Molecular mechanisms of proliferation in endometrial tumour cells

Ana-Maria Bamberger¹, Christoph M. Bamberger and Heinrich M. Schulte

IHF, Institute for Hormone and Fertility Research, University of Hamburg, Grandweg 64, 22529 Hamburg, Germany

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The human endometrium normally undergoes a cyclic proliferation process followed by differentiation under the influence of ovarian steroids and locally produced growth and differentiation factors. Understanding of the molecular mechanisms involved in controlling these processes is of great interest, since imbalances between proliferation- and differentiation-promoting signals can have pathophysiological consequences ranging from infertility to endometrial hyperplasia and tumour formation. The present work reviews aspects of the role played by oncogenes and ovarian steroid receptors in modulating proliferation of endometrial tumour cells. The expression pattern and possible roles of protein kinase C (PKC) subunits are discussed in the context of response-specificity of endometrial tumour cells to tumour-promoting agents such as 12-O-tetradecanoyl-phorbol acetate (TPA) and possible implications for anti-tumour therapy.

Key words: carcinoma/endometrium/oncogenes/protein kinase C/steroid receptors

Introduction

The human endometrium normally undergoes a cyclic proliferation process followed by differentiation under the influence of ovarian steroids and locally produced growth and differentiation factors (Clarke and Sutherland, 1991; Giudice, 1994). The tight regulation of these processes is essential, and imbalances between proliferation- and differentiation-promoting signals have pathophysiological consequences ranging from infertility to endometrial hyperplasia or even tumour formation (Clarke and Sutherland, 1991).

Endometrial carcinoma is the most frequent invasive malignancy of the female genital tract in developed countries (Kurman, 1987; Marth and Daxenbichler, 1996). Despite this fact, knowledge of the molecular mechanisms involved in the pathophysiology of this tumour is much less compared with other malignancies, such as breast or prostate cancer.

The normally cycling endometrium is both a source of and a target for several growth factors and cytokines which, by activating their receptors, are essentially implicated in the normal proliferation and differentiation of the endometrium required for successful implantation (for a review, see Giudice, 1994). For example, epidermal growth factor (EGF) and insulin-like growth factor I (IGF-I) have been implicated as mediators of oestradiol actions in the uterus (Murphy et al., 1987; Nelson et al., 1991). Oestrogen treatment has been shown to enhance uterine expression of both EGF and EGF receptor, a structural homologue of the c-Erb b oncogene product (Giudice, 1994), and to increase expression of IGF-I and IGF-II mRNA in the uterus (Giudice, 1994). Also, heparin-binding epidermal growth factor-like growth factor (HB-EGF), a member of the EGF family which has been found to mediate the mitogenic effects of oestrogen on the endometrial epithelial cells and of progesterone on stromal cells, has been found to be expressed in the human endometrium throughout the menstrual cycle (Birdsall et al., 1996). Such growth factors, which act normally in the endometrium to regulate proliferation and differentiation can, under certain circumstances, act as oncogenes and/or become targets of overactivation through steroid hormones and transcription factors. In addition, they may induce uncontrolled proliferation, thus playing an important role in the pathogenesis of endometrial cancer (see below; for a review, see Beato, 1996).

¹To whom correspondence should be addressed: Tel: 0049/40/561-908-88; Fax: 049/40/561-908-64
The endometrium is a classical target tissue for ovarian steroids. Both epithelial and stromal cells contain oestrogen receptors (OR) and progesterone receptors (PR) (Press et al., 1988), and the ovarian steroids play an essential role in regulating growth and differentiation of endometrial cells. This influence is partially preserved in endometrial tumours, especially in early and well-differentiated stages which are more frequently receptor-positive than the more advanced stages (Ehrlich et al., 1988; Marth and Daxenbichler, 1996).

**Role of steroid receptors and oncogenes**

The OR and PR belong to the large family of nuclear hormone receptors, which also includes the receptors for other steroid hormones, thyroid hormone, vitamin D, retinoic acid, and for as yet unidentified ligands (‘orphan receptors’) (Evans, 1988; Jensen, 1991; Truss and Beato, 1993; Tsai and O’Malley, 1994). These receptors are ligand-activated transcription factors which share a characteristic three-domain structure: the highly conserved DNA-binding domain (DBD), consisting of two zinc fingers, the N-terminal transactivation domain, and the C-terminal ligand-binding domain. These receptors are located inside the cell, and they can interact at different levels with signalling cascades initiated by membrane receptors which transduce extracellular signals by means of protein phosphorylation by serine/threonine or tyrosine protein kinases, finally modulating the activity of transcriptional regulators within the nucleus (Hill and Treisman, 1995).

Several oncogenes have been suggested to play a role in endometrial cancer (Beato, 1996). Among them are activated Ki-ras, erb-B2 (HER/neu) and the overexpression of the EGF receptor, which correlate with the lack of PR and poor prognosis (Bigsby et al., 1992). Also, suppression of IGF-I binding protein and high expression levels of c-fms mRNA, the receptor for colony stimulating factor (CSF)-1, have been described (for a review, see Beato, 1996). Nuclear proto-oncogenes belonging to the activating protein-1 (AP-1) family of transcription factors are also overexpressed in endometrial cancer cells, especially in response to oestrogen treatment (Sakakibara et al., 1992). The composite transcription factor AP-1 is the prototype of a mitogen-activated trans-activator, and its transcriptional activity is believed to reflect cell proliferation in many tissues (Miller et al., 1984; Schutte et al., 1989; Angel and Karin, 1991). AP-1 is a homo- or heterodimeric DNA-binding protein composed of either two Jun family proteins or one Jun and one Fos family protein (Ransone and Verma, 1990; Angel and Karin, 1991). The activity of this transcription factor complex is modulated by growth factors, cytokines and tumour promoters that activate protein kinase C (PKC), such as the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA) (Ransone and Verma, 1990; Angel and Karin, 1991). The activated AP-1 dimer binds to specific
DNA sequences in the regulatory regions of mitogen-responsive genes, so-called TPA response elements (TRE) (Angel et al., 1987; Lee et al., 1987) (Figure 1).

Because cell proliferation and differentiation are not regulated independently, it is conceivable that factors modulating these processes interact with each other. It has been shown for instance, that the AP-1 transcription factor and the nuclear receptor superfamily interact at different levels (Pfahl, 1993; Hyder et al., 1994). For instance, the ligand-activated glucocorticoid receptor (GR) can suppress AP-1 activity on TRE-dependent promoters (Jonat et al., 1990; Lucibello et al., 1990; Schüle et al., 1990; Yang-Yen et al., 1990). This effect does not require DNA binding by the receptor, and cross-linking studies indicate that it is due to direct protein–protein interactions between the receptor protein and either c-Jun or c-Fos (Yang-Yen et al., 1990). Similar results have been reported for other nuclear receptors, such as the retinoic acid (Schüle et al., 1991; Yang-Yen et al., 1991) and the thyroid receptors (Zhang et al., 1991).

It has been shown that oestrogen induces c-fos mRNA both in rat (Loose-Mitchel et al., 1988; Cicatiello et al., 1992) and human uterus and endometrial cancer cells, as mentioned above (Sakakibara et al., 1992), and this induction has been implicated as a possible mechanism by which oestrogens stimulate growth of endometrial epithelia. The induction could be explained by the presence of an oestrogen response element (ORE) binding site in the c-Fos promoter, which binds both OR and AP-1 (Weisz and Rosales, 1990). OR and AP-1 have also been shown to interact on other promoters, such as the ovalbumin gene promoter, where a half-palindromic ORE that mediates cell-specific trans-activation by the OR was shown to bind Jun and Fos and also to be responsible for induction by TPA (Gaub et al., 1990). Tzuckerman et al. (1991) have shown that in MCF-7 breast cancer cells, in which TPA induces proliferation arrest while oestriadiol induces proliferation, TPA or c-Jun and c-Fos inhibit oestriadiol-dependent OR activity on an ORE-containing promoter. In addition, OR mRNA was downregulated by TPA within 6 h. Gel shift experiments demonstrated that Jun reduced OR binding to DNA, and OR reduced Jun binding to AP-1 sites at excess levels of the antagonizing component. However, this interaction seems to be more complex, because in other cell lines, such as HeLa and CV1 cells, OR did not antagonize AP-1, and low concentrations of OR could even stimulate TPA activation of an AP-1-CAT reporter plasmid (Pfahl, 1993; Tzukerman et al., 1991).

Mutual antagonism between OR and AP-1 was reported by Doucas et al. (1991) in MCF-7 cells, where overexpression of c-Jun of c-Fos proteins but not Jun D inhibited OR activity. These interactions are even more complex at the level of endometrial cells and endometrial tumour cells, where one has to take into account the additional influences played by the progesterone receptor, the promoter of which is induced by OR (Kastner et al., 1990). Regarding induction of AP-1 mRNA by OR, it has been shown that progesterone can antagonize induction of c-fos mRNA by oestradiol in both rat and mouse uterus (Kirkland et al., 1992). The interaction between PR and AP-1 has been less studied in vitro. Shemshedini et al. (1991) investigated the effect of c-Jun and/or c-Fos co-expression on transcription repression by PR, androgen receptor (AR) and GR using three different reporter genes and four different cell lines, and found that actions of the receptors as well as Jun and Fos appear to be cell type-, receptor- and promoter-specific.

Concerning PR, Shemshedini et al. (1991) found that, in HeLa cells, activation of the MMTV promoter by PR can be inhibited by c-Fos, but not by c-Jun. In breast cancer cells bearing endogenous PR it was found that c-Fos inhibits PR activity on GRE-tk–CAT in T47D, but not in MCF-7 cells. Another group found that, in T47D cells, progesterin increased c-jun mRNA levels, but decreased overall AP-1 activity, as measured by transient transfection of an AP-1-regulated reporter plasmid (Alkhalaf and Murphy, 1992). In Ishikawa human endometrial adenocarcinoma cells, progesterin treatment results in growth inhibition (Alkhalaf et al., 1993). Under these conditions, it was found that progesterin decreased c-jun mRNA and protein levels, as well as overall AP-1 activity. In HEC-50 cells, a decrease in c-jun mRNA without a decrease in AP-1 activity was observed. When c-jun was overexpressed in Ishikawa cells by transient transfection, an increase in cell numbers in response to progesterin treatment was observed.

These data indicate that altered c-jun activity can alter the proliferative response to progesterins in endometrial tumour cells. Results in HEC-1B cells also showed that the PR can modulate the induction of AP-1 activity by TPA, and progesterin treatment is followed by a reduction of AP-1 activity in transfection experiments (Bamberger et al., 1996b). These studies indicate the complexity of interactions involving the ovarian steroid receptors with the AP-1 transcription factor and the importance of these interactions in understanding the mechanisms regulating proliferation in steroid-dependent tumours, such as endometrial cancer.

The molecular pattern of response-specificity is rendered even more complex by the cell type-specific expression of the enzymes implicated in activating the AP-1 transcription factor family. PKC, a serine/threonine
kinase, mediates the effects of a large number of hormones, growth factors and cytokines and is thus considered a key factor in the regulation of cellular proliferation and differentiation (Clemens et al., 1992; Würtzner et al., 1995; Kratzeanier et al., 1996). PKC is generally thought to be activated by signal transduction systems that produce diacylglycerol (DAG), such as certain tyrosine kinase and G protein-coupled receptors (Bell, 1986; Clemens et al., 1992; Hug and Sarre, 1993; Dekker and Parker, 1994). PKC is also the major target for the well-known tumour-promoting agent TPA, a phorbol ester which acts in a fashion very similar to DAG (Blumberg et al., 1984; Nishizuka, 1984; Bell and Burns, 1991; Gschwendt et al., 1991). The downstream targets of activated PKC are only partially identified and include other protein kinases (Dekker and Parker, 1994; Y amagushi et al., 1995) and AP-1 (Angel and Karin, 1991; Boyle et al., 1991; Karin, 1995).

The original concept of TPA and thus PKC being purely involved in cellular proliferation was questioned by numerous studies showing cell type-specific inhibition of cell proliferation in response to TPA (Vandenbarg et al., 1984; Exley et al., 1987; Issandou et al., 1988; Choi et al., 1990; Oberg et al., 1991; Misiek et al., 1993; Murray et al., 1993; Bouche et al., 1995). Over the past years, a growing number of PKC isoforms exhibiting specific expression patterns and functional properties have been identified, thus providing a possible molecular explanation for the observed differences in TPA responsiveness (Clemens et al., 1992; Wetsel et al., 1992; Hug and Sarre, 1993; Dekker and Parker, 1994). The PKC family now comprises at least 12 members, which can be divided into two groups (Hug and Sarre, 1993), the Ca²⁺-dependent or conventional PKCs (cPKCs) α, βI, βII and γ, and the Ca²⁺-independent or novel PKCs (nPKCs) δ, ε, ζ, η, θ, τ, λ and μ. cPKCs do not require the presence of Ca²⁺ for activation by phorbol esters; however, Ca²⁺ lowers the concentration of phorbol ester necessary to obtain full activity (Ryves et al., 1991). The members of the nPKC group do not require Ca²⁺ for activation, but need phosphatidylserine as a cofactor, except for PKC ζ which exhibits low but constitutive activator-independent kinase activity (Liyanage et al., 1992; McGlynn et al., 1992).

The α, βI, βII, δ, ε and ζ isoforms seem to be widely distributed (Nishizuka, 1988; Wada et al., 1989; Wetsel et al., 1992; Hug and Sarre, 1993). However, most cell types express only a certain subset of these isotypes (Wetsel et al., 1992; Hug and Sarre, 1993). PKC γ has so far only been found in neural tissues (Nishizuka, 1988; Wada et al., 1989; Wetsel et al., 1992; Hug and Sarre, 1993), where it appears to play a distinct role in the regulation of GABA receptor activity (Harris et al., 1995). Information on PKCs η, θ, τ, λ and μ is as yet very limited.

To study the functional role of differential PKC isoform expression in uterine tumour progression, we have compared the proliferative response to TPA, changes in cell morphology induced by TPA, and the PKC isoform expression pattern in two uterine tumour cell lines of different origin (Bamberger et al., 1996a). The moderately differentiated endometrial HEC-1-B adenocarcinoma cell line showed a marked increase in proliferative activity and a profound morphological change in response to TPA. In contrast, TPA did not induce cell proliferation and/or morphological changes in the well-differentiated SKUT-1-B mixed mesodermal cell line. Analysis of the PKC isoform expression profile by Western blot revealed that PKC α, βI, δ, ε and ζ were expressed at a much higher level in HEC-1-B as compared with SKUT-1-B cells. PKC βI was the only isoenzyme to be expressed equally in both cell lines.

These data indicate that the expression level of PKC isoforms can predict the proliferative response to phorbol esters and, possibly, other mitogenic signals in uterine cancer cells. Cellular proliferation and differentiation may not only depend on overall PKC activity, but also on the expression of certain PKC isoforms. In Chinese hamster ovary (CHO) cells, for instance, overexpression of PKC α and δ induced MAP kinase activity and resulted in the appearance of a transformed, tetrakaryotic phenotype (Yamagushi et al., 1995). PKC α and δ are also the predominant PKC isoenzymes in HEC-1-B cells, which have also been shown to be tetrakaryotic (Kuramoto, 1972).

The observed correlation between TPA-induced proliferative activity and PKC isoenzyme expression in endometrial tumour cells is particularly interesting, since this tissue normally undergoes proliferation and differentiation in a cyclic fashion. Unopposed oestrogen keeps endometrial cells in the proliferative phase, finally leading to the formation of endometrial tumours. The molecular mechanisms underlying these processes are poorly understood. Therefore, it will be interesting to determine the PKC isoenzyme expression pattern in the normal uterus at different points of the cycle. Furthermore, analysing the PKC expression profile in uterine tumours will contribute to our understanding of tumorigenesis in this organ. Another very interesting subject of investigation will be the interactions of PKC isoforms and ovarian steroid receptors in the context of endometrial tumorigenesis. So far, several links between PKC and the OR have been described (Hähnel and Gschwendt, 1995): oestradiol upregulates PKC in the ovary, anterior pituitary
and mammalian tissue, while tamoxifen inhibits PKC. Furthermore, activation of PKC leads to a marked decrease of OR mRNA and protein in human breast cancer cells and other cell lines, and inhibition or downregulation of PKC enhances OR binding. Our data indicate that the PKC expression pattern may predict the proliferative potential of uterine tumours and thus be a useful marker to classify these tumours in a clinical setting.

Conclusions

The molecular and functional heterogeneity of the PKC family make them attractive targets for anti-cancer drug development (Basu, 1993). As isoform-specific PKC activators and inhibitors will become available as therapeutic agents, analysis of the PKC isoenzyme profile in uterine tumours will be essential to establish tumour-specific treatment protocols. Clearly, further studies are necessary to clarify the multiple levels of interaction between the steroid receptors and the PKC/AP-1 pathway and the specific roles played by these interactions in the pathogenesis of hormone-dependent cancers such as endometrial cancer.

References


