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Perspectives and Commentaries

Plasminogen Activator and Cancer

M. J. DUFFY*† and P. O'GRADY‡

*Department of Nuclear Medicine, St. Vincent's Hospital, Dublin 4, Ireland and ‡Departments of Medicine and Surgery, University College Dublin, Dublin 4, Ireland

Abstract—*Plasminogen activator is a protease which catalyses the conversion of the inactive plasminogen to the active plasmin. Most transformed cell lines and solid tumors produce elevated levels of plasminogen activator compared with non-transformed counterparts. This increased synthesis of plasminogen activator may play a role in tumorigenesis, cancer invasion and metastasis. Measurement of plasminogen activator in tumor extracts and body fluids may provide diagnostic and prognostic information. Finally, since plasminogen activator is an estradiol-inducible enzyme, its measurement in breast carcinomas might be a marker for a functional estrogen receptor.*

INTRODUCTION

PLASMINOGEN activators (PA) are serine proteolytic enzymes found in nearly all animal tissues and many body fluids. The PA group of enzymes are often divided into 4 main groups: circulating PA (blood), tissue PA, urinary PA (urokinase) and tissue culture PA. At present we do not know if these PA molecules are the products of different genes. The best-known function of PA is to catalyze the conversion of the inactive plasminogen to the active plasmin. PA accomplishes this reaction by catalyzing the hydrolysis of the arg-560-val-561 peptide bond in plasminogen. Plasmin, in contrast to PA, is a broad specific enzyme and breaks down lys-lys bonds in a variety of proteins (for a review see ref. [1]). Clinically, one of the most important substrates appears to be fibrin clots. Thus the main role of circulating PA/plasmin is thought to be clot dissolution.

The function of cellular PA or urokinase is less clear. Recently, however, a cellular substrate for urokinase was identified in human fibroblasts. The substrate was a protein with a molecular weight of 66,000 daltons and was found in the pericellular matrix [2]. The significance of the degradation of this protein by urokinase remains to be determined. Biological processes in which cellular PA has been implicated include prohormone conversion, macrophage migration, ovulation in mammals, blastocyst implantation during early embryonic development, mammary gland involution and neoplasia (for a review see ref. [3]). The function of this paper is to critically review the latter topic, i.e. the relationship between PA and cancer.

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†To whom correspondence and requests for reprints should be addressed.

NATURE OF PA PRODUCED BY CANCER CELLS

The PA found in cancer cells is often described as being either urokinase-like or non-urokinase-like. This distinction is based on the differential reaction of antibodies to the two forms of PA activity. Using this approach, it appears that most tumors synthesise primarily a urokinase-like enzyme. For example, in human lung tumors greater than 90% of PA resembles urokinase, whereas in normal lung tissue less than 50% resembles the urokinase form [4]. Similarly, in human breast carcinomas approximately 80% of PA activity is inhibited by antibodies to urokinase, whereas in normal breast only 62% of PA activity is inhibited [5]. Malignancies of the ovary [6] and pancreas [7] also produce mainly a urokinase-type PA. Tumors of the brain, on the other hand, appear to synthesise a non-urokinase-like PA [8].

Why some carcinomas such as those of the lung and breast form mostly a urokinase-like enzyme

whereas the corresponding normal tissue contains lower proportions of this fraction is unknown. The higher proportion of urokinase-like activity in tumors may, however, relate to contamination of these tissues with varying amounts of white cells. Alternatively, it might reflect altered gene expression or differential processing of possible pro-PA peptides. These two phenomena are well-known characteristics of malignant tissue.

Separation of tumor PA into urokinase- and non-urokinase-like forms is probably over-simplistic. Shyamala and Dickerman noted that 6 different proteases with PA activity were secreted by MCF-7 breast cancer cells in culture. The molecular weight of these proteases varied from 65,000 to 25,000. All but one of these proteins, the Mr = 59,000 form, were antigenically similar to urokinase [9]. Medium from a lung tumor cell line has been reported to contain a urokinase-type plasminogen activator with a molecular weight of between one and two million daltons. This high-molecular-weight enzyme could not be dissociated into subunits by either SDS or 8M urea [10].

ELEVATED PA LEVELS PRODUCED BY TRANSFORMED CELL LINES

Most but not all transformed cell lines show an increased secretion of PA activity when compared with the corresponding non-transformed cells. The increased release of PA occurs whether the transformation is accomplished by chemicals, oncogenic DNA or RNA viruses. Unkeless *et al.* [11] have shown that cultured chick embryo fibroblasts possessed no measurable plasminogen-dependent fibrinolytic activity. However, after infection with either wild-type Rous sarcoma virus (RSV) or temperature-sensitive mutants of the virus grown at transforming temperature, marked fibrinolysis was observed. In contrast, no elevated fibrinolytic activity was seen in cells infected with a temperature-sensitive RSV mutant, cytocidal RNA or DNA viruses or non-transforming strains of avian leukosis virus. The increased PA levels therefore appear to be related to transformation rather than to virus infection or cell lysis.

Tumor promoters such as phorbol myristate acetate (PMA) can also increase levels of PA [12]. This has been shown to occur in both normal chick embryo fibroblasts and synergistically in the same fibroblasts transformed with Rous sarcoma virus. In the latter system the morphological changes resulting from PMA treatment can be prevented by inhibitors of PA. In contrast, inhibitors of other proteolytic enzymes such as trypsin, chymotrypsin, elastase, thrombin and plasmin were ineffective in preventing the PMA-induced cellular alterations. Since both the nature of the inhibitors and the concentrations used to prevent the morphological changes correlated with the inhibition of these compounds on PA, it was concluded that PA, independent of plasmin, brought about the morphological changes [12].

While the above data suggest a strong association between PA secretion and transformation, it should be stated that not all transformed cells release high levels of PA. In particular, transformed cell lines maintained for a long time in culture appear to lose their ability to produce PA [13].

ELEVATED PA LEVELS PRODUCED BY SOLID TUMORS

Compared with normal control tissues, a variety of human cancers show elevated tissue levels of PA. These include carcinoma of the colon, lung, breast, cervix, prostate and malignant melanoma [4, 5, 14, 15]. In addition, a number of tumors grown in culture release more PA activity than normal tissue adjacent to the tumor. Lung tumor explants released about 40 times more PA activity into medium than did normal lung [16]. In contrast, tissue PA extracted by Triton X-100 was only about 3 times greater in the malignant lung tissue than in normal tissue [16].

In mouse and rat mammary tumors PA activity levels were 12–150- and 2–24-fold higher than the highest levels seen in normal lactating and involuting glands respectively [17]. Furthermore, greater amounts of PA were released from organ cultures of these animal tumors than from the corresponding normal tissue. Of interest was the finding that PA secretion by these tumors was more resistant to hydrocortisone than was secretion from normal mammary tissue [17]. In contrast to animal breast tumors, human breast carcinomas failed to release higher levels of PA *in vitro* than did normal breast tissue [18]. The human breast tumors did, however, secrete greater amounts of another proteolytic enzyme, i.e. cathepsin B, than was found with control tissue [18].

CORRELATION OF PA LEVELS WITH TUMORIGENESIS

Data presented above show that both transformed cells *in vitro* and solid tumors *in vivo* possess higher levels of PA activity than their normal counterparts. However, these findings are not specific for PA; many enzymes, especially in solid tumors, have higher activities than are found in control surrounding tissue. Perhaps what is more important than finding high levels of PA in transformed malignant cells is whether these high levels have any role in tumorigenesis,

invasion or metastases. Some evidence exists that PA indeed may have a role in these processes.

In some transformed cell lines the secretion of PA has been shown to correlate with a number of transformation parameters, including the growth of these cells in soft agar [19], their increased rate of migration [20] and their ability to form tumors in immunosuppressed animals [19]. However, as mentioned above, high levels of PA production is not consistently linked with malignant transformation and cells maintained for long periods in culture can cease to secrete high levels of PA without losing tumorigenicity.

Some of the best evidence supporting a role for PA in carcinogenesis has come from work with strains of tumorigenic and non-tumorigenic melanoma cells [21]. One clone of these cells, B_5 59, is highly tumorigenic in mice. If, however, these cells are grown in the presence of BrdU, their morphological appearance is altered and they lose the ability to induce tumors in mice. Moreover, the ability of these cells to digest fibrin and their ability to form tumors declines in parallel. On return of these cells to media without BrdU, fibrinolytic activity, tumorigenicity and normal morphology were restored. Thus in these melanoma cells PA production appears to correlate with tumorigenicity. However, whether the cells grown in BrdU actually lose their ability to synthesis PA or produce excess of an inhibitor is presently unknown. At least they do not release an inhibitor of either PA or plasmin into the growth media.

In contrast to these results linking PA with tumorigenicity, Nicolson *et al.* [22] found no significant differences in PA activity between B16 melanoma cells with different metastatic potentials.

CORRELATION OF PA LEVELS WITH INVASION AND METASTASIS

In 1973 Peterson *et al.* [23] showed that a correlation existed between high levels of fibrinolysis and local invasion and metastasis in human breast cancer. A similar relationship was later shown between fibrinolysis and intravascular tumor growth in epidermoid lung carcinomas [24]. More recently Markus and coworkers [15] found a significant correlation between tissue PA levels and local invasion in colonic carcinomas. However, these workers were unable to demonstrate a relationship between PA levels and local invasion in either lung [4] or breast cancer [5]. No data have yet appeared on a correlation between levels of PA secreted by tumors and cancer spread.

More direct evidence that PA enzymes play a role in metastases has come from experiments involving administration of urokinase to tumor-bearing animals. Kodama and Tanaka [25] have shown that administration of urokinase to rabbits with V2 carcinomas enhanced growth and metastases. In another study urokinase enhanced spontaneous pulmonary metastases, while tranexamic acid, an inhibitor of plasmin, prevented metastatic formation [26]. In 1968 Peterson showed that the growth rate of two transplantable mouse tumors was enhanced by induced fibrinolysis [27]. Other studies have also shown inhibitory effects of tranexamic acid and epsilon-amino caproic acid (another inhibitor of fibrinolysis) on tumor growth and metastases of breast carcinomas in syngeneic mice [28]. Preliminary data suggest that tranexamic acid may have a therapeutic role in advanced human ovarian cancer [29].

Despite these findings it is not clear how exactly tranexamic acid is acting in inhibiting tumor growth and metastases. For example, it may inhibit other proteolytic enzymes in addition to plasmin, or may inhibit tumor vascularization [30, 31]. Finally, the growth of at least one animal tumor, a chemically-induced sarcoma, was not influenced by tranexamic acid [28].

There are a number of reasons to suggest why the PA/plasmin system may be important in tumor invasion. Firstly, as mentioned in the introduction, PA appears to be involved in normal physiological events associated with invasion and tissue destruction. Secondly, due to the catalytic nature of PA and the high level of plasminogen found in extracellular spaces, large amounts of plasmin could be generated locally. Thirdly, PA appears to be able to activate collagenase [32]. Evidence exists that collagenase also plays a role in tumor invasion and metastases [33]. Thus PA could play a role in tumor spread by activating not only plasmin but also collagenase. It should be said that basement membranes are poor substrates for the PA/plasmin system, although non-collagenous components may be degraded by these enzymes [34].

Other actions of the PA/plasmin system which might relate to its role as a mediator of tumor invasion and/or metastasis include its ability to stimulate cell division [35], modify cell surfaces [36], enhance cellular migration [20] or digest fibrin surrounding tumors [37].

POSSIBLE CLINICAL SIGNIFICANCE OF PA MEASUREMENTS

PA is an estrogen-inducible enzyme both in rat uteri [38] and human breast cancer cells in culture [39]. The enzyme is thus a potential marker for a functional estradiol receptor in human breast cancer and possibly in other estrogen-dependent

carcinomas. Indeed, two recent papers have reported a correlation between estradiol receptors and PA in mammary carcinoma biopsies [40, 41]. Evers *et al.* [5], however, found no significant correlation between PA and estradiol receptors. The reasons for the conflict between these results are unknown. It may, however, relate to the relatively small number of biopsies studied by the latter group, 44, whereas in the other two reports greater than 100 tumors were included. It should also be stated that Evers *et al.* extracted PA with Triton X-100, while in the studies showing positive correlation between ER and PA, soluble fractions of cell extracts were used to assay the enzyme. The results on whether PA values, in addition to estradiol receptor results, can increase our ability to predict hormone-dependent breast malignancies over the estradiol receptor alone is awaited with interest. If PA should turn out to be a marker for a functional estradiol receptor in breast carcinomas, its assay should be considerably simpler and cheaper than that of the progesterone receptor.

Separation of breast tumor PA into its different molecular forms may also provide clinically useful information. Using polyacrylamide gel electrophoresis, Yang *et al.* [42] divided breast carcinoma extracts into four groups with respect to isoenzymes of PA. Group A expressed little or no PA activity, group B expressed primarily a 70-K form, group C expressed both 70-K and 55-K forms and group D expressed mostly the 55-K form. Two years after primary surgery, patients with phenotypes A, B, C and D had shown 0, 8.9, 36.5 and 49% disease recurrence respectively ($P < 0.004$). These results show that in order to obtain meaningful results with PA it may be necessary to fractionate the enzyme into its different molecular forms.

A totally different potential clinical role for urokinese-like PA has been suggested by Niklasson *et al.* [43]. Using radioimmunoassay, these workers showed that this enzyme was considerably higher in uterine aspirates from patients with endometrial neoplasia than from patients with either normal or benign endometrial histology. They concluded that measurement of urokinase-like PA in uterine fluid might be useful when combined with other parameters of malignancy in screening for endometrial carcinoma.

Few reports have appeared on the measurement of PA in blood from patients with cancer. However, as far back as 1953 Tagnon *et al.* [44] showed that fibrinolytic activity in blood was increased in some patients with prostatic cancer. Later Burchardt and Marseek [45] proposed that the increased PA activity in blood could be used as a diagnostic test for cancer of the prostate. In contrast to these findings reporting higher levels of fibrinolytic activity in blood from cancer patients compared with controls, Rennie and Ogston found that fibrinolytic activity was lower in blood from patients with a variety of different cancers than in age-matched controls [46]. Moreover, the activity in patients with advanced disease was lower than in those with localized cancer. More recently, Colombi *et al.* [47] have shown, using isoelectrofocusing, that the urokinase-like forms of PA are absent or decreased in plasma from patients with breast cancer compared with healthy controls. The recent development of a radioimmunoassay for plasma urokinase [48] should stimulate research into the possibility of using this form of PA as a tumor marker.

CONCLUSION

PA is an enzyme intimately associated with cancer. It is increased in cells transformed *in vitro* by a variety of agents. Increased levels of the enzyme are also found in many solid tumors. High levels of PA may be important in tumorigenesis, tumor invasion and metastases. If these findings are confirmed, inhibition of PA activity could be a method of controlling cancer growth. The increased PA produced by many tumors may allow this enzyme to be used as a tumor marker. However, many conflicting reports will have to be resolved before PA can be used as a tumor marker. Some of the conflicting results may relate to different or incomplete extraction of the enzyme, or inaccurate enzyme assays due to the presence of endogenous inhibitors or multiple forms of this enzyme. Future work should therefore concentrate on optimum extraction methods and assay of specific molecular forms by immunoassay.

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