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PROGNOSTIC VALUE OF pS2 PROTEIN AND RECEPTORS FOR EPIDERMAL GROWTH FACTOR (EGF-R), INSULIN-LIKE GROWTH FACTOR-1 (IGF-1-R) AND SOMATOSTATIN (SS-R) IN PATIENTS WITH BREAST AND OVARIAN CANCER

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Summary—The prognostic value of EGF-R, IGF-1-R and SS-R, and of cytosolic estrogen-regulated pS2 protein, was studied in patients (pts) with primary breast and advanced ovarian cancer. Ovarian cancer tissues were negative for pS2 (by immunoradiometric assay) IGF-1-R and EGF-R contents (by ligand binding assay, LBA) were of no or moderate prognostic value for breast cancer pts ($n = 214$). For advanced ovarian cancer pts, EGF-R content determined by LBA ($n = 55$) showed no prognostic value, whereas EGF-R status ($n = 35$) determined by immunohistochemistry (MoAb 2E9) significantly correlated with progression of disease ($P < 0.05$). In breast cancer pts, both SS-R and pS2 showed no association with tumor size, nodal status and grade. For pS2 the best cut-off level with respect to relapse-free (RFS) and overall survival (OS) was found to be 11 ng/mg protein. Both SS-R (1 g% SS-R+, $n = 135$; $P < 0.04$) and pS2 (27% pS2+, $n = 197$; $P < 0.001$), which were mainly positive in ER+ tumors, were of prognostic value, especially within the subgroups with ER+/PgR+ tumors. Also within N+ and No pts the 5-yr RFS and OS showed a difference between pS2+ and pS2− (33 and 54% for N+, and 31 and 13% difference for No pts). In summary, SS-R and pS2 are valuable prognosticators in breast cancer pts, and prognostic significance of EGF-R in ovarian cancer pts needs further study.

INTRODUCTION

In the last 5 yr much attention has been focussed on the role of autocrine or paracrine growth factors and their receptors in the development and biology of cancer. Both the presence and the levels of specific growth factor receptors on tumor cells may be of importance in the clinical outcome of several malignancies. With respect to prognosis in breast cancer, Sainsbury *et al.* [1] described that high levels of the epidermal growth factor receptor (EGF-R) may indicate a shorter relapse-free and overall survival. In addition, high levels of EGF-R were associated with a lack of response to endocrine therapy in recurrent disease [2]. However, with respect to prognosis contradictory results have been described since the initial report by Sainsbury *et al.* [1], and the prognostic value of EGF-R in subgroups of breast cancer patients is currently under debate [1, 3–7]. In ovarian cancer only very limited data are available, and interestingly, high levels of EGF-R may indicate a better response of the patients to chemotherapy [8].

Receptors for another growth factor which may act in an autocrine or paracrine way, i.e. the insulin-like growth factor-1 receptor (IGF-1-R), were found in the majority of primary human breast tumors analyzed [4, 9–11]. The prognostic value of the IGF-1-R in breast cancer could not be established in our series of patients [4]. In analogy with the EGF-R in breast cancer, there is also controversy for the prognostic value of IGF-1-R [12]. With respect to the presence of IGF-1-R in ovarian cancer only preliminary data have been reported, indicating that IGF-1-R was present in all ovarian tumors studied [13].

Recently, two other proteins which may be markers for breast tumor differentiation, the

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somatostatin-receptor (SS-R) and the estrogen-regulated pS2-protein, have been described [14–16].

This study describes the EGF-R, IGF-1-R, SS-R and pS2-protein levels in human primary breast and ovarian tumors, and we have correlated these findings with patients' prognosis.

RESULTS AND DISCUSSION

Epidermal growth factor receptor

Breast cancer. Various reports have described EGF-receptors in human primary breast cancers. More than 20 laboratories have used different techniques to measure EGF-R and have used different cut-off levels to discriminate between EGF-R+ and EGF-R− as well. In general, EGF-R positivity was observed in 41–90% of ER-negative and in 6–47% of ER-positive tumors. There is no agreement regarding the relationship between EGF-R and tumor size, lymph node status, differentiation grade, ploidy, proliferation indices and age of the patient. So far, five groups reported the prognostic value of EGF-R in breast cancer [1–6]. Sainsbury *et al.* [1] reported a highly significant prognostic value of EGF-R. In their series of patients the EGF-R was the most important variable in predicting (relapse-free) survival, far outweighing the ER-status of the tumor. However, three other studies with longer follow-up periods were not able to confirm this high significance [3–5]. Macias *et al.* [3] described a non-significant difference in relapse rate between patients with EGF-R+ and EGF-R− tumors after 6 yr of follow-up. Grimaux *et al.* [5], and our group [4], found only a tendency towards a significant prognostic value for EGF-R with respect to survival. In 55 patients with node-positive (N+) tumors, Grimaux *et al.* [5] only found a borderline significant ($P = 0.051$) prognostic value when overall survival was analyzed at 40 months (the same follow-up period as in the study of Sainsbury *et al.* [1]), but failed to predict long-term outcome. In contrast to the results of Sainsbury *et al.* [1], showing the best discriminative effects in lymph-node negative (No) and ER− patients, our data show the highest significance in N+ and ER+ patients [4]. In the study of Grimaux *et al.* [5], EGF-R+/ER− patients had the lowest survival probability, as in the study of Sainsbury *et al.*, but statistical significance was not reached ($P = 0.06$). Coombes *et al.* [6] described that there was no statistically significant difference in relapse-free survival between patients with tumors which were positive for EGF-R transcripts (55% of 64 tumors) and which were negative for EGF-R transcripts.

Together, the above described discrepancies with respect to EGF-R and prognosis in breast cancer, and with respect to the various controversies between EGF-R and other tumor and patient characteristics makes a common EGF-R assay with proper quality control necessary. In this respect, the recently developed EGF-R assay [17], which makes use of hydroxylapatite adsorption to separate the receptor-bound from the unbound EGF, and has recently been adapted by the EORTC receptor group as the method of preference to measure EGF-R, may prove to be very helpful.

Ovarian cancer. In ovarian cancer data on EGF-R are very limited. Bauknecht *et al.* [8] and Battaglia *et al.* [18] reported EGF-R positivity in 36% ($n = 101$) and 75% ($n = 24$), respectively. Importantly, the presence of EGF-R appeared to be of prognostic significance in the studies of Bauknecht *et al.* [8, 19]. EGF-R positivity, measured by biochemical techniques, suggested a more favorable response to chemotherapy in ovarian carcinomas [8]. Recently two preliminary reports, in which the EGF-R status was established by immunohistochemical techniques, showed that EGF-R positivity of the tumor was associated with a worse prognosis [20, 21]. In the study of Berchuck *et al.* [21], 79% (58/73) of the cancers were found to be positive for EGF-R. The survival of the 58 patients with EGF-R+ tumors was significantly shorter than that of the 15 patients whose cancers did not express EGF-R ($P < 0.05$). In our own study [20] with 35 patients with advanced ovarian cancer, 12 had an early stage disease (5 EGF-R−, 7 EGF-R+), and all of them were still alive with no evidence of disease. All 23 patients with advanced disease were treated with cisplatinum-containing chemotherapy. Of these 14 had relapsed or developed progressive disease, and 9 out of 23 were without evidence of disease. Of the 14 patients with a relapse or with progressive disease, 12 had EGF-R+ tumors and only two had EGF-R− tumors. In contrast, only one out of the nine patients with no evidence of disease was EGF-R positive ($P < 0.05$). EGF-R status did not correlate with clinical parameters of importance, like residual tumor mass following surgery, ascitis and histological grade. The discrepancy between the study of Bauknecht *et al.* [8] and the two

other studies, including our own, i.e. on the one hand high EGF-R is favorable and on the other hand low EGF-R is favorable, may be due to the different techniques used to assay EGF-R (ligand binding assay versus immunohistochemistry). More studies with a common standardized methodology for measurement of EGF-R, and a larger number of patients, are therefore needed before firm conclusions can be drawn.

Insulin-like growth factor-1 receptor

Breast cancer. Receptors for IGF-1 in human breast tumors have so far been reported by three different laboratories [9–11] in 50–67, 93 and 93% of the cancers, respectively. IGF-1 binding was less frequently observed in benign breast disease (43%) and normal breast tissue [22]. All three groups of investigators demonstrated a positive relationship between IGF-1-R and steroid receptor levels of the tumor. However, in our study on the prognostic value of IGF-1-R in a series of 214 patients, we did not observe any relationship between IGF-1-R and (disease free) survival [4]. No association was found between IGF-1-R and EGF-R [4, 9], lymph-node status [4, 9], tumor size [4], differentiation grade [4, 9] or menopausal status [4]. In contrast to our results, Peyrat *et al.* [12] were able to demonstrate a significant prognostic benefit for breast cancer patients whose tumors were IGF-1-R positive.

Ovarian cancer. With respect to the incidence and levels of IGF-1-R in ovarian cancers, only preliminary data have been reported by us [13]. Using autoradiography on 5 μm cryostat sections, we were able to demonstrate IGF-1-R in all ovarian cancer samples studied. Tissue sections derived from tumor tissues showed a higher expression (varying from 2+ to 4+) of IGF-1-R when compared to sections derived from normal ovarian tissues (1+ to 2+). Positiveness observed in serous, mucinous, endometrioid and clear cell tumors all expressed the same degree of density (2+ to 4+). High expression of IGF-1-R was predominantly associated with epithelial tumor cells. No specific IGF-1-R localization was present in surrounding connective tissue ($n = 50$). Also when measured by ligand binding assay on membrane preparations, we could demonstrate IGF-1-R in all carcinoma tissues examined by Scatchard analysis (B_{max}: median 55, range 6–220 fmol/mg protein; K_d: median 0.3, range 0.1–0.8 nM, $n = 22$). These levels were higher than that found in benign tumors ($n = 10$) and normal ($n = 7$) ovarian tissues (median B_{max}: 21 and 26 fmol/mg protein, respectively). The prognostic significance of IGF-1-R in ovarian carcinoma awaits further study with a larger number of patients.

Somatostatin receptor

Breast cancer. Various endocrine or endocrine-related tumors have been shown to express SS-R [14, 23]. Recently it was shown that a subpopulation of breast tumors also express SS-R [14, 15]. Importantly, we have shown that somatostatin and its analogs were able to inhibit breast cancer cell proliferation *in vitro* in a direct way, probably by direct action through the somatostatin receptor [24]. These studies have recently been confirmed by two other groups of investigators [25, 26]. We have therefore studied human primary breast tumor biopsies for the presence of SS-R by autoradiography. Of 135 tumor biopsies examined, 26 (19%) were positive for SS-R. Patients with SS-R+ tumors showed a significantly longer relapse-free survival than patients with SS-R− tumors (Fig. 1, $P = 0.035$). With respect to overall survival, the difference was not yet statistically significant after a 5-yr follow-up period. Apart from the prognostic significance of SS-R, the presence of SS-R may also have therapeutic consequences, i.e. a direct growth inhibitory effect of SS-analog treatment in analogy with the reported effects *in vitro* [24–26]. In addition, SS-analog treatment may cause breast tumor growth inhibition by endocrine effects (lowering of plasma GH and as a result of IGF-1; see for review [27]). Indeed, SS-analog treatment has been shown to cause breast tumor growth inhibition *in vivo* in the nude mouse [28], and in mice bearing MTX mammary carcinoma [29]. The

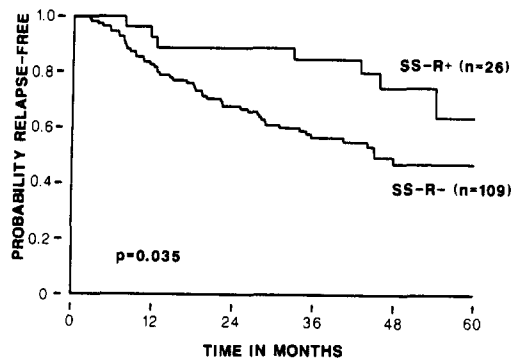

Fig. 1. Actuarial relapse-free survival analysis of human breast cancer patients stratified by somatostatin receptor status.

incidence of SS-R positivity (19%) in our series of tumors is lower than has been reported by Fekete and coworkers [30], who showed a 35% SS-R positivity using a biochemical technique (ligand binding assay on membrane preparations). This difference in incidence of SS-R in breast tumors is most probably due to the heterogeneity of tissue distribution of SS-R. For SS-R autoradiography small cryostat sections (mean surface area: approx. 14 mm^2) were analyzed, and for ligand binding assays on membrane preparations (as described by Fekete *et al.* [30]) an estimate of the SS-R status will be obtained which is more representative for the total tumor biopsy. Indeed, we have recently shown that heterogeneity of tissue distribution of SS-R in breast tumors exists [31]. Analysis of 72 large cryostat sections (mean surface area: approx. 180 mm^2) revealed a topographical heterogeneity of SS-R in breast tumor biopsies in more than 50% of the cases analyzed. In addition, in 33/72 (46%) of these large cryostat sections areas of epithelial cells which are positive for SS-R were found [31]. This is more than twice the incidence that has been observed for small cryostat sections, which means that analyses of small cryostat sections of tissues with an heterogeneous distribution of SS-R will probably result in a significant amount of false negative results [31].

Ovarian cancer. For ovarian cancer, only very limited data are available with respect to the presence of SS-R. It was recently reported that ovarian cancer tissues contain specific SS-analog binding sites when analyzed by ligand binding assay on membrane preparations [23]. We were able to confirm the presence of SS-R in some ovarian tumors by SS-R autoradiography (unpublished data).

pS2-protein

Breast cancer. In analogy with the PgR [32, 33], the specific transcription of the estrogen-regulated pS2-gene may reflect a more intact ER-machinery. The pS2-gene was initially characterized as a gene expressed specifically by estrogens in breast cancer cells *in vitro* [34, 35]. The pS2-protein is an 84 amino-acid-long secretory protein of unknown function. Using a cDNA probe for pS2 mRNA and specific polyclonal antibodies against the pS2-protein, it was shown that pS2 was predominantly expressed in ER+ primary breast tumors, and that pS2 expression was virtually absent in ER− breast tumors [16]. Interestingly, no significant staining of pS2-protein was observed in a variety of normal human specimens (such as colon, pancreas, liver, lung, prostate, kidney, endometrium, ovary and adrenals) [36]. However, pS2-protein was specifically expressed and secreted by ER− epithelial cells of the mucosa of the normal stomach of both female and male individuals [36].

In a collaborative study [37] we have analyzed 205 breast tumor specimens for the amount of cytosolic pS2-protein with a radiometric immunoassay (ELSA-PS2™, CIS Bioindustries, Gif-sur-Yvette, France). The levels of pS2-protein varied from 0 to 274 ng/mg protein (median 3.6). Higher levels of pS2-protein were found in ER+ breast tumors when compared to ER− tumors (Fig. 2). Isotonic regression analysis, as previously described for ER and PgR [38], was used to search for a cut-off value for pS2 to enable us to classify tumors as negative or positive for pS2. With both endpoints relapse-free survival (RFS) and overall survival (OS), a significant jump in average time to failure was found when pS2 values were above 11 ng/mg protein (Table 1). This value was therefore chosen as cut-off point to discriminate between pS2+ and pS2−. Patients with pS2− tumors experienced a significantly shorter RFS and OS ($P < 0.0001$). Also in multivariate analysis, after adjustment for tumor size, lymph-node status and ER-status, pS2 negativity was associated with earlier recurrence and death. As shown before with different techniques [16], pS2 positivity (55/205; 27%) was almost exclusively confined to the subclass of ER+ tumors (53/55; 96%). The death rate for patients with pS2+

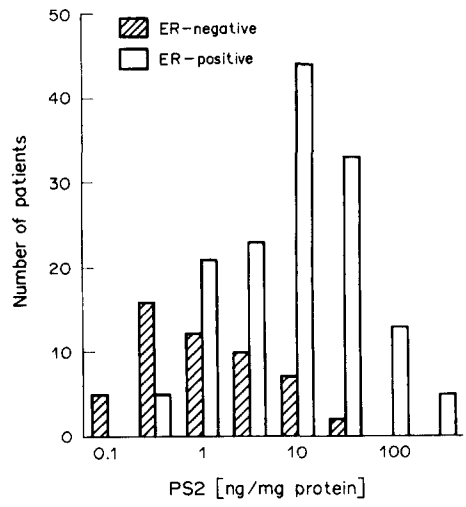

Fig. 2. Distribution of pS2-protein (PS2) over ER-negative and ER-positive breast tumor biopsies.

Table 1. Establishment of cut-off values for pS2-protein by isotonic regression analysis

Range of pS2 (ng/mg protein)	n	Number of failures	Average time to failure (months)
Relapse-free survival			
<0.2	32	18	42.4
0.3–0.9	35	19	55.6
1.0–2.9	34	16	73.1
3.0–6.2	36	17	73.8
6.3–11	22	10	77.2
11.2–16	11	4	113
17.6–274	44	9	231.7
Overall survival			
<0.2	32	15	63.7
0.3–2.6	64	30	89
2.7–2.9	5	2	92.5
3.0–5.1	31	13	98
5.3–8.5	20	7	110.3
8.6–11	7	3	122.3
11.2–16	11	2	269.5
17.6–45.2	33	4	437.3
47.9–274	11	1	550

tumors was one-tenth of the death rate in patients with ER−/pS2− tumors. Also, importantly, in subclasses of both node-negative, node-positive and ER+ patients, a very strong prognostic power of the pS2-status was found. Five-year OS was 97% in ER+/PgR+/pS2+ patients and only 54% in ER+/PgR+/pS2− patients. In the known good-prognosis group of node-negative patients, pS2 appeared a powerful prognosticator and pS2 negativity allowed identification of a subgroup of patients with a bad prognosis (58% for pS2− vs 89% for pS2+, a 31% difference in 5-yr RFS). In addition, in the known bad-prognosis group of node-positive patients, pS2 positivity could identify a subgroup of patients with a good prognosis (5-yr OS: 88% for pS2+ vs 34% for pS2−; a 54% difference) [37].

Ovarian cancer. In a collaborative study with Drs P. Seguin and J. Fauque (CIS Bioindustries, Bagnols, France), 26 ovarian cancer tissues were analyzed for their cytosolic content of pS2-protein by the ELSA-PS2™ assay. All pS2-protein values were below the level of 11 ng/mg protein (median: 0.03 ng pS2/mg protein), which was found to be the clinically relevant cut-off point for breast tumors.

CONCLUSIONS

In our own studies, analyzing the incidence and the prognostic value of receptors for EGF, IGF-1 and somatostatin, and of pS2-protein, in human ovarian tumor samples, the incidence of pS2-protein and somatostatin receptor positivity in ovarian tumor samples was too low to justify analysis for possible prognostic significance. EGF-R status, measured by immunohistochemistry but not by ligand binding assay, was able to identify a subgroup of patients who responded more favorably to cisplatinum-containing chemotherapy.

In primary breast tumor biopsies, the pS2-protein status was the most powerful single prognosticator [39]. In addition, the somatostatin receptor status appeared of prognostic significance in analysis of relapse-free survival. Receptors for EGF and IGF-1 did not show a significant prognostic power in our series of breast cancer patients. A combination of prognostic factors (e.g. pS2 and ER and/or PgR status) can further improve the discriminative efficacy of single prognosticators.

With respect to consequences for treatment decisions it awaits further study whether node-negative and node-positive patients with pS2 negative tumors will benefit from aggressive adjuvant therapy (with or without the addition or hormonal therapy), and if patients with pS2 positive tumors ought not to be treated, or only with hormonal agents.

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