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Prognostic Significance of pS2 mRNA in Breast Cancer

S.J. Wysocki, B.J. Iacopetta and D.M. Ingram

The oestrogen-inducible pS2 protein has previously been associated with good prognosis for breast cancer patients. In 1987–1988 a series of 145 primary breast cancers were examined for pS2 mRNA using northern blots. On recent examination of mortality data, we were unable to find any association between tumour pS2 positivity and patient survival. One patient in 6 died within 5 years of surgery, regardless of pS2 status. In the oestrogen receptor positive/progesterone receptor positive tumour subgroup of patients, we found no evidence of increased survival for pS2-positive tumours. These results do not support use of pS2 as an indicator of increased survival in an average breast cancer patient population.
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INTRODUCTION

THE IDENTIFICATION of a gene coding for an oestrogen-inducible protein termed pS2 in breast cancers led to anticipation that expression of the pS2 gene would help in the identification of patients likely to respond to hormone therapy [1]. Preliminary investigations supporting this experimental approach have been published [2–4], although more recent data are less supportive of pS2 as a predictor of endocrine response [5]. A large retrospective study demonstrated that the measurement of pS2 protein in breast cancers provided independent prognostic information about patient outcome [6]. In accordance with this finding, high pS2 gene expression was shown to be an indicator of good prognosis for breast cancer patients [7]. However, the value of pS2 estimation in breast cancers has been challenged by several groups [8–10] and is now somewhat controversial. The latter studies all used immunohistochemistry, whereas investigators supporting pS2 estimation used an immunoradiometric assay of pS2 protein and northern blot analysis of pS2 mRNA. The question still remains as to whether differences in methodology are responsible for inconsistent results regarding the usefulness of pS2 quantitation in breast cancers. In the present study, we have correlated pS2 mRNA in breast cancers with patient survival at 5 years after surgery.

Correspondence to S.J. Wysocki.
The authors are at the Departments of Surgery, University of Western Australia, Fremantle Hospital, Fremantle, 6160 and Queen Elizabeth II Medical Centre, Nedlands 6009, Western Australia, Australia.
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PATIENTS AND METHODS

A total of 150 primary breast cancers were collected from Western Australian women who underwent surgical excision of these tumours during the period 1987–1988. 5 of the patients died within 5 years from causes other than disseminated breast cancer and were censored. The average age of the remaining 145 breast cancer patients was 56 years (range 27–88) with 41% of the patients less than 50 years of age. Tumours were frozen in liquid nitrogen prior to quantitation of pS2 mRNA using standard northern blot methodology [11]. A RNA ladder (Gibco BRL, U.S.A.) was used in sizing of bands on gels. Intensities of pS2 mRNA bands were visually assessed and assigned a signal strength on a graded scale from very weak to very strong, taking into account the relative signal for the ubiquitous 36B4 mRNA from the same specimen [12]. Tumours displaying strong and very strong mRNA signals were judged to be positive for pS2 and those with weaker signals were classed as negative. Oestrogen receptor (ER) and progesterone receptor (PR) measurements were carried out using an enzyme immunoassay kit (Abbott Laboratories, Chicago, Illinois, U.S.A.) and expressed in fmol/mg protein. Tumours were ER+ if 4 fmol/mg protein or greater were detected, and PR+ if 10 fmol/mg protein or greater were detected. Patients dying from breast cancer within 5 years of surgery were identified from a Death Register

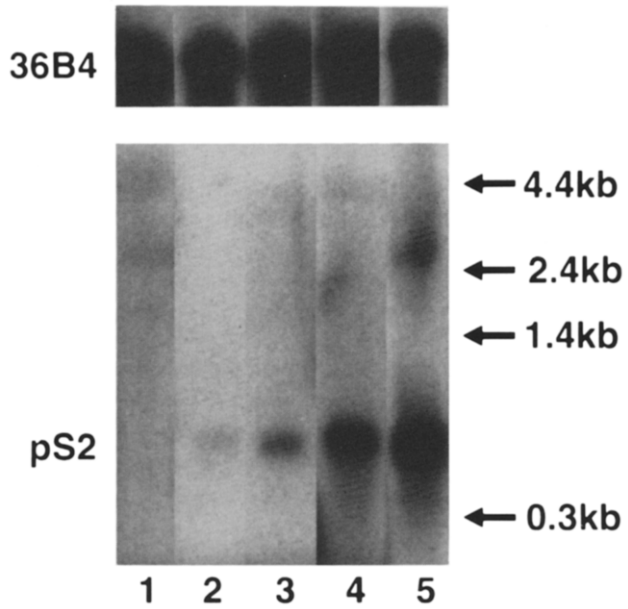

Figure 1. Northern blot analyses of pS2 mRNA in breast cancers. Lane 1: 73 years old, ER not detected (nd), PR nd, pS2 nd, dead at 11 months; lane 2: 39 years, ER nd, PR 5 fmol/mg, pS2 weak, alive at 5 years; lane 3: 48 years, ER 63 fmol/mg, PR 14 fmol/mg, pS2 weak to moderate, alive at 5 years; lane 4: 62 years, ER 22 fmol/mg, PR 215 fmol/mg, pS2 strong, dead at 2 years 7 months; lane 5: 74 years, ER 104 fmol/mg, PR 7 fmol/mg, pS2 very strong, alive at 5 years. Lanes 1–3 were pS2-negative, lanes 4 and 5 were pS2-positive.

supplied by the Registrar General's Office of Western Australia which provides the date and certified cause of death. Statistical analysis was performed using a χ^2 test with Yates correction.

RESULTS

Expression of the pS2 gene in primary breast cancers was quite variable, as shown in Figure 1. We found that 34% (49/145) of tumours from breast cancer patients were positive for pS2 mRNA. This compares with previous reported values of 32% [7] and 49% [1] for pS2 gene expression in primary breast cancers, and demonstrates that the cut-off threshold chosen was within the range used in these earlier studies. When we examined our data to see whether the presence of high pS2 mRNA in the primary breast cancer was an indicator of a good patient prognosis, we found no evidence to support this hypothesis. In our series, approximately 1 in 6 patients with breast cancer died within 5 years of surgery, regardless of pS2 mRNA status in the primary tumour (Table 1). There was, however, a borderline association of poor patient prognosis with ER negativity in the breast cancer ($P < 0.046$). Examination of the correlation

Table 1. Association of pS2 mRNA and ER in primary breast cancers with overall patient survival at 5 years

	pS2 mRNA		ER	
	+	−	+	−
Patients deceased (*n*)	8	15	13	10
Patients alive (*n*)	41	81	96	26
χ^2 test with Yates correction	$P = 0.90$		$P = 0.046$	

*Table 2. Relationship of pS2 mRNA to steroid receptor status in breast cancers: patient deaths in subgroups**

Steroid receptor status ER	PR	pS2 mRNA +	−	Total
+	+	43 (6)	46 (5)	89 (11)
+	−	3 (1)	17 (1)	20 (2)
−	+	2 (1)	7 (3)	9 (4)
−	−	1 (0)	26 (6)	27 (6)

*Number of deceased patients in each subgroup are shown in parentheses.

between pS2 mRNA status and prognosis in the ER+/PR+ subgroup of tumours showed that approximately 1 patient in 7 with an ER+/PR+/pS2+ tumour died within 5 years of surgery, whereas for ER+/PR+/pS2− tumours the figure was 1 patient in 9 deceased (Table 2). We found that pS2 mRNA positivity in ER+/PR+ tumours did not improve overall patient survival at 5 years.

DISCUSSION

The management of breast cancer patients would be somewhat simplified if there was a single test which would identify patients at high risk for disease recurrence and death. Foekens and colleagues appeared to describe such a test when they identified patients with poor prognosis by measuring cytosolic pS2 protein status in primary breast cancers [6]. Their findings were supported by the observation that detection of pS2 mRNA in breast cancers was associated with freedom from recurrent disease [7]. In the latter study, only 1 patient in 25 with pS2 mRNA in their primary tumour had recurrent disease at 31 months. By contrast, we found that 10% of patients (5/49) with a positive pS2 mRNA status in the primary tumour had died from breast cancer disease at 30 months follow-up. Our mortality figures for patients with pS2-negative breast cancers were essentially the same: (9%, 9/96).

The cause of the discrepancy between our results and those of Thompson and colleagues [7] is not obvious to us. Approximately one in three tumours demonstrated positivity for pS2 mRNA in the two series (34 and 32%) suggesting that an inappropriate choice of cut-off threshold was not the reason for the difference in results. We found no prognostic value for pS2 mRNA determination in breast tumours in assessing overall patient survival at 5 years. However, it should be emphasized that we were studying an average population of breast cancer patients with much lower mortality figures than the series of patients studied by Foekens and colleagues (19% uncensored versus 35%). When examining subgroups of patients with ER+/PR+ tumours, we found no evidence of increased survival for patients whose tumours were also pS2 positive. These observations contradict those of Foekens and colleagues who reported that patients with ER+/PR+/pS2+ tumours had a 6-fold increased chance of survival at 5 years compared to those with ER+/PR+/pS2− tumours [6].

Discussion of the usefulness of pS2 mRNA or protein estimation as an indicator for survival of breast cancer patients is hampered by the range of quantitative methods used in relevant studies and lack of patient follow-up. In the current study, we documented 8 patients with breast cancers containing high levels of pS2 mRNA who subsequently died within 5 years of surgery. 2 more patients from our series have recently died from breast

cancer (5 years 2 months, 5 years 6 months), and both had tumours with abundant pS2 gene expression. We, therefore, agree with Cappelletti and colleagues [10] that considerable caution must be exercised before acceptance of pS2 as a prognostic indicator of patient survival in breast cancer, particularly bearing in mind the well-known heterogeneity of these cancers at the cellular level.

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Feature Articles

Cancer and the Heat Shock Response

K.J. Fuller, R.D. Issels, D.O. Slosman, J.-G. Guillet, T. Soussi and B.S. Polla

HEAT SHOCK proteins (HSP) represent one of the most conserved groups of proteins throughout evolution [reviewed in Refs 1 and 2]. They have been found in all organisms examined to date including prokaryotes, yeast and plants, as well as higher eukaryotes. Although first identified in response to heat shock (HS), the wide range of stimuli able to cause induction (including oxidative injury, sodium arsenite, heavy metals, amino acid analogues and serum deprivation) has led to the concept that HSPs are part of a larger group of "stress proteins". Many members of HSP families are also expressed under normal conditions in a cell-cycle dependent manner [2, 3].

Both under normal conditions and in response to stress, HSPs are implicated in protein–protein interactions such as folding, translocation and prevention of inappropriate protein aggregation [1, 4]. Many features of the functions, regulation and expression of HSP suggest they play a role in cancer (Figure 1). In this review, we address the biological aspects of HS, i.e. HSP expression in tumours, their involvement in apoptosis, interactions with proto-oncogenes and *TP53*, as well as their role as tumour antigens and the clinical aspects of HS, such as its use in cancer therapy (hyperthermia).

Correspondence to B.S. Polla in Paris.
K.J. Fuller and B.S. Polla are at the Allergy Unit and D.O. Slosman is at the Nuclear Medicine Division, University Hospital, Geneva, Switzerland; R.D. Issels is at the GSF-Institut für Klinische Hämatologie and Medizinische Klinik III, Universitäts Klinikum, 81377 Munich, Germany; T. Soussi is at INSERM U301, Institute of Molecular Genetics and J.-G. Guillet is at INSERM U152, CHU Cochin Port-Royal, Paris; and B.S. Polla is at the Laboratoire de Physiologie Respiratoire, CHU Cochin Port-Royal, Paris, France.
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THE HS RESPONSE

Introduction: classification of HSPs and possible roles in cancer

The HSPs are classified on the basis of their molecular weight as determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). There are five main families: low molecular weight, hsp65, hsp70, hsp90 and hsp100. Each family is comprised of