

Thymidine phosphorylase expression in normal and hyperplastic endometrium

Efthimios Sivridis, Alexandra Giatromanolaki, Michael I Koukourakis, Roy Bicknell, Adrian L Harris, Kevin C Gatter

Abstract

Aims—To investigate the expression of thymidine phosphorylase (TP), a known angiogenic factor for endothelial cells, in normally cycling endometrium and various forms of endometrial hyperplasia.

Methods—TP expression was assessed with the P-GF.44C monoclonal antibody, using the alkaline phosphatase anti-alkaline phosphatase method. Ninety two normal and hyperplastic endometria were studied.

Results—In normal proliferative endometrium, TP is found exclusively in the basal layer and the inner third of the functionalis; expression is cytoplasmic in glandular epithelium and nuclear in stromal cells. It is invariably patchy. This immunohistochemical picture remains almost unaltered during the early and mid secretory phase of the normal menstrual cycle but, most impressively, TP is expressed uniformly in the epithelium of all endometrial glands towards the end of the cycle. At this stage, expression is mixed nuclear/cytoplasmic and there is very little stromal nuclear staining. In simple endometrial hyperplasia, the staining pattern for TP is identical to normal proliferative endometrium, with a distribution that is usually limited to a few rather weakly proliferating glands and to the adjacent periglandular stroma of the deep endometrium. The distribution is more extensive in complex and atypical endometrial hyperplasias, where a mixed nuclear/cytoplasmic pattern usually prevails over the pure cytoplasmic reaction.

Conclusions—TP is expressed consistently in normal and hyperplastic endometrium, suggesting a role in physiological and pathological angiogenesis. In normal endometrium, TP has a definite pattern of distribution, which is dependent on the phase of the menstrual cycle, whereas in all forms of endometrial hyperplasia the enzyme is randomly distributed and lacks an orderly pattern.

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Keywords: thymidine phosphorylase; normal endometrium; hyperplastic endometrium

Unlike other tissues and organs of the body, human endometrium undergoes cyclical changes, approximately each month, during the reproductive years.¹ These endometrial changes, which are under the control of the ovarian hormones, constitute the menstrual cycle and include the regeneration and growth of the shed endometrium, its maturation and

secretory transformation and, in the case of conceptual failure, disintegration and menstrual bleeding.

The high metabolic activity of the endometrium is ensured by a rich arterial supply. Indeed, an integral part of the menstrual cycle is the phenomenon of angiogenesis—the formation of new blood vessels from a pre-existing vascular network² in the basalis. Angiogenesis is a complex multistep process involving extracellular matrix remodelling, endothelial cell proliferation and migration, capillary differentiation, and anastomosis.³⁻⁴ Factors promoting angiogenesis in the endometrium include basic fibroblast growth factor (bFGF),⁵⁻⁶ transforming growth factor α (TGF- α)⁷ and TGF- β ,⁸ vascular endothelial growth factor (VEGF),⁹ and thymidine phosphorylase (TP), also known as platelet derived endothelial cell growth factor (PD-ECGF).⁴ This enzyme is specifically involved in the reversible dephosphorylation of thymidine to thymine and 2-deoxy-D-ribose-1-phosphate¹⁰ and is an angiogenic factor for endometrial carcinomas.¹¹ The precise mechanism by which TP promotes angiogenesis is not known but the metabolic product 2-deoxy-D-ribose has been shown to stimulate endothelial cell migration.^{4,12} It is possible that 2-deoxy-D-ribose acts on endothelial cells through a cell surface receptor.¹²

TP is widely expressed in normal tissues, including the human endometrium.^{4,13} However, although the morphological and functional changes in the normal endometrium, together with the related fluctuating values of the ovarian hormones and their receptors, have been studied extensively,¹⁴⁻¹⁶ little is known about the immunohistochemical expression of angiogenic factors in this tissue during the various phases of a normal menstrual cycle.¹³ Equally obscure remains their status in relation to endometrial hyperplasias, these being common gynaecological disorders in every day practice.

Our study set out to investigate the cellular and tissue distribution of TP in the normally cycling endometrium and in simple, complex, and atypical endometrial hyperplasias.

Material and methods

Ninety two normal and hyperplastic endometria were studied. These comprised: 21 normal endometria of early (seven), mid (seven), and late (seven) proliferative phase; 21 normal endometria of early (seven), mid (seven), and late (seven) secretory phase; 20 simple hyperplasias, 15 complex hyperplasias, and 15 atypical hyperplasias. The specimens were retrieved from the files of the department of pathology, Democritus University of Thrace. They had

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been fixed in 10% formalin and processed to paraffin wax. Sections were cut at 5 µm thickness and stained with haematoxylin and eosin.

The histologically normal endometria were obtained from premenopausal women, age 32–44 years, who had undergone hysterectomy for non-endometrial disease, usually for uterine leiomyomas. In all cases, the date of the last menstrual period was available. None of the patients had received hormone treatment. The normal endometria had been “dated” on haematoxylin and eosin stained sections using the histological criteria of Noyes *et al.*¹⁴

The endometrial hyperplasias were derived from premenopausal and perimenopausal women, age 33 to 49 years, who had been subjected to hysterectomy for episodic bleeding, often heavy and prolonged. They were classified as suggested by Norris and colleagues¹⁷ and Buckley and Fox.¹⁶

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TP expression was assessed with the P-GF.44C monoclonal antibody⁴ using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method. In brief, sections were dewaxed and rehydrated sequentially in 100%, 95%, 80%, 70%, 50% ethanol and, finally, distilled water. The P-GF.44C monoclonal antibody (undiluted supernatant) was applied at room temperature for 30 minutes and washed in Tris buffered saline (TBS). Rabbit antimouse antibody (1/50; Dako, Glostrup, Denmark) was applied for 30 minutes, followed by mouse APAAP complex (1/1) for 30 minutes. After washing in TBS, the last two steps were repeated for 10 minutes each. The colour was developed by 15 minutes incubation with new Fuchsin solution. Normal mouse IgG was substituted for primary antibody as the negative control (same concentration as the test antibody). Stromal macrophages were used as a positive internal control.¹⁸

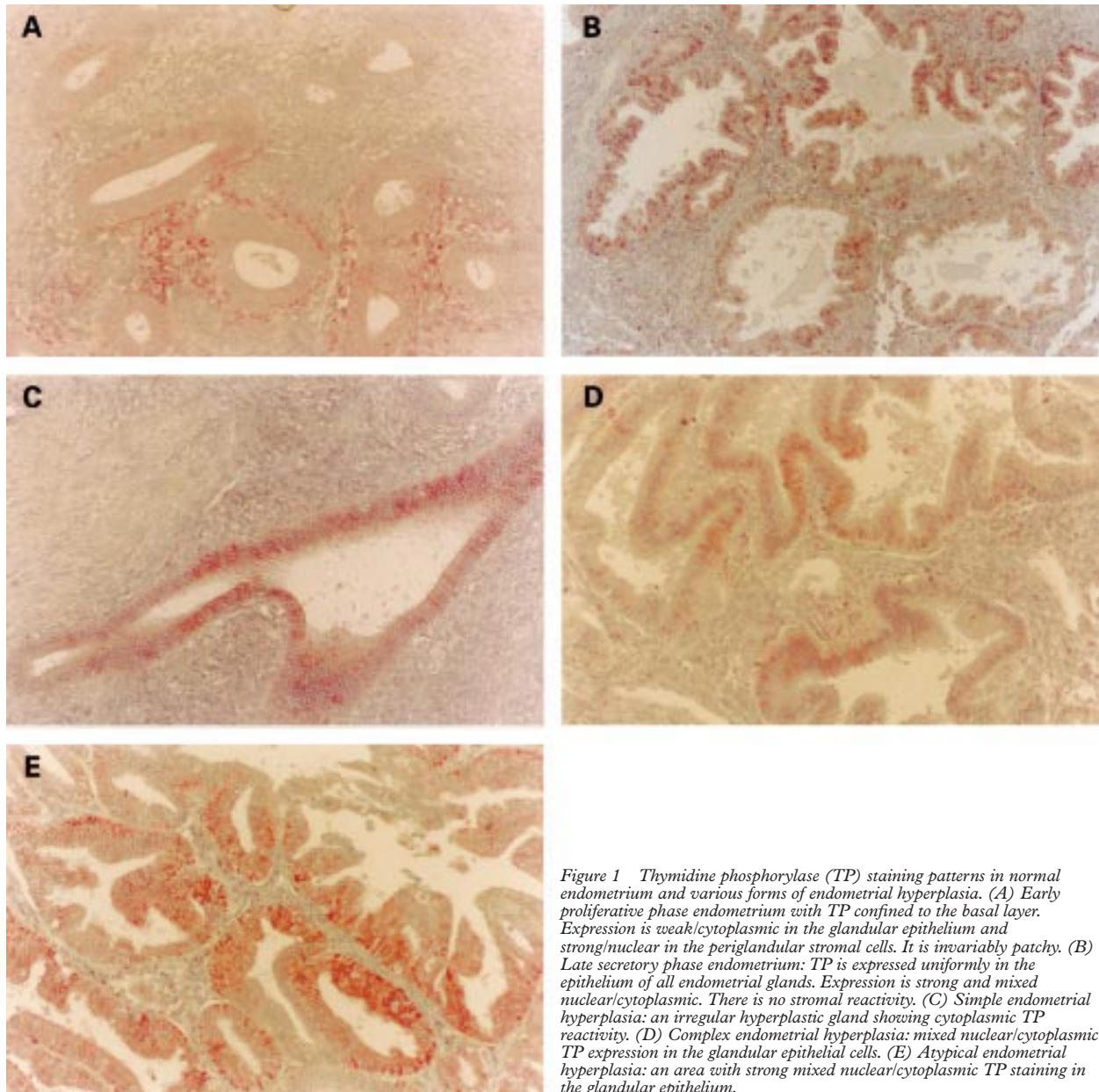


Figure 1 Thymidine phosphorylase (TP) staining patterns in normal endometrium and various forms of endometrial hyperplasia. (A) Early proliferative phase endometrium with TP confined to the basal layer. Expression is weak/cytoplasmic in the glandular epithelium and strong/nuclear in the periglandular stromal cells. It is invariably patchy. (B) Late secretory phase endometrium: TP is expressed uniformly in the epithelium of all endometrial glands. Expression is strong and mixed nuclear/cytoplasmic. There is no stromal reactivity. (C) Simple endometrial hyperplasia: an irregular hyperplastic gland showing cytoplasmic TP reactivity. (D) Complex endometrial hyperplasia: mixed nuclear/cytoplasmic TP expression in the glandular epithelial cells. (E) Atypical endometrial hyperplasia: an area with strong mixed nuclear/cytoplasmic TP staining in the glandular epithelium.

TP expression in normal and hyperplastic tissues was assessed by cellular distribution (nuclear or cytoplasmic) and staining intensity: negative (-), weak (\pm), or strong (+).

Results

TP was found consistently in normal endometrium. In the early and mid proliferative phase of a normal menstrual cycle, TP was found exclusively in the basal endometrium: glands and periglandular stroma (fig 1A). Expression was cytoplasmic in the glandular epithelial cells and nuclear in the periglandular stromal cells. Interestingly, all of the cells in TP positive glands expressed the enzyme. Cytoplasmic staining was very weak, whereas nuclear staining was usually strong. It was characteristically patchy, involving < 30% of the basal endometrial glands and stroma. In the late proliferative phase endometrium, the reactivity of the epithelial cells extended towards the inner third of the functional layer.

This immunohistochemical picture remained, by and large, unchanged during the early and mid secretory phase; however, in the late secretory phase of the cycle, cytoplasmic TP was expressed uniformly by all epithelial cells in all endometrial glands throughout the endometrium (fig 1B). At this stage, the pattern of enzyme expression was mixed nuclear/cytoplasmic, and the reaction was strong, in contrast to the remaining phases of the menstrual cycle, where the intensity of staining was, in general, weak. Stromal cell reactivity was not a feature.

The pattern of staining was similar in simple endometrial hyperplasia to that seen in the normal proliferative phase endometrium (fig 1C). However, in simple hyperplasia cytoplasmic reactivity was much stronger and involved random, small groups of weakly proliferating glands of the deep endometrium, occupying an area of < 5% of the total. By contrast, nuclear staining of the periglandular stroma was weak and present even less frequently.

TP was also expressed in complex endometrial hyperplasias (fig 1D). Immunohistochemical staining was cytoplasmic (four of 15) or mixed nuclear/cytoplasmic (11 of 15), and varied in intensity within and between individual cases. Focal areas of stromal cells showed nuclear staining which was, in most cases, weak.

Atypical hyperplasias showed mixed nuclear/cytoplasmic staining (15 of 15), involving a variable number of "hyperplastic" glands (fig 1E), which were distributed randomly in the endometrium over an area not exceeding 25% of the uterine mucosa.

In many cases, there was some variation of immunostaining between individual epithelial cells within glands and also from one gland to another in any one specimen.

Interestingly, scattered phosphorylase laden macrophages were seen throughout the normal endometrium, without any apparent cycle dependent changes. Similar cells were also seen in the various forms of endometrial hyperplasia.

Endothelial and smooth muscle cells in the media of occasional blood vessels were positive for TP, with a frequency of approximately 10%

(four of 42 normal endometria, five of 50 hyperplastic endometria), and TP was expressed with an almost equal frequency in scattered myometrial cells (six of 42 normal endometria, six of 50 hyperplastic endometria). No association between staining of blood vessels or myocytes and the phase of the menstrual cycle or the type of endometrial hyperplasia was noted.

Discussion

We examined the expression of TP in the endometrium by an immunohistochemical technique, using a specific monoclonal antibody to thymidine phosphorylase.⁴ This monoclonal antibody detected platelet derived endothelial cell growth factor or TP (PD-ECGF/TP). We found that TP is invariably expressed in the normal endometrium and in the various forms of endometrial hyperplasia.

In normal endometrium, TP expression has a cyclical pattern, which mirrors the changing endometrial patterns of the menstrual cycle. Thus, at the beginning of the cycle, TP is found in the basal layer, where it forms patches of positively stained endometrial glands and stroma. Expression is weak/cytoplasmic in epithelium and strong/nuclear in stromal cells. Towards the end of the menstrual cycle, epithelial but not stromal expression is extended throughout the entire endometrium, involving all glands, and epithelial expression is strong and mixed nuclear/cytoplasmic.

Comparable menstrual cycle related changes have been reported recently by Zhang *et al*,¹³ who described a shift in TP expression from the endometrial stroma to the endometrial glands as the cycle advances. The intensity of the epithelial staining was greatest in the basalis adjacent to myometrium and diminished towards the endometrial surface. Fox *et al*,⁴ who studied TP expression in a series of normal tissues, including human endometrium, noted that the enzyme is found in both endometrial glands and periglandular stroma, and observed both cytoplasmic and nuclear staining. It is possible that such cyclical changes in TP expression are under the control of ovarian hormones.¹³

Interestingly, TP is not the only angiogenic factor whose concentrations vary during the course of the menstrual cycle: cyclical patterns of endometrial activity for VEGF have also been reported.⁹ VEGF was expressed in normal glandular epithelium and, to a lesser extent, in stromal cells throughout the cycle; its activity was highest at the beginning (menstrual and early proliferative phase) and towards the end of the menstrual cycle (late secretory phase).

TP is, of course, an angiogenic factor and as such promotes the formation of new blood vessels from a pre-existing network of capillaries.^{12, 19} The new blood vessels, which are most important for the regeneration and growth of the shed endometrium, arise from the straight arteries in the boundary region between myometrium and basal layer. At this part of the uterine mucosa, coordinated nuclear and cytoplasmic TP activity, regulated by stromal and

epithelial cells, respectively, seems to be needed for the formation of new spiral arteries. This is consistent with the finding that TP expression, and apparently that of the endometrial stroma, is maximal in areas of poor perfusion and necrosis, where hypoxia prevails;²⁰ that is, in the endometrium after menstruation. Later in the course of the menstrual cycle and, specifically during the late secretory phase, maturation of the muscular coat occurs,¹⁶ which is also dependent on combined nuclear/cytoplasmic secretion of thymidine phosphorylase, this being produced mainly, if not exclusively, by the glandular epithelium. It has been suggested that in the nucleus TP might regulate thymidine concentrations for DNA synthesis, whereas in the cytoplasm it might control other effects via different enzyme systems, such as thymidylate synthase and ribonucleotide reductase.⁴ Whether cytoplasmic or nuclear, the enzyme is located intracellularly, whereas its effects are extracellular—on the nearby capillary endothelium; therefore, the angiogenic stimulus must be mediated by a paracrine pathway.

It is notable that the cytoplasmic expression of TP is not associated with an intense proliferative activity, because it is usually seen in the weakly proliferating glands of the basal endometrium and in those of the late secretory phase. This is equally true for all forms of endometrial hyperplasia where TP expression was not more prominent than in normally cycling late proliferative endometrium and, certainly, was less conspicuous than in late secretory endometrium. Indeed, TP expression was focal and of limited extent in all forms of endometrial hyperplasia, usually being linked with the less intensively proliferating endometrial glands. This is in contrast to other tissues of the body where the hyperplastic process is constantly associated with higher TP activity.¹⁹ Perhaps hyperplastic endometrial tissues are more sensitive to TP enzymatic activity, or other angiogenic factor(s), such as VEGF, are equally important for the formation of new blood vessels in endometrial hyperplasias.

We did not investigate microvessel density in our study; however, in the light of the report of Morgan *et al*,²¹ it appears that normal secretory phase endometrium is more vascular than normal proliferative endometrium. This finding is in accordance with our observation that epithelial/cytoplasmic TP expression increases from proliferative to secretory phase endometrium. The fact that stromal/nuclear TP expression prevails in the basalis of the early proliferative endometrium suggests that this type of enzymatic activity is important in the induction of neo-angiogenesis, at least in normal endometrium. In this context, it appears that stromal TP positivity is oestrogen, rather than cycle (time), related because it is not only seen in the normal proliferative endometrium, but also in all forms of endometrial hyperplasia, although to a lesser extent. The high microvessel density recorded in hyperplastic endometria in the study of Morgan and colleagues²¹ contrasts with the limited expression of TP found in similar tissues in our study. This discrepancy might be explained by the aforementioned assumptions of

an increased sensitivity of hyperplastic tissues to thymidine phosphorylase and/or the concomitant action of other, possibly more potent, angiogenic factor(s).

Stromal macrophages, which are scattered in the endometrial layers, express TP throughout the menstrual cycle, but this enzymatic activity was not found to be cycle related.^{4–13} Such TP laden macrophages, which are recruited not only in the normal endometrium, but also in wound healing, the inflammatory response, and in several tumours,^{12–18–22} were thought to play an important role in the regulation of angiogenesis.^{10–23} If this is true, enzyme production might not simply be regulated by ovarian hormones, but might also be regulated by cytokines released at high concentrations by endometrial macrophages.^{4–13} Other factors may be equally important. Thus, the treatment of normal glandular epithelial cells with physiological concentrations of progesterone or transforming growth factor β 1 alone did not have an effect on TP expression, but the combined delivery of both at the same dose induced a 48-fold increase in expression.¹³ Furthermore, the combination of tumour necrosis factor α and interferon γ was reported to induce an even greater expression (205-fold) of the enzyme.¹⁵

In summary, the expression of TP in the normal endometrium has well defined cellular and tissue patterns that are dependent on the phase of the menstrual cycle. TP is also expressed in all forms of endometrial hyperplasia, where it has a random distribution, lacking an orderly pattern. We conclude that the consistent expression of TP in the endometrium is suggestive of a role in physiological and pathological angiogenesis.

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