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ORIGINAL ARTICLE

Overexpression of PTK6 (breast tumor kinase) protein—a prognostic factor for long-term breast cancer survival—is not due to gene amplification

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Abstract In a previous retrospective study, we demonstrated the prognostic value of protein tyrosine kinase 6 (PTK6) protein expression in breast carcinomas. Here, we analyzed PTK6 gene amplification using fluorescence in situ hybridization technique in a cohort of 426 invasive breast carcinomas and compared it with PTK6 expression level as well as with the clinical outcome of patients. Forty-five percent of tumors show increased PTK6 gene copy numbers when compared to normal tissue. Most of these, however, were related to chromosome 20 polysomy (30%), while gene amplification accounted for only 15%. Only "low level" amplification of the PTK6 gene, with up to eight signals per nucleus, was found. The PTK6 cytogenetic status (normal, gene amplification, polysomy 20) was not associated with histopathological parameters or with the protein expression of HER receptors. No statistical association was identified between PTK6 gene status and expression level. Further, the PTK6 gene status does not influence the disease-free survival of patients at ≥240 months. Based on these results, we state that the PTK6 overexpression is not essentially attributed to gene amplification, and the PTK6 protein expression—but not gene status—is of prognostic value in breast carcinomas. PTK6 protein overexpression may result from polysomy 20 in a minority of the tumors. In a marked proportion of tumors, however, the overexpression is likely to be caused by posttranscriptional regulation mechanisms.

Keywords PTK6 (BRK) · Gene amplification · FISH · Overexpression · Breast cancer · Prognosis

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Introduction

Gene amplification represents a key mechanism leading to gene activation and overexpression [1]. Amplification is widely observed in human tumors where it represents an oncogene-activating genetic mechanism [2] that may indicate an aggressive behavior of the tumor and a poor prognosis [3]. Among the various proto-oncogenes amplified in primary breast cancer, HER2/neu, located on chromosome 17q11–12, is the best studied [4]. Approximately 20–25% of primary breast cancers show amplification of the Her2/neu proto-oncogene with amplification values in many cases up to 20 gene copies [4]. Her2/neu overexpression/amplification is associated with worse prognosis [5, 6]. Anti-HER2/neu receptor antibody therapy with Trastuzumab induces tumor regression in approximately 30–35% of patients with HER2-amplified metastatic breast cancer [7]. This response rate indicates that additional signaling molecules may influence the biological response to Trastuzumab.

Signal transduction of the HER receptor family (EGFR/HER1, HER2/neu, HER3, and HER4) is thought to be transmitted through the action of nonreceptor protein tyrosine kinases (TK) in the cytoplasm. These TK proteins frequently show alterations in their activities in human tumorigenesis [8], and selective inhibitors of TKs are being investigated for the treatment of cancers [9]. One of these TKs is protein tyrosine kinase 6 (PTK6, BRK), a cytoplasmic tyrosine kinase, which is not only overexpressed in two thirds of breast carcinomas [10–15] but is also overexpressed in colon and prostate tumors and several cancer cell lines [11, 12, 16–18]. In vitro studies to define the physiological role and to identify the PTK6 interaction partners confirmed that PTK6 functions downstream of the HER receptors [18, 19]. This makes PTK6 an interesting tumor marker and a potential new therapy target.

Knowledge of PTK6 expression in tumor tissues is limited, although PTK6 overexpression is established in breast carcinogenesis [15, 19, 20]. High levels of PTK6 are also observed in differentiating noncancerous epithelial tissues such as the gastrointestinal tract, skin [12, 21], and prostate [17]. The extent of PTK6 expression may be influenced by the level of differentiation of breast tissue with evidence for an association with estrogen receptor expression [22] and with the expression of HER receptors [13, 15]. More significantly, the expression of PTK6 has been shown to be a prognostic indicator of outcome in invasive breast carcinomas [15].

The mechanisms behind increases in PTK6 protein overexpression as well as its role in tumorigenesis and prognosis are not understood. We are not aware of studies of PTK6 protein expression or gene status in relation to clinical outcome in invasive breast carcinomas. We conducted a study to compare the prognostic impact of PTK6 gene copy number and protein expression. We performed fluorescence in situ hybridization (FISH) for PTK6 and for the chromosome 20 alpha satellite and studied associations between the FISH signal counts and histopathological and immunohistochemical parameters as well as with the clinical course of disease. We demonstrate that increased PTK6 gene copy number is mainly attributed to polysomy of chromosome 20, not to gene amplification, and that there is no statistical association between PTK6 gene status and PTK6 protein levels. Furthermore, unlike PTK6 protein levels, gene status is not a significant predictor of disease-free survival.

Material and methods

Tumor samples and patient data

Formalin-fixed and paraffin-embedded archival material was obtained from 426 patients with invasive breast carcinomas, as recently described [23]. This cohort contains 244 lymph node negative and 182 node positive tumors, and a total of 234 tumors were less than 2 cm in size. The median follow-up of patients was 80 months (mean 94, maximum 264 months) with 121 (28%) of the patients showing disease recurrence by distant metastases and/or local recurrence. In addition to the standard histopathological parameters (lymph node status, tumor size, and histological grade and type), immunohistochemical data were available for HER2, HER3, HER4, PTK6, estrogen receptor (ER), and progesterone receptor (PrR).

Tissue microarrays (TMAs) were produced as described [15] using a tissue-arraying instrument (Beecher Instruments Inc., Silver Spring, MD, USA). Five-micrometer sections were cut and transferred to adhesive slides using the "paraffin-tape-transfer system" (Instrumedics, Hackensack, NJ, USA). Hematoxylin- and eosin-stained sections from the TMAs, as well as from the original paraffin block, were reexamined to validate representative sampling.

FISH

FISH was performed on 5-μm thick TMA sections. Pretreatment of the sections was done by heating the slides for 12 min in a microwave oven (750 W), digestion with Pronase E (0.05%) for 6 min, and denaturation at 75°C for 15 min, as described elsewhere [24].

The DNA hybridization probes were obtained from Chrombios (Molecular Cytogenetics Chrombios, Raubling, Germany). The PTK6 probe was labeled with Cy3 (red fluorescence), and the centromere 20 probe (20q11.21, CEP20) was labeled with FITC (green fluorescence).

Counterstaining of the sections was done using 4',6-diamidino-2-phenylindole (DAPI). The probes were applied sequentially; after the first hybridization using the PTK6 probe, slides were washed as described [24] and the hybridization procedure repeated using the centromeric probe.

Image acquisition and evaluation of FISH signals

The hybridized slides were viewed under a confocal laser scanning microscope (LSM 510, Zeiss, Jena, Germany) equipped with the Apotome Extension (Zeiss Vision) and appropriate filters [24]. Images were captured using a C-Apochromat ×63/1.2 W objective, and 3-dimensional image projections (AxioVision software) were used for PTK6 signal counting. Multiple tumor areas were scored from each case, and FISH signals were evaluated by counting at least 50 non-overlapping tumor nuclei. Signals were also counted in normal tissues involved in the TMAs, with a mean signal count of 1.54 (± 0.15 SD). Consequently, the presence of ≤2 PTK6 signals per nucleus (corresponding to mean + 3 times SD) were considered normal.

The signals for centromere 20 were counted directly under the microscope and registered as normal (≤2 signals per nucleus) or as increased (>2 signals per nucleus).

The ratio PTK6/CEP20 was interpreted as normal (PTK6 and CEP20≤2 signals), as polysomy of chromosome 20 (>2 signals for PTK6 and for CEP20) or as gene amplification (>2 PTK6 signals and ≤2 CEP20 signals).

Quantitative mRNA analysis

From 90 randomly selected cases, total RNA was isolated and purified from paraffin sections as described elsewhere [13], and RNA was reverse-transcribed using Superscript II Reverse Transcriptase (Invitrogen) according to manufacturer's directions. Intron-spanning primers and probes (FAM™ dye-labeled) were obtained from Applied Biosystems (Assay-on-Demand™, PE Applied Biosystems, PTK6: HS00178742). For the TBP reference gene (*T*ATA-*B*ox binding protein), intron-spanning primers and oligonucleotide probes with 5' fluorescent reporter dye (FAM) and a 3' quencher dye (TAMRA) were designed (Primer-Express software, PE Applied Biosystems, Weiterstadt, Germany) [13]. Polymerase chain reaction (PCR) amplifications were performed as described elsewhere [13], following established criteria. Relative expression levels for the target gene were determined by the standard curve method using a reference RNA (MCF7 breast cancer cell line) [13], and the target amount was divided by the endogenous reference amount to obtain a normalized target value. Final results are expressed as *N*-fold differences in expression relative to the TBP gene. In normal tissues, the mean PTK6 mRNA expression was 0.55 (± 0.8). Therefore, values ≥10-fold (corresponding to mean +2-fold logarithmic SD) were considered as increased mRNA expression.

Statistics

The Spearman rank correlation test was used to examine the relationship between two markers and between markers and clinical parameters. Statistical significance was considered at the $p \leq 0.05$ level.

Survival analysis was performed using disease-free survival, which was defined as the interval from the date of surgery to the first loco-regional recurrence and/or distant metastases.

For univariate survival analysis, Kaplan–Meier curves were calculated, and differences between strata were tested with the log-rank Chi-Square value. Multivariate analyses were performed using Cox proportional hazards regression and a combined stepwise selection algorithm (SAS Inst., Cary, NC, USA). All parameters reaching a significance level of $p \leq 0.15$ in univariate analysis were offered to multivariate analysis. In all other tests, statistical significance was considered proven if $p \leq 0.05$.

Results

PTK6 gene amplification and polysomy 20 in invasive breast cancer

PTK6 FISH signals were detectable in 389 of the tumors examined. A normal PTK6 gene copy number (signal frequency ≤2 signals/nucleus) was found in 55% (n=213) of the invasive breast carcinomas (Table 1). An increased PTK6 gene copy number (>2 signals, as defined in normal tissue) was present in 45% (n=171) of the tumors. In most of these cases, overrepresentation of the gene copy number was caused by polysomy of chromosome 20 (30%); gene amplification was seen in only 15% (Table 1). In Fig. 1, examples for polysomy 20 (a) and for PTK6 amplification (b) are shown. The highest PTK6 gene copy number in our tumors was eight signals/nucleus (corresponding to a 4-fold

Table 1 Frequency of PTK6 gene copy number in comparison with the cytogenetic status

PTK6 signal frequency	Percent of cases	Cytogenetic status*	Percent of cases
≤2.0	55% (n=213)	n	55%
>2.0	45% (n=171)	Polysomy	30% (n=115)
		Amplification	15% (n=56)

Cytogenetic status = ratio of PTK6/CEP20 signals

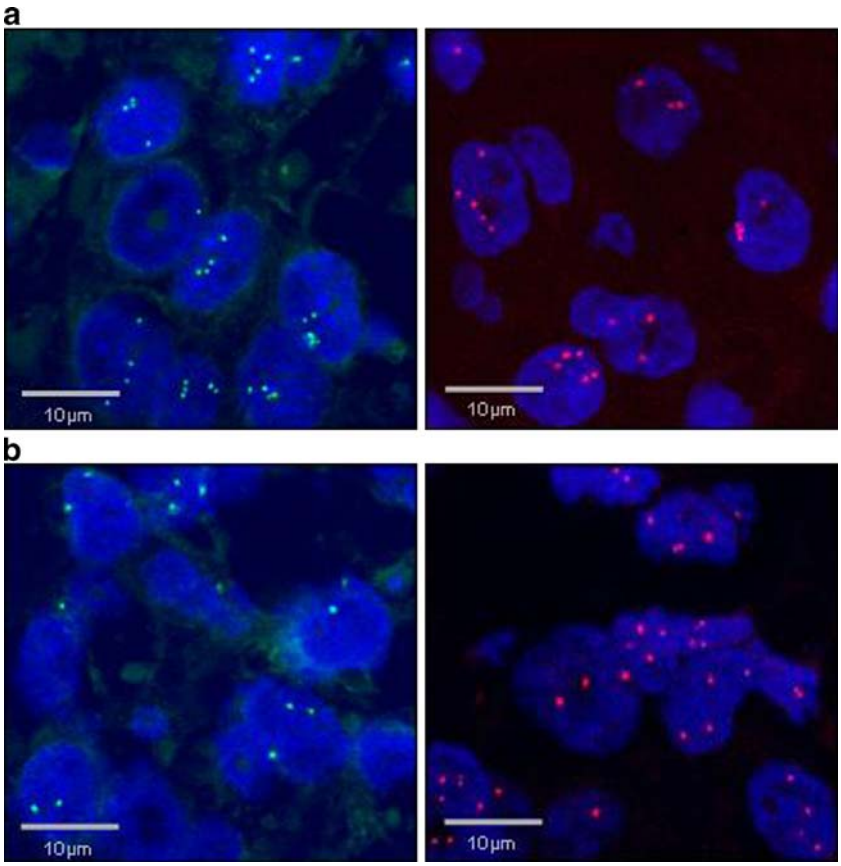

Fig. 1 Examples for an increased PTK6 signal count in breast cancer, caused by polysomy 20 (**a**) or by PTK6 gene amplification (**b**). PTK6 signals = red fluorescence; CEP20 signals = green fluorescence; counterstaining: DAPI

gene dosis); no higher levels of amplification were found. The majority of tumors with an increased signal count showed between two and four signals (n=150); only a minority of cases showed >4 signals (n=18). Therefore, in comparison to other gene amplifications like the HER2 receptor in breast cancer, the PTK6 gene is amplified only on low level.

Correlations between FISH signal counts and other parameters

As shown in Table 2, the PTK6 signal count was significantly associated ($p \leq 0.05$) with ER status, histological grade, and tumor size. The number of tumors with normal and increased signal counts in the three different histological grades is given in Table 3. Most of the cases with normal PTK6 signal counts are classified as grade 1 or 2 (n=152 vs. n=37), whereas the majority of cases with increased signals are grade 2 or 3 (n=142 vs. n=35). The number of centromeric CEP20 signals correlated with tumor size and histological grade. The cytogenetic status (ratio PTK6/CEP20) does not show association with any of the histopathological parameters (Table 2).

The number of PTK6 gene copies was significantly correlated with the immunohistochemical staining intensities of HER3 and HER4 (Table 4), and CEP20 signals revealed significant correlation with HER2, HER3, and HER4 receptor staining. The ratio of PTK6/CEP20 signals does not show any significant correlation with the immunohistochemical parameters (Table 4). Remarkably, there was neither any statistical association between PTK6 IHC and PTK6 FISH gene copy numbers (p=0.5).

To compare gene copy number with mRNA expression and with protein expression, we performed quantitative RT-

Table 2 Associations between FISH signals (PTK6, CEP20, ratio PTK6/CEP20), the PTK6 mRNA, and the histopathological parameters

	Lymph node status	Histological grade	Tumor size	ER	PrR
PTK6 FISH	0.2	**0.0015** 0.17	**0.02** 0.12	**0.003** 0.15	0.15
FISH CEP20	0.6	**0.023** 0.13	**0.036** 0.12	0.06	0.8
ratio PTK6/CEP20	0.2	0.6	0.9	0.4	0.4
PTK6 mRNA	0.3	0.2	0.99	**0.009** 0.27	**0.011** 0.27

Significant correlations are highlighted in bold, and in addition, the Spearman correlation coefficients are given

Table 3 Number of tumors with normal and increased PTK6 signal counts in the three different histological grades

Grade	≤2 signals/nucleus (n=189)	>2 signals/nucleus (n=177)
1	50 (26.4%)	35 (19.8%)
2	102 (54.0%)	82 (46.3%)
3	37 (19.6%)	60 (33.9%)

Spearman rank correlation test p=0.02

PCR for PTK6 in 90 of our tumors. An increased expression (≥10-fold) was found in 71% of cases. PTK6 mRNA expression showed significant association with the expression of ER and PrR ($p \leq 0.01$; Table 2), and at least a trend (p=0.08) was indicated between PTK6 mRNA and PTK6 IHC (Table 4). No significant association was found between mRNA expression and the FISH results (PTK6 signals, p=0.95; CEP20 signals, p=0.6; ratio PTK6/CEP20, p=0.85).

Correlations with the disease-free survival of patients

As recently published by us, the PTK6 protein expression showed significant correlation with the disease-free survival of patients in multivariate analysis, together with the lymph node status and the tumor size [23]. In the study presented here, which was performed on the same tumor cohort, no statistical significant association was present for normal (≤2) or increased (>2) PTK6 signal counts and the clinical course (p=0.36). Further, neither CEP20 signals nor the ratio PTK6/CEP20 shows association with the clinical course of patients in univariate analysis. Parameters, positively correlated with a disease-free survival of patients in univariate analysis, were lymph node status and tumor size ($p \leq 0.0001$) and histological grade (p=0.002), and an inverse association was found for PTK6 IHC ($p \leq 0.0001$), progesterone receptor status (p=0.02), and HER4 staining intensity (p=0.03), as published else [23].

In multivariate analysis for a disease-free survival of patients, all parameters reaching a significance level of ≤0.15 in univariate analysis were offered. We found an independent prognostic value for lymph node status (p=0.0003, relative risk 2.13), protein expression of PTK6 (p=0.0007, 0.62), and tumor size (p=0.003, 1.55) [23]. The FISH values (PTK6, CEP20, PTK6/CEP20) as well as the mRNA expression values do not influence this result due to insufficient significance.

Discussion

PTK6 is overexpressed in about two thirds of breast carcinomas [10–15], but the mechanisms behind this overexpression are still unknown, and the physiological role of PTK6 in overexpressing cells is not defined. In this study, we show that most tumors with increased PTK6 gene copies are attributed to polysomy of chromosome 20, not to gene amplification, and that there is no statistical association between PTK6 gene status and protein or mRNA expression. Hence, besides an increased dose caused by polysomy 20, there may be additional mechanisms contributing to overexpression as, for example, posttranscriptional regulation mechanisms.

Gene amplification is one major mechanism to increase gene dosage and has importance for both prognosis and targeted therapy. For example, amplification of HER2, MYC, and CCND1 have been reported to be significantly associated with high grade in breast cancer, and a decrease of survival was observed with increasing number of co-amplifications [25]. Amplification is also one of the underlying causes of resistance to therapy [4] as, for example, BCR-ABL gene amplification in patients with chronic myeloid leukemia [26] or clinical resistance to 5-fluorouracil in liver metastases [26].

Although enhanced expression is often the consequence of gene amplification, there are alternative pathways to increase protein expression such as activating regulatory mutations or posttranscriptional and posttranslational mechanisms [4] and, especially in the case of PTK6, via activation by other kinases. Protein overexpression without gene amplification was also described for HER2 protein in some urothelial carcinomas [27] and for hypoxia inducible factor-1alpha in human breast cancer [28].

About half of the tumors analyzed in our study showed increased PTK6 signals; however, most of them had 2–4 signals and only a minority had >4 PTK6 signals. This and, in particular, that most tumors with increased PTK6 signals are caused by polysomy let as state that PTK6 amplification in breast cancers is present only on low level and that it cannot account for the protein overexpression found in two thirds of the tumors. There are also indications that

Table 4 Associations between FISH signals (PTK6, CEP20, ratio PTK6/CEP20), the PTK6 mRNA expression, and the immunohistochemically determined expression of HER2, HER3, HER4, PTK6, and the hormone receptors

	HER2	HER3	HER4	PTK6 IHC
PTK6 FISH	0.2	**0.01** −0.15	**0.01** −0.13	0.5
FISH CEP20	**0.04** −0.11	**<.0001** −0.46	**<.0001** −0.29	0.7
ratio PTK6/ Cep20	0.7	0.5	0.6	0.4
PTK6 mRNA	0.8	0.1	0.4	0.08

Significant correlations are highlighted in bold, and in addition, the Spearman correlation coefficients are given

chromosomal polysomy may, to a certain degree, cause protein overexpression, as has been reported for chromosome 17 polysomy and HER2 in breast carcinomas [29, 30] and in esophageal and gastric carcinomas [31]. In the current study, the majority of tumors with increased PTK6 signals exhibited polysomy 20.

We found no association between the signal ratio PTK6/CEP20 and tumor size or histological grade, whereas both PTK6 and CEP20 signals showed a significant correlation with these histopathological parameters. This indicates that increased signal counts are predominantly found in high histological grades and in tumors larger in size. The cytogenetic status, as described by the ratio PTK6/CEP20, is indicative for gene amplification and does not show significant correlation. Consequently, these associations with histopathological parameters are based upon polysomy of chromosome 20, not on gene amplification. Likewise, the PTK6/CEP20 ratio was not associated with the HER2, HER3, or HER4 protein expression, whereas PTK6 signals as well as CEP20 signals showed significant inverse correlations with HER receptor expressions, indicating that polysomy 20 is associated with normal or low HER receptor expression. There is growing evidence in the literature that HER3 overexpression is associated with worse, HER4 with good prognosis [32, 33]. Some other studies, however, have indicated that HER3 overexpression may be a good prognostic factor [34]. In our tumor cohort, both markers showed a similar (inverse) tendency in univariate survival analysis, although HER3 did not reach significance. This explains the inverse correlation of CEP20 and PTK6 signals with HER3 and HER4. It is remarkable that there was no statistical association between PTK6 IHC or mRNA and the PTK6 gene status. This further emphasizes that overrepresentation of PTK6 appears at the messenger RNA or protein level. Consequently, the altered synthesis and degradation rates of the gene products seem to play a crucial role.

We further show that PTK6 protein expression—but not PTK6 gene status or mRNA expression—is significantly associated with the clinical course of patients. So far, no other study described PTK6 FISH analysis on breast carcinomas with correlation with the clinical outcome. Only one study [9] reported that six of seven high-grade ovarian carcinomas showed PTK6 amplification with an up to 3-fold increase of the PTK6 gene. The authors concluded that gene amplification may be responsible for the observed protein overexpression in ovarian tumors. However, such a small cohort may not allow to draw the conclusion that increased gene dosage may be the reason for protein overexpression. Further, in comparison to our results here with a maximum of eight PTK6 gene signals (corresponding to a 4-fold gene dosage), the level of gene dosage in ovarian carcinomas was up to 3-fold [9]. Compared to other oncogene amplifications in breast cancer, e.g., HER2/neu with >20 gene copies, we would classify those PTK6 gene increases from both studies only as "low level" amplifications. After all, such low-level amplifications in only part of our tumors (15%) cannot account for PTK6 protein overexpression in two thirds of breast carcinomas.

PTK6 has been linked to proliferation of carcinoma cells [35] and represents a potential target for the development of novel cancer therapies based on interfering with its functions [35]. Therefore, it is important to understand how it contributes to the phenotypic effect. Here, we showed that gene amplification cannot account for the protein overexpression in two thirds of breast cancers. Further studies, however, are necessary to define protein–protein interactions and to further uncover therapeutic opportunities.

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References

1. Savelyeva L, Schwab M (2001) Amplification of oncogenes revisited: from expression profiling to clinical application. Cancer Lett 167:115–123
2. Imoto I, Yang ZQ, Pimkhaokham A et al (2001) Identification of cIAP1 as a candidate target gene within an amplicon at 11q22 in esophageal squamous cell carcinomas. Cancer Res 61:6629–6634
3. Ohta JI, Miyoshi Y, Uemura H et al (2001) Fluorescence in situ hybridization evaluation of c-erbB-2 gene amplification and chromosomal anomalies in bladder cancer. Clin Cancer Res 7:2463–2467
4. Schwab M (1998) Amplification of oncogenes in human cancer cells. Bioessays 20:473–479
5. Slamon DJ (1990) Studies of the HER-2/neu proto-oncogene in human breast cancer. Cancer Invest 8:253
6. Jukkola A, Bloigu R, Soini Y et al (2001) c-erbB-2 positivity is a factor for poor prognosis in breast cancer and poor response to hormonal or chemotherapy treatment in advanced disease. Eur J Cancer 37:347–354
7. Hsieh AC, Moasser MM (2007) Targeting HER proteins in cancer therapy and the role of the non-target HER3. Br J Cancer 97:453–457
8. Hahn WC, Weinberg RA (2002) Rules for making human tumor cells. N Engl J Med 347:1593–1603
9. Schmandt RE, Bennett M, Clifford S et al (2006) The BRK tyrosine kinase is expressed in high-grade serous carcinoma of the ovary. Cancer Biol Ther 5:1136–1141
10. Mitchell PJ, Barker KT, Martindale JE et al (1994) Cloning and characterisation of cDNAs encoding a novel non-receptor tyrosine kinase, brk, expressed in human breast tumours. Oncogene 9:2383–2390

11. Barker KT, Jackson LE, Crompton MR (1997) BRK tyrosine kinase expression in a high proportion of human breast carcinomas. Oncogene 15:799–805
12. Llor X, Serfas MS, Bie W et al (1999) BRK/Sik expression in the gastrointestinal tract and in colon tumors. Clin Cancer Res 5:1767–1777
13. Born M, Quintanilla-Fend L, Braselmann H et al (2005) Simultaneous over-expression of the Her2/neu and PTK6 tyrosine kinases in archival invasive ductal breast carcinomas. J Pathol 205:592–596
14. Ostrander JH, Daniel AR, Lofgren K et al (2007) Breast tumor kinase (protein tyrosine kinase 6) regulates heregulin-induced activation of ERK5 and p38 MAP kinases in breast cancer cells. Cancer Res 67:4199–4209
15. Aubele M, Auer G, Walch AK et al (2007) PTK (protein tyrosine kinase)-6 and HER2 and 4, but not HER1 and 3 predict long-term survival in breast carcinomas. Br J Cancer 96:801–807
16. Meric F, Lee WP, Sahin A et al (2002) Expression profile of tyrosine kinases in breast cancer. Clin Cancer Res 8:361–367
17. Derry JJ, Prins GS, Ray V et al (2003) Altered localization and activity of the intracellular tyrosine kinase BRK/Sik in prostate tumor cells. Oncogene 22:4212–4220
18. Kamalati T, Jolin HE, Fry MJ et al (2000) Expression of the BRK tyrosine kinase in mammary epithelial cells enhances the coupling of EGF signalling to PI 3-kinase and Akt, via erbB3 phosphorylation. Oncogene 19:5471–5476
19. Zhang P, Ostrander JH, Faivre EJ et al (2005) Regulated association of protein kinase B/Akt with breast tumor kinase. J Biol Chem 280:1982–1991
20. Petro BJ, Tan RC, Tyner AL et al (2004) Differential expression of the non-receptor tyrosine kinase BRK in oral squamous cell carcinoma and normal oral epithelium. Oral Oncol 40:1040–1047
21. Haegebarth A, Heap D, Bie W et al (2004) The nuclear tyrosine kinase BRK/Sik phosphorylates and inhibits the RNA-binding activities of the Sam68-like mammalian proteins SLM-1 and SLM-2. J Biol Chem 279:54398–54404
22. Zhao C, Yasui K, Lee CJ et al (2003) Elevated expression levels of NCOA3, TOP1, and TFAP2C in breast tumors as predictors of poor prognosis. Cancer 98:18–23
23. Aubele M, Walch AK, Ludyga N et al (2008) Prognostic value of protein tyrosine kinase 6 (PTK6) for long-term survival of breast cancer patients. Br J Cancer 99:1089–1095
24. Walch A, Bink K, Hutzler P et al (2001) Sequential multilocus fluorescence in situ hybridization can detect complex patterns of increased gene dosage at the single cell level in tissue sections. Lab Invest 81:1457–1459
25. Al-Kuraya K, Schraml P, Torhorst J et al (2004) Prognostic relevance of gene amplifications and coamplifications in breast cancer. Cancer Res 64:8534–8540
26. Gorre ME, Mohammed M, Ellwood K et al (2001) Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 293:876–880
27. Caner V, Turk NS, Duzcan F et al (2008) No strong association between HER-2/neu protein overexpression and gene amplification in high-grade invasive urothelial carcinomas. Pathol Oncol Res 14(3):261–266
28. Vleugel MM, Bos R, Buerger H et al (2004) No amplifications of hypoxia-inducible factor-1alpha gene in invasive breast cancer: a tissue microarray study. Cell Oncol 26:347–351
29. Hyun CL, Lee HE, Kim KS et al (2008) The effect of chromosome 17 polysomy on HER-2/neu status in breast cancer. J Clin Pathol 61:317–321
30. Lal P, Salazar PA, Ladanyi M et al (2003) Impact of polysomy 17 on HER-2/neu immunohistochemistry in breast carcinomas without HER-2/neu gene amplification. J Mol Diagn 5:155–159
31. Bizari L, Borim AA, Leite KR et al (2006) Alterations of the CCND1 and HER-2/neu (ERBB2) proteins in esophageal and gastric cancers. Cancer Genet Cytogenet 165:41–50
32. Tovey S, Dunne B, Witton CJ et al (2005) Can molecular markers predict when to implement treatment with aromatase inhibitors in invasive breast cancer? Clin Cancer Res 11:4835–4842
33. Koutras AK, Fountzilas G, Kalogeras KT et al (2009) The upgraded role of HER3 and HER4 receptors in breast cancer. Crit Rev Oncol Hematol, in press
34. Pawlowski V, Revillion F, Hebbar M et al (2000) Prognostic value of the type I growth factor receptors in a large series of human primary breast cancers quantified with a real-time reverse transcription-polymerase chain reaction assay. Clin Cancer Res 6:4217–4225
35. Harvey AJ, Crompton MR (2003) Use of RNA interference to validate Brk as a novel therapeutic target in breast cancer: Brk promotes breast carcinoma cell proliferation. Oncogene 22:5006–5010

Statement

Ethical approval for this study was obtained from the Ethics Committee of the Medical Faculty of the Technische Universität München, Germany.