

Detection of pS2 Messenger RNA in Gynecological Cancers

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ABSTRACT

Estrogen-inducible pS2 mRNA was previously detected in human cancer cell lines the growth of which was sensitive to estrogen. In the present study, the expression of the pS2 gene was analyzed in 111 gynecological carcinomas. The pS2 message was detected in greatest abundance in 6 primary carcinomas of the ovary (6 of 29), 4 of these being mucinous cystadenocarcinomas. A secondary carcinoma of the ovary, and another of the omentum (1 of 4), also contained detectable levels of pS2 mRNA. Weak pS2 mRNA signals were occasionally observed in endometrial (2 of 55) and cervical carcinomas (2 of 33) as well. There was a poor correlation between estrogen receptor and pS2 mRNA in ovarian carcinomas.

INTRODUCTION

The pS2 cDNA² was first cloned from a human breast cancer cell line, MCF-7 (1). Expression of the pS2 gene in this cell line is under the direct control of estradiol at the level of transcription (2) and therefore provides a model of estrogen regulation of gene expression in human tissues. The function of pS2 protein remains unknown (3), although its primary structure has been elucidated (4). It has extensive homology with pig pancreatic spasmodic polypeptide and shares immunological properties with human epidermal growth factor (5, 6). On the basis of experiments demonstrating that pancreatic spasmodic polypeptide has growth-stimulating properties on colon and breast cancer cell lines (7), it has been suggested that pS2 protein may be a member of a new family of growth factors (8). In an extensive study of estrogen-regulated mRNAs in human breast cancer cells, pS2 mRNA was one of 3 RNAs to be detected only in estrogen-responsive cell lines (9). These data, together with results of analyses of pS2 mRNA in breast tumors (10), indicate that measurement of pS2 mRNA is likely to provide a useful marker of estrogen responsiveness of breast cancers.

Cancers of the ovary and the breast appear to share common causative determinants, possibly of a hormonal nature (11). For example, women with breast cancer are twice as likely to subsequently develop a separate primary cancer of the ovary, and women with a cancer of the ovary are 3-4 times more likely to subsequently develop a separate primary cancer of the breast (12). Bearing in mind that hormones, particularly estrogens, may play a role in the genesis of some gynecological cancers, we have examined a range of gynecological cancers for the presence of pS2 mRNA. In addition, we have measured ER and PR concentrations in these tumors.

MATERIALS AND METHODS

Samples. Carcinoma specimens were frozen in liquid nitrogen as soon as possible after excision and stored in a -70°C freezer until use.

Received 7/14/89; revised 11/8/89.

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² The abbreviations used are: cDNA, complementary DNA; ER, estrogen receptor; PR, progesterone receptor; SDS, sodium dodecyl sulfate; SSC, standard saline citrate; 0.15 M NaCl, 0.015 M sodium citrate; EGF, epidermal growth factor.

Plasmid DNAs. The recombinant plasmid DNAs used in this study, pS2 and 36B4, were provided by P. Chambon. 36B4 mRNA is ubiquitous in human tissues and is used as an internal control.

RNA Extraction and Northern Blot Analysis. Tissue powder prepared by microdismembration (Braun Micro-dismembrator II, 10 s) of about 200 mg of sliced, frozen gynecological carcinoma tissue (-70°C) was extracted using a guanidinium isothiocyanate procedure (13) to yield a total RNA preparation. RNA preparations were dissolved in 0.5% SDS (10-50 µl), and aliquots (17-50 µg RNA) were glyoxalated (14) prior to electrophoresis (1.2% agarose, 10 mM sodium phosphate, pH 7.0, 2 mg/ml sodium iodoacetate) and capillary blotting to Zeta-Probe membranes using 5 mM NaOH (15). Membranes were hybridized using a filter paper sandwich technique. The hybridization solution consisted of 50% deionized formamide, 2x(0.18 M NaCl, 10 mM sodium phosphate, pH 7.7, 1 mM EDTA), 7% SDS, 0.5% bovine lacto transfer technique optimizer or Carnation instant nonfat dry milk, 1% polyethylene glycol 20,000, and 0.5 mg/ml denatured salmon sperm DNA. DNA probes, pS2 and 36B4, were labeled with [α -³²P]dCTP by nick translation (16) using a modified method which enabled a high efficiency of label incorporation.³ Probe specific activity was 2 x 10⁸ dpm/µg DNA, and the concentration of labeled cDNA in the hybridization solution was 40 ng/ml. Prior to addition to hybridization solution, labeled cDNA probes were partially acid hydrolyzed (3 µl 4 M HCl added to reaction mixture, room temperature incubation, 10 min) and then alkali denatured (9 µl 4 M NaOH, 10 min). Hybridizations were at 50°C overnight. Membranes were washed briefly in 2x SSC, then shaken for 15 min in 2x SSC, 0.1% SDS at room temperature, followed by a further shaking in 0.2x SSC, 1% SDS at 65°C and a final rinse in 0.5x SSC, 0.1% SDS. Washed membranes were then exposed to Kodak X-Omat AR film in a Kodak X-Omatic regular cassette with intensifying screens at -70°C. The initial hybridization of a blotted membrane was with ³²P-labeled pS2 cDNA. Membranes were then stripped and reused to hybridize with labeled 36B4 DNA. To strip, membranes were treated twice for 20 min with 100 ml 0.1x SSC, 0.1% SDS which had been brought to a boil. Autoradiography following hybridization with pS2 cDNA was for 3-4 days and 1 day after hybridization with 36B4 cDNA. Relative intensities of mRNA bands were visually assessed and assigned a signal strength on a 5-grade scale (very weak, weak, moderate, strong, and very strong) taking into account intensities of bands for the ubiquitous 36B4 mRNA.

Assay of ER and PR. ER and PR were estimated by a dextran-coated charcoal steroid-binding assay (17) or by a solid phase enzyme immunoassay (Abbott Laboratories, Diagnostic Division, Chicago, IL).

RESULTS

Presence of pS2 mRNA in Gynecological Carcinomas. Total RNA extracts were isolated from 111 gynecological carcinomas and analyzed by Northern blotting. Examples of autoradiograms are shown in Fig. 1. A single pS2 mRNA band (0.6 kilobases) of varying intensity was clearly visible in a number of gynecological carcinomas (*Lanes 1, 4, 5, and 7*). Details of the carcinomas in which pS2 mRNA has been found are presented in Table 1. There were 6 mucinous cystadenocarcinomas of the ovary in our series (6 of 29), and 4 of these contained pS2 mRNA. Levels of pS2 mRNA in these 4 tumors were higher than those generally observed for other pS2 mRNA-positive gynecological carcinomas. By contrast, pS2 mRNA was demonstrated in only 1 of 14 serous cystadenocarcinomas

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Fig. 1. Northern blot analyses of pS2 and 36B4 mRNA in various gynecological and breast carcinomas. *A* and *B* were generated by overlaying 2 autoradiography films. In *A* registration of 2 films has been offset in order to prevent the faint pS2 bands from being obliterated by degraded 36B4 mRNA. The true position of mRNA bands relative to each other is shown in *B*. The sources of the RNA preparations were as indicated: *Lane 1*, carcinoma of the cervix; *Lanes 2 and 3*, carcinomas of the breast; *Lane 4*, mesonephroid carcinoma of the ovary; *Lane 5*, adenocarcinoma of the omentum; *Lane 6*, carcinoma of the breast; *Lanes 7 and 8*, mucinous cystadenocarcinomas of the ovary; *Lane 9*, serous cystadenocarcinoma of the ovary; *Lane 10*, carcinoma of the breast. Further details are shown in Table 1. *kb*, kilobases.

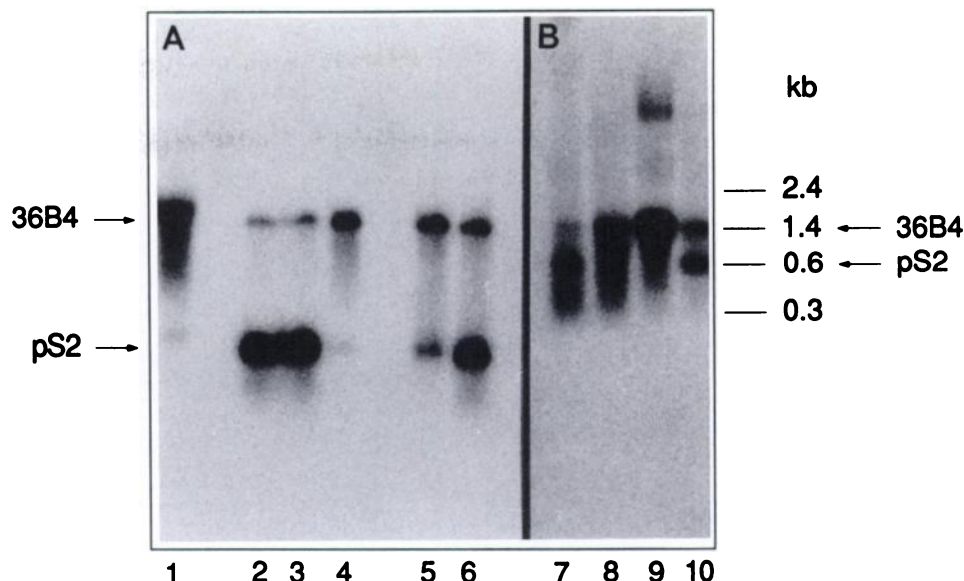


Table 1 Features of gynecological carcinomas containing pS2 mRNA

Details of other carcinomas whose RNA extracts were run in Fig. 1 are shown in the lower section. Receptor values are in fmol/mg protein.

Patient code	Location of tumor	Lane no. in Fig. 1	RNA (μ g)	Histological characteristics	ER	PR	Intensity of pS2 band
Fs	Cervix	1	25	Small cell, nonkeratinizing squamous cell carcinoma with adenocarcinoma <i>in situ</i>	ND ^a	ND	Weak
Hn	Ovary	4	23	Mesonephroid, poorly differentiated	16 ^b	8 ^b	Weak
Fn	Omentum	5	23	Adenocarcinoma secondary of unknown origin	6	ND	Moderate
Ti	Ovary	7	17	MCAC, grade I	ND ^b	ND ^b	Moderate to strong
Fe	Ovary	19	19	MCAC	4	ND	Strong
Mn	Ovary	19	19	MCAC, well differentiated	ND ^b	ND ^b	Moderate
Cy	Ovary	46	46	MCAC, poorly differentiated	ND ^b	ND ^b	Weak to moderate
Bl	Ovary	47	47	SCAC, grade II	181	ND	Weak
Wn	Ovary	16	16	Adenocarcinoma secondary from colon, well-differentiated	ND	ND	Very weak
Hh	Cervix	50	50	Large cell, nonkeratinizing squamous cell carcinoma	93	41	Very weak
Re	Endometrium	23	23	EAC, grade II	132	252	Very weak
Cy	Endometrium	50	50	EAC, grade II	20	ND	Very weak
Ft	Breast	2, 3	23	IDCB	245 ^b	198 ^b	Very strong
Mr	Breast	6	20	IDCB	305 ^b	96 ^b	Very strong
Se	Ovary	8	50	MCAC	65 ^b	255 ^b	ND
Ml	Ovary	9	50	SCAC	123	ND	ND
Ee	Breast	10	23	IDCB	>500	16	Moderate to strong

^a ND, not detected; MCAC, mucinous cystadenocarcinoma; EAC, endometrial adenocarcinoma; IDCB, infiltrating ductal carcinoma of the breast; SCAC, serous cystadenocarcinoma.

^b Dextran-coated charcoal steroid-binding assay. Other determinations of ER and PR were by the Abbott kit method.

(14 of 29), and the pS2 signal in this tumor was quite weak. A weak pS2 mRNA signal was also displayed by 1 of the 2 mesonephroid carcinomas in our study. We detected pS2 mRNA in 2 of 23 cervical carcinomas examined. One cervical carcinoma with a slightly stronger pS2 mRNA signal was a squamous cell carcinoma with an adenocarcinoma *in situ*. Like Rio *et al.* (10), we have had difficulty finding pS2 mRNA in endometrial carcinomas, observing only a very faint signal for this message in 2 of 55 of these tumors. Two carcinoma secondaries contained detectable pS2 mRNA. One was an ovarian carcinoma probably originating from a primary colonic carcinoma, and the second was a omental metastasis of unknown origin.

Lack of Correlation of ER and pS2 mRNA. Unexpectedly, 3 of the mucinous ovarian carcinomas which expressed the pS2 gene lacked ER (Table 1, patients Ti, Mn, and Cy). Two additional primary carcinomas of the ovary with detectable levels of pS2 mRNA (patients Hn and Fe) had fairly low levels of ER (<20 fmol/mg). In all 5 of 6 pS2 mRNA-positive primary carcinomas of the ovary lacked the capacity to bind estradiol or

did so very weakly. Previous studies had shown that up to 50% of ovarian cancers demonstrated a high capacity to bind estradiol (18, 19). In our own series 17 of 29 (59%) ovarian tumors had high levels of ER (>20 fmol/mg) which is in good agreement with earlier observations (18, 20). In addition to the primary carcinomas of the ovary, a cervical carcinoma (patient Fs) and an ovarian metastasis from a colonic carcinoma (patient Wn) also demonstrated a weak pS2 mRNA signal in the absence of ER.

DISCUSSION

Earlier work indicated that pS2 gene expression was confined to a breast cancer cell line, MCF-7, and to some breast cancers (21). Further study extended distribution of pS2 mRNA to lymph node metastases derived from primary breast tumors (10). The discovery that pS2 protein was secreted by normal stomach mucosa demonstrated that the protein was not exclusive to breast cancers and metastases (3). Human gastric cancer cell lines also secreted large amounts of a protein with immunological properties similar to those of pS2 protein (6). The

present study extends the known distribution of pS2 gene expression to ovarian cancers and, less frequently, to cancers of the cervix and endometrium. Highest levels of pS2 mRNA were detected in 4 of 6 ovarian cancers with a histological type designated as mucinous cystadenocarcinoma. Mucinous ovarian carcinomas are generally 3–4 times less common than serous ovarian carcinomas (22). It is intriguing that pS2 gene expression was associated with mucin-producing ovarian carcinomas bearing in mind that pS2 protein has been detected in stomach mucosa and in salivary glands (3), both of which produce mucin. In agreement with earlier reports (3, 21), we were unable to detect pS2 mRNA in normal ovarian, cervical, and endometrial tissues.

The expression of the pS2 gene in stomach mucosa must be estrogen independent since no ER could be detected in mucosal cells (3). The lack of correlation between pS2 mRNA and ER in mucinous cystadenocarcinomas reported in this study resembles findings observed for stomach mucosa. The overall poor correlation of these parameters in gynecological cancers is in sharp contrast to the high degree of correlation observed for breast carcinomas (10, 23). An explanation as to how estrogen-independent expression of the pS2 gene can occur was provided by the demonstration that epidermal growth factor (23, 24) and molecules involved in the signal transduction of growth factors (23) all stimulated pS2 mRNA production by MCF-7 cells. A similar increase in expression of the pS2 gene by MCF-7 cells was obtained when cells were treated with insulin (24). The binding of insulin and EGF to their respective receptors leading to tyrosine-specific phosphorylation of proteins (25, 26) may contribute in turn to a common signal-transduction pathway linking the receptor-bound ligands to pS2 gene expression.

Although the regulation of the pS2 gene has proved to be more complex than originally thought, the involvement of an estrogen-dependent mechanism prior to a response to oncogenes or growth factors may still operate in breast carcinomas (23). However, the data obtained in the present study are not in accord with an ER-mediated mechanism being of prime importance to pS2 gene expression in ovarian carcinomas. Should this finding be confirmed, it follows that the presence of pS2 mRNA is unlikely to provide a direct marker of estrogen responsiveness of ovarian cancers. Since many ER- ovarian tumors contain EGF receptors (27), it is possible that EGF is responsible for pS2 mRNA production by ovarian tumors. It remains to be seen whether the detection of pS2 mRNA in gynecological cancers is of prognostic significance.

ACKNOWLEDGMENTS

We thank Professor P. Chambon, Institut de Chimie Biologique, Faculté de Médecine, Strasbourg, France, for donation of pS2 and 36B4 cDNAs. We also thank Dr. Simon Knowles for permission to use histological data, Dr. Steven Wilkinson for helpful discussion of the manuscript, and Ella Twaddle for receptor assays.

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