Eur J Cancer Clin Oncol, Vol. 25, No. 3, pp. 565–569, 1989.
Printed in Great Britain

0277-5379/89$3.00 + 0.00

Sounding Board

Plasminogen Activators in (Pre)malignant Conditions of the Colorectum

H.W. VERSPAGET,* J.H. VERHEIJEN,† P.A.F. DE BRUIN,* G. GRIFFIOEN* and C.B.H.W. LAMERS*

**Department of Gastroenterology and Hepatology, University Hospital Leiden and †Gaubius Institute, Health Research Division TNO Leiden, The Netherlands*

Abstract—*Plasminogen activators have been implicated in the process of tumour growth, invasion and metastatic spread. Recent studies indicate that urokinase (u-PA) is the major type of plasminogen activator correlated with the evolution of adenocarcinomas of the colon. Comparable changes in the plasminogen activator profile have been found in premalignant conditions of the colon as observed in adenocarcinomas. The feasibility of determining plasminogen activators in endoscopical biopsies may provide diagnostic opportunities for the detection of early malignant changes in the human colon.*

PLASMINOGEN ACTIVATORS

PLASMINOGEN ACTIVATORS are currently distinguished into two types: the urokinase-type plasminogen activator (u-PA) and the tissue-type plasminogen activator (t-PA). They show considerable structural homology although their molecular weights, immunological properties and functional activities are different [1].

u-PA has a molecular weight of 54 kDa. It is synthesized as a single-chain polypeptide proenzyme (pro-u-PA, molecular weight 54 kDa) with virtually no enzymatic activity [2]. This proenzyme form is probably the only intracellular form of u-PA. Activation, catalysed by plasmin, is achieved by cleavage of a single peptide bond, resulting in a molecule consisting of two chains connected by a disulphide bridge. In the extracellular fluid, both pro-u-PA and u-PA, and a smaller form of u-PA, molecular weight 33 kDa, are found [3]. Active u-PA can be bound (and inactivated) by plasminogen activator inhibitor (PAI). However, PAI does not bind to pro-u-PA [4].

The other plasminogen activator, t-PA, is a glycoprotein with a molecular weight of 70 kDa. Its catalytic activity is largely dependent on binding to fibrin and thus plays an important role in fibrinolysis. The principal site of t-PA synthesis appears to be the endothelium. Several types of inhibitors for plasminogen activator as well as for plasmin are known to be present in plasma in significant quantities [5]. The most important inhibitor of plasminogen activators is the endothelial inhibitor (PAI-1).

Accepted 21 October 1988.
Correspondence: Dr. H.W. Verspaget, M.Sc., Ph.D., Department of Gastroenterology and Hepatology, University Hospital, Building 1, C4-PO12, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands.

PLASMINOGEN ACTIVATORS AND MALIGNANCY

A direct relation between changes in the expression of plasminogen activators and malignant derailment of cells has been shown to exist [6]. Transformation by oncogenic viruses or incubation with tumour promotors induce an increased PA activity [7, 8]. Also neoplastic human cell cultures have been shown to exhibit characteristic changes in the plasminogen activator synthesis and secretion after stimulation with tumour promoting agents [9–12]. Human tumours of different origin like breast, lung, prostate and colon demonstrate altered fibrinolytic activity compared with their normal tissue counterparts. Depending on the method used, the fibrin plate assay, which is especially sensitive to t-PA, or the azocaseinolytic assay, which is more sensitive to u-PA, and material analysed a decreased or augmented total fibrinolytic activity in tumours was found [10, 13–19].

These earlier studies did not always discriminate between u-PA and t-PA, but since inhibitory antibodies to u-PA or t-PA have become available, the total measured plasminogen activating activity can be divided into activities caused by the two activators [20, 21]. Discrimination between t-PA and u-PA based on their different molecular weights is also possible using gel electrophoresis in PA gels in the presence of SDS followed by zymographic detection on a fibrin and plasminogen containing underlay gel [22]. Plasminogen activator activities become visible by the disappearance of the insoluble fibrin layer in the underlay. A purely immunological approach of measuring u-PA and t-PA antigens by an ELISA is a third possibility of discriminating between the two activators. From studies utilizing these methods, it has become clear that, at least in adenocarcinomas, u-PA is the activator type primarily related to malignancy.

PLASMINOGEN ACTIVATORS IN (PRE)MALIGNANT CONDITIONS OF THE COLON

In colorectal carcinomas, plasminogen activator activity could largely be ascribed to u-PA while this activity in the normal mucosa consisted nearly totally of t-PA. The measured u-PA activity in tissue homogenates was increased approximately five-fold in the tumours when compared to the normal mucosa [23, 24]. In short term organ cultures tumours were found to secrete almost 50 times more u-PA than normal mucosa [25]. In contrast, t-PA activity was decreased to one third of that in the normal tissue [23, 24].

Adenomatous polyps of the colon are generally considered as precursor lesions to adenocarcinomas. They are neoplastic proliferations of colonic epithelial cells, are often pedunculated and often protrude into the colonic lumen. Adenomatous polyps are characterized by dysplastic changes in the epithelial cell layer in which a malignancy may develop [26]. Flow cytometric DNA analysis studies revealed aneuploid cells in the intestinal tissue of this disorder [27]. Both u-PA and t-PA activities were also determined in adenomatous polyps and compared to those of normal colonic mucosa and adenocarcinomas. In the polyps, u-PA activity was found to be intermediate and significantly different to the activities in normal mucosa and adenocarcinomas. The activity of t-PA in the polyps was decreased compared to the level in the normal mucosa to a similar level as found in the carcinomas [23, 28, 29]. Not only the enzyme activities but also the antigen concentration of u-PA and t-PA in adenomatous polyps were evaluated. In polyps and carcinomas, the increase of u-PA antigen was clearly much stronger than the parallel increase of u-PA activity [23, 30]. It was found that, of the u-PA in polyps and tumours, approx. 75% is present in the proenzyme form, not recognized by the enzyme activity assay [30], and only 25% in the active form. The antigen levels of u-PA and t-PA were not affected by the presence of complexes with inhibitors in the tissues, which were mainly complexes with t-PA. Only the decreased t-PA activity could partly be attributed to inhibitor complexes. Because the increase of u-PA is so strong when compared to normal tissue (five-fold in the polyp group, and ten-fold in the carcinoma group), and because t-PA antigen, like t-PA activity, decreases, a discrimination between normal mucosa, premalignant polyps and carcinomas can readily be obtained on the basis of the two antigen determinations.

The main finding was, however, that the occurrence of invasion in a polyp (the actual malignancy) coincided with a discrete rise of the u-PA antigen concentration [30]. It was concluded that during the adenoma–carcinoma sequence, two discrete changes of the plasminogen activator pattern could be observed: the first at adenoma formation from the normal mucosa, featuring a u-PA increase and a t-PA decrease, and the second on the occurrence of invasion in a polyp, with a further rise of u-PA to a level also found in grown-out adenocarcinomas.

In the colon, several affections are known to be premalignant, in other words, to increase the chance to develop a malignancy. It is now generally accepted that by far the most common precursor lesion for a colorectal carcinoma is the adenomatous polyp while also long standing chronic inflammatory bowel disease (IBD) with dysplastic changes in the epithelial cell layer is known to increase the cancer risk. In the case of IBD, the precancerous lesion would not necessarily be polypoid, but could be any site of the colonic mucosa with severe dysplasia [31–37]. Only a few studies have been performed to determine PAs in this premalignant condition. In chronic inflammatory bowel disease studies on the fibrinolytic activity of inflamed mucosa in the 1970s showed enhanced fibrinolysis and led to some studies on antifibrinolytic therapy [38–41]. No studies were performed, however, in relation with malignancy risk in this disease.

IBD comprises Crohn's disease (CD) and ulcerative colitis (UC). In the literature, indications are found that, on the one hand, an increased fibrinolysis in the colonic mucosa might be responsible for an increased bleeding incidence, while on the other hand, an altered plasminogen activator pattern might be related to an increased cancer risk. t-PA levels (activity as well as antigen) were found to be lower in the inflamed mucosa than in mucosa without inflammatory changes and in mucosa of controls, in addition the decrease in t-PA was related to the severity of the inflammatory process. u-PA antigen, however, was increased with increasing

degree of inflammation, although this was not mirrored in a parallel increase of u-PA enzyme activity, caused by the presence of pro-u-PA and complexes of u-PA with specific inhibitors [42, 43]. The changes in the plasminogen activator pattern of inflamed IBD tissue are thus comparable with those in adenomatous polyps, and may possibly point to the premalignant character of IBD. On the basis of the data from this study, it was however impossible to rule out a more direct relationship between the changes and the inflammatory process.

Recently, a study was performed to evaluate the possible application of plasminogen activator measurements in the diagnosis of malignant, and perhaps premalignant, conditions of the gastrointestinal tract. Activities and antigens of u-PA and t-PA in endoscopic biopsies of gastrointestinal malignancies were compared to the same parameters in the subsequent resection specimens of the same patients. A second comparison was made between the results of the plasminogen activators and the results of a histological evaluation of equivalent biopsies by the pathologist. It became clear that the plasminogen activator determinations in the biopsies showed a good general agreement with those in the corresponding resection samples. Virtually all carcinomas showed the characteristic strong increase of u-PA antigen and the decrease of t-PA antigen with respect to the normal mucosa, both in biopsies and resections as well as in gastric and colonic tumours. In comparison with histology, malignancy was identified by PA measurements in two patients which was not noticed in the histological tissue sections of the biopsies [44].

ROLE OF PLASMINOGEN ACTIVATORS IN THE PATHOGENESIS AND INVASIVENESS OF TUMOURS

Transformation of cells by tumour viruses and tumour promoting agents is usually followed by changes in the production and secretion of PA [6–8]. The exact nature and origin of this shift in quantitative and qualitative (t-PA and u-PA) PA activity is not known but as a consequence these cells react upon stimulation with an enhanced secretion of PA. This process is followed by a breakdown of the extracellular matrix and associated with tissue remodelling and malignant-cell migration [3]. The evidence for the involvement of the fibrinolysis in growth and spread of tumours has been reviewed by Peterson [45]. Ossowski and Reich [46] showed that antibodies to u-PA inhibited the metastasis of a human tumour cell line in a chicken embryo model. Recently, Ossowski [47] showed that the dissemination of the human tumour cell line was prevented by the inhibition of the u-PA dependent intravasation of these cells. These were direct indications of a role for plasminogen activators in the mechanism of tumour spread. Moreover, human primary colon carcinomas xenografted into nude mice were found to be modulated in their growth by plasminogen activators [48].

In a review [6], Markus suggested mechanisms by which plasminogen activator could be used by the tumour for invasive growth and metastasis formation. For invasion, plasminogen activator is secreted by the tumour cells to activate extracellular plasminogen. The formed plasmin could degrade matrix proteins, like laminin and fibronectin, or activate latent collagenase for the same purpose [49]. Thereby, the extracellular matrix is dissolved and the tumour cells can invade the host tissue. The cells responsible for the PA secretion were shown to be the tumour cells when immunohistochemical staining for u-PA was used [25, 50], although earlier studies showed that using the fibrin overlay technique (Todd slides) only the perivascular zone (t-PA type) had fibrinolytic activity [18, 19]. Besides the tumour and endothelium, mononuclear phagocytes and epithelial cells have been shown to produce a considerable amount of PA [51, 52].

There is also evidence that the PAs of the tumours affect the host immune response, especially the lymphocyte cytotoxicity [53, 54]. This PA activity might contribute to the escape of the tumour from the host's control and subsequent metastasis formation. From these observations it emerges that PAs play an important role in the development and invasiveness of tumours in general.

CONCLUSIONS

The occurrence of malignancies in the gastrointestinal tract is closely connected with an increase of the u-PA concentration and activity. This increase can, to a lesser extent, already be observed in premalignant lesions as adenomatous polyps but also in severe inflammatory bowel disease. Since such changes can also be found in small endoscopical biopsies, these clear and early occurring changes may find an application in the early diagnosis of gastrointestinal malignancies, in addition to a thorough clinical evaluation.

Acknowledgements—The authors are indebted to Loes Niepoth and Maritza Koster-de Vreese for secretarial help. Supported in part by the grant IKW 85-87 from the Netherlands Cancer Foundation KWF.

REFERENCES

1. Blasi F, Riccio A, Sebastio G. Human plasminogen activators. Genes and proteins structure. In: Quagliariello E, Palmieri F, Blasi, eds. *Horizons in Biochemistry and Biophysics.* Vol. 8 *Human Genes and Disease.* New York, Wiley, 1986, Vol. 8, 377–414.

2. Pannell R, Gurewich V. Activation of plasminogen by single-chain urokinase or by two-chain urokinase—a demonstration that single-chain urokinase has a low catalytic activity (pro-urokinase). *Blood* 1987, **69**, 22–26.
3. Danø K, Andreasen PA, Grøndahl-Hansen J, Kristensen P, Nielsen LS, Skriver L. Plasminogen activators, tissue degradation and cancer. *Adv Cancer Res* 1985, **44**, 139–166.
4. Andreasen PA, Nielsen LS, Kristensen P, Grøndahl-Hansen J, Skriver L, Danø K. Plasminogen activator inhibitor from human fibrosarcoma cells binds urokinase-type plasminogen activator, but not its proenzyme. *J Biol Chem* 1986, **261**, 7644–7651.
5. Sprengers ED, Kluft C. Plasminogen activator inhibitors. *Blood* 1987, **69**, 381–387.
6. Markus G. Plasminogen activators in malignant growth. In: Davidson JF, Bachman F, Bouvier CA, Kruithof RO, eds. *Progress in Fibrinolysis*. Churchill Livingstone, 1984, Vol. VI, 587–604.
7. Unkeless JC, Tobia A, Ossowski L, Quigley JP, Rifkin B, Reich E. An enzymatic function associated with transformation of fibroblasts by oncogenic viruses. I. Chick embryo fibroblasts cultures transformed by avian RNA tumour viruses. *J Exp Med* 1973, **137**, 85–111.
8. Wigler M, Weinstein IB. Tumour promotor induces plasminogen activator. *Nature* 1976, **259**, 232–233.
9. Rifkin DB, Loeb JN, Moore G, Rerdi E. Properties of plasminogen activators formed by neoplastic human cell cultures. *J Exp Med* 174, **139**, 1317–1328.
10. Wilson EL, Dowle E. Secretion of plasminogen activator by normal, reactive and neoplastic human tissues cultured *in vitro*. *Int J Cancer* 1978, **23**, 390–399.
11. Friedman EA. Differential response of premalignant epithelial cell classes to phorbol ester tumour promotors and to deoxycholic acid. *Cancer Res* 1981, **41**, 4588–4599.
12. Friedman EA, Urmacher C, Winawer D. A model for human colon carcinoma evolution based on the differential response of cultured preneoplastic, premalignant and malignant cells to 12-*O*-tetradecanoylphorbol-13-acetate. *Cancer Res* 1984, **44**, 1568–1578.
13. Cliffton EE, Grossi CE. Fibrinolytic activity of human tumours as measured by the fibrin plate method. *Cancer* 1955, **8**, 1146–1154.
14. Evers JL, Patel J, Madeja JM *et al.* Plasminogen activator activity and composition in human breast cancer. *Cancer Res* 1982, **42**, 219–226.
15. Camiolo SM, Markus G, Evers JL *et al.* Plasminogen activator content of neoplastic and benign human prostate tissues; fibrin augmentation of an activator activity. *Int J Cancer* 1981, **27**, 191–198.
16. Markus G, Takita H, Camiolo SM, Corasanti JG, Evers JL, Hobika GH. Content and characterization of plasminogen activators in human lung tumours and normal lung tissue. *Cancer Res* 1980, **40**, 841–848.
17. Nagy B, Ban J, Brdar B. Fibrinolysis associated with human neoplasia: production of plasminogen activator by human tumours. *Int J Cancer* 1977, **19**, 614–620.
18. Szczepánski M, Lucer C, Zawadzki J, Tolloczko T. Procoagulant and fibrinolytic activities of gastric and colorectal cancer. *Int J Cancer* 1982, **30**, 329–333.
19. Franklin JD, Gervin AS, Bowers DG, Lynch JB. Fibrinolytic activity in human neoplasms. *Plast Reconstr Surg* 1978, **61**, 241–249.
20. Corasanti JG, Celik C, Camiolo SM *et al.* Plasminogen activator content of human colon tumours and normal mucosa: separation of enzymes and partial purification. *J Natl Cancer Inst* 1980, **65**, 345–352.
21. Vetterlein D, Young PL, Bell TE, Robin R. Immunological characterization of multiple molecular weight forms of human cell plasminogen activator. *J Biol Chem* 1979, **254**, 575–578.
22. Granelli-Piperno A, Reich E. A study of proteases and protease–inhibitor complexes in biological fluids. *J Exp Med* 1978, **148**, 223–234.
23. Gelister JSK, Mahmoud M, Lewin MR, Gaffney PJ, Boulos PB. Plasminogen activators in human colorectal neoplasia. *Br Med J* 1986, **293**, 728–731.
24. de Bruin PAF, Vespaget HW, Griffioen G, Nap M, Verheijen JH, Lamers CBHW. Plasminogen activator activity and composition in human colorectal carcinomas. *Fibrinolysis* 1987, **1**, 57–62.
25. Markus G, Camiolo SM, Kohga S, Madeja JM, Mittelman A. Plasminogen activator secretion of human tumours in short-term organ culture, including a comparison of primary and metastatic colon tumours. *Cancer Res* 1983, **43**, 5517–5525.
26. Muto T, Bussey HJR, Morson BC. The evolution of cancer of the colon and rectum. *Cancer* 1975, **36**, 2251–2270.
27. van den Ingh HF, Griffioen G, Cornelisse CJ. Flow cytometric detection and aneuploidy in colorectal adenomas. *Cancer Res* 1985, **45**, 3392–3397.
28. de Bruin PAF, Griffioen G, Verspaget HW, Verheijen JH, Lamers CBHW. Plasminogen activators and tumour development in the human colon: activity levels in normal mucosa, adenomatous polyps, and adenocarcinomas. *Cancer Res* 1987, **47**, 4654–4657.
29. Verspaget HW, de Bruin PAF, Verheijen JH, Griffioen G, Lamers CBHW. Plasminogen activators in human colorectal neoplasia. *Br Med J* 1986, **293**, 1056.

30. de Bruin PAF, Griffioen G, Verspaget HW *et al.* Plasminogen activator profiles in neoplastic tissue of the human colon. *Cancer Res* 1988, **48**, 4520–4524.
31. Swinson CM, Hall PJ, Bedford PA, Booth CC. HLA antigens in coeliac disease associated with malignancy. *Gut* 1983, **24**, 925–928.
32. Enterline HT, Evans GW, Mercudo-Lugo R, Miller L, Fitts WT. Malignant potential of adenomas of colon and rectum. *JAMA* 1962 **179**, 322–330.
33. Gyde S, Prior P, McCartney JC, Thompson H, Waterhouse JAH, Allan RN. Malignancy in Crohn's disease. *Gut* 1980, **21**, 1024–1029.
34. Greenstein AJ, Sachar DB, Smith H, Janowitz HD, Aufses AH. A comparison of cancer risk in Crohn's disease and ulcerative colitis. *Cancer* 1981, **48**, 2742–2745.
35. Hammarberg C, Slezak P, Tribukait B. Early detection of malignancy in ulcerative colitis: a flow-cytometric DNA study. *Cancer* 1984, **53**, 291–295.
36. Riddell RH, Ransokoff DF, Fenoglio CM, Ahrén C, Hamilton SR, Sommers SC. Dysplasia in inflammatory bowel disease. *Human Pathol* 1983, **14**, 931–968.
37. McKinley MJ, Budman DR, Kahn E. High grade dysplasia in Crohn's colitis characterized by flow cytometry. *J Clin Gastroenterol* 1987, **9**, 452–455.
38. Kwaan HC, Cocco A, Mendeloff I, Astrup T. Fibrinolytic activity in the normal and inflamed rectal mucosa. *Scand J Gastroenterol* 1969, **4**, 441–445.
39. Swan CHJ, Alexander Williams J, Cooke WT. Fibrinolysis in colonic disease. *Gut* 1970, **11**, 588–591.
40. Salter RF, Read AE. Epsilon-aminocaproic acid therapy in ulcerative colitis. *Gut* 1970, **11**, 585–587.
41. Hollanders P, Thompson JH, Schofield PF. Tranexamic acid therapy in ulcerative colitis. *Postgr Med J* 1982, **58**, 87–91.
42. Verspaget HW, de Bruin PAF, Weterman IT, Verheijen JH, Nap M, Lamers CBHW. Changes in plasminogen activator activity in inflammatory bowel disease (IBD). *Gut* 1986, **27**, 630.
43. de Bruin PAF, Crama-Bohbouth G, Verspaget HW *et al.* Plasminogen activators in the intestine of patients with inflammatory bowel disease. *Thromb Haemostas* 1988, **60**, 262–266.
44. de Bruin PAF. *Plasminogen Activators in (Pre)malignant Conditions of the Human Colon.* ICG Printing, Dordrecht, 1988.
45. Peterson H. Fibrinolysis and antifibrinolytic drugs in the growth and spread of tumours. *Cancer Treat Rev* 1977, **4**, 213–217.
46. Ossowski L, Reich E. Antibodies to plasminogen activator inhibit tumour metastasis. *Cell* 1983, **35**, 611–619.
47. Ossowski L. Plasminogen activator dependent pathways in the dissimination of human tumor cells in the chick embryo. *Cell* 1988, **52**, 321–328.
48. Cajot JF, Sordat B, Bachmann F. Human primary colon carcinomas xenografted into nude mice. II: Modulation of tumor plasminogen activator activity by the host tissue environment. *JNCI* 1986, **77**, 1099–1105.
49. Saksela O. Plasminogen activation and regulation of pericellular proteolysis. *Biochim Biophys Acta* 1985, **823**, 35–65.
50. Kohga S, Harvey SR, Weaver RM, Markus G. Localization of plasminogen activators in human colon cancer by immunoperoxiase staining. *Cancer Res* 1985, **45**, 1787–1796.
51. Ragsdale CG, Arend WP. Characteristics of fibrinolytic enzyme release from human monocytes. *Clin Exp Immunol* 1981, **46**, 214–224.
52. Doe WF, Dorsman F. Chronic inflammatory bowel disease—increased plasminogen activator secretion by mononuclear phagocytes. *Clin Exp Immunol* 1982, **48**, 256–260.
53. Sundar SK, Bergeron J, Menezes J. Purified plasminogen activating factor produced by malignant lymphoid cells abrogates lymphocytes cytotoxicity. *Clin Exp Immunol* 1984, **56**, 701–708.
54. Wainberg MA, Israel E, Margolese GR. Further studies on the mitogenic and immune modulating effects of plasminogen activator. *Immunology* 1982, **45**, 715–720.