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PRECLINICAL STUDY

Divergent effects of insulin-like growth factor-1 receptor expression on prognosis of estrogen receptor positive versus triple negative invasive ductal breast carcinoma

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Abstract The insulin-like growth factor type 1 receptor (IGF1R) is involved in progression of breast cancer and resistance to systemic treatment. Targeting IGF1R signaling may, therefore, be beneficial in systemic treatment. We report the effect of IGF1R expression on prognosis in invasive ductal breast carcinoma (IDC), the most common type of breast cancer. Immunohistochemistry was performed on tumor tissue of a consecutive cohort of 429 female patients treated for operable primary IDC. Associations between IGF1R expression with clinicopathological parameters, disease free survival (DFS) and breast cancer specific survival (BCSS) were evaluated by multivariate analyses focusing on ER-positive and triple negative IDC (TN-IDC). To enlarge the TN-IDCs cohort, we analyzed a combined dataset of 51 TN-IDC tumors from our series with 64 TN-IDCs with similar clinicopathological parameters. Patients with tumors expressing cytoplasmic IGF1R have a longer DFS and BCSS (DFS: HR 0.46, 95% CI 0.27–0.49, $P = 0.005$, BCSS: HR 0.38, 95% CI 0.19–0.74, $P = 0.005$). This effect was most prominent in ER-positive tumors. However, in a combined series of 105 TN-IDCs cytoplasmic IGF1R expression was associated with a shorter DFS (HR = 2.29, 95% CI 1.08–4.84, $P = 0.03$), also when combined in a multivariate model, including well-known prognostic factors (HR 2.06; 95% CI 0.95–4.47; $P = 0.07$). IGF1R expression in ER-positive IDC is strongly related to a favorable DFS and BCSS, but to a shorter DFS in TN-IDC tumors. This divergent effect of IGF1R expression in subgroups of IDC may affect selection of patients for IGF1R targeted therapy.

Keywords Insulin-like growth factor type 1 receptor · Breast cancer · Ductal type · Prognosis · Triple negative breast tumors · Estrogen receptor

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Introduction

Signaling via the insulin-like growth factor type 1 receptor (IGF1R) plays a crucial role in proliferation, cell survival, invasion, and metastatic behavior of many cancers, including breast cancer [1, 2]. In addition, IGF1R is implicated in resistance to chemotherapy, hormonal therapy, Human Epidermal Growth Factor Receptor 2 (HER2) and Epidermal Growth Factor Receptor (EGFR) directed therapies [3–5].

IGF1R is a transmembrane tyrosine kinase receptor activated by binding of IGF-1 or -2 [6]. Subsequently, signaling occurs via Src homology 2 domain-containing protein, insulin receptor substrates, phosphatidylinositol-3-kinase/Akt, and mitogen-activated protein kinase pathways [7–9]. High IGF1R expression levels have been reported in a wide range of human malignancies, but correlations with tumor characteristics and outcome are infrequently found [10]. Based on in vitro and in vivo data with IGF1R antibodies and tyrosine kinase inhibitors, responses are expected in a variety of carcinomas [3, 11–14]. IGF1R targeted therapy in breast cancer is particularly interesting, because synergy with anti-hormonal therapy and targeting of related receptor tyrosine kinases HER2 and EGFR has been shown [15–17]. There is evidence coming from cell line studies suggesting IGF1R-signaling has different effects on cellular characteristics like proliferation and migration in ER-negative tumors as opposed to ER-positive tumors [18–20]. However, it is still unclear which factors contribute to sensitivity to anti-IGF1R directed therapy as no biomarker is available yet to select patients and tumor types.

Breast cancer is a heterogeneous disease with different sensitivity to drug treatment and outcome between different histological and molecular subtypes [21, 22]. Previous studies showed high IGF1R expression in human breast carcinomas,[23–27] and several studies showed correlations of IGF1R expression with estrogen receptor expression and well-differentiated carcinomas [23, 27, 28]. A special subgroup of breast carcinomas, called triple negatives (TNs), defined by absence of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression, has a worse prognosis and lacks a scaffold for conventional targeted treatments [29]. Identification of new targets in TNs are warranted to extend the armamentarium for this relatively poor prognostic subgroup of breast cancer. The IGF1R could be a target and thereby providing new treatment options.

In the current study, IGF1R expression was investigated in a consecutive cohort of patients with invasive ductal carcinomas (IDCs) of the breast. In this way, the value of IGF1R expression in the most prevalent histological subtype can be related to patient and tumor characteristics, molecular markers, and prognosis. For above-mentioned reasons special attention will be put on TN-IDCs. For the latter, we combined two datasets of TN-IDCs with similar clinicopathological parameters.

Patients, materials, and methods

Patients

A consecutive cohort of 429 female patients treated for a primary local or locally advanced invasive ductal breast carcinoma, as defined by the WHO classification [30] at the University Medical Center Groningen between January 1996 and December 2005 was included in this study. In this same period 26 patients presented with a synchronous ($n = 4$) or metachronous ($n = 18$) contralateral breast tumor or a recidive tumor ($n = 4$), these patients were not included in our cohort.

All patients received local treatment, i.e., breast conserving therapy including radiotherapy or ablative surgery, both with lymph node staging (sentinel node procedure and/or axillary dissection). Local therapy was followed by chemotherapy in 118 patients (96/118 anthracycline-containing chemotherapy (AC/FEC/CAF); 20/118 cyclophosphamide, methotrexate, and fluorouracil (CMF); 2/118 taxane based). In 55 patients chemotherapy was combined with endocrine treatment, 97 other patients received only endocrine systemic treatment (total $n = 152$; 121/146 tamoxifen; 24/146 aromatase inhibitor; in 7 patients who remained premenopausal after received chemotherapy, a LHRH analogue was added). Only 4 patients received herceptin. Patient and tumor characteristics and data on follow-up were obtained from hospital records (Table 1). The medical ethical committee of the University Medical Center Groningen approved this study.

To study a subgroup of patients in more detail, 51 triple negative breast carcinomas (TN-IDCs) from the cohort described above (Groningen dataset) were combined with a previously described cohort of 71 TN tumors (Amsterdam dataset) [31], from which 64 were invasive ductal carcinomas and evaluable for IGF1R expression in our study. The two populations were similar with regard to patient and tumor characteristics, local and systemic therapy and survival (Supplementary Table 1).

Characterization of breast carcinomas by immunohistochemistry

A tissue microarray was constructed as previously described [32, 33]. TMA sections were stained with antibodies against IGF1R, insulin receptor (IR), phosphorylated-Akt (p-Akt), ER, PR, and HER2. Specific details of the

Table 1 Patient and tumor characteristics

	$N = 429$	%
Age at diagnosis (years)		
Median, range	59 (27–91)	
Menopausal status at diagnosis		
Post	290	68
Pre	139	32
Tumor size		
0–2 cm	228	54.4
>2–5 cm	161	38.3
>5 cm	31	7.4
Missing	9	
Differentiation grade		
Well	113	27
Moderate	182	42
Poor	131	31
Missing	3	
IDC	244	57
IDC and DCIS	185	43
Axillary nodal status		
Positive	194	46
Missing	11	
Surgical therapy		
BCT	224	52
Mastectomy	205	48
Radiotherapy	276	64
Adjuvant systemic treatment	215	50
Chemotherapy only	63	15
Hormonal therapy only	97	23
Chemotherapy combined with hormonal therapy	55	13
Adjuvant chemotherapy	118	28
Anthracycline-containing	96	
CMF	20	
Taxane based	2	
Adjuvant hormonal therapy	152	35
Tamoxifen	121	
Aromatase inhibitor	24	
LHRH analogue	7	
Follow-up (months)		
Median, range	55 (0–134)	
Locoregional recurrence	18	4
Distant metastasis	55	12
Breast cancer related death	36	8

IDC invasive ductal carcinoma no special type, *DCIS* ductal carcinoma in situ, *BCT* breast conserving therapy

antibodies and their antigen retrieval methods used are summarized in Supplementary Table 2. The immunohistochemical methods in general were previously described by van der Vegt et al. [34] for ER, PR, and HER2 staining.

Scoring of the immunohistochemical staining was performed by a consultant breast pathologist (JW). ER and PR were assessed based on the percentage of tumor cells showing positive nuclear staining and were considered positive if nuclear staining was present in ≥10% of the cells, according to Dutch guidelines (www.oncoline.nl). HER2 expression was scored as follows: 0 for no staining at all or membrane staining in <10% of the tumor cells; 1+ for a faint/barely perceptible partial membrane staining in >10% of the tumor cells; 2+ for weak to moderate complete membrane staining in >10% of the tumor cells; 3+ for strong complete membrane staining in >10%. HER2 was considered positive if the score was 3+. IR and p-Akt were considered positive if either membranous or cytoplasmic staining was present in ≥10% of the cells.

IGF1R expression, analogue to HER2 expression, was considered positive with strong complete staining pattern in >10% of tumor cells as depicted in Fig. 1. To confirm the robustness of the staining pattern for IGF1R using the 24–31 antibody, a tissue microarray of 50 consecutive invasive breast carcinomas was stained using the same clone provided by another supplier (clone 24–31, Neomarkers, Fremont CA, USA; dilution 1/800). Each individual core of both stained tissue microarrays can be seen at http://telepathology.nki.nl (Login: igf1r, Password: nkiavl). For all stainings, the highest score out of three cores from the same tumor was used for the analysis. If one or two cores out of three failed, the highest value of the remaining core(s) was included in the analysis. Only the invasive tumor component was considered when judging the staining. When no (invasive) tumor cells were available in the sample, the result was considered 'missing' in the statistical analysis.

Statistical analysis

Since the clinical relevance of IGF1R localization is yet unknown, separate analyses were conducted for 1. positive cytoplasmic IGF1R staining vs. no cytoplasmic staining, 2. positive membranous vs. no membranous staining and 3. any positive staining (cytoplasmic, membranous, or both) vs. negative staining pattern (further addressed as "overall IGF1R expression").

Univariate and multiple logistic regression analyses were performed to evaluate the associations of IGF1R expression with clinical (age, menopausal status, lymph node status, surgical, and adjuvant therapy), tumor (size and differentiation grade), and immunohistochemical (ER, PR, IR, HER2, and p-Akt) characteristics. Multivariate Cox regression analysis was used to explore the relationship between IGF1R and the following survival endpoints; (1) disease free survival (DFS), defined as time to any

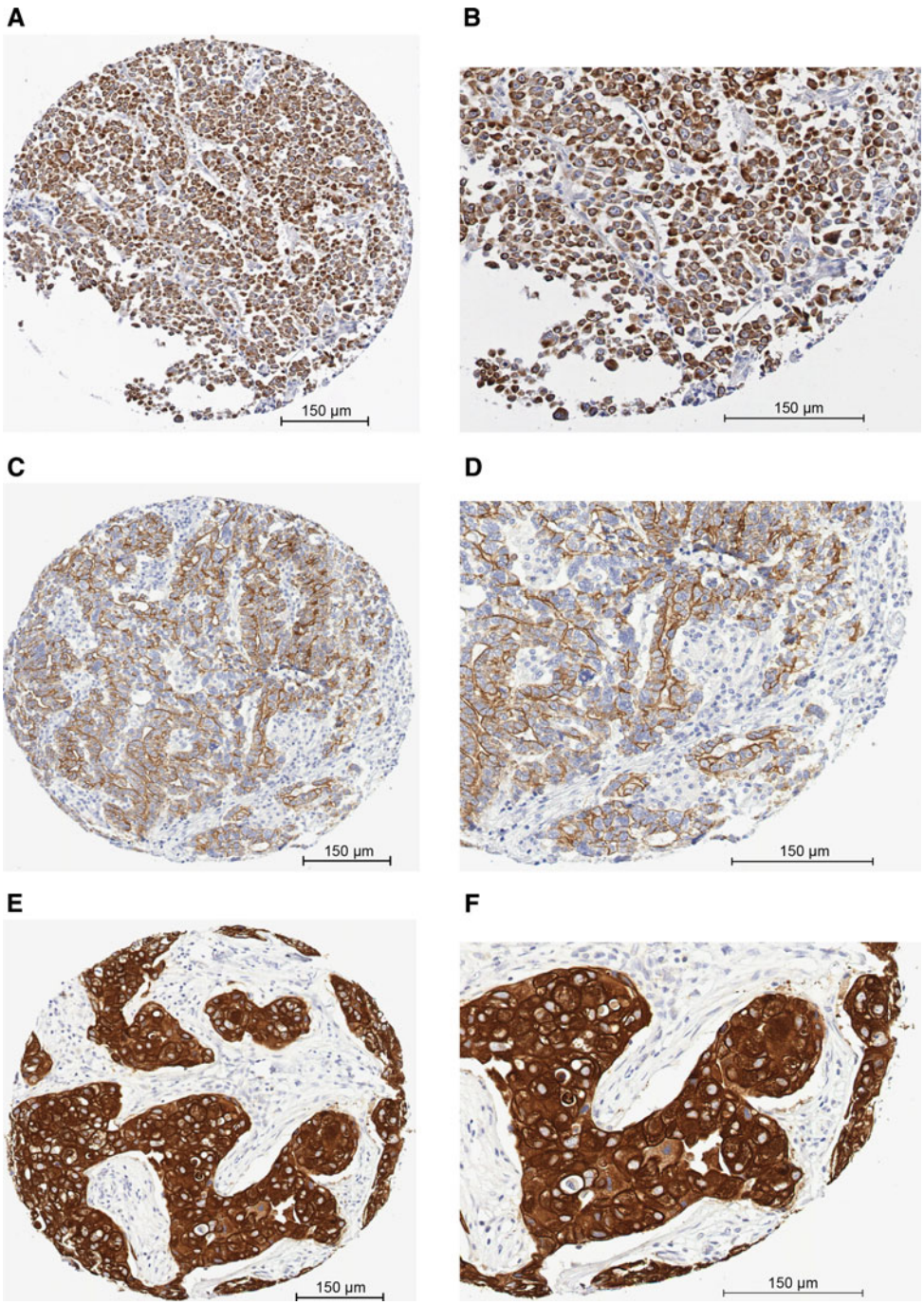

Fig. 1 **a**–**f** Location patterns of IGF-1R immunohistochemical staining: **a**, **b** cytoplasmic (magnification ×10, ×20); **c**, **d** membranous (×10, ×20), and **e**, **f** membranous and cytoplasmic (×10, ×20)

recurrence (local or regional or distant), and (2) breast cancer specific survival (BCSS). Clinicopathological variables that had a significant univariate association (Tables 2, 3) were entered simultaneously in a multivariate Cox regression model. Log-rank tests were performed to evaluate the difference in group-specific survival which is illustrated by Kaplan–Meier plots.

All statistical analyses were performed using the statistical package SPSS 16.0 (SPSS inc., Chicago, IL, USA).

Findings

Immunohistochemistry

Immunohistochemistry for IGF1R could be evaluated in 368 cases (86%). In case IGF1R was present, the intensity of the staining was strong and uniformly present in all tumor cells in 295 cases (80%). Pure membranous IGF1R expression was present in 8%, pure cytoplasmic in 21%,

Table 2 Univariate Cox regression analysis of clinicopathological variables and disease free survival (DFS)

	HR	95% CI	*P* value
Age	0.98	0.96–1.0	0.07
Menopausal status			
Postmenopausal	1		0.06
Premenopausal	1.61	0.98–2.81	
Tumor size			
0–2 cm	1		<0.001
>2–5 cm	2.07	1.15–3.75	0.02
>5 cm	5.62	2.59–12.19	<0.001
Differentiation grade			
Well	1		<0.001
Moderate	2.49	0.93–6.64	0.07
Poor	5.87	2.28 – 15.09	<0.001
Axillary nodal status			
Negative	1		0.06
Positive	1.75	0.98–2.99	
Surgical therapy			
BCT	1		0.02
Mastectomy	1.89	1.2–3.26	
Radiotherapy			
No	1		0.4
Yes	1.33	0.74–2.37	
Adjuvant chemotherapy			
No	1		0.03
Yes	1.84	1.08–3.14	
Adjuvant hormonal therapy			
No	1		1.0
Yes	1.23	0.72–2.10	
ER			
Negative	1		0.002
Positive	0.43	0.25–0.74	
PR			
Negative	1		<0.001
Positive	0.36	0.21–0.63	
HER2			
Negative	1		0.03
Positive	2.46	1.13–5.45	
Cytoplasmic IGF1R			
Negative	1		0.005
Positive	0.46	0.27–0.79	
Membranous IGF1R			
Negative	1		0.2
Positive	1.42	0.80–2.49	
Overall IGF1R expression			
Negative	1		0.4
Positive	0.76	0.41–1.43	
IR			
Negative	1		0.2
Positive	0.65	0.33–1.30	
p-AKT			
Negative	1		0.6
Positive	0.78	0.31–1.96	

BCT breast conserving therapy

and both membranous and cytoplasmic staining in 51%. An overview of other assessed immunohistochemical markers is summarized in Supplementary Table 3.

Association of IGF1R staining pattern with clinicopathological parameters

The univariate association of IGF1R cytoplasmic and membranous expression with patient and tumor characteristics is summarized in Supplementary Table 4. In a multiple logistic regression model, including tumor size, differentiation grade of the tumor, ER and PR expression, ER was strongly associated with IGF1R cytoplasmic expression (OR = 4.18; 95% CI 2.07–8.44, $P < 0.001$) and IGF1R overall staining (OR = 3.22; 95% CI 1.54–6.74, $P = 0.002$). No relation with p-Akt or IR expression was found.

Prognostic relevance of IGF1R staining pattern

Cytoplasmic, but not membranous or overall IGF1R expression was strongly associated with prolonged DFS (HR 0.46, 95% CI 0.27–0.49, $P = 0.005$) and increased BCSS (HR 0.38, 95% CI 0.19–0.74, $P = 0.005$) (Figure 2).

Tumor size, differentiation grade, and PR were multivariately significant factors for DFS (Table 4). Interestingly, while in ER-positive tumors cytoplasmic IGF1R expression is a favorable prognostic factor, in ER-negative tumors this effect is lost and membranous staining is related to a worse prognosis (DFS) (log-rank $P = 0.02$) (Fig. 3).

Subsequently, significant univariate parameters for BCSS (Table 3) were analyzed by multivariate Cox regression in which differentiation grade and HER2 were prognostic factors (Supplementary Table 5).

Effect of IGF1R expression in triple negative breast carcinomas

This interesting result, i.e., a possible opposite effect of IGF1R expression on prognosis of ER-positive versus

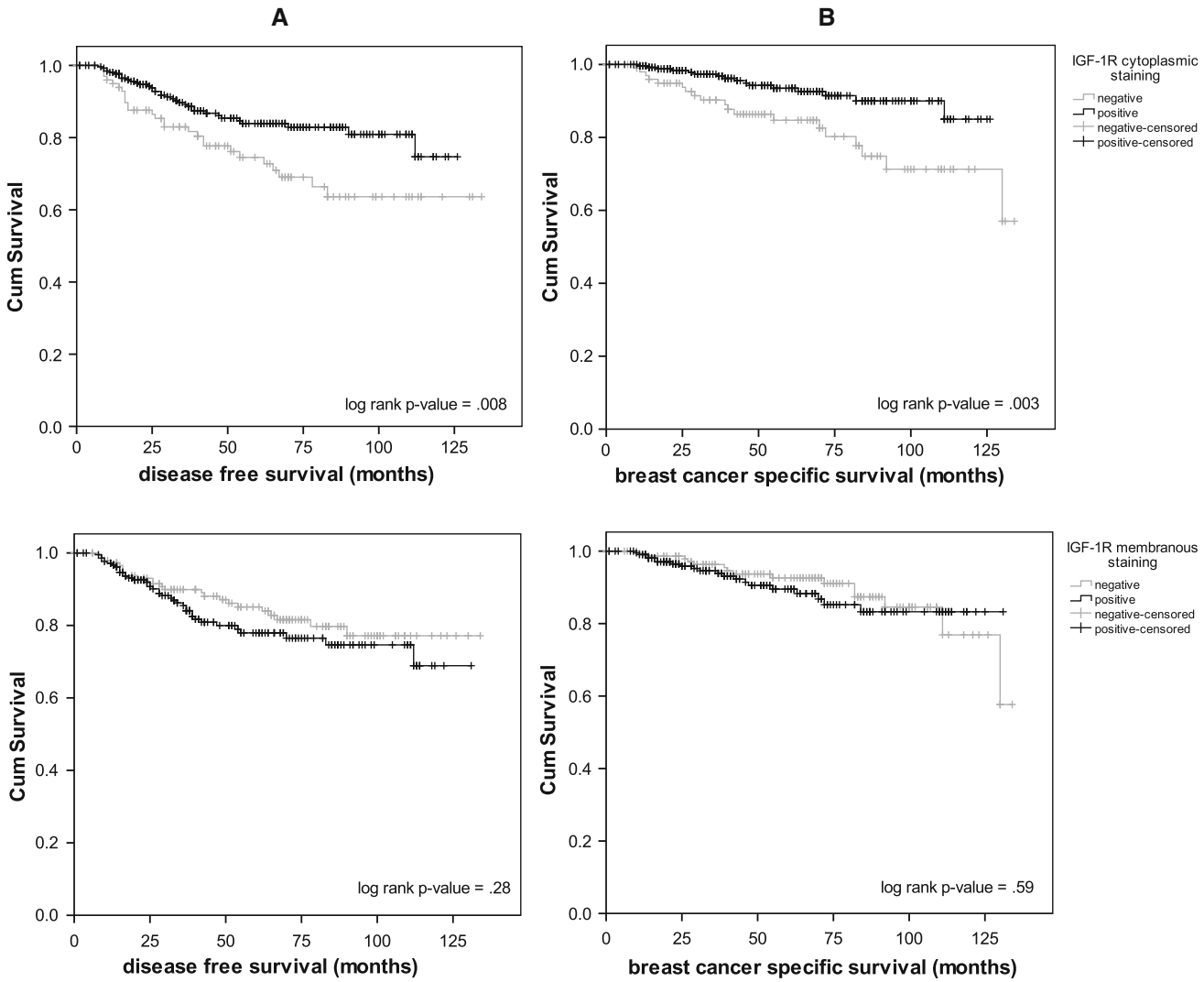

Fig. 2 Kaplan–Meier plots of associations of IGF1R expression with disease free survival (**a**) and breast cancer specific survival (**b**) in months

ER-negative tumors, led us to investigate the effect of IGF1R expression in a cohort of TN-IDCs. Because there were only 51 such cases in our cohort (University Medical Center Groningen; referred to as Groningen dataset), we combined these with a previously described cohort of 64 evaluable cases of TN-IDCs from another center (Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital; referred to as Amsterdam dataset) [31] with similar patient and tumor characteristics and survival (Supplementary Table 1). For the Amsterdam cohort, the majority of patients was treated by breast conserving therapy (BCT) including post-operative radiotherapy, whereas for the Groningen dataset approximately halve of the patients were treated by BCT. Immunohistochemistry for IGF1R could be evaluated in 105 triple negative cases (91%). IGF1R was expressed in 52 cases (45%). Pure cytoplasmic IGF1R expression was present in 11 cases (10%) and pure membranous IGF1R expression in 11 cases (10%). In total, 30 cases (26%) had combined membranous and cytoplasmic staining. We found no association between membranous or cytoplasmic IGF1R expression and clinicopathological parameters.

In the combined patient cohort of 105 TN-IDCs, cytoplasmic IGF1R expression has an unfavorable prognostic impact on DFS (cytoplasmic: HR = 2.29, 95% CI 1.08–4.84, $P = 0.03$; membranous: HR 1.98; 95% CI 0.94–4.17, $P = 0.07$). Comparable direction and magnitude of effects were seen in both triple negative databases separately (cytoplasmic HR 2.99 and 1.83 resp.; membranous HR 1.58 and 1.83 resp.), although not consistently statistically significant, most likely due to lower numbers in the separate databases, and thus reduced power. In a multivariate analysis, including all well-known prognostic clinicopathological parameters, cytoplasmic IGF1R expression was a near-significant prognostic factor for DFS (HR 2.06; 95% CI 0.95–4.47; $P = 0.07$) (Table 5).These same associations were also found when only time to distant metastasis

Table 3 Multivariate Cox Regression on disease free survival (number of events = 61; number of patients = 341)

	HR	95% CI	*P* value
Tumor size			
0–2 cm	1		0.05
>2–5 cm	1.29	0.71–2.35	0.41
>5 cm	2.92	1.21–7.05	0.02
Differentiation grade			
Well	1		0.02
Moderate	2.45	0.92–6.57	0.08
Poor	3.98	1.44–10.97	0.008
Surgical therapy			
Mastectomy	1		0.74
BCT	0.91	0.51–1.61	
Adjuvant chemotherapy			
No	1		0.56
Yes	1.17	0.69–2.00	
ER			
Negative	1		0.44
Positive	1.34	0.64–2.80	
PR			
Negative	1		0.02
Positive	0.47	0.26–0.87	
HER2			
Negative	1		0.32
Positive	1.54	0.67–3.60	
Cytoplasmic IGF1R			
Negative	1		0.09
Positive	0.61	0.34–1.09	

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was tested (cytoplasmic: HR 2.69, 95% CI 1.16–6.22, $P = 0.02$; membranous: HR 2.74, 95% CI 1.18–6.32, $P = 0.02$). Both cytoplasmic (HR 2.43, 95% CI 1.02–5.8, $P = 0.04$) and membranous staining (HR 2.64, 95% CI 1.11–6.31, $P = 0.03$) were multivariately related to time to distant metastases. No association between IGF1R expression and BCSS could be demonstrated in triple negative cases.

Discussion

In this study, we found that cytoplasmic expression of IGF1R in ER-positive invasive ductal breast carcinomas is associated to a more favorable prognosis, while in triple negative breast carcinomas IGF1R expression is associated to a poor outcome. This study underlines that the clinical impact of transmembrane tyrosine kinase receptor expression should be interpreted in the context of the carcinoma subtype, such as co-expression with hormone receptors. We found IGF1R expression in 80% in our series of invasive ductal breast carcinomas (IDC). The majority of these tumors (62%) had combined cytoplasmic and membranous staining. Since the clinical relevance of IGF1R localization is yet unknown and our goal of the present study was evaluation of a candidate prognostic biomarker that would be clinical applicable, separate analyses were conducted for cytoplasmic, membranous, and overall staining. Cytoplasmic IGF1R staining highly correlated with hormone receptor expression, well differentiated breast carcinomas, and to a more favorable prognosis. This prognostic effect occurred specifically in ER-positive breast carcinomas. Since cytoplasmic staining patterns of tumor samples incorporated on the same TMA-slide show differential staining intensity and subsequent significant correlation with biologic plausible factors, we considered this not an artifact but a relevant observation which should not a priori be ignored. Moreover, the robustness of IGF1R staining intensity and staining pattern was confirmed using an antibody of the same clone provided by another supplier on a tissue microarray of 50 randomly chosen invasive breast carcinomas.

Although the biologic significance of cytoplasmic localization of the IGF1R has received little attention in the literature up till now, cytoplasmic localization has been observed before. Confocal microscopy images studying subcellular localization of IGF1R show cytoplasmic staining [35, 36]. Some have reported cytoplasmic IGF1R staining by IHC [37, 38], while others have chosen to ignore cytoplasmic staining, although endoplasmatic staining had been present [39]. Biologically cytoplasmic IGF1R has been linked to mutant receptors which show abolished cell surface expression and endoplasmatic retention of the receptor due to failure to traffic to the cell membrane [40]. Also, accumulation of IGF1R into the cytoplasm has been observed after internalization and intracellular trafficking induced by binding of ligand or antibodies, resulting in receptor degradation [36]. Most of the biologic effects of the IGF1R have been ascribed to its tyrosine kinase activity, which propagates signaling through the phosphatidylinositol 3-kinase and mitogen-activated protein kinase pathways. Recently, however, IGF1R translocation to the nucleus has been described and shown to activate transcription but not alter its kinase-dependent signaling [41, 42]. This is analogue to the earlier observation that ErbB family proteins are expressed in the nucleus and can function as transcriptional regulators [43]. Although cytoplasmic or nuclear HER2 staining by IHC in clinical tumor samples is not considered to be relevant for prognosis or selection of HER2 targeted therapy, the biological relevance of nucleocytoplasmic trafficking has been accepted.

In our samples, nuclear IGF1R expression did not become evident. Of particular interest is the relation of IGF1R localization with ER expression. Interaction of

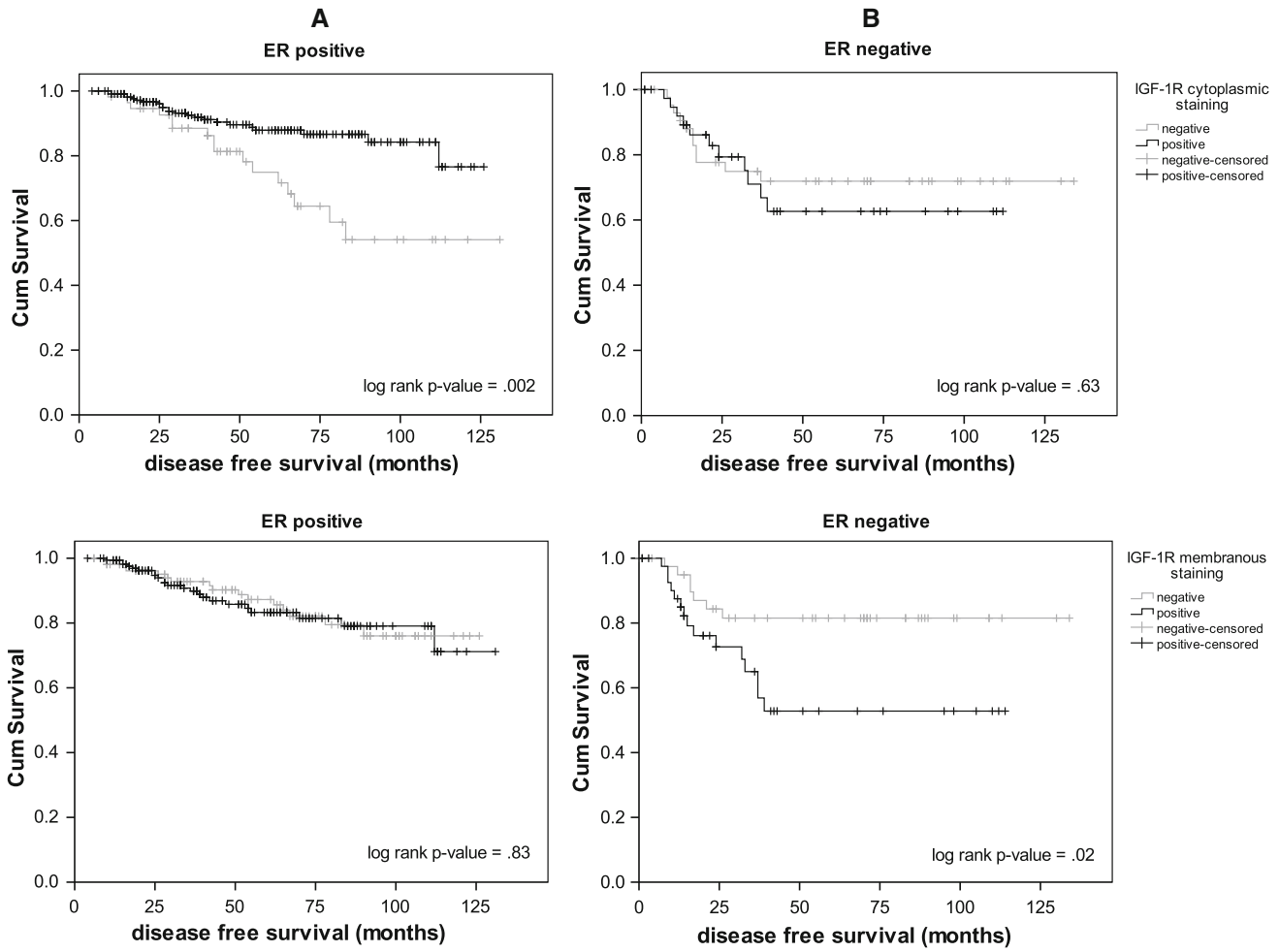

Fig. 3 Kaplan–Meier plots of group-specific associations of IGF1R expression with disease free survival in ER-positive (**a**) and ER-negative (**b**) breast carcinomas

IGF1R and ER has been shown in numerous studies, but this interaction also involves physical co-localization. Reported co-localization of the ER, which is generally localized in the nucleus, with IGF1R at the cell membrane underscores the diversity of receptor localization [35].

Taken together, these data suggest that protein localization of tyrosine kinase receptors is a highly dynamic biological process. The specific details of functional implications of IGF1R cytoplasmic localization need to be appreciated in future studies.

In previous studies, similar associations of ER and IGF1R expression were reported [27, 28, 44], e.g., Ueda et al. [27] described a favorable prognosis for IGF1R expression in ER-positive carcinomas. In triple negative breast carcinomas (TN-IDCs) we found IGF1R expression in 65% of the cases. In these tumors cytoplasmic staining showed some relation to shorter DFS, although no effect was found on overall survival rates. In line with our results, Railo et al. [45] reported similar worse survival rates in ER-negative IGF1R expressing breast carcinomas.

Our observation of a different association between IGF1R expression and prognosis in ER-positive versus ER-negative carcinomas is supported by in vitro studies. In breast cancer cell lines IGF1R and ER synergistically stimulate proliferation [20], but in the absence of ER, IGF1R activation fails to induce mitogenesis, while its migratory actions are retained [18, 19].

High circulating IGF1 levels are by now a well-established risk factor in women for developing breast cancer [46, 47], and a most recent collaborative analysis of 17 prospective studies established that the risk-association is confined to ER-positive tumors [48]. These data strongly support a notion of altered biologic and prognostic significance of IGF1 and IGF1R in ER-positive vs. ER-negative tumors as presented in our study.

Most likely, IGF1R expressing, ER-positive carcinomas represent a large subclass of breast carcinomas with presumably a distinct biology and an excellent prognosis. It could be hypothesized that well-differentiated, ER-positive breast cancers retain physiological growth control by ER

Table 4 Univariate Cox regression analysis of patient, tumor and treatment characteristics with Breast Cancer Specific Survival (BCSS)

	HR	95% CI	*P* value
Age	0.97	0.94 – 1.0	0.03
Menopausal status			
Premenopausal	1		0.02
Postmenopausal	0.45	0.23–0.88	
Tumor size			
0–2 cm	1		<0.001
>2–5 cm	2.59	1.17–5.73	0.02
>5 cm	7.41	2.84–19.33	<0.001
Differentiation grade			
Well	1		<0.001
Moderate	6.78	0.87–52.51	0.07
Poor	22.71	3.07–168.05	0.002
Axillary nodal status			
Negative	1		0.01
Positive	2.43	1.22–4.86	
Surgical therapy			
BCT	1		0.3
Mastectomy	1.43	0.73–2.78	
Radiotherapy			
No	1		0.6
Yes	1.30	0.64–2.64	
Adjuvant chemotherapy			
No	1		0.002
Yes	2.87	1.49–5.52	
Adjuvant hormonal therapy			
No	1		0.8
Yes	1.07	0.54–2.11	
ER			
Negative	1		<0.001
Positive	0.26	0.13–0.52	
PR			
Negative	1		0.02
Positive	0.45	0.23–0.88	
HER2			
Negative	1		0.003
Positive	3.81	1.57–9.23	
Cytoplasmic IGF1R			
Negative	1		0.005
Positive	0.38	0.19–0.74	
Membranous IGF1R			
Negative	1		0.6
Positive	1.19	0.61–2.34	
Overall IGF1R expression			
Negative	1		0.08
Positive	0.53	0.26–1.09	
IR			
Negative	1		0.3
Positive	0.65	0.29–1.49	
p-AKT			
Negative	1		0.5
Positive	0.73	0.22–2.38	

BCT breast conserving therapy

Table 5 Multivariate Cox regression on disease free survival in triple negative invasive ductal breast carcinomas (number of events = 28, number of patients = 101)

	HR	95% CI	*P* value
Tumor size			
<2 cm	1		0.15
≥2 cm	2.08	0.78–5.56	
Differentiation grade			
Well/Moderate	1		0.19
Poor	3.87	0.53–28.5	
Axillary nodal status			
Negative	1		0.85
Positive	1.08	0.51–2.30	
Cytoplasmic IGF1R			
Negative	1		0.07
Positive	2.06	0.95–4.47	

and IGF1R signaling, while in ER-negative carcinomas IGF1R expression confers primarily metastatic capacities.

Hormone receptor positive invasive ductal breast cancers are nowadays treated with adjuvant tamoxifen and/or aromatase inhibitors during 5 years or longer. Long-term survival benefit of hormonal therapy is reported to be 4–12% [21]. Because IGF1R and hormone receptors closely interact, a combination of hormonal therapy with IGF1R drugs may help to block all mitogenic hormonal responses. Also, as modulations of the IGF1R system have been implicated in development of resistance to endocrine therapies, IGF1R directed therapy may overcome or prevent development of hormone therapy resistance [4, 49, 50]. Indeed, synergistic effects of IGF1R targeting drugs with hormonal therapies have been reported in vivo [3]. Therefore, we suppose that IGF1R directed therapy may complement therapy for hormone receptor positive breast cancers, in particular in case of constitutive or acquired resistance to endocrine therapy.

Our patient cohort covers a considerable time span, in which the indications and therapeutic options for surgical

and systemic treatment have further been developed and adjusted. Surgical treatment (mastectomy vs. lumpectomy followed by radiation), received chemotherapy, and hormonal therapy were included in our multivariate survival models and group by group analysis was conducted for anthracycline-containing chemotherapy versus no chemotherapy and for tamoxifen versus no hormonal treatment (data not shown). Herein we found no evidence that the survival benefit of IGF1R expression on prognosis in our study was compounded by interaction with adjuvant systemic therapy. However, changing chemotherapy regimens, different hormonal treatment and shifting indications for adjuvant treatment over this period of time could not entirely be accounted for in our models.

In the Amsterdam dataset of TN-IDC more patients were treated with breast conserving surgery followed by radiation than in our cohort. Breast conserving surgical therapy bears a slightly increased risk on local recurrence of the tumor. However, our finding of an unfavorable prognostic impact of IGF1R on DFS in this population held true when time to distant metastasis was tested and was not attributed to local recurrences.

The present database was considered less appropriate for studying effects on prognosis in HER2 positive tumors, because herceptin had not yet been introduced as a standard adjuvant systemic treatment in this patient group during the inclusion period of this study. Also, correlations with HER2 may be inaccurate, since only 3+ HER2 cases were considered positive, and 2+ cases were not subjected to FISH analysis.

Activation of PI3K signaling, which among multiple other signals is induced by IGF1R, occurs often in breast carcinomas [51] and has been related to limited sensitivity to HER2 directed therapy [52]. We, however, found no correlation of IGF1R expression with staining of phosphorylated-Akt or PTEN, neither with RNA expression of PI3K pathway related genes (data not shown). Therefore, we could not show PI3K to be a dominant pathway emerging from high IGF1R expression and we can only speculate on the functional significance of positive IGF1R expression. Since an IGF-1 signature has been published predicting worse prognosis of ER-positive breast carcinomas [53] it is of interest to test whether the presence of such a signature is related to IGF1R expression on the protein level.

As the IGF1R is highly homologous to the IR, and they share several functions [1], IR expression was studied but no relation with clinicopathological parameters or IGF1R expression pattern could be shown. Although there are data suggesting IR is also a favorable prognostic factor [54, 55] we could not reproduce these results. Among other explanations, using antibodies with different specificity for (splice variants) of IR and IGF1R-IR hybrids may have played a role.

Interestingly, Law et al. [56] reported that phosphorylated IGF1R and insulin receptor (IR) can be found in all breast carcinoma subtypes and is related to a poor survival. Whether detection of phosphorylated IGF1R and IR outperforms the detection of IGF1R expression as presented here as a biomarker is subject of ongoing studies.

In TN-IDCs, inhibition of the IGF1R-mediated signaling is likely to have a clear beneficial effect as has been shown for a mouse model carrying basal type breast carcinomas [57]. Within this perspective, clinical phase trials of IGF1R antibodies and tyrosine kinase inhibitors or antibodies blocking the IGF1R signaling should focus both on ER-positive as well as TN-IDCs. With regard to the importance of cytoplasmic IGF1R expression, treatment with tyrosine kinase inhibitors may be advantageous over antibodies. The clinical development of antibodies against the IGF1R membrane receptor, however, is far ahead of the tyrosine kinase inhibitors, which is also understandable with regard to the expected side effects of such a treatment.

In conclusion, IGF1R expression in invasive ductal breast carcinomas is a strong favorable prognostic factor in ER-positive, but highly likely an unfavorable factor in TN-IDCs. The findings of our study combined with those of others strongly suggest that targeting IGF1R signaling is likely to be a promising treatment strategy of IGF1R expressing breast carcinomas. Clearly, further validation studies according to well established criteria are required to implement the IGF1R as a biomarker that could assist making a rational choice for IGF1R targeted therapy [58].

Conflict of interest No potential conflict of interest relevant to this article was reported.

References

1. Hartog H, Wesseling J, Boezen HM, van der Graaf WT (2007) The insulin-like growth factor 1 receptor in cancer: old focus, new future. Eur J Cancer 43:1895–1904
2. Pollak M (2008) Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer 8:915–928
3. Cohen BD, Baker DA, Soderstrom C, Tkalcevic G, Rossi AM, Miller PE, Tengowski MW, Wang F, Gualberto A, Beebe JS, Moyer JD (2005) Combination therapy enhances the inhibition of tumor growth with the fully human anti-type 1 insulin-like growth factor receptor monoclonal antibody CP-751, 871. Clin Cancer Res 11:2063–2073
4. Milano A, Dal LagoL, Sotiriou C, Piccart M, Cardoso F (2006) What clinicians need to know about antioestrogen resistance in breast cancer therapy. Eur J Cancer 42:2692–2705
5. Nahta R, Yuan LX, Zhang B, Kobayashi R, Esteva FJ (2005) Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. Cancer Res 65:11118–11128
6. Hubbard SR, Miller WT (2007) Receptor tyrosine kinases: mechanisms of activation and signaling. Curr Opin Cell Biol 19:117–123

7. Dupont J, LeRoith D (2001) Insulin and insulin-like growth factor I receptors: similarities and differences in signal transduction. Horm Res 55(2):22–26
8. Jones JI, Clemmons DR (1995) Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 16:3–34
9. Weber MM, Fottner C, Liu SB, Jung MC, Engelhardt D, Baretton GB (2002) Overexpression of the insulin-like growth factor I receptor in human colon carcinomas. Cancer 95:2086–2095
10. Chong YM, Colston K, Jiang WG, Sharma AK, Mokbel K (2006) The relationship between the insulin-like growth factor-1 system and the oestrogen metabolising enzymes in breast cancer tissue and its adjacent non-cancerous tissue. Breast Cancer Res Treat 99:275–288
11. Garcia-Echeverria C, Pearson MA, Marti A, Meyer T, Mestan J, Zimmermann J, Gao J, Brueggen J, Capraro HG, Cozens R, Evans DB, Fabbro D, Furet P, Porta DG, Liebetanz J, Martiny-Baron G, Ruetz S, Hofmann F (2004) In vivo antitumor activity of NVP-AEW541-A novel, potent, and selective inhibitor of the IGF-IR kinase. Cancer Cell 5:231–239
12. Haluska P, Carboni JM, Loegering DA, Lee FY, Wittman M, Saulnier MG, Frennesson DB, Kalli KR, Conover CA, Attar RM, Kaufmann SH, Gottardis M, Erlichman C (2006) In vitro and in vivo antitumor effects of the dual insulin-like growth factor-I/insulin receptor inhibitor, BMS-554417. Cancer Res 66:362–371
13. Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Akiyama M, Hideshima T, Chauhan D, Joseph M, Libermann TA, Garcia-Echeverria C, Pearson MA, Hofmann F, Anderson KC, Kung AL (2004) Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors. Cancer Cell 5:221–230
14. Pandini G, Wurch T, Akla B, Corvaia N, Belfiore A, Goetsch L (2007) Functional responses and in vivo anti-tumour activity of h7C10: a humanised monoclonal antibody with neutralising activity against the insulin-like growth factor-1 (IGF-1) receptor and insulin/IGF-1 hybrid receptors. Eur J Cancer 43:1318–1327
15. Camirand A, Lu Y, Pollak M (2002) Co-targeting HER2/ErbB2 and insulin-like growth factor-1 receptors causes synergistic inhibition of growth in HER2-overexpressing breast cancer cells. Med Sci Monit 8:BR521–BR526
16. Chakraborty AK, Liang K, DiGiovanna MP (2008) Co-targeting insulin-like growth factor I receptor and HER2: dramatic effects of HER2 inhibitors on nonoverexpressing breast cancer. Cancer Res 68:1538–1545
17. Jones HE, Goddard L, Gee JM, Hiscox S, Rubini M, Barrow D, Knowlden JM, Williams S, Wakeling AE, Nicholson RI (2004) Insulin-like growth factor-I receptor signalling and acquired resistance to gefitinib (ZD1839; Iressa) in human breast and prostate cancer cells. Endocr Relat Cancer 11:793–814
18. Bartucci M, Morelli C, Mauro L, Ando S, Surmacz E (2001) Differential insulin-like growth factor I receptor signaling and function in estrogen receptor (ER)-positive MCF-7 and ER-negative MDA-MB-231 breast cancer cells. Cancer Res 61: 6747–6754
19. Oesterreich S, Zhang P, Guler RL, Sun X, Curran EM, Welshons WV, Osborne CK, Lee AV (2001) Re-expression of estrogen receptor alpha in estrogen receptor alpha-negative MCF-7 cells restores both estrogen and insulin-like growth factor-mediated signaling and growth. Cancer Res 61:5771–5777
20. Yee D, Lee AV (2000) Crosstalk between the insulin-like growth factors and estrogens in breast cancer. J Mammary Gland Biol Neoplasia 5:107–115
21. Early Breast Cancer Trialists' Collaborative Group (EBCTCG) (2005) Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365:1687–1717
22. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lonning PE, Brown PO, Borresen-Dale AL, Botstein D (2003) Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA 100:8418–8423
23. Happerfield LC, Miles DW, Barnes DM, Thomsen LL, Smith P, Hanby A (1997) The localization of the insulin-like growth factor receptor 1 (IGFR-1) in benign and malignant breast tissue. J Pathol 183:412–417
24. Koda M, Przystupa W, Jarzabek K, Wincewicz A, Kanczuga-Koda L, Tomaszewski J, Sulkowska M, Wolczynski S, Sulkowski S (2005) Expression of insulin-like growth factor-I receptor, estrogen receptor alpha, Bcl-2 and Bax proteins in human breast cancer. Oncol Rep 14:93–98
25. Nielsen TO, Andrews HN, Cheang M, Kucab JE, Hsu FD, Ragaz J, Gilks CB, Makretsov N, Bajdik CD, Brookes C, Neckers LM, Evdokimova V, Huntsman DG, Dunn SE (2004) Expression of the insulin-like growth factor I receptor and urokinase plasminogen activator in breast cancer is associated with poor survival: potential for intervention with 17-allylamino geldanamycin. Cancer Res 64:286–291
26. Shimizu C, Hasegawa T, Tani Y, Takahashi F, Takeuchi M, Watanabe T, Ando M, Katsumata N, Fujiwara Y (2004) Expression of insulin-like growth factor 1 receptor in primary breast cancer: immunohistochemical analysis. Hum Pathol 35:1537–1542
27. Ueda S, Tsuda H, Sato K, Takeuchi H, Shigekawa T, Matsubara O, Hiraide H, Mochizuki H (2006) Alternative tyrosine phosphorylation of signaling kinases according to hormone receptor status in breast cancer overexpressing the insulin-like growth factor receptor type 1. Cancer Sci 97:597–604
28. Schnarr B, Strunz K, Ohsam J, Benner A, Wacker J, Mayer D (2000) Down-regulation of insulin-like growth factor-I receptor and insulin receptor substrate-1 expression in advanced human breast cancer. Int J Cancer 89:506–513
29. Fadare O, Tavassoli FA (2008) Clinical and pathologic aspects of basal-like breast cancers. Nat Clin Pract Oncol 5:149–159
30. Tavassoli FA, Devilee P (2003) World Health Organization classification of tumours pathology and genetics of tumours of the breast and female genital organs. IARC Press, Lyon
31. Kreike B, van Kouwenhove M, Horlings H, Weigelt B, Peterse H, Bartelink H, van de Vijver MJ (2007) Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas. Breast Cancer Res 9:R65
32. Camp RL, Charette LA, Rimm DL (2000) Validation of tissue microarray technology in breast carcinoma. Lab Invest 80:1943–1949
33. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP (1998) Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 4:844–847
34. van der Vegt B, de Roos MA, Peterse JL, Patriarca C, Hilkens J, de Bock GH, Wesseling J (2007) The expression pattern of MUC1 (EMA) is related to tumour characteristics and clinical outcome of invasive ductal breast carcinoma. Histopathology 51:322–335
35. Song RX, Chen Y, Zhang Z, Bao Y, Yue W, Wang JP, Fan P, Santen RJ (2010) Estrogen utilization of IGF-1-R and EGF-R to signal in breast cancer cells. J Steroid Biochem Mol Biol 118:219–230
36. Broussas M, Dupont J, Gonzalez A, Blaecke A, Fournier M, Corvaia N, Goetsch L (2009) Molecular mechanisms involved in activity of h7C10, a humanized monoclonal antibody, to IGF-1 receptor. Int J Cancer 124:2281–2293
37. Lee CY, Jeon JH, Kim HJ, Shin DH, Roh TW, Ahn CM, Chang YS (2008) Clinical significance of insulin-like growth factor-1 receptor expression in stage I non-small-cell lung cancer: immunohistochemical analysis. Korean J Intern Med 23:116–120

38. Cunningham MP, Essapen S, Thomas H, Green M, Lovell DP, Topham C, Marks C, Modjtahedi H (2006) Coexpression of the IGF-IR, EGFR and HER-2 is common in colorectal cancer patients. Int J Oncol 28:329–335
39. Nakamura M, Miyamoto S, Maeda H, Zhang SC, Sangai T, Ishii G, Hasebe T, Endoh Y, Saito N, Asaka M, Ochiai A (2004) Low levels of insulin-like growth factor type 1 receptor expression at cancer cell membrane predict liver metastasis in Dukes' C human colorectal cancers. Clin Cancer Res 10:8434–8441
40. Wallborn T, Wuller S, Klammt J, Kruis T, Kratzsch J, Schmidt G, Schlicke M, Muller E, van de Leur HS, Kiess W, Pfaffle R (2010) A heterozygous mutation of the insulin-like growth factor-I receptor causes retention of the nascent protein in the endoplasmic reticulum and results in intrauterine and postnatal growth retardation. J Clin Endocrinol Metab 95:2316–2324
41. Sehat B, Tofigh A, Lin Y, Trocme E, Liljedahl U, Lagergren J, Larsson O (2010) SUMOylation mediates the nuclear translocation and signaling of the IGF-1 receptor. Sci Signal 3:ra10
42. Aleksic T, Chitnis MM, Perestenko OV, Gao S, Thomas PH, Turner GD, Protheroe AS, Howarth M, Macaulay VM (2010) Type 1 insulin-like growth factor receptor translocates to the nucleus of human tumor cells. Cancer Res 70:6412–6419
43. Wang Y, Hailey J, Williams D, Wang Y, Lipari P, Malkowski M, Wang X, Xie L, Li G, Saha D, Ling WL, Cannon-Carlson S, Greenberg R, Ramos RA, Shields R, Presta L, Brams P, Bishop WR, Pachter JA (2005) Inhibition of insulin-like growth factor-I receptor (IGF-IR) signaling and tumor cell growth by a fully human neutralizing anti-IGF-IR antibody. Mol Cancer Ther 4:1214–1221
44. Peyrat JP, Bonneterre J, Beuscart R, Djiane J, Demaille A (1988) Insulin-like growth factor 1 receptors in human breast cancer and their relation to estradiol and progesterone receptors. Cancer Res 48:6429–6433
45. Railo MJ, Von Smitten K, Pekonen F (1994) The prognostic value of insulin-like growth factor-I in breast cancer patients. Results of a follow-up study on 126 patients. Eur J Cancer 30A:307–311
46. Renehan AG, Zwahlen M, Minder C, O'dwyer ST, Shalet SM, Egger M (2004) Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 363:1346–1353
47. Renehan AG, Egger M, Minder C, O'dwyer ST, Shalet SM, Zwahlen M (2005) IGF-I, IGF binding protein-3 and breast cancer risk: comparison of 3 meta-analyses. Int J Cancer 115:1006–1007
48. Key TJ, Appleby PN, Reeves GK, Roddam AW (2010) Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. Lancet Oncol 11:530–542
49. Parisot JP, Leeding KS, Hu XF, DeLuise M, Zalcberg JR, Bach LA (1999) Induction of insulin-like growth factor binding protein expression by ICI 182, 780 in a tamoxifen-resistant human breast cancer cell line. Breast Cancer Res Treat 55:231–242
50. Parisot JP, Hu XF, DeLuise M, Zalcberg JR (1999) Altered expression of the IGF-1 receptor in a tamoxifen-resistant human breast cancer cell line. Br J Cancer 79:693–700
51. Brugge J, Hung MC, Mills GB (2007) A new mutational AKTivation in the PI3K pathway. Cancer Cell 12:104–107
52. Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, Linn SC, Gonzalez-Angulo AM, Stemke-Hale K, Hauptmann M, Beijersbergen RL, Mills GB, van de Vijver MJ, Bernards R (2007) A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 12:395–402
53. Creighton CJ, Casa A, Lazard Z, Huang S, Tsimelzon A, Hilsenbeck SG, Osborne CK, Lee AV (2008) Insulin-like growth factor-I activates gene transcription programs strongly associated with poor breast cancer prognosis. J Clin Oncol 26:4078–4085
54. Mulligan AM, O'Malley FP, Ennis M, Fantus IG, Goodwin PJ (2007) Insulin receptor is an independent predictor of a favorable outcome in early stage breast cancer. Breast Cancer Res Treat 106:39–47
55. Mathieu MC, Clark GM, Allred DC, Goldfine ID, Vigneri R (1997) Insulin receptor expression and clinical outcome in node-negative breast cancer. Proc Assoc Am Physicians 109:565–571
56. Law JH, Habibi G, Hu K, Masoudi H, Wang MY, Stratford AL, Park E, Gee JM, Finlay P, Jones HE, Nicholson RI, Carboni J, Gottardis M, Pollak M, Dunn SE (2008) Phosphorylated insulin-like growth factor-i/insulin receptor is present in all breast cancer subtypes and is related to poor survival. Cancer Res 68: 10238–10246
57. Klinakis A, Szabolcs M, Chen G, Xuan S, Hibshoosh H, Efstratiadis A (2009) Igf1r as a therapeutic target in a mouse model of basal-like breast cancer. Proc Natl Acad Sci USA 106:2359–2364
58. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM (2005) Reporting recommendations for tumor marker prognostic studies. J Clin Oncol 23:9067–9072