Overexpression of P70 S6 kinase protein is associated with increased risk of locoregional recurrence in node-negative premenopausal early breast cancer patients

JA van der Hage1,2,3, LJCM van den Broek2, C Legrand1, PC Clahsen2, CJA Bosch4, EC Robanus-Maandag4, CJH van de Velde1 and MJ van de Vijver5,4

1Department of Surgery, Leiden University Medical Center, Albinusdreef 2, 2300 RC Leiden, The Netherlands; 2Department of Pathology, Leiden University Medical Center, Albinusdreef 2, 2300 RC Leiden, The Netherlands; 3EORTC Data Center, Avenue E. Mounier B3 b11, 1200 Brussels, Belgium; 4Department of Pathology and Division of Experimental Therapy, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

The RPS6KB1 gene is located at 17q23 and amplified in approximately 10% of breast carcinomas and has been found associated with poor prognosis. We studied the prognostic significance of P70 S6 kinase protein (PS6K) overexpression in a series of 452 node-negative premenopausal early-stage breast cancer patients (median follow-up: 10.8 years). Immunohistochemistry was used to assess PS6K expression in the primary tumour, which had previously been analysed for a panel of established prognostic factors in breast cancer. In a univariate analysis, PS6K overexpression was associated with worse distant disease-free survival as well as impaired locoregional control (HR 1.80, P 0.006, respectively). In a multivariate analysis including other prognostic factors, PS6K overexpression remained an independent predictor for poor locoregional control (RR 2.67, P 0.003). To our knowledge, P70 S6 kinase protein is the first oncogenic marker that has prognostic impact on locoregional control and therefore may have clinical implications in determining the local treatment strategy in early-stage breast cancer patients.

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The treatment of breast cancer is guided by risk factors. Approximately 70% of all node-negative breast cancer patients can be cured by locoregional therapy alone. This automatically implies that the remaining 30% of these patients will develop a recurrence despite adequate locoregional therapy. Currently, treatment strategy in breast cancer is based upon tumour stage, grade, and hormone receptor status. Additional prognostic factors are greatly needed, first to select those patients who might benefit from adjuvant systemic therapy and second to optimise locoregional therapy in order to avoid locoregional recurrences.

The prognostic significance of a considerable number of tumour markers has already been investigated but to date, none of these factors can be used to guide the treatment of primary breast cancer.

A recent study by Barlund et al (2000a) demonstrated that amplification of a putative tumour marker called P70 S6 kinase protein (PS6K) might be associated with poor outcome in breast cancer. In addition, the authors reported that RPS6KB1 gene amplification and PS6K overexpression are significantly correlated.

The RPS6KB1 gene is located at 17q23 and amplified in approximately 10% of all primary breast cancer cases. PS6K is a ribosomal protein that is involved in the progression from the G1 to S phase of the cell cycle. It is rapidly activated in response to mitogenic stimuli, for example, growth factors, cytokines, and oncogene products (Grove et al, 1991; Lane et al, 1993; Chou et al, 1995; Grammer et al, 1996; Thomas et al, 1997; Couch et al, 1999; Barlund et al, 2000b; Wu et al, 2000; Latham et al, 2001; Monni et al, 2001; Andersen et al, 2002; Li et al, 2002; Sinclair et al, 2002; Sinclair et al, 2003).

To study the significance of P70 S6 kinase protein compared with other established prognostic factors in breast cancer, we have tested the prognostic significance of PS6K overexpression in a subset of node-negative premenopausal early breast cancer patients. In this series, we have shown previously that premenopausal node-negative breast cancer patients whose tumours show p53 accumulation have a poor response to one cycle of adjuvant chemotherapy, whereas patients whose tumours have no accumulation of p53 benefit from adjuvant chemotherapy. In addition, we showed Ki-67 overexpression, negative ER status, and young age (<43 years) to be associated with worse prognosis (Clahsen et al, 1998).

PATIENTS AND METHODS

Patients

All patients were drawn from EORTC trial 10854 (1986–1991, median follow up 10.8 years). This trial, which randomised 2795 patients, studied whether one course of peri-operative chemother-
apy given directly after surgery yields better results in terms of treatment outcome than surgery alone. Peri-operative chemotherapy consisted of one single course of doxorubicin 50 mg m\(^{-2}\), 5-fluorouracil 600 mg m\(^{-2}\), and cyclophosphamide 600 mg m\(^{-2}\) (FAC), administered intravenously within 36h after surgery. Axillary lymph node-positive premenopausal patients in the peri-operative chemotherapy group were recommended to receive five additional cycles of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) postoperatively. Node-positive patients, younger than 50 years, who did not receive peri-operative chemotherapy, were advised to be treated with one conventional course of FAC followed by five cycles of CMF after surgery. At randomisation, patients were stratified for institution, age (<50 years or >50 years), and surgical procedure (breast conserving therapy and modified radical mastectomy). Prolonged adjuvant systemic treatment was left to the discretion of the local investigator (van der Hage et al., 2001).

In total, 676 node-negative premenopausal patients were enrolled in this trial and representative tumour material was collected for 452 patients. Tumour material consisted of formalin-fixed, paraffin-embedded tumour blocks. Tumours were histologically typed and graded (Elston et al., 1991) centrally by one pathologist; immunohistochemistry to assess the expression of various proteins has been performed. Results of these studies have been reported previously (Clahsen et al., 1998). For the present study, assays were reviewed simultaneously by two investigators (MJ Van de Vijver, JA Van der Hage) who had to come to an agreement in case of any uncertainties. During the evaluation of the results, the investigators were blinded for the clinical outcome of the patients.

**p70 S6 kinase protein expression**

A standard indirect immunoperoxidase protocol with a 3,3'-diaminobenzidine/imidazole solution as a chromogen was used. Before incubation with the primary antibody, antigen retrieval was done by boiling the sections in 10 mM citrate buffer for 10 min using a microwave oven. PS6K expression was determined using a polyclonal anti-p70 s6k antibody (sc-230 Santa Cruz Biotechnology, Santa Cruz, USA) (1:1000 dilution in PBS containing 1% bovine serum albumin). PS6K staining was evaluated in tumour cells and in normal ductal epithelial cells. PS6K staining was scored categorical as: 0 = no staining; 1 = weak cytoplasmic staining; 2 = moderate cytoplasmic staining; 3 = strong cytoplasmic staining. In all cases analysed, the staining was homogeneously distributed in the normal cells and also in the tumour cells. If the difference in staining score between the tumour cells and the normal epithelial ducts was greater or equal than two, tumours were deemed PS6K positive.

**RPS6KB1 gene amplification**

Two-colour FISH of tumor interphase nuclei was performed according to the ERBB2 short protocol of Ventana Medical Systems, Inc. (Tucson, AZ, USA). The Spectrum Orange-labelled chromosome 17 centromeric probe was purchased from Vysis, Inc. (Downers Grove, IL, USA), the unlabelled bacterial artificial chromosome (BAC) clones for PS6K was isolated from a BAC library (RPCI-13 BAC library, Research Genetics, Inc.). Fluorescent signals were counted in 2 × 20 non-overlapping nuclei per component. Mapping of the PS6K BAC was verified by FISH on metaphase chromosomes.

**Other tumour markers**

Previously, tumour sections had been stained and analysed for oestrogen and progesterone receptor status, Ki-67 positivity, PI3

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**Table I** Patient characteristics

|                      | N = 452 |
|----------------------|---------|
| **Age (year)**       | Median (range) 44 (24–63) |
| **Local treatment (N (%))** | Breast-conserving therapy 368 (81) | Mastectomy 84 (19) |
| **Tumour diameter (N (%))** | ≤2 cm 278 (62) | >2 cm 148 (33) |
| **Histologic tumour type (N (%))** | Infiltrating ductal 316 (70) | Infiltrating lobular 34 (8) |
| **Histologic tumour grade (N (%))** | I 155 (34) | II 144 (32) | III 131 (21) |
| **ER status (N (%))** | Positive 390 (86) | Negative 46 (10) | Unknown 16 (4) |
| **PgR status (N (%))** | Positive 329 (73) | Negative 106 (23) | Unknown 17 (4) |
| **HER2 overexpression (N (%))** | Negative 380 (84) | Positive 60 (13) | Unknown 12 (3) |
| **p53 expression (N (%))** | Negative 359 (79) | Positive 81 (18) | Unknown 12 (3) |
| **Ki-67 (N (%))** | Negative 217 (48) | Positive 215 (48) | Unknown 20 (4) |
| **PS6K** | Negative 391 (87) | Positive 39 (9) | Unknown 22 (5) |

**Statistical methods**

This analysis was based on locoregional control, distant-disease free survival, and overall survival. Locoregional recurrence was defined as the time to locoregional recurrence as a first event. Locoregional recurrences occurring simultaneously or after the diagnosis of distant metastasis or contralateral breast cancer or a secondary primary tumour were censored. Distant disease-free survival was defined as the time to distant metastasis or death, whichever of the events happened first. All variables were first analysed for their prognostic importance in a univariate analysis.
Eight potential prognostic variables were considered: PS6K (negative vs positive), ER status (negative vs positive), PgR status (negative vs positive), HER2 overexpression (negative vs positive), Ki67 (negative, i.e. ≤ 20% of positive tumour cells, vs positive, > 20% positive tumour cells), histologic tumour grade (grade I vs grade II vs grade III), tumour diameter (T ≤ 2 cm vs T > 2 cm), and p53 (negative vs positive).

To test the independent prognostic significance of PS6K overexpression, we included PS6K together with the previously tested markers into a multivariate Cox regression analysis for overall survival, progression-free survival, distant disease-free survival, and locoregional control. Only markers that were significant predictors in the univariate analysis were included in the multivariate analysis.

A Cox proportional hazards model was used for the univariate and multivariate analyses (Cox, 1972). For factors with only two levels the second one was compared to the first one, while for factors with more than two levels dummy variables were used to compare each level to the first one. Patients who had missing information for any of the variables in the analysis were excluded when this variable was included in the model. All tests were two-sided with a 5% alpha level.

RESULTS

Patient characteristics are listed in Table 1. At the time of the analysis, the median follow-up period was 10.8 years, 80 (18%) of

| Table 2 Event rates | Number of events |
|---------------------|------------------|
|                     | N = 452          |
| Local treatment (N (%)) | Overall survival | Locoregional recurrence (first event) | Distant disease-free survival |
| Breast-conserving therapy  | 368 (81) | 65 (18) | 58 (16) | 102 (28) |
| Mastectomy             | 84 (19)  | 15 (18) | 9 (11)  | 24 (29)  |
| Tumour diameter (N (%)) |         |       |        |          |
| ≤ 2 cm                 | 278 (62) | 34 (12) | 41 (15) | 68 (24)  |
| > 2 cm                 | 148 (33) | 40 (27) | 21 (14) | 51 (34)  |
| Unknown                | 26 (6)   | 6 (23)  | 5 (19)  | 7 (27)   |
| Histologic tumour type (N (%)) |       |        |        |          |
| Infiltrating ductal    | 316 (70) | 60 (19) | 48 (15) | 91 (29)  |
| Infiltrating lobular   | 34 (8)   | 4 (12)  | 9 (26)  | 7 (21)   |
| Other                  | 91 (20)  | 14 (15) | 8 (9)   | 25 (27)  |
| Unknown                | 11 (2)   | 2 (18)  | 2 (18)  | 3 (27)   |
| Histologic tumour grade (N (%)) |       |        |        |          |
| I                      | 155 (34) | 10 (6)  | 23 (15) | 30 (19)  |
| II                     | 144 (32) | 30 (21) | 27 (19) | 45 (31)  |
| III                    | 131 (21) | 36 (27) | 14 (11) | 47 (36)  |
| Unknown                | 22 (5)   | 4 (18)  | 3 (14)  | 4 (18)   |
| ER status (N (%))      |         |        |        |          |
| Positive               | 390 (86) | 64 (16) | 60 (15) | 104 (27) |
| Negative               | 46 (10)  | 13 (28) | 4 (9)   | 18 (39)  |
| Unknown                | 16 (4)   | 3 (19)  | 3 (19)  | 4 (25)   |
| PgR status (N (%))     |         |        |        |          |
| Positive               | 329 (73) | 5 (16)  | 53 (16) | 90 (27)  |
| Negative               | 106 (23) | 27 (25) | 12 (11) | 33 (31)  |
| Unknown                | 17 (4)   | 2 (12)  | 2 (12)  | 3 (18)   |
| HER2 overexpression (N (%)) |       |        |        |          |
| Negative               | 380 (84) | 66 (17) | 52 (14) | 107 (28) |
| Positive               | 60 (13)  | 12 (20) | 13 (22) | 16 (27)  |
| Unknown                | 12 (3)   | 2 (17)  | 2 (17)  | 3 (25)   |
| P53 expression (N (%)) |         |        |        |          |
| Negative               | 359 (79) | 59 (16) | 47 (13) | 99 (28)  |
| Positive               | 81 (18)  | 19 (23) | 18 (22) | 24 (30)  |
| Unknown                | 12 (3)   | 2 (17)  | 2 (17)  | 3 (25)   |
| Ki-67 (N (%))           |         |        |        |          |
| Negative               | 217 (48) | 19 (9)  | 36 (17) | 43 (20)  |
| Positive               | 215 (48) | 59 (27) | 27 (13) | 78 (36)  |
| Unknown                | 20 (4)   | 2 (10)  | 4 (20)  | 5 (25)   |
| PS6K                   |         |        |        |          |
| Negative               | 391 (87) | 66 (17) | 52 (13) | 102 (26) |
| Positive               | 39 (9)   | 8 (21)  | 11 (28) | 17 (44)  |
| Unknown                | 22 (5)   | 6 (27)  | 4 (18)  | 7 (32)   |
the 452 patients had died, 126 (29%) patients had experienced distant metastases or death, and 67 (15%) patients experienced a locoregional recurrence as first event (see Table 2). PS6K expression levels could be assessed in 430 tumours. In all, 39 tumours (9%) showed PS6K overexpression (Table 1). Examples of PS6K overexpression are shown in Figure 1A & B.

**Univariate analyses**

In the univariate analyses, we could not confirm a significant association between PS6K overexpression and overall survival (Table 3). However, PS6K overexpression was a significant predictor for increased risk of locoregional recurrence (HR 2.50, 95% CI 1.30–4.81, \(P = 0.006\)) and of developing distant metastases (HR 1.80, 95% CI 1.08–3.01, \(P = 0.025\)).

**Multivariate analyses**

Apart from PS6K, p53 was the only significant risk factor for locoregional recurrence in the univariate analysis. When including these two factors in a multivariate model, PS6K appears as the only independent prognostic factor for locoregional control predicting a poor control rate in PS6K overexpressing cases (HR 2.67, 95% CI 1.39–5.14, \(P = 0.003\), Table 4).

Variables significantly associated with distant disease-free survival in the univariate analysis were PS6K, ER status, Ki-67, grade, and tumour diameter. In a multivariate model including all these factors, Ki-67 overexpression was the only independent prognostic factor associated with poor distant disease-free survival (HR 1.79, 95% CI 1.11–2.91, \(P = 0.018\), Table 4). PS6K as a prognostic factor did not remain significant in the multivariate analysis. In addition, Ki-67

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**Figure 1** (A & B) Two cases of PS6K overexpressing breast cancer tumours.
Table 3  Univariate analyses all patients (N = 430)

| Marker | Hazard ratio | 95% CI | P-value |
|--------|--------------|--------|---------|
| PS6K+  | 2.50         | 1.30–4.81 | 0.006   |
| ER+    | 1.38         | 0.50–3.79  | 0.536   |
| PgR+   | 1.31         | 0.70–2.45  | 0.403   |
| HER2+  | 1.64         | 0.89–3.01  | 0.111   |
| Ki 67+ | 0.96         | 0.58–1.58  | 0.856   |

Table 4  Multivariate analyses all patients (N = 430)

| Marker | Hazard ratio | 95% CI | P-value |
|--------|--------------|--------|---------|
| PS6K+  | 2.67         | 1.39–5.14 | 0.003   |
| p53 positivity | 1.67 | 0.95–2.96 | 0.076   |

Correlation between HER2 and PS6K

As the PS6K gene and the HER2 gene are both located on chromosome 17, and amplification has been reported to occur in both genes simultaneously, we studied the correlation of PS6K expression and HER2 expression and between PS6K expression and Ki67 expression, respectively. Based on available data, we found a significant association between PS6K and HER2 expression (Fisher’s exact test (two sided) P = 0.01), whereas no significant association was found between PS6K positivity and Ki67 positivity (Fisher’s exact test (two sided) P = 0.24).

DISCUSSION

We have found that P70 S6 kinase protein overexpression in breast cancer is associated with increased risk of locoregional recurrence. To our knowledge, no other oncogenic markers as predictors of locoregional recurrence have been identified previously. At present, the common risk factors for local control after breast-conserving treatment are: patient age, margin status, and the presence of an extensive intraductal component (De la Rochefordiere et al, 1993; Elkhuizen et al, 1998; Voogd et al, 1999, 2001). The addition of new predictive markers for locoregional recurrence may help in guiding the optimal type of local therapy. This is of particular importance since local therapy does not only have an impact on locoregional control but also on survival (Shukla et al, 1999; Early Breast Cancer Trialists’ Collaborative Group (EBCTCG), 2000).

P70 S6 kinase protein overexpression was associated with an increased risk of locoregional recurrence when all patients were analysed. The majority of the patients (N = 368) underwent breast-conserving treatment. When these patients were analysed separately, PS6K remained an independent predictor of locoregional recurrence. In the univariate analysis, p53 overexpression was also associated with an increased risk of locoregional recurrence (HR 1.85, P = 0.027); however, this was not the case for the subset of patients who underwent breast-conserving therapy (data not shown). In addition, 5-year follow-up results concerning the impact of p53 and PS6K status on locoregional control
Table 5  Pts who underwent breast-conserving therapy

| Patient characteristics | N = 368 | Overall survival | Locoregional recurrence (first event) | Distant disease-free survival |
|-------------------------|---------|-----------------|--------------------------------------|-----------------------------|
|                         |         | Number of events |                                     |                             |
| Tumour diameter (N (%)) |         |                 |                                      |                             |
| ≥2 cm                   | 249 (68)| 32 (13)         | 38 (15)                              | 59 (24)                     |
| >2 cm                   | 100 (27)| 27 (27)         | 15 (15)                              | 36 (36)                     |
| Unknown                 | 19 (5)  | 6 (32)          | 5 (26)                               | 7 (37)                      |
| Histologic tumour type (N (%)) | | | | |
| Infiltrating ductal     | 260 (71)| 48 (18)         | 41 (16)                              | 73 (28)                     |
| Infiltrating lobular    | 22 (6)  | 2 (9)           | 7 (32)                               | 3 (14)                      |
| Other                   | 79 (21) | 13 (16)         | 8 (10)                               | 23 (29)                     |
| Unknown                 | 7 (2)   | 2 (29)          | 2 (29)                               | 3 (43)                      |
| Histologic tumour grade (N (%)) | | | | |
| I                       | 124 (34)| 6 (2)           | 19 (15)                              | 21 (17)                     |
| II                      | 121 (33)| 18 (21)        | 25 (21)                              | 40 (33)                     |
| III                     | 107 (29)| 30 (28)        | 11 (10)                              | 37 (35)                     |
| Unknown                 | 16 (4)  | 3 (19)          | 3 (19)                               | 4 (25)                      |
| ER status (N (%))       |         |                 |                                      |                             |
| Positive                | 319 (87)| 52 (16)         | 51 (16)                              | 85 (27)                     |
| Negative                | 38 (10) | 10 (26)         | 4 (11)                               | 13 (34)                     |
| Unknown                 | 11 (3)  | 3 (27)          | 3 (27)                               | 4 (37)                      |
| PgR status (N (%))      |         |                 |                                      |                             |
| Positive                | 268 (73)| 45 (17)         | 45 (17)                              | 77 (29)                     |
| Negative                | 87 (24) | 19 (21)         | 11 (13)                              | 22 (25)                     |
| Unknown                 | 13 (4)  | 2 (15)          | 2 (15)                               | 3 (23)                      |
| HER2 overexpression (N (%)) | | | | |
| Positive                | 312 (85)| 54 (17)         | 44 (14)                              | 87 (28)                     |
| Negative                | 48 (13) | 9 (19)          | 12 (25)                              | 12 (25)                     |
| Unknown                 | 8 (2)   | 2 (25)          | 2 (25)                               | 3 (36)                      |
| p53 expression (N (%))  |         |                 |                                      |                             |
| Positive                | 290 (79)| 47 (16)         | 42 (14)                              | 77 (27)                     |
| Negative                | 70 (19) | 16 (23)         | 14 (20)                              | 22 (31)                     |
| Unknown                 | 8 (2)   | 2 (25)          | 2 (25)                               | 3 (36)                      |
| Ki-67 (N (%))           |         |                 |                                      |                             |
| Negative                | 175 (48)| 16 (9)          | 32 (18)                              | 33 (19)                     |
| Positive                | 181 (49)| 47 (26)         | 23 (13)                              | 65 (36)                     |
| Unknown                 | 12 (3)  | 2 (17)          | 3 (25)                               | 4 (33)                      |
| PS6K                    |         |                 |                                      |                             |
| Negative                | 322 (88)| 54 (17)         | 45 (14)                              | 83 (26)                     |
| Positive                | 8 (8)   | 7 (23)          | 10 (33)                              | 13 (43)                     |
| Unknown                 | 16 (4)  | 4 (25)          | 3 (19)                               | 6 (38)                      |

Table 6  Univariate analyses (Pts who received breast-conserving therapy n = 368)

| Marker | Locoregional control | Distant disease-free survival | Overall survival |
|--------|-----------------------|------------------------------|------------------|
|        | Hazard ratio | 95% CI | P-value | Hazard ratio | 95% CI | P-value | Hazard ratio | 95% CI | P-value |
| PS6K+  | 2.83        | 1.42–5.62 | 0.003 | 1.80        | 1.00–3.23 | 0.049 | 1.44        | 0.66–3.18 | 0.360 |
| ER+    | 1.22        | 0.44–3.38 | 0.701 | 0.65        | 0.36–1.16 | 0.146 | 0.54        | 0.27–1.06 | 0.071 |
| PgR+   | 1.29        | 0.66–2.48 | 0.461 | 1.09        | 0.68–1.75 | 0.728 | 0.77        | 0.45–1.33 | 0.346 |
| HER2+  | 1.79        | 0.94–3.39  | 0.075 | 0.86        | 0.47–1.57 | 0.617 | 1.07        | 0.53–2.16 | 0.862 |
| Ki-67+ | 0.86        | 0.50–1.47  | 0.582 | 2.31        | 1.52–3.52 | <0.0001 | 3.40        | 1.93–6.02 | <0.0001 |
| Grade  | I vs I      | 0.58 | 0.87–2.86 | 0.136 | 2.18        | 1.29–3.70 | 0.004 | 4.94        | 2.03–12.01 | 0.0004 |
|        | II vs II    | 0.86 | 0.41–1.80 | 0.683 | 2.52        | 1.47–4.30 | 0.0007 | 7.12        | 2.96–17.11 | <0.0001 |
| Diameter | R>2 cm    | 1.14 | 0.63–2.07 | 0.670 | 1.72        | 1.14–2.60 | 0.011 | 2.24        | 1.34–3.75 | 0.002 |
| p53+   | 1.47        | 0.81–2.70 | 0.209 | 1.20        | 0.74–1.92 | 0.460 | 1.44        | 0.82–2.54 | 0.209 |
Table 7 Multivariate analyses (Pts who received breast conserving therapy n = 368)

| Marker                        | Risk ratio | 95% CI       | P-value |
|-------------------------------|------------|--------------|---------|
| Locoregional recurrence       | 2.83       | 1.42–5.62    | 0.003   |
| Distant disease-free survival |            |              |         |
| PS6K+                         | 1.54       | 0.83–2.88    | 0.174   |
| Grade III vs I/I              | 1.17       | 0.83–1.64    | 0.369   |
| Diameter > 2 cm               | 1.24       | 0.78–1.98    | 0.365   |
| Overall survival              |            |              |         |
| K67+                          | 2.01       | 0.96–4.22    | 0.066   |
| Grade III vs I/I              | 1.63       | 1.04–2.53    | 0.032   |
| Diameter > 2 cm               | 1.44       | 0.83–2.48    | 0.194   |

Several studies have examined the relationship between PS6K overexpression and local breast tumour recurrence. A case–control study of 66 women with local breast tumour relapse following lumpectomy and radiation therapy showed that PS6K overexpression was an independent predictive factor for ipsilateral breast tumour recurrence (IBTR) (Noguchi et al, 1997). Recent studies conducted by Turner et al (2000) and Zellars et al (2000) demonstrated predictive significance of PS6K overexpression for locoregional recurrence in patients who underwent breast-conserving therapy, as well as in patients who underwent mastectomy. Turner and colleagues showed in a matched case-control study comprising 47 cases and 47 controls that overexpression of PS6K had prognostic significance in respect to IBTR following lumpectomy and radiotherapy (P = 0.003). Zellars and co-workers demonstrated in 1530 mastectomy-treated breast cancer patients of whom 259 received adjuvant radiotherapy that PS6K overexpression was independently associated with a significantly increased local failure rate in patients treated with mastectomy, with (RR 2.5, 95% CI 1.1–5.7) or without (RR 1.7, 95% CI 1.2–2.4) radiotherapy. Although, in our series, PS6K lost its prognostic significance in the multivariate analysis, a trend still remained, suggesting worse locoregional recurrence rates in PS6K-overexpressing tumours (RR 1.67, 95% CI 0.95–2.96).

Barlund et al (2000a) analysed RPS6KB1 amplification using FISH in 668 informative primary breast tumours. In all, 9% of the tumours showed amplification of the RPS6KB1 gene. In their series, PS6K was significantly associated with poor survival (P = 0.0021). In addition, the authors analysed overexpression in a subset of 455 primary breast tumours. P70 S6 kinase protein staining of cytoplasm was subjectively scored into four groups: negative (no staining), weak, moderate, or strong staining. For statistical analyses, the data were combined into two groups: low expression (negative or weak staining) and high expression (moderate or strong staining). High expression was seen in 15.6%. There was a statistically significant association between RPS6KB1 amplification and high P70 S6 kinase protein expression (P = 0.0004), with 41% of the amplified tumours (FISH) exhibiting high PS6K expression, and overexpression of PS6K was associated with poor survival (P = 0.0083) as well. Our results suggest an even stronger association between amplification and expression, albeit with not enough data to make a sound statistical comparison. Moreover, the authors found that patients showing both PS6K and HER2 amplification had a significant worse prognosis in terms of breast cancer-specific survival than those with no amplification or amplification of only one of the genes.

These results together with our data suggest that P70 S6 kinase protein overexpression may be an important predictor of not only worse survival but also of poor locoregional control.

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