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Oestrogen Receptor Independent Expression of Progestin Receptors in Human Meningioma—a Review

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Human meningiomas are rich in progestin receptors (PR), which are expressed in this tissue in an oestrogen independent fashion. In the search for an explanation of this observation, the existence of a protein in human meningioma cytosol which is capable of binding to a synthetic oestrogen responsive element (ERE) has been demonstrated. Using reverse transcriptase, PCR mRNA encoding for the wild-type oestrogen receptor (ER) was found. In addition, several splice variants of ER mRNA have been identified in human meningioma tissue, including variants lacking exons 4, 5 and 7. We found the ERΔ4 protein to have no transcriptional activity and the ERΔ7 protein reportedly is dominant negative. These mutants therefore probably are not responsible for the autonomous PR synthesis in human meningioma. The ERΔ5 protein, by contrast, has been reported to have oestrogen independent transcriptional activity and it is tempting to speculate that this protein is similar or identical to the ERE binding protein we have found in human meningioma. The role of wild type ER mRNA is presently unclear. Activation of other signal transduction pathways in meningioma does not lead to an increased PR concentration. The promoter area of the meningioma PR gene should be investigated for the possible sensitivity to other transcription factors.

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INTRODUCTION

Meningiomas are tumours which can arise from the arachnoid cells of the leptomeninges. Although the vast majority of meningiomas are histologically benign, their localization within the skull and the concomitant compression of brain tissue makes them potentially lethal. Surgical removal of the tumours is the primary treatment, although this can be extremely challenging in view of the invasion of the tumour in bone, cerebral arteries, venous sinuses or cranial nerves. Surgery thus is curative in 68–80% of patients. Adequate treatment for recurrent meningiomas is not readily available in view of the low sensitivity for ionizing radiation and chemotherapy, but even after partial removal of the tumour patients may manage well for years [1].

Human meningiomas are considered to be potentially hormone sensitive tumours. Several epidemiological findings support this statement. Firstly, the incidence of meningioma is considerably higher in women than in men [2]. Secondly, meningiomas may aggravate reversibly during pregnancy and in the luteal phase of the menstrual cycle, i.e. in periods of relative progesterone excess [3]. Thirdly, epidemiology suggests that meningiomas and breast cancer are associated and the meningioma are a preferred localization for intracranial breast cancer metastases.

We have been studying the expression of steroid hormone receptors in human meningioma in an attempt to explain the apparent hormone sensitivity of this tumour. The purpose of the present communication was to review our studies in this intriguing area of research.

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STEROID RECEPTORS IN HUMAN MENINGIOMA

In view of the potential hormone sensitivity of meningiomas, many groups have attempted to demonstrate the presence of steroid receptors in cytosols of these tissues. Initially, and with the use of single dose saturation assays, the presence of both oestrogen (ER) and progestin receptors (PR) in human meningioma tissue was reported [4, 5]. Other studies, using more appropriate techniques like Scatchard plot analysis, confirmed the presence of PR but questioned the presence of ER [6]. Today consensus exists with respect to the receptor phenotype of human meningiomas: most tumours contain PR in considerable quantity, but essentially lack ER. The receptor phenotyping of our series of meningiomas is compared to that of a large series of breast cancer specimens in Fig. 1. When ER occur in meningiomas, their concentration is invariably low.

We have scrutinized our experimental techniques, but found no evidence to suggest that the virtual absence of ER from meningioma cytosols could be attributed to resistance of receptors to extraction; occurrence of ER in only a minority of the cells [7]; proteolytic degradation of ER during the experiments; or metabolism of the ligand during the incubation. At that stage, we could not rule out the possible existence of ER with impaired steroid binding, which as a consequence of this impairment would escape detection by the ligand binding techniques used.

Alternatively, the properties of the PR in meningioma cytosol do not differ from that of PR in "classical" oestrogen target tissues like breast and uterus with respect to affinity for the ligand; ligand specificity; nuclear localization; molecular mass; sedimentation in sucrose gradients; and recognition by monoclonal antibodies to the human progestin receptor [8, 9]. Based on these findings we have concluded that PR are expressed in an oestrogen independent way in most meningiomas. The oestrogen independent expression of PR in brain tumours is specific for meningiomas since PR could not be identified in other brain tumours [8]

The presence of PR in meningiomas raised two questions, i.e. (i) can the presence of PR be used in a therapeutic approach to benefit those patients in whom complete surgical removal of the tumour is not possible?; and (ii) how is the expression of these PR triggered?

The approach to the first of these questions has been *in vitro* and *in vivo*. Cell culture studies have shown that primary cultures of human meningiomas respond to the presence of the antiprogestin RU-486 (mifepristone) with a decrease in DNA synthesis [10, 11]. Clinical studies have shown that a number of patients may benefit from treatment with this drug [12, 13]. Although the endocrine treatment of meningioma is not established as well as that of breast cancer, the treatment is slowly gaining popularity.

The second question, i.e. the regulation of PR expression, required a molecular biological approach since at the receptor protein level all possibilities had been explored and new insight into the mechanism of action of steroid hormone receptors was becoming available. We hypothesized that one of the reasons for the apparent autonomous expression of PR could be the presence of a mutant form of the ER, which was incapable of binding its ligand, but would still be able to act as a transcription factor. Such an "ER-like protein" would escape detection by ligand binding assays and immunological assays employing antibodies directed against the steroid binding domain. For such a protein to be transcriptionally active, binding to the estrogen responsive element (ERE) would be a prerequisite.

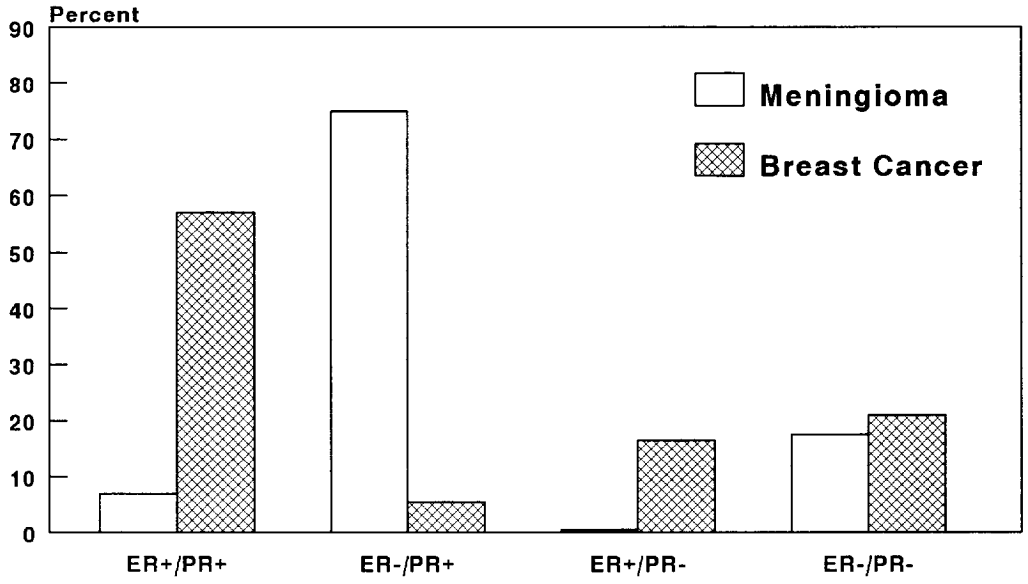

Fig. 1. ER and PR phenotypes of 188 human meningioma and 2647 breast cancer cytosols.

Table 1. Nucleotide sequences for the probes used in the identification of the ERE binding protein in human meningioma [14]. Consensus sequences are underlined. The differences between the ERE and PRE are in bold

ERE: 5′-GATCCGTCAGGTCACAGTGACCTGATCCATC-3′
PRE: 5′-CCAAAGTCAGAACACAGTGTTCTGATCAAG-3′
Sp1 binding site: 5′-CAAAGTCTGGGCGGGCCGATCAAG-3′

IDENTIFICATION OF A SOLUBLE ERE-BINDING PROTEIN

Since a classical ERE was not found in the promoter region of the PR gene and the functional ERE was also not known, we turn to the classical ERE from the *Xenopus* vitellogenin A2 gene. This ERE was synthesized, as were the progestin responsive element (PRE) and the Sp1 binding site consensus sequence. The nucleotide sequences of these probes are given in Table 1. Radioactively labelled ERE was incubated with a high salt extract from meningioma tissue and the reaction mixture was subjected to a band shift assay using polyacrylamide electrophoresis. For comparison, extracts from the MCF-7 human breast cancer cell line and a solid breast cancer specimen were used. Sp1 binding was used to verify the validity of the experiments.

The ERE was found to bind to proteins in all meningioma extracts tested, irrespective of the receptor phenotype of the tissue, as shown in Table 2 [14]. Competition experiments with radioinert PRE demonstrated the specificity of the binding. The nature of the protein binding to the ERE was established as "ER-like" since an antibody directed against an epitope on the A/B domain of the human ER was found to inhibit complex formation. The presence of the ER-like protein *per se* was not sufficient for the induction of PR synthesis since the protein was also detected in the PR negative meningiomas.

ABERRANT ER mRNAs

The second approach we used was to try to identify mRNAs encoding mutant forms of the ER. Reasoning that if the presence of such mRNAs was the cause for the apparent autonomous expression of PR in meningiomas the DNA binding of such mutants would be unaltered and aberrations were to be expected in the regions D through F of the ER, we prepared cDNA from meningioma tissues and amplified this by PCR using primers in exons 2 and 6; and 4 and 8 respectively. Much to our surprise, analysis of the PCR products resulted in identification of a mRNA encoding wild-type ER in both instances [15]. In addition to this wild type mRNA the presence of two aberrant mRNA species was observed. In subsequent experiments these were found to be splice variants, i.e. one lacking exon 4 and one lacking exon 7 [16]. An aberrant ER-mRNA lacking exon 7 had been found to yield a dominant negative protein which thus would suppress ER function [17]. Therefore this mutant could not be taken to be responsible for the autonomous expression PR in meningioma and this variant was not further investigated. The existence of ER mutants lacking exon 4 had not yet been described, although Pfeffer *et al.* [18] and Skipper *et al.* [19] made independent observations of a similar aberrant ER mRNA in human breast cancer cells and lizard brain, respectively.

Table 2. Occurrence of an ERE-binding protein in 0.4 M KCl of human meningioma tissues [14]

ER/PR phenotype	No. with ERE-binding protein/No. tested
ER − /PR −	3/3
ER − /PR +	8/8
ER + /PR +	3/3

The protein translated from a mRNA lacking of exon 4 would be in frame and leave the zinc fingers as well as a large part of the hormone binding domain intact. Such a protein, however, would not bind heat shock protein 90 and defects in hormone binding could be expected based upon deletion of part of the hormone binding domain. The putative properties made this protein a possible candidate as the inducer of PR synthesis in meningioma and therefore we decided to express the protein and to characterize its transcriptional activity.

CHARACTERIZATION OF ER VARIANT MISSING EXON 4

The transactivational properties of the ERΔ4 were investigated by coexpression in P19EC embryo carcinoma and JEG3 human choriocarcinoma cells of ERΔ4 with an oxytocin promoter containing an ERE [20]. Irrespective of the presence of hormones in the culture medium, no transcription activation of the mutant was observed. The wild-type ER, by contrast, clearly showed an oestradiol dependent stimulation of the oxytocin promoter. Cotransfection of wild-type and mutant receptor showed that the mutant is unable to interfere with the action of the wild-type receptor. Following *in vitro* translation, this could be attributed to the fact that the mutant protein, which was recognized on Western blots by anti-ER antibodies H222 and H226, was unable to bind to DNA, as judged by its inability to bind to the *Xenopus* vitelogenin A2 gene ERE, and also did not form heterodimers with the wild type ER. We concluded that the product of the ERΔ4 mutant is a silent variant [20]. It is therefore unlikely that this mutant protein is involved in the autonomous synthesis of PR in human meningiomas.

ALTERNATIVE MECHANISMS FOR PR INDUCTION

Although initially the involvement of ER in PR synthesis was dogmatic [21], it was established later

that PR synthesis is not exclusively under estrogenic control. Activation of other signal transduction pathways was shown to activate PR synthesis in uterine cells [22, 23]. We, therefore, investigated whether an increase in intracellular cAMP or activation of tyrosine kinase of Ca^{2+} and phospholipid dependent protein kinases would have a similar effect in cultured meningioma cells. In agreement with previous results [10], primary cultures from six meningiomas with cytosolic PR concentrations ranging from <15 to 267 were found to lose their PR. The presence of 1 nM epidermal growth factor could not prevent this, indicating that activation of tyrosine kinase dependent protein kinase is incapable of inducing PR synthesis. Also forskolin and TPA, used to increase intracellular cAMP and activate Ca^{2+} and phospholipid dependent protein kinase C respectively, were ineffective in restoring PR synthesis. The validity of the above mentioned experiments has been ascertained by studying effects of the respective agents on the synthesis of pS2 in MCF-7 breast cancer cells. In these cells the presence of oestradiol, forskolin and TPA all increased the cytosolic pS2 concentration. In meningioma cytosols no pS2 was present and no pS2 induction was observed in cultured meningioma cells.

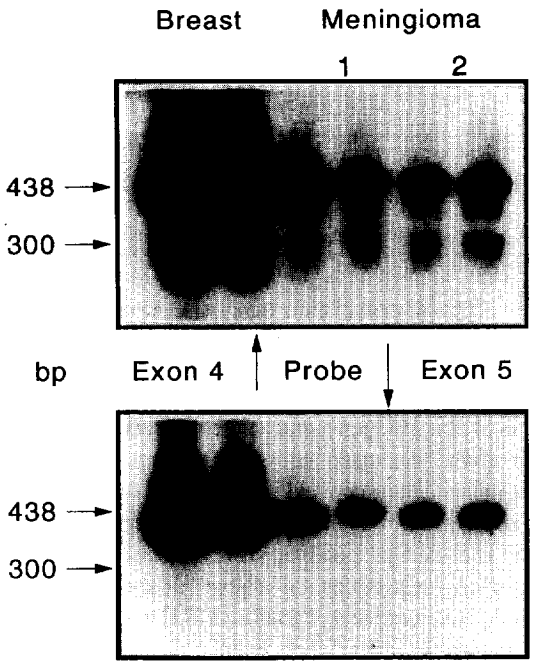

Fig. 2. Expression of ER transcripts in one breast cancer and two human meningioma specimens. cDNA was prepared and exon 5 was amplified with primers in exons 4 and 6. Following electrophoretic separation, the products were hybridized with probes in exons 4 (left) and 5 (right), respectively. Wild-type product is shown at 438 bp. Differential hybridization with probes recognizing sequences in exons 4 and 5 respectively of the product at 300 bp demonstrates the presence of a mRNA product lacking exon 5. All lanes are in duplicate.

EPILOG

Although we and others have been studying the expression of PR in meningioma for many years now, the reason for this phenomenon is still not clear. A number of questions emerge from the above review of our studies. The first and maybe the most pertinent question regards the observation that rt-PCR revealed the presence of mRNA for wild-type ER [15, 16]. At the protein level most meningiomas are ER negative both by ligand binding and immuno(histo)chemistry. We have amplified the ER mRNA encoding amino acids 178–562 and found the sequence of the wild type product to be identical to the published sequence. We, therefore, rule out the possibility that a point mutation in exons 2–8 renders the protein undetectable by the assays used. Alternative explanations which at present cannot be ruled out are a relatively rapid turnover of ER protein in meningioma tissue or a protein concentration below the respective detection limits. Based on the results of experiments in which human meningioma and myometrium cytosols were mixed, we found no evidence for the presence in meningioma of a higher proteolytic turnover. Thus the option of a more rapid turnover of ER in meningioma tissue may not be valid.

The second question emerging is the relationship between the ERE binding protein and the ER mRNA variants identified. It is tempting to speculate that the protein is encoded by one of the mutant mRNAs. The product of ERΔ4, however, was found to be transcriptionally inactive [20], whereas ERΔ7 was reported to be dominant negative [17]. This means that ERΔ7 could actually block low levels of wt-ER. The only ER variant identified so far with a positive effect is ERΔ5 [24]. Only very recently we were able to obtain evidence to suggest that this variant also prevails in meningioma (Fig. 2). It remains to be established whether ERΔ5 encodes the ERE binding protein we have found. This is one of our next challenges.

The third open question relates to the PR gene. If the meningioma PR protein is indeed as normal as we have referred from our studies, why is its synthesis not stimulated by any of the agents we tested? EGF for instance has recently been shown to be able to trigger transcription activation through ER [25]. It is conceivable that the cellular and promoter context of the PR gene in meningioma is not appropriate. Tzukerman *et al.* [26] showed evidence to suggest that TAF-1 is the major transcriptional activator for the ER. The presence of ER mutants with defects in exon 1 and/or the part of exon 2 which we did not yet investigate could be an explanation for this possibility. Finally, mutants like the ERΔ4 might act through novel EREs which only recently started to be identified [27]. As the human PR is devoid of a full consensus ERE the possibility of ER mutants acting through such novel responsive elements should not yet be dismissed.

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