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INSULIN-LIKE GROWTH FACTOR 1 RECEPTORS (IGF1-R) AND IGF1 IN HUMAN BREAST TUMORS

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Summary—To appreciate the IGF1 sensitivity of breast tumors we detected IGF1-R with a biochemical assay (RRA). We then localized and quantified IGF1-R on frozen tissue sections by histo-autoradiographic analysis (HAA). In some cases, the IGF1 and IGF1-R mRNA expression were studied by Northern blot analysis. We also studied the IGF1 plasma concentration in primary breast cancers compared to controls. IGF1-R (RRA) were found in 87% ($n = 297$) of the breast cancers. The mean geometric value was 3.87% (specific binding as percentage of total radioactivity); we found a highly significant correlation between IGF1-R and ER on the one hand ($P = 0.0001$) and PgR on the other ($P = 0.0001$) (Spearman test). The presence of IGF1-R was associated with a better prognosis, either on relapse-free survival (actuarial analysis: $P = 0.004$; Cox analysis: $P = 0.005$) or overall survival (respectively $P = 0.003$; $P = 0.005$). The median duration of follow-up was 30 months. By Cox analysis IGF1-R was a better prognostic factor than ER and PgR. In a series of 77 cases of benign breast disease only 47% (36/77) were positive; the mean geometric level was 1.8%. The HAA IGF1-R quantification in 20 breast carcinomas and 12 cases of benign breast disease confirmed the RRA results and demonstrated that the labeling was localized on the epithelial component. In four breat cancers, we did not detect IGF1 mRNA; IGF1-R probe demonstrated two major mRNAs of 11 and 7 kB. Finally we found that IGF1 plasma level was higher in breast cancer patients than in healthy controls of the same age. These results show that IGF1 is implicated in breast cancer growth and suggest that anti-IGF1 treatment might be useful in human breast cancer: for this reason, we and others carried out a phase II clinical trial with somatostatin.

INTRODUCTION

It is demonstrated that the growth of breast cancer is not only stimulated by hormones such as estradiol but also by growth factors. Epidermal growth factor (EGF) (as well as transforming growth factor α), fibroblast growth factor (FGF) and insulin-like growth factor 2 (IGF2) act via the autocrine pathway; platelet-derived growth factor (PDGF) is produced by breast cancer cells but acts on stromal cells (Fig. 1).

The well-known physiological role of insulin-like growth factor 1 (IGF1) is to act, with growth hormone, on skeletal development via the endocrine pathway [1, 2].

In breast cancer IGF1 stimulates the growth of various cell lines [3–7]. Immunoreactive IGF1 had been found in the medium conditioned by breast cancer cell lines [5, 8, 9] but the very recent demonstration of the absence of IGF1 mRNA in these cells [10] suggests that this "immunoreactive IGF1" represents an IGF1-related protein. These cell-lines have been shown to produce binding proteins which can also interfere in the RIA for IGF1 [11–13]. It has been proved that breast cancer cell-lines bind IGF1; variations in the sensitivity to IGF1 correlate with IGF1 cell binding [3, 4]. These results are confirmed using microsomal membrane preparation where the IGF1 receptor (IGF1-R) determination is not hampered by binding proteins [14–16].

We present here the results we have obtained assaying IGF1-R in human breast cancer tumors.

IGF1-R IN BREAST CANCERS

We determined the characteristics of IGF1-R in breast cancer [17] demonstrating the presence of one high affinity binding site. Chemical cross-linking of [^{125}I] IGF1 and subsequent

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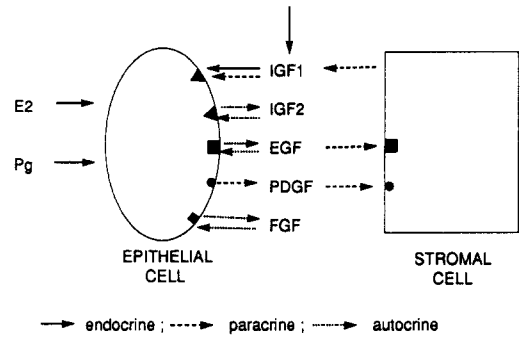

Fig. 1. Growth factors and breast cancer.

SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) revealed one band with an apparent molecular weight of 130,000. The study of the specificity showed that IGF2 was a good competitor (and could act via the IGF1-R) whereas insulin competed with a lower potency.

IGF1-R was then assayed in a series of 297 female breast cancers. All the breast cancer patients underwent surgery for loco-regional disease in the "Centre Oscar Lambret" from January 1986. For the binding test, 400 μg of membrane proteins were incubated for 5 h at 4°C with approx. 200,000 cpm of iodinated IGF1 in the presence or absence of an excess of IGF1. The final incubation volume was adjusted to 0.5 ml with Tris $MgCl_2$ buffer containing 0.1% bovine serum albumin (fraction V, ref. A 3912, Sigma Chemical Company, St Louis) and PMSF 0.1 mM. Duplicates were used for total and non-specific determinations. In each series, a characterized pool of cell membrane receptors (BT-20) was included to ensure the assay quality control. A tumor was considered as positive when the binding was greater or equal to 1% (specific binding/total radioactivity ratio in percent).

Both estradiol receptor (ER) and progesterone receptor (PgR) were determined by the DCC method [18]. ER and PgR were found in respectively 71 and 61% of the cases. IGF1-R were found in 87% of the cases. Similar results were found by other groups [19–21]. The geometric mean value was 3.87% (range 1–23%). A positive correlation between IGF1 and age was found (Spearman test: $P = 0.015$). No difference was found between N+ and N− patients or according to tumor diameter. IGF1-R were less frequently found in GHP3 than in GHP1 or GHP2 (χ^2 test: $P = 0.05$). No difference was observed according to ductular or lobular origin of the tumor. Relation between IGF1-R and ER or PgR are shown in Table 1.

IGF1-R IN BENIGN BREAST DISEASES

The characteristics of IGF1-R in benign breast diseases [22] were the same as in breast cancer. IGF1-R were detected in 47% (36/77) of these tumors. The geometric mean level of IGF1-R was 1.8% ranging from 1.2 to 2.7%. The percentage of positive tumors and the IGF1-R concentration were significantly lower in these tumors than in breast cancer. In five normal breasts we detected low levels or no IGF1-R.

IGF1-R HISTO-AUTORADIOGRAPHY

It was realized on frozen sections (7 μm) of 20 primary breast cancers; 12 benign breast diseases and two normal breasts. Frozen sections were processed as described by Haour *et al.* [23]. The slide-mounted sections were pre-incubated for 10 min at 20°C in Tris-HCl buffer (50 mM, pH 7.4) containing $CaCl_2$ (2 mM) and KCl (5 mM). The sections were then washed twice with Tris-HCl buffer (50 mM). Incubation was achieved at 20°C for 30 min in Tris-HCl (120 mM, pH 7.4) containing 0.1% BSA and [^{125}I]IGF1 (10^6 cpm/ml). Non-specific binding was determined in presence of 10^{-7} M IGF1. Following incubation, the sections were washed three times for 10 min in ice-cold Tris-HCl buffer (50 mM), dried, and exposed for 7 days to Amersham film (3 H-hyperfilm), The films were processed and the relative grain density was quantified by computerized densitometry using an image analyzer (RAG 200, BIOCOM, Les Ulis, France). Standardization of binding was achieved according to the method of Haour *et al.* [23] and values expressed as fentomoles per milligram protein.

The histo-autoradiography demonstrated that the IFG1 binding was precisely localized on the epithelial component. Moreover the quantification of IGF1-R using this technique led to results that are highly-correlated to those obtained by the classical biochemical method (Spearman rank correlation coefficient: 0.76, $n = 38$).

Table 1. Positive correlations (Spearman test) between IGF1-R and ER or PgR

Patients		P
Whole	IGF1-R ER	0.0001
	IGF1-R PgR	0.0001
Post-menopausal	IGF1-R ER	<0.001
	IGF1-R PgR	<0.001
Post-menopausal	IGF1-R ER	0.003
	IGF1-R PgR	NS

PROGNOSIS STUDIES

In actuarial survival studies a longer relapse free survival (RFS) was found in patients with IGF1-R than in those without ($P = 0.014$, Table 2) (277 patients, median duration of follow-up: 40 months); this result confirmed our previous one with a shorter duration of follow-up [24]. ER and PgR was prognostic factors on RFS too. IGF1-R was a prognostic factor in the node positive (N+) or PgR− subgroups. The positive prognostic value of IGF1-R on RFS was confirmed by Cox analysis ($P = 0.016$, Table 3). The IGF1-R prognostic value was lower than those of ER and histoprognostic grading (GHP) and had the same value as PgR, tumor diameter and N. Interestingly the combination of IGF1-R, GHP and N led to a very high evaluation of the prognosis.

In actuarial studies a longer overall survival (OS) was found in patients with IGF1-R than in those without ($P = 0.007$, Table 2) (291 patients, median duration of follow-up: 40 months). IGF1-R was a prognostic factor in the N+ or PgR− subgroups. The positive prognostic value of IGF1-R on OS was confirmed by Cox analysis ($P = 0.009$, Table 3). The IGF1-R prognostic value was lower than that of ER and had the same value than PgR, tumor diameter, N and GHP. The combination of IGF1-R, GHP and N had a very high prognostic value.

IGF1 PLASMA CONCENTRATIONS IN BREAST CANCERS

For IGF1 plasma determination we used two RIA procedures. The first one was that of Furnaletto [25] who allowed unextracted serum to incubate for 72 h at 4°C with the antibody before the addition of [^{125}I] IGF1 (IGF1 NE); the second one was that proposed by Daughaday *et al.* [26] which, thanks to an acid ethanol extracted serum, avoids, at least partially, interferences induced by quantitative and qualitative variations in the IGF1 combining protein complex (IGF1 E). The antiserum used for the radioimmunoassay was a gift from Drs L. Underwood and J. Van Wyk, who had standardized the technique [25]; it was distributed by the Hormone Distribution Program of NIDDK through the National Hormone and Pituitary Program (U.S.A.). The IGF1 for standards was purchased for Amersham (ARN 4010, Amersham, France). As the repartition of IGF1 plasma concentrations was not normal, for comparison between IGF1 in different populations, a non-parametric test was utilized. Aging in women was a major factor of IGF1 decrease: for this reason comparison was done between same age populations. We found that plasma IGF1 concentrations were higher in primary breast cancer patients ($n = 47$, age > 30 yr) than in controls ($n = 134$, age > 30 yr). These results were true considering either IGF1 NE ($P = 0.001$) or IGF1 E ($P = 0.018$).

Table 2. Prognostic value of IGF1-R and other factors in actuarial survival studies

	RFS	OS
IGF1-R	0.014	0.007
ER	0.002	0.0008
PgR	0.02	0.01
IGF1-R N+	0.0003	0.0006
IGF1-R PgR−	0.011	0.006

Table 3. Prognostic value of IGF1-R and other factors by Cox analysis

	RFS	OS
IGF1-R	0.016	0.009
ER	0.002	0.001
PgR	0.022	0.012
Tumor diameter	0.018	0.006
N	0.032	0.010
GHP	0.004	0.008
IGF1-R GHP N	0.0002	0.0004

SPECIFIC TREATMENTS

We hypothesized that treatments with drugs decreasing IGF1 could be beneficial to breast cancer patients. For this reason we carried out a first phase II trial to test the effect of somatostatin that decreases GH, indirectly IGF1 and inhibits directly breast cancer cell growth (Fig. 2) [27].

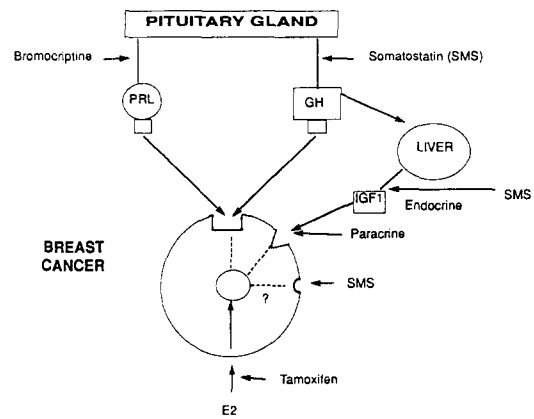

Fig. 2. Breast cancer IGF1-GH- and PRL-dependence and possible treatments (PRL = prolactin, GH = growth hormone, E2 = estradiol).

In this first trial post-menopausal patients with advanced breast cancer were twice daily subcutaneously injected with 0.1 mg of the somatostatin analogue SMS-201-995 (Sandoz, Basle). In the 14 evaluable patients we did not observe any complete or partial response but only three stabilizations. The IGF1 plasma concentrations of these patients were only partially decreased.

CONCLUSION

The IGF1 stimulation of the proliferation of cultured human breast cancer cells had been found. Our demonstration of high levels of IGF1-R in breast cancer biopsies, specially compared to lower levels in benign breast diseases, demonstrates that IGF1 is a possible important growth factor of breast cancer. These results are emphasized by our recent observation of elevated IGF1 plasma concentrations in breast cancer patients compared to controls. In breast cancer IGF1-R are correlated with ER and PgR and their presence is associated to a better prognostic. These results suggest the existence of a subgroup of breast cancers that contained ER, PgR, IGF1-R and prolactin/growth hormone receptors [28], and with a better prognostic. Following the example of tamoxifen, it could be useful to treat these tumors with specific drugs (Fig 2). The first trials with low doses of somatostatin [27] or with the prolactin inhibitor bromocriptin [29] were disappointing. It appears necessary to test now an anti-prolactinic drug combined to high doses of somatostatin.

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REFERENCES

1. Furnaletto R. W., Underwood L. E., Van Wyk J. J. and D'Ercole A. J.: Estimation of somatomedin C levels in normals and patients with pituitary disease by radioimmunoassay. *J. Clin. Invest.* **60** (1977) 648–657.
2. Zapf J. and Froesch E. R.: Pathophysiological and clinical aspects of the insulin-like growth factors. *Hormone Res.* **24** (1986) 160–165.
3. Furnaletto R. W. and Di Carlo J. N.: Somatomedin C receptors and growth effects in human breast cells maintained in long-term tissue culture. *Cancer Res.* **44** (1984) 2122-2128.
4. Myal Y., Shiu R. P. C., Bhaumick B. and Bala M.: Receptor binding and growth promoting activity of insulin-like growth factor in human breast cancer cells (T47-D) in culture. *Cancer Res.* **44** (1984) 5486–5490.
5. Huff K. K., Kaufman D., Gabbay K. H., Spencer E. M., Lippman M. E. and Dickson R. B.: Human breast cancer cells secrete an insulin-like growth factor I related polypeptide. *Cancer Res.* **46** (1986) 4613–4619.
6. Dickson R. B. and Lippman M. E.: Estrogenic regulation of growth and polypeptide growth factor secretion in human breast carcinoma. *Endocr. Rev.* **8** (1987) 29–43.
7. Karey K. P. and Sirbasku D. A.: Differential responsiveness of human breast cancer cell lines MCF-7 and T47-D to growth factors and 17-b estradiol. *Cancer Res.* **48** (1988) 4083-4092.
8. Baxter R. C., Maitland J. E., Raisur R. L., Reddel R. and Sutherland R. L.: High molecular weight somatomedin-C (IGF1) from T47-D human mammary carcinoma cells: immunoreactivity and bioactivity. In *Insulin-like Growth Factors—Somatomedins* (Edited by Spencer E. M.) Walter de Gruyter, Berlin (1983) pp. 615–678.
9. Minuto F., Delmonte P., Barreca A., Nicolin A. and Giordano G.: Partial characterization of somatomedin-C like immunoreactivity secreted by breast cancer cells *in vitro*. *Mol Cell. Endocrinol.* **54** (1987) 179–184.
10. Yee D., Palk S., Lebovic G. S. Marcus R. R., Favoni R. E., Cullen K. J., Lippman M. and Rosen N.: Analysis of insulin-like growth factor I gene expression in malignancy: evidence for a paracrine role in human breast cancer. *Mol. Endocrinol.* **3** (1989) 509–517.
11. De Leon D. D., Wilson D. M., Bakker B., Lamsoni G., Hintz R. L. and Rosenfeld R. G.: Characterization of insulin-like growth factor binding proteins from human breast cancer cells. *Mol. Endocrinol.* **3** (1989) 567–574.
12. Yee D., Favoni R. E., Lupu R., Cullen K. J., Lebovic G. S., Huff K. K., Lee P. D. K., Lee Y. L., Powell D. R., Dickson R. B., Rosen N. and Lippman M. E. The insulin-like growth factor binding protein BP-25 is expressed by human breast cancer cells. *Biochem. Biophys. Res. Commun.* **158** (1989) 38–44.
13. Daughaday W. H., Kapadia M. and Mariz I.: Serum somatomedin binding proteins: physiologic significance and interference in radioligand assay. *J. Lab. Clin. Med.* **109** (1987) 355–369.
14. De Leon D. D., Bakker B., Wilson D. M., Hintz R. L. and Rosenfeld R. G.: Demonstration of insulin-like growth factor (IGF-1 and -2) receptors and binding protein in human breast cancer cell lines. *Biochem. Biophys. Res. Commun.* **152** (1988) 398-405.
15. Pollak M. N. Polychronakos C., Yousefi S. and Richard M.: Characterization of insulin-like growth factor I (IGF-1) receptors of human breast cancer cells. *Biochem. Biophys. Res. Commun.* **154** (1988) 326–331.
16. Peyrat J. P., Bonneterre J., Dusanter-Fourt I., Leroy-Martin B., Djiane J. and Demaille A.: Characterization of insulin-like growth factor 1 receptors (IGF1-R) in human breast cancer cell lines. *Bull. Cancer* **76** (1989) 311-319.
17. Peyrat J. P., Bonneterre J., Beuscart R., Djiane J. and Demaille A.: Insulin-like growth factor I receptor (IGF1-R) in human breast cancer. Relation to estradiol and progesterone receptors. *Cancer Res.* **48** (1988) 6429–6433.
18. Koenders T. and Thorpe S. M.: On behalf of the EORTC receptor group standardization of steroid receptor assays in human breast cancer. IV: long-term within and between laboratory variation of estrogen and progesterone receptor assay. *Eur. J. Cancer Clin. Oncol.* **22** (1986) 945–953.
19. Pollak M. N., Perdue J. F., Margolese R. G., Baer K. and Richard M.: Presence of somatomedin receptors on primary human breast and colon carcinomas. *Cancer Lett.* **38** (1987) 223–230.
20. Pekonen F., Partanen S., Mäkinen T. and Rutanen E. M.: Receptors for epidermal growth factor and

insulin-like growth factor I and their relation to steroid receptors in human breast cancer. *Cancer Res.* **48** (1988) 1343–1347.

21. Foekens J. A., Portengen H., Van Putten W. L. J., Trapman A. M. A. C., Reubi J. C., Alexieva-Figush J. and Klijn J. G. M.: Prognostic value of receptors for insulin-like growth factor 1, somatostatin and epidermal growth factor in human breast cancer. *Cancer Res.* **49** (1989) 7002–7009.
22. Peyrat J. P., Bonneterre J., Laurent J. C., Louchez M. M., Amrani S., Leroy-Martin B., Vilain M. O., Delobelle A. and Demaille A.: Presence and characterization of insulin-like growth factor 1 receptors in human benign breast disease. *Eur. J. Cancer Clin. Oncol.* **24** (1988) 1425–1431.
23. Haour F., Dussaillant M., Leblanc P. and Rostene W.: Mise en évidence et répartition topographique des récepteurs du LH-RH chez le rat mâle normal et castré au niveau du système nerveux central. *C.R. Acad. Sci. Paris* **305** (1987) 41–42.
24. Bonneterre J., Peyrat J. P. Beuscart R. and Demaille A.: Prognostic significance of IGF1 receptors in human breast cancer. *Cancer Res.* **50** (1990) In press.
25. Furnaletto R. W., Underwood L. E., Van Wyk J. J. and d'Ercole A. J.: Estimation of somatomedin-C levels in normals and patients with pituitary disease by radioimmunoassay. *J. Clin. Invest.* **60** (1977) 648–657.
26. Daughaday W. H., Mariz I. K. and Blethen S. L.: Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radioreceptor and radiommunoassay of somatomedin in native and acid ethanol-extracted serum. *J. Clin. Endocrinol. Metab.* **51** (1980) 781–788.
27. Vennin Ph., Peyrat J. P., Bonneterre J., Louchez M. M., Harris A. G. and Demaille A.: Effect of the long-acting somatostatin analogue SMS 201–995 (Sandostatin) in advanced breast cancer. *Anticancer Res.* **9** (1989) 153–156.
28. Bonneterre J., Peyrat J. P., Beuscart R., Lefebvre J. and Demaille A.: Prognostic significance of prolactin receptors in human breast cancer. *Cancer Res.* **47** (1987) 4724–4728.
29. Bonneterre J., Mauriac L., Weber B., Roche H., Fargeot P., Tubiana-Hulin M., Sevin M., Chollet P. and Cappelaere P.: Tamoxifen plus bromocriptine versus tamoxifen plus placebo in advanced breast cancer: results of a double blind multicentre clinical trial. *Eur. J. Cancer Clin. Oncol.* **24** (1988) 1851–1853.