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Invited Review

Expression of the oestrogen responsive protein pS2 in human breast cancer

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Summary. The trefoil peptide pS2 was discovered in a breast cancer cell line as a result of its oestrogen responsive character. The expression of pS2 in breast tumours *in vivo* is also likely to be an oestrogenic effect and as such, the presence of pS2 in oestrogen receptor positive breast cancer is evidence of an intact oestrogen response pathway and an indicator of putative hormone responsiveness. Consistent with this, clinical studies of breast cancer have revealed a correlation between pS2 expression and favourable tumour characteristics as well as response to endocrine therapy.

Key words: Breast Neoplasms, pS2-protein-(estrogen induced)

Introduction

Human breast cancer is frequently responsive to the ovarian steroid hormones oestrogen and progesterone and manipulation of the endocrine environment of a tumour can induce regression in some circumstances. Breast cancer is more likely to respond to endocrine therapy if the specific nuclear receptors for oestrogen and progesterone are expressed however there is not a simple relationship between receptor expression and hormone responsiveness as a proportion of receptor positive tumours are resistant to this form of treatment (Horwitz, 1981; McGuire et al., 1991). It is important therefore to identify downstream products of hormone receptor action in order to gain insight into the mechanisms of hormone resistance in breast cancer and to predict more accurately which patients are likely to respond to endocrine therapy. pS2 is a protein whose expression in breast cancer is induced by oestrogenic stimulation of the oestrogen receptor (ER). Although the function of pS2 in the breast and its role, if any, in mediating oestrogen effects is not known, its expression is a useful marker of a functional oestrogen response pathway and therefore potential hormone responsiveness in breast tumors.

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pS2 protein

pS2 belongs to a family of proteins known as trefoil peptides because of their distinctive three loop structure formed by disulfide bonds between six similarly placed cysteine residues (Thim, 1989). Trefoil peptides in humans are principally found in the gastrointestinal tract (Chinery and Coffey, 1996) and pS2 is expressed in normal mucosa of the gastric body and antrum and is also secreted into the gastric lumen (Rio et al., 1988). The role of pS2 in the gastrointestinal tract is uncertain but studies in which the protein was overexpressed in the jejunum of mice suggest it may be involved in mucosal defence (Playford et al., 1996). Adenomatous hyperplasia and malignancy in the stomach of pS2 knock-out mice imply a role in regulating cellular proliferation or even tumour suppression in this site (Lefebvre et al., 1996).

ps2 is encoded by a gene located on chromosome 21q22 (Moisan et al., 1988), close to the gene encoding human spasmolytic protein, another trefoil peptide (Tomasetto et al., 1992). The gene is divided into 3 coding exons (Jeltsch et al., 1987) and the coding sequence is 252 nucleotides in length (Jakowlew et al., 1984). The sequence predicts for a protein of 84 amino acids however features of the amino terminus suggested the presence of a signal peptide (Jakowlew et al., 1984) and subsequent studies proved that 24 amino acids were cleaved prior to export (Nunez et al., 1987; Mori et al., 1988, 1990).

The oestrogen responsive nature of pS2 in human breast cancer

Despite its prominent expression in the gut, pS2 was actually first discovered in breast cancer and it was the oestrogen responsive character of pS2 in this context which brought it to attention.

In 1982 Masiakowski et al. reported experiments where differential hybridisation of a cDNA library

Table 1. Immunohistochemical studies of pS2 expression in breast cancer.

STUDY	INCLUSION CRITERIA	CUT OFF FOR POSITIVITY	n	% POSITIVE
1. Henry et al.,1991	unselected primaries	4%	172	67%
2. Dookeran et al.,1993	unselected primaries	10%	178	77%
3. Detre et al.,1994	unselected primaries	10%	30	43%
4. Hurlimann et al., 1993	IDC only	any positive cells	196	50%
5. Koerner et al.,1992	IDC or ILC only	any positive cells	97	66%
6. Pallud et al.,1993	patients without distant metastases	5%	145	53%
7. Soubeyran et al., 1995	without distant metastases	1%	942	73%
8. Cappelletti et al, 1992	lymph node negative, no distant metastases	5%	200	56%
9. Thor et al.,1992	no distant metastases, no synchronous primaries	5%	279	49%
10. Luqmani et al., 1993	56 primary, 14 metastatic deposits, went on to metastatic or locally advanced disease	staining intensity index of 25	70	36%
11. Schwartz et al., 1991	70 primary, went on to advanced breast cancer. CNS or immeasurable metastases excluded.	10%	70	29%

IDC: infiltrating ductal carcinoma; ILC: infiltrating lobular carcinoma; n: number of patients.

Table 2. Measurement of pS2 in breast cancer by radioimmunoassay.

STUDY	INCLUSION CRITERIA	CUT OFF FOR POSITIVITY (ng/mg protein)	n	% POSITIVE	RANGE (ng/mg protein)	MEDIAN (ng/mg protein)
1. Foekens et al., 1990	unselected primaries	11	205	27	0-274	3.6
2. Stonelake et al.,1994	clinically lymph node negative	1	83	51		
3. Spyratos et al., 1994	without distant metastases	1.9	319	70	0-700	6.4
4. Foekens et al., 1994	patients developed recurrence within follow-up period	<2 2 - 10 >10	230	40 16 44	0-599	7.2
5. Gion et al., 1993	under 75 years without distant metastases	4	446	60		
6. Foekens et al., 1993	without distant metastases	2	710	61	0-773	4.9
7. Correale et al., 1993	unselected primaries	5	100	50	0-215	5
8. Speiser et al., 1994	without distant metastases	2	354	63	0-653	5

n: number of patients.

derived from the MCF-7 breast cancer cell line was performed with cDNA probes prepared from cells grown in normal medium or else medium stripped of steroids. Using this strategy, 4 colonies designated pS1-pS4, containing inserts whose expression was stimulated by steroids, were isolated. The authors considered that these were derived from a single gene and used the colony with the longest insert, pS2, to show that oestrogen alone could increase its expression (Masiakowski et al., 1982). Subsequently others cloned the same gene (Prud'homme et al., 1985; May and Westley, 1986; Manning et al., 1988; Skilton et al., 1989) and it has been referred to variously as pNR-2 (May and Westley, 1986), pLIV-2 (Manning et al., 1988), Md2 (Skilton et al., 1989) and BCEI (Moisan et al., 1988).

The increase in pS2 expression by oestradiol in breast cancer cells *in vitro* is a rapid, direct transcriptional effect (Brown et al., 1984) which can be abrogated by antioestrogens (May and Westley, 1987; Weaver et al., 1988). Its molecular basis has been attributed to a modified oestrogen responsive element in the 5' flanking region of the gene which is identical to the palindromic consensus oestrogen responsive element except for a single base alteration (Berry et al., 1989).

There is evidence from clinical studies of pS2 expression in breast tumours that suggests the protein is induced by oestrogen *in vivo* also. This comes initially from the consistent demonstration of a correlation between the presence of pS2 and the oestrogen receptor (ER) in breast cancer (Rio et al., 1987; Henry et al., 1989, 1991; Foekens et al., 1990, 1993, 1994; Cappelletti et al., 1992; Koerner et al., 1992; Predine et al., 1992; Thor et al., 1992 Correale et al., 1993; Gion et al., 1993; Hurlimann et al., 1993; Manning et al., 1993; Pallud et al., 1993; Thompson et al., 1993; Speiser et al., 1994; Spyratos et al., 1994; Stonelake et al., 1994; Soubeyran et al., 1995). Thus tumours which have the potential to respond to oestrogen are more likely to be

Table 3. Coexpression of ER, PR and pS2 in breast cancer.

	n	ER+ PR+ pS2+ (%)	ER+ PR- pS2+ (%)	ER+ PR+ pS2- (%)	ER+ PR- pS2- (%)	ER- PR+ pS2+ (%)	ER- PR- pS2+ (%)	ER- PR+ pS2- (%)	ER- PR- pS2- (%)
Rio et al., 1987	180	44	4	19	4	0.5	0.5	0	27
Foekens et al., 1990	205	19	7	26	22	0.5	0.5	1	24
Koerner et al., 1992	97	46	15	11	4	0	4	2	16
Gion et al., 1993	446	39	9	18	8	5	3	4	15
Pallud et al., 1993	122	36	12	28	6	0	5	2	12
Correale et al., 1993	100	43	3	17	12	1	3	6	15
Stonelake et al., 1994	83	35	12	19	6	1	2	1	23
Wysoki et al., 1994	145	30	2	32	12	1	1	5	18
Speiser et al., 1994	354	44	10	18	6	4	5	4	11
mean %		37	8	21	9	2	3	3	18

n: number of patients.

pS2 positive. In addition, some authors have reported a positive correlation between the relative quantities of ER and pS2 protein in breast tumours (Rio et al., 1987; Detre et al., 1994; Stonelake et al., 1994) although this has not been a consistent finding (Gion et al., 1993; Manning et al., 1993). Additional evidence of oestrogen induction of pS2 expression *in vivo* is that tumours which are exposed to higher levels of oestrogen, that is in premenopausal women, are more likely to be pS2 positive (Pallud et al., 1993; Spyratos et al., 1994) or else express higher levels of the protein (Henry et al., 1989; Foekens et al., 1990; Predine et al., 1992; Gion et al., 1993).

There is not however a simple relationship between the expression of pS2 and exposure to oestrogen in breast cancer even *in vitro*. In addition to being oestrogen responsive, the 5' flanking region of the gene has constitutive enhancer activity (Berry et al., 1989) and is responsive to epidermal growth factor (EGF), c-Ha-*ras* oncoprotein, c-*jun* and the tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Nunez et al., 1989). Levels of pS2 in MCF-7 cells are increased also by the growth factors EGF, insulin, insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (FGF) (Cavailles et al., 1989) and by the tumour promoter TPA (Cavailles et al., 1989; Nunez et al., 1989). In addition methylation of cytosines in the 5' flanking region of the pS2 gene may be implicated in pS2 expression as these were mostly methylated in the pS2 negative BT20 cell line and unmethylated in pS2 positive MCF-7 cells (Martin et al., 1995). Similarly in clinical studies, despite the well-established relationship between ER and pS2 it is consistently reported that a proportion of ER negative tumours express pS2 (Table 3) and it is possible therefore that factors other than oestrogen are inducing pS2 expression these cases.

The function of pS2 in the breast is not known. Speculation that it may be an autocrine growth factor (Jakowlew et al., 1984) has been refuted by studies in breast cancer cell lines (Davidson et al., 1986; Kida et al., 1989) and also transgenic mice (Tomasetto et al., 1989). However, despite the paucity of information on pS2 function, its importance in breast cancer is a consequence of its oestrogen responsive character as this allows the protein to serve as a marker of ER function and therefore potential hormone responsiveness.

Expression of pS2 in breast cancer

Since its discovery in a breast cancer cell line, the expression and significance of pS2 in clinical breast tumours has been extensively studied. In addition, low level expression in the normal breast (Piggott et al., 1991; Koerner et al., 1992; Predine et al., 1992; Hahnel et al., 1993; Luqmani et al., 1993; Pallud et al., 1993) and relatively high levels in carcinoma *in situ* of the breast have been documented (Inaji et al., 1993; Luqmani et al., 1993; Pallud et al., 1993).

The most commonly used methods for measuring pS2 in breast tumours have been determination of the percentage of pS2 positive cells by immunohistochemical staining of paraffin-embedded tumour sections or radioimmunoassay performed on breast tumour cytosols. In studies where the two techniques have been compared a good correlation has been reported (Robbins et al., 1993; Detre et al., 1994; Soubeyran et al., 1995). In both instances there has been little uniformity in the cut-off level used to designate a case as "positive" and this is likely to contribute to the variation in reported rates of pS2 positivity: in a series of immunohistochemical studies the percentage of tumours regarded as pS2 positive ranged from 29-77% (mean 54.5%) Table 1 and in studies using radioimmunoassay 27-70 % (mean 55%) Table 2.

Immunohistochemical staining of tumour sections for pS2 reveals a distinctive appearance. The protein is located in the cytoplasm and typically there is marked cell to cell variation in staining intensity (Rio et al., 1987). Perinuclear accentuation of staining attributed to accumulation of pS2 in the Golgi complex has been

described (Rio et al., 1987) and in some studies, specific cytoplasmic membrane staining in a proportion of cases has also been documented (Dookeran et al., 1993; Pallud et al., 1993; Soubeyran et al., 1995). Immunoreactive material within glandular structures in tumours has been reported also (Cappelletti et al., 1992; Soubeyran et al., 1995).

Consistent with the relationship between ER and pS2 expression in breast cancer, pS2 is generally associated with pathological features of good prognosis. Thus a number of studies have reported that smaller tumours are more likely to be pS2 positive (Henry et al., 1991; Cappelletti et al., 1992; Hurlimann et al., 1993; Stonelake et al., 1994; Soubeyran et al., 1995) and also that grade is inversely related to pS2 expression (Henry et al., 1991; Predine et al., 1992; Thor et al., 1992; Dookeran et al., 1993; Foekens et al., 1993; Pallud et al., 1993; Speiser et al., 1994; Spyratos et al., 1994; Stonelake et al., 1994; Soubeyran et al., 1995). Cappelletti et al. (1992) reported that tumours with a low proliferation rate were more likely to be pS2 positive; however this has not been corroborated by other studies (Correale et al., 1993; Manning et al., 1993). There is no apparent relationship between pS2 expression and the presence of lymph node metastases (Henry et al., 1989; Foekens et al., 1990, 1993; Schwartz et al., 1991 Predine et al., 1992; Thor et al., 1992; Correale et al., 1993; Dookeran et al., 1993; Hurlimann et al., 1993; Speiser et al., 1994; Spyratos et al., 1994; Soubeyran et al., 1995).

Co-expression of PR and pS2 in breast cancer

Expression of the human progesterone receptor (PR) is induced by oestrogenic stimulation of ER (Clarke, 1993), and the presence of PR in ER positive breast tumours is associated with a higher rate of response to therapeutic endocrine agents (Horwitz, 1981; McGuire et al., 1991). PR has therefore been the archetypal marker of a functional ER and is commonly routinely assayed in breast tumours. The presence of ER and PR in breast cancer are however imperfect predictors of endocrine responsiveness as approximately 25% of tumours which contain both receptors will be clinically hormone resistant (Horwitz, 1981; McGuire et al., 1991). The basis of this receptor positive though hormone resistant phenotype is not known and in this context determination of the combined ER, PR and pS2 profile of a tumour is important if it provides additional insight into receptor function and hormone sensitivity.

Studies which have reported on the relationship between PR and pS2 expression in breast cancer (Foekens et al., 1990, 1993, 1994; Schwartz et al., 1991; Cappelletti et al., 1992; Koerner et al., 1992; Predine et al., 1992; Correale et al., 1993; Dookeran et al., 1993; Gion et al., 1993; Pallud et al., 1993; Detre et al., 1994; Speiser et al., 1994; Spyratos et al., 1994; Stonelake et al., 1994; Soubeyran et al., 1995) have in the main found that the expression of these two proteins is positively correlated (Foekens et al., 1990, 1993, 1994; Cappelletti et al., 1992; Koerner et al., 1992; Predine et al., 1992; Correale et al., 1993; Gion et al., 1993; Detre et al., 1994; Speiser et al., 1994; Spyratos et al., 1994; Stonelake et al., 1994; Soubeyran et al., 1995). As expression of each of these proteins is related to the presence of ER however, it is likely that ER acts as a confounding variable in this relationship and the apparent association between PR and pS2 is a function of the influence of oestrogen up-regulating the two proteins. The only study to take account of this is Koerner et al. (1992) who found that there was a significant relationship between PR and pS2 but that this did not hold true if the analysis was confined to ER positive cases.

Simultaneous measurement of ER, PR and pS2 in breast cancer reveals a complex picture. Despite the fact that both PR and pS2 are oestrogen responsive, it is consistently found that ER positive tumours may express one but not the other protein (Table 3) and if only one protein is present, it is more likely to be PR than pS2. The discordance between PR and pS2 expression implies that oestrogenic stimulation of the two proteins is distinct. Consistent with this hypothesis is the finding that oestrogen up-regulates expression of pS2 but not PR in some tamoxifen resistant varieties of MCF-7 breast cancer cells (Davidson et al., 1986; Lykkesfeldt et al., 1994) and PR but not pS2 in an oestrogen-independent subclone (Cho et al., 1991). It is possible also in these tumours that factors other than oestrogenic stimulation are responsible for the induction of either PR or pS2 in a situation where ER is not functioning and the occurrence of cases which are ER negative but PR and/or pS2 positive testify to this possibility. Taken together however, the evidence suggests that expression of PR and/or pS2 is a marker of a functional ER in breast cancer and tumours which are ER positive but fail to express both proteins may have aberrant responsiveness.

pS2 expression in breast cancer and response to endocrine therapy

The clinical usefulness of pS2 is dependent on the ability of the protein to predict whether a tumour is likely to respond to endocrine therapy. In particular, it is of interest to know whether pS2 status provides additional information if the ER or ER/PR content of a tumour is known.

There are a number of studies which have reported on the relationship between pS2 status and response to endocrine therapy on relapse (Table 4). These have returned largely inconsistent results. The studies are retrospective and in the main, patient numbers are small. In some reports the well-established relationship between ER/PR expression and endocrine response did not hold true (Henry et al., 1991; Schwartz et al., 1991), calling into question the reliability of conclusions made in these studies about the predictive value of pS2.

In the largest study to date, Foekens et al. (1994) examined pS2 expression by radioimmunoassay in

Table 4. Summary of studies examining tumour pS2 expression and patient response to endocrine therapy at relapse.

STUDY	n	pS2 EXPRESSION vs ENDOCRINE RESPONSE	COMMENTS
1. Henry et al., 1991	35	yes	No correlation between ER and response
2. Skilton et al., 1989	21	yes	ER correlated with response
3. Schwartz et al., 1991	72	yes, however the relationship was not significant in a multivariate analysis including ER and PR	No correlation between ER/PR and response No relationship between ER and pS2
4. Luqmani et al., 1993	56 primary 14 metastatic	No	ER/PR correlated with response
5. Foekens et al., 1994	230	No	ER/PR related to response

n: number of patients.

tumours from 230 patients who developed recurrent disease during follow up and were treated with hormonal therapy. In this study, tumours with the highest level of pS2 had a higher rate of response than those with lower pS2 content but this difference was not statistically significant. In a multivariate analysis, pS2 status was not an independent predictor of progression-free survival but did confer a significant advantage to patients whose tumours contained only intermediate levels of ER and PR. It was concluded that knowledge of pS2 expression may contribute refinement of clinical information provided by measurement of receptors (Foekens et al., 1994). Three studies have reported improved survival associated with pS2 expression in patients given adjuvant endocrine therapy (Predine et al., 1992; Spyratos et al., 1994; Stonelake et al., 1994).

In postmenopausal women given tamoxifen as neoadjuvant treatment a correlation between tumour pS2 expression and response has also been reported (Wilson et al., 1994; Soubeyran et al., 1996). In the study by Soubeyran et al. (1996) immunohistochemical staining for ER and pS2 were the only independent predictors of response in a multivariate analysis although in this report ER expression was not related to PR or pS2 suggesting that the cohort studied may not have been typical of breast cancers in general (Soubeyran et al., 1996).

Current evidence does therefore favour a relationship between pS2 expression in breast tumours and response to endocrine therapy and reinforces the evidence that pS2 is an indicator of ER function in human breast cancer.

pS2 as a prognostic indicator

There has been considerable interest in whether or not pS2 expression in breast cancers may be a useful predictor of survival. Its association with ER and some other favourable prognostic features suggests *a priori* that it is more likely to be expressed in tumours of patients who do well, and demonstrates the need for multivariate analysis including all of these factors to determine whether pS2 provides additional information.

A number of studies have reported that pS2 expression confers a survival advantage (Foekens et al., 1990, 1993; Gion et al., 1993; Thompson et al., 1993; Spyratos et al., 1994), however others have not observed this relationship (Henry et al., 1991; Thor et al., 1992; Dookeran et al., 1993; Hurlimann et al., 1993; Speiser et al., 1994; Wysocki et al., 1994; Soubeyran et al., 1995). In addition, in ER negative tumours, one group have reported an association between pS2 positivity and higher likelihood of relapse (Cappelletti et al., 1992).

It is likely that methodological differences may be contributing to this inconsistency as most studies which have used radioimmunoassay to measure pS2 have reported a survival advantage associated with presence of the protein (Foekens et al., 1990, 1993; Gion et al., 1993; Spyratos et al., 1994), whereas immunohistochemical studies have not corroborated this finding (Henry et al., 1991; Thor et al., 1992; Dookeran et al., 1993; Hurlimann et al., 1993; Soubeyran et al., 1995). It is not clear why radioimmunoassay and immunohistochemistry should reveal different associations between pS2 expression and prognosis. One potential explanation is that pS2 positive cells would be diluted by those which were pS2 negative in cell extracts used for radioimmunoassay, with consequent lowering of the sensitivity. It is notable in this regard that the median values of pS2 measured by radioimmunoassay was close to the limit of detection in a number of published studies (Table 2). More pertinent to the issue of why the studies reveal different associations between pS2 expression and prognosis is the length of follow-up which tended to be longer in studies which reported on immunohistochemical measurement of pS2 than those where radioimmunoassay was used.

Whether or not pS2 expression in breast cancer is an independent marker of good prognosis is therefore unclear at the present time. On balance it seems that technical aspects of pS2 measurement can influence the value of pS2 as a prognostic indicator and hence if there is a relationship between pS2 expression and prognosis in breast cancer, it is not a strong one.

Conclusion

pS2 is of proven value as a marker of oestrogen responsiveness *in vitro* and similarly has a role in clinical studies of ER function in breast cancer. Its utility as an additional marker of putative hormonal sensitivity

in breast cancer management is supported by the published evidence although the value of pS2 as a prognostic indicator is less certain. Results of future studies on the function of pS2 in the breast and its role in mediating oestrogenic effects are likely to provide useful insight into the mechanisms of hormonal action in breast tumours and means by which these can be intercepted to therapeutic effect.

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