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Expression of the *PIP/GCDFP-15* gene and survival in breast cancer

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Abstract Expression of the PIP/GCDFP-15 gene was determined by measuring PIP/GCDFP-15-mRNA in breast carcinomas of 91 patients. The patients were followed-up for an average of 47 months after initial diagnosis and treatment of the disease. There were no deaths in the group of 14 patients with tumours of high PIP/GCDFP-15-mRNA levels, while 16 of 77 patients of the group with low PIP/GCDFP-15-mRNA tumour levels died. A similar advantage for high PIP/GCDFP-mRNA expression was observed with regard to disease free survival.

Key words Breast cancer · Survival · Prolactin-inducible protein · Gross cystic disease fluid protein

Introduction

Expression of the gene for prolactin-inducible protein (PIP) [8], also known as gross cystic disease fluid protein (GCDFP-15) [9] is regarded as a marker for apocrine differentiation [6].[1] Its role in breast carcinoma is still not well understood. *PIP/GCDFP-15* expression has been measured in normal breast tissue, and in benign and malignant breast tumours. The proportion of these tissues reported to contain either *PIP/GCDFP-15*-mRNA or GCDFP-15 protein varies substantially. Murphy et al. [8] detected *PIP/GCDFP-15*-mRNA in 10 of 20 primary carcinomas. Two samples of nonmalignant breast tissue showing cystic and proliferative disease were also positive, but one normal breast tissue sample and a human breast epithelial cell line (HBL100) contained no detectable *PIP/GCDFP-15*-mRNA.

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[1] In this paper the term GCDFP-15 is used to indicate the protein. For the corresponding gene the term is PIP/GCDFP-15

In another small series, Pagani et al. [10] observed *PIP/GCDFP-15*-mRNA expression in 17 of 33 breast carcinomas. In a larger sample of breast cancer biopsies [4] we found *PIP/GCDFP-15*-mRNA in 29 of 85 (34%) of primary carcinomas, with an additional 13% of borderline cases, and in 53% of carcinomas *PIP/GCDFP-15*-mRNA was not detected. In breast cancer metastases *PIP/GCDFP-15*-mRNA was significantly less frequently detectable (3 of 19, or 16%), while uninvolved breast tissue from mastectomies for breast carcinoma expressed PIP/GCDFP-15-mRNA significantly more often (35 of 71, or 49%), or, if borderline positive cases were included 51 of 71, or 72%. In patients where both primary tumour and metastatic tumour were available for study, the *PIP/GCDFP-15* results were usually similar in both tissues.

GCDFP-15 protein has been assessed by immunohistochemical methods in most studies. Mazoujian et al. [7] found positive staining for GCDFP-15 in 299 of 539 primary breast carcinomas (55%) and also showed that in lymph node metastases a smaller proportion of cases stained positive (64 of 137, 47%). In the majority of cases where both primary and metastatic tumour (in axillary lymph nodes) were studied there was no difference in staining for GCDFP-15; if there was a difference, the metastasis had usually lost GCDFP-15 expression, while positive staining in metastases from a negative primary tumour was rare.

Of the 105 breast carcinomas studied by Wick et al. [11], 72% scored positive. There were no cases in which immunoreactive primary tumours lost their ability to express GCDFP-15 in metastases or in which immunonegative lesions acquired GCDFP-15 after secondary spread. A still higher proportion of breast carcinomas reacting with GCDFP anti-serum was observed by Le Doussal et al. [5], namely 136 of 161 cases (84%). None of the 14 samples of normal breast tissue that had been peritumoural or peripheral to adenofibromas was positive. Of 10 adenofibromas without apocrine features, 4 were positive, as were over 60% of 32 cases of atypical hyperplasia. In a study by Bundred et al. [1] in which GCDFP-15

was measured by radioimmunoassay in the culture medium of 117 breast cancer explants, GCDFP-15 was detected in 90% of tumours.

Pagani et al. [10] compared immunohistochemistry of GCDFP-15 protein with *PIP/GCDFP-15*-mRNA by Northern blot. There was agreement between immunohistochemistry and Northern blot in 25 of 33 cases; discordant results were mostly positive by immunohistochemistry and negative by Northern blot, but the reverse was also found.

Discrepancies in published results are also seen with regard to the correlations of *PIP/GCDFP-15* expression in breast carcinoma with oestrogen and progesterone receptors, with the histology of the tumours, and with clinical information. Several authors have studied the relation between *PIP/GCDFP-15* expression in the tumour and disease-free survival or overall survival of breast cancer patients. Bundred et al. [1] followed 97 women with early breast cancer for at least 5 years, and found no correlation between GCDFP-15 secretion from tumour explants in tissue culture and patient survival. Mazoujian et al. [7] analysed sets of 126 and 267 patients, who were followed up for 14 and 7 years, respectively, and found that a positive stain for GCDFP-15 in the primary carcinoma was not related to risk of recurrence or survival. In contrast, Pagani et al. [10] observed a highly significant correlation between *PIP/GCDFP-15*-mRNA expression in 33 primary breast carcinomas and disease-free survival of the patients during an average follow-up period of 44 months. Using the immunohistochemistry results for GCDFP-15 in the same tumours the level of statistical significance was lower by a factor of about 3.

In an earlier study we examined the expression of several hormone-regulated genes, including *PIP/GCDFP-15*, in primary breast cancer, metastatic breast cancer and uninvolved breast tissue from mastectomies for cancer [4]. The patients concerned have now been followed up for an average of 47 months from the initial diagnosis and treatment of the disease. The expression of *PIP/GCDFP-15*-mRNA in the primary tumour was related to the clinical information on disease-free survival and overall survival, and the results are reported here. One of the patients in the original study was not available for follow-up; 6 additional patients were included in the present study.

Patients and methods

Patients

The patients underwent surgery for primary breast cancer between July 1989 and July 1991. Tissue samples were submitted for histopathology and steroid receptor assays, and these specimens were also used for the isolation of RNA for the study of *PIP/GCDFP-15* expression.

Follow up of the patients was conducted by the surgeon, the outpatient clinic of the hospital or the general medical practitioner. Follow-up information was obtained from medical records or by written contact with the doctor who regularly saw the patient. The information included the date when the patient was last seen and, if applicable, the date of recurrent carcinoma, the site of the recurrence, the date of death and whether death was due to cancer or to other causes. Some uncertain dates were confirmed with the death register of the State Health Department.

No attempt was made to standardise the treatment of patients following surgery. A population-based study of all cases of breast cancer diagnosed in Western Australia in 1989 [2] showed that 92% of node-positive and 23% of node-negative patients had adjuvant systemic therapy. Tamoxifen was prescribed as part of adjuvant therapy in 93% of those over 50 years, and cyclophosphamide, methotrexate and 5-fluorouracil in 71% of those under 50 years. A procedure involving breast conservation was performed in 31% of patients with operable tumours. If axillary dissection or sampling was part of such treatment, 94% of those under 50 years and 75% of those 50 years and over had radiotherapy to the residual breast. There is no reason to believe that these figures were different for 1990 or 1991. A summary of the patients in the present study and their tumour characteristics is given in Table 1.

Methods

Mastectomy or excision biopsy specimens from patients with breast carcinoma were studied. The tissues were dissected by pa-

Table 1 Characteristics of breast carcinomas studied

Tumour size	29	Less than 20 mm
	55	20 mm or more
	7	Not recorded
Axillary lymph node status	46	Node negative
	43	Node positive
	2	Not done
Histological type	76	Invasive ductal carcinomas
	6	Invasive lobular carcinomas
	7	Ductal carcinomas in situ
	1	Colloid carcinoma
	1	Clear cell carcinoma
Histological grade	7	Grade I
	29	Grade II
	34	Grade III
	6	Invasive ductal carcinomas, not graded
	15	Grading not applicable
Receptor status	68	Oestrogen receptor positive (>4 fmol/mg protein)
	23	Oestrogen receptor negative
	56	Progesterone receptor positive (>10 fmol/mg protein)
	35	Progesterone receptor negative

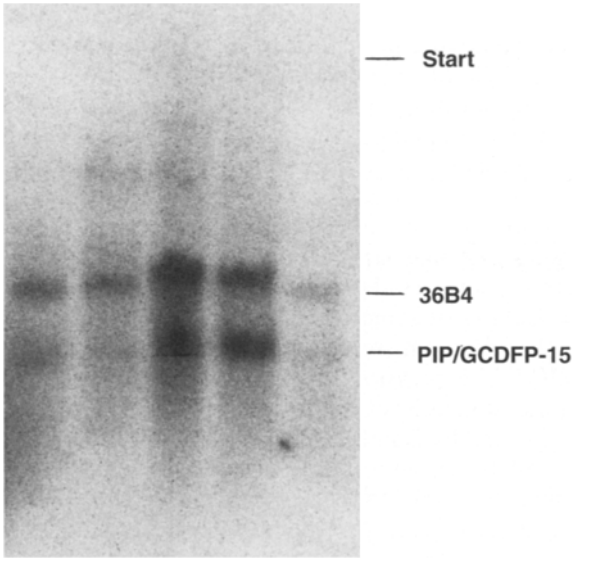

Fig. 1 Composite figure of a Northern blot showing *PIP/GCDFP-15*-mRNA and 36B4-mRNA for four primary breast carcinomas

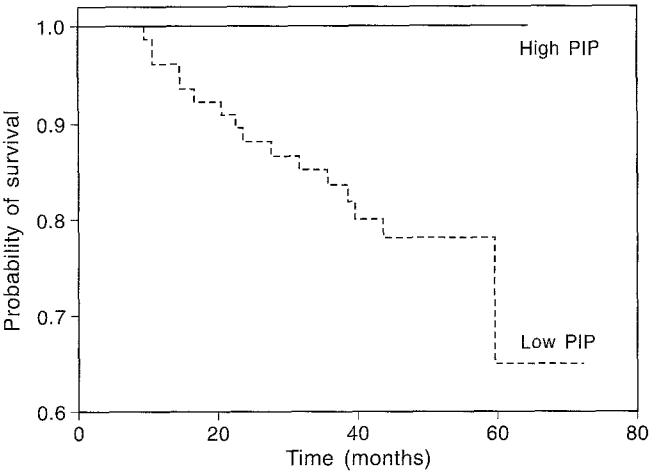

Fig. 2 Kaplan-Meier survival analysis of patients with primary breast cancer expressing high or low levels of *PIP/GCDFP-15*-mRNA

thologists, who selected representative samples for histopathology and biochemical studies. the carcinomas were graded and classified according to conventional systems. Oestrogen and progesterone receptors were estimated in tumour cytosols by means of commercial kits (Abbott). *PIP/GCDFP-15* expression was determined by Northern blotting, and the relative intensities of the mRNA bands were assessed visually with due consideration for the intensities of the ubiquitous 36B4 bands. The approximate sizes of the mRNAs detected by the procedures were 0.9 kb for *PIP/GCDFP-15* and 1.4 kb for 36B4. Details of the procedures and references can be found in our previous report [4]. A typical result is shown in Fig. 1.

Statistical analysis included Fisher's exact test, Chi-square and Kaplan-Meier, using the computer programs SPSS and Epistat (Trag L Gustafson, Round Rock, Tex.).

Results

The correlation between *PIP/GCDFP-15*-mRNA expression and survival is summarised in Table 2. There were 67 patients who remained disease-free during the follow-up period; 24 patients had recurrent disease, and 16 of these died during follow-up. For statistical evaluation, negative and weakly positive *PIP/GCDFP-15*-mRNA results (−, ±, +) were combined and compared with strongly positive results (2+ or more). With this grouping 77 tumours (85%) had low *PIP/GCDFP-15*-mRNA and 14 (15%) had high *PIP/GCDFP-15*-mRNA. During the mean follow-up period of 47 months there were no deaths in the high *PIP/GCDFP-15* group, while 16 patients (21%) with low *PIP/GCDFP-15* carcinomas died of the disease. The difference is of borderline significance according to Fisher's exact test (P=0.052). A similar result was obtained when disease-free survival was compared for patients with low and high *PIP/GCDFP-15*-mRNA tumours. There was only 1 patient in the high *PIP/GCDFP-15* group who had recurrent disease during the follow-up period, while 23 of the 77 patients with low *PIP/GCDFP-15* tumours suffered recurrent breast carcinoma (P=0.066). The survival curves (Kaplan-Meier) of patients whose breast carcinomas had low or high *PIP/GCDFP-15*-mRNA levels are shown in Fig. 2. For comparison with a well-established prognostic indicator, survival curves for lymph-node-positive and lymph-node-negative breast cancer patients were determined (details not shown). Kaplan-Meier analysis demonstrated a highly significant survival advantage of lymph-node-negative patients, both for disease-free survival and for overall survival (P=0.005 and 0.003), respectively.

Discussion

Only one other paper [10] deals with the relation between *PIP/GCDFP-15*-mRNA expression in breast carcinomas and survival. Pagani et al. and this study find that the presence of *PIP/GCDFP-15*-mRNA in the primary tumour is a good prognostic sign for the patient. The two studies are comparable in terms of length of fol-

Table 2 *PIP/GCDFP-15*-mRNA in breast carcinoma cytosols and survival. The semi-quantitative assessment of PIP/GCDFP-15-mRNA on Northern blots is based on comparison with the constant expression of 36B4

	Intensity of *PIP/GCDFP-15*-mRNA					
	−	±	+	++	+++	++++
Disease-free (67)	34	7	13	7	3	3
Recurrent disease, alive (8)	6	1	0	0	0	1
Dead (16)	10	3	3	0	0	0

Table 3 *PIP/GCDFP-15*-mRNA level in the tumour tissue and survival (*Low PIP/GCDFP-15*-mRNA −, ±, +, *high PIP mRNA* 2+ or more)

	Low *PIP/GCDFP-15*-mRNA	High *PIP/GCDFP-15*-mRNA
Alive	61	14
Dead	16	0
	P=0.052	
Disease-free	54	13
Recurrent disease	23	1
	P=0.066	

lowup of patients and proportion of *PIP/GCDFP-15*-mRNA-negative tumours. In our study we had more small tumours (35% versus 21%), more node-negative tumours (52% versus 33%), more well-differentiated tumours (10% versus 0%) and more patients who remained disease-free during the follow-up period (74% versus 58%).

In our follow-up of 91 cases there were no deaths in the group with high *PIP/GCDFP-15*-mRNA carcinomas, while the patients with low *PIP/GCDFP-15*-mRNA tumours had a 5-year survival probability of about 65%. Disease-free survival in the high *PIP/GCDFP-15*-mRNA group was about 90%, while it was 65% in the low *PIP/GCDFP-15*-mRNA group. Both disease-free survival and overall survival differences were only of borderline significance (see Table 3).

There are several possible explanations for the conflicting results on the relation between *PIP/GCDFP-15* expression and survival. First, the method used by Bundred et al. detected GCDFP-15 in 90% of breast carcinomas, while with the methods used by the other investigators only 52–55% of tumours were classified as positive [4, 7, 10]. Secondly, the number of patients studied was relatively small in one study [10]. The follow-up period in this study was similar to ours, and we also found differences between the *PIP/GCDFP-15*-positive and *PIP/GCDFP-15*-negative groups in terms of both disease-free survival and overall survival in our larger series, but the differences were of borderline significance. Mazoujian et al. [7] found no relation between GCDFP-15 staining and risk of recurrence or survival. One of the reasons for the discrepancy between the long- and short-term follow-up observations could be that the advantage of *PIP/GCDFP-15*-positive carcinomas gradually diminishes with time and the survival curves of positive and negative carcinomas converge if followed for an extended period. This has been observed for the prognostic value of the oestrogen receptor [3]. The papers by Bundred et al. [1] and Mazoujian et al. [7] do not present the survival curves. The curves shown in the paper by Pagani et al. [10] could possibly converge with time. In our *PIP/GCDFP-15*-positive group no deaths were observed and only 1 recurrence, and the survival curves do not support the notion of convergence with time. Thirdly, there are differences in the relative proportions of histological types of cancer studied. In Mazoujian et al.'s [7] sample, 19% of tumours were ductal carcinomas in situ, while in Pagani et al.'s [10] study there were none, and in ours the proportion was 8%. Invasive ductal carcinomas represented 88% and 84%, respectively, in the last two studies mentioned, but only 67% in the first. A number of other factors, such as lymph node status, histological grade, tumour size, and oestrogen and progesterone receptor status, could not be compared because details were lacking in some of the reports. Furthermore, there may be different results according to whether *PIP/GCDFP-15* expression is determined at the mRNA level or at the protein level. The results reported by Mazoujian et al. [7] and by Bundred et al. [1] are based on measurements of the protein, while in both Pagani et al.'s [10] and this study mRNA assays were used in assessment of the relation between *PIP/GCDFP-15* expression and survival. Finally, the manner in which low and high results were separated differed between the authors using protein assays and those using mRNA measurements. Mazoujian et al. [7] and Bundred et al. [1] regarded a tumour as positive if GCDFP-15 was detectable, while Pagani et al. [10], like us, compared survival for tumours below and above a specified *PIP/GCDFP-15* content so that the low *PIP/GCDFP-15* group contained not only tumours in which *PIP/GCDFP-15* was not detectable, but also weakly positive tumours.

In conclusion, it appears that expression of *PIP/GCDFP-15*-mRNA in breast cancer tissue is associated with a survival advantage, and that further studies are therefore indicated. The estimation of *PIP/GCDFP-15*-mRNA, however, is not suitable for routine assays in a larger number of tumours. The correlation between *PIP/GCDFP-15*-mRNA assays and assays measuring PIP/GCDFP-15 protein by radioimmunoassay or enzyme immunoassay should be further characterised so that mRNA assays can be replaced by protein estimations, which are suitable for automation and large-scale studies.

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