Ferno M, Olsson H, Sigurdsson H: c-myc amplification is an independent prognostic factor in postmenopausal breast cancer. Int J Cancer 1992, 9:687–691
16. Hyytinen E, Visakorpi T, Kallioniemi A, Kallioniemi OP, Isola JJ: Improved technique for analysis of formalin-fixed, paraffin-embedded tumors by fluorescence in situ hybridization. Cytometry 1994, 16: 93–99
17. Asano K, Vornlocher HP, Richter-Cook NJ, Merrick WC, Hinnebusch AG, Hershey JW: Structure of cDNAs encoding human eukaryotic initiation factor 3 subunits: possible roles in RNA binding and macromolecular assembly. J Biol Chem 1997, 272:27042–27052
18. Kallioniemi A, Kallioniemi OP, Piper J, Tanner M, Stokke T, Chen L, Smith HS, Pinkel D, Gray JW, Waldman FM: Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. Proc Natl Acad Sci USA 1994, 91:2156–2160
19. Tanner MM, Tirkkonen M, Kallioniemi A, Isola J, Kuukasjarvi T, Collins C, Kowbel D, Guan XY, Trent J, Gray JW, Meltzer P, Kallioniemi OP: Independent amplification and frequent co-amplification of three nonsyntenic regions on the long arm of chromosome 20 in human breast cancer. Cancer Res 1996, 56:3441–3445
20. Bieche I, Tomasetto C, Regnier CH, Moog-Lutz C, Rio MC, Lidereau R: Two distinct amplified regions at 17q11–q21 involved in human primary breast cancer. Cancer Res 1996, 56:3886–3890
21. Karlseder J, Zeillinger R, Schneeberger C, Czerwenka K, Speiser P, Kubista E, Birnbaum D, Gaudray P, Theillet C: Patterns of DNA amplification at band q13 of chromosome 11 in human breast cancer. Genes Chromosomes Cancer 1994, 9:42–48
22. Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM, Meltzer PS: AIB1, a steroid receptor coactivator amplified in breast, and ovarian cancer. Science 1997, 277:965–968
23. Collins C, Rommens JM, Kowbel D, Godfrey T, Tanner M, Hwang SI, Polikoff D, Nonet G, Cochran J, Myambo K, Jay KE, Froula J, Cloutier T, Kuo WL, Yaswen P, Dairkee S, Giovanola J, Hutchinson GB, Isola J, Kallioniemi OP, Palazzolo M, Martin C, Ericsson C, Pinkel D, Gray JW: Positional cloning of ZNF217 and NABC1: genes amplified at 20q13.2 and overexpressed in breast carcinoma. Proc Natl Acad Sci USA 1998, 95:8703–8708
24. Koivisto P, Kononen J, Palmberg C, Tammela T, Hyytinen E, Isola J, Trapman J, Cleutjens K, Noordzij A, Visakorpi T, Kallioniemi OP: Androgen receptor gene amplification: a possible molecular mechanism for androgen deprivation therapy failure in prostate cancer. Cancer Res 1997, 57:314–319
25. Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinanen R, Palmberg C, Palotie A, Tammela T, Isola J, Kallioniemi OP: In vivo amplification of the androgen receptor gene, and progression of human prostate cancer. Nat Genet 1995, 9:401–406
26. Kainu T, Kononen J, Johansson O, Olsson H, Borg A, Isola J: Detection of germline BRCA1 mutations in breast cancer patients by quantitative messenger RNA in situ hybridization. Cancer Res 1996, 56: 2912–2915
27. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, Press MF: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989, 244:707–712
28. Fukuchi-Shimogori T, Ishii I, Kashiwagi K, Mashiba H, Ekimoto H, Igarashi K: Malignant transformation by overproduction of translation initiation factor eIF4G. Cancer Res 1997, 57:5041–5044
29. Koromilas AE, Lazaris-Karatzas A, Sonenberg N: mRNAs containing extensive secondary structure in their 5′ non-coding region traslate efficiently in cells overexpressing initation factor eIF-4E. EMBO J 1992, 11:4153–4158
30. Lazaris-Karatzas A, Sonenberg N: The mRNA 5′ cap-binding protein, eIF-4E, cooperates with v-myc or E1A in the transformation of primary rodent fibroblasts. Mol Cell Biol 1992, 12:1234–1238
31. Lazaris-Karatzas A, Smith MR, Frederickson RM, Jaramillo ML, Liu YL, Kung HF, Sonenberg N: Ras mediates translation initation factor 4E-induced malignant transformation. Genes Dev 1992, 6:1631–1642
32. Rosenwald IB, Rhoads DB, Callanan LD, Isselbacher KJ, Schmidt EV: Increased expression of eukaryotic translation initiation factors eIF-4E and eIF-2 α in response to growth induction by c-myc. Proc Natl Acad Sci USA 1993, 90:6175–6178
33. Tatsuka M, Mitsui H, Wada M, Nagata A, Nojimata H, Okayama H: Elongation factor-1α determines susceptibility to transformation. Nature 1992, 359:333–336
34. Shen R, Su ZZ, Olsson CA, Fisher PB: Identification of the human prostatic carcinoma oncogene PTI-1 by rapid expression cloning and differential RNA display. Proc Natl Acad Sci USA 1995, 92:6778–6782
35. Asano K, Merrick WC, Hershey JW: The translation initiation factor eIF3–p48 subunit is encoded by int-6, a site of frequent integration by the mouse mammary tumor virus genome. J Biol Chem 1997, 272: 23477–23480
36. Miyazaki S, Imatani A, Ballard L, Marchetti A, Buttitta F, Albertsen H, Nevanlinna HA, Gallahan D, Callahan R: The chromosome location of the human homolog of the mouse mammary tumor- associated gene INT6 and its status in human breast carcinomas. Genomics 1997, 46:155–158
37. Brass N, Heckel D, Sahin U, Pfreundschuh M, Sybrecht GW, Meese E: Translation initiation factor eIF-4γ is encoded by an amplified gene and induces an immune response in squamous cell lung carcinoma. Hum Mol Genet 1997, 6:33–39
38. Scott PA, Smith K, Poulsom R, De Benedetti A, Bicknell R, Harris AL: Differential expression of vascular endothelial growth factor mRNA versus protein isoform expression in human breast cancer and relationship to eIF-4E. Br J Cancer 1998, 77:2120–2128
39. Hershey JW, Asano K, Naranda T, Vornlocher HP, Hanachi P, Merrick WC: Conservation and diversity in the structure of translation initiation factor eIF3 from humans and yeast. Biochimie 1996, 78:903–907