Occurrence of Components of Fibrinolytic Pathways in Situ in Laryngeal Cancer

Marek Z. Wojtukiewicz, M.D.,[1] Ewa Sierko, M.D.,[2] Leo R. Zacharski, M.D.,[3] Malgorzata Rózanska-Kudelska, M.D.,[4] and Lech Zimnoch, M.D.[5]

ABSTRACT

Malignancy is characterized by the occurrence of components of coagulation reaction pathways in situ within tumor tissues detectable immunohistochemically. However, tumors vary in the details of this coagulation-cancer interaction. We have previously described tumor cell–associated tissue factor (TF), factor (F) VII, and F X in laryngeal carcinoma tissues. Fibrinogen and F XIIIa were found in the tumor connective tissue. Tissue factor pathway inhibitor (TFPI) occurred in the tumor connective tissue and on microvascular endothelial cells and normal squamous epithelial cells but not in the tumor cells. Fibrin (thrombin-cleaved fibrinogen) existed at the host-tumor interface and the margins of tumor nodules consistent with an active tumor cell–associated clotting pathway in this tumor type. Studies were extended here to detect components of fibrinolytic pathways. Plasminogen and tissue plasminogen activator (t-PA) were detected on laryngeal tumor cells, particularly in more well-differentiated cases. Low-molecular-weight urokinase plasminogen activator (LMW u-PA) was primarily a feature of more undifferentiated laryngeal carcinoma cells. Staining to a lesser extent was found for high-molecular-weight u-PA (HMW u-PA) on tumor cells and various normal cell types in the tumor tissue. Relatively weak and variable tumor cell staining was found for plasminogen activator inhibitors (PAI) 1, 2, and 3. Trace staining was found for u-PA receptor (u-PAR) in differentiated tumor cells. The significance of coagulation and fibrinolytic pathways present in situ to the economy of laryngeal carcinoma remains to be determined.

KEYWORDS: Laryngeal cancer, blood coagulation, fibrinolysis

Objectives: Upon completion of the article the reader should be able to (1) identify components of the fibrinolytic system found in tumor tissue of laryngeal cancer and (2) state the potential clinical consequences of these observations.

Accreditation: Tufts University School of Medicine (TUSM) is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians. TUSM takes responsibility for the content, quality, and scientific integrity of this CME activity.

Credit: TUSM designates this educational activity for a maximum of 1 Category 1 credit toward the AMA Physicians Recognition Award. Each physician should claim only those credits that he/she actually spent in the activity.

Modulation of Malignant Growth by the Coagulation Mechanism and Anticoagulants; Editor in Chief, Eberhard F. Mammen, M.D.; Guest Editor, Leo R. Zacharski, M.D. *Seminars in Thrombosis and Hemostasis*, volume 29, number 3, 2003. Address for correspondence and reprint requests: Marek Z. Wojtukiewicz, M.D., Department of Oncology, Medical Academy, 12 Ogrodowa Street, 15–027 Bialystok, Poland. E-mail: mwojtuk@polbox.com. [1]Professor of Medicine and Oncology, Department of Oncology, Medical Academy; Departments of [2]Oncology, [4]Otolaryngology, and [5]Pathomorphology, Medical Academy, Bialystok, Poland; [3]Department of Medicine, Dartmouth Medical School, Hanover, New Hampshire and the Department of Veterans Affairs Medical and Regional Office Center, White River Junction, Vermont. 0094-6176,p;2003,29,03, 317,320,ftx,en;sth00890x.

Coagulopathies commonly complicate the course of malignancy.[1] They are usually defined in the fluid phase in the form of blood coagulation test abnormalities.[1–3] However, coagulation reactions are also known to occur in the solid phase in situ within various solid tumors, although much less is known about these.[3–8] Based on existing data, it appears that heterogeneous mechanisms define this coagulation-cancer interaction in situ. Whereas some tumor types exhibit a tumor cell–associated coagulation pathway with fibrin formation, others appear to express primarily u-PA.[9–13]

Laryngeal cancer is the most frequently diagnosed malignancy of the head and neck region. Treatment success is strongly related to tumor stage at presentation, and advanced disease is associated with a poor prognosis. The course of laryngeal carcinoma may be complicated by thromboembolism.[14] In an attempt to define the role of the tumor cell in coagulation activation in laryngeal cancer, we previously demonstrated (using immunohistochemical techniques) the presence of TF, F VII, and F X on laryngeal tumor cells.[7] Fibrinogen was plentiful in the tumor connective tissue, and fibrin (thrombin-cleaved fibrinogen) was present at the host-tumor interface. TFPI that inhibits TF/F VIIa/F Xa complex activity was found in the tissues but not associated with the tumor cells that are thus apparently responsible for solid phase, cell-associated coagulation activation in this tumor type. The present study was undertaken to extend the database on the coagulation-cancer interaction in laryngeal cancer by searching for components of the fibrinolytic pathways in situ in this tumor type.

MATERIALS AND METHODS

Tissue specimens were obtained during surgical resection of squamous cell laryngeal cancer from 37 previously untreated patients. Samples were fixed according to AMeX method[15] and then paraffin embedded. Normal tissues derived from the tumor-free surgical margins served as controls. Staining procedures and controls for the avidin-biotin complex (ABC) technique using reagents (Vectastain Kits, Vector Laboratories, Burlingame, California) were reported elsewhere.[16] Procedures applied antibodies purchased from American Diagnostica (Greenwich, Connecticut) and Dade Behring Diagnostics (Deerfield, Illinois). Monoclonal antibodies (mAb) used were directed against PAI-1 and PAI-2. A panel of polyclonal antibodies (pAb) was also used, including antibodies directed against plasminogen, t-PA, PAI-3, HMW u-PA, LMW u-PA, and u-PAR. Antibodies reacting with the epitopes of HMW u-PA, LMW u-PA, and PAI-3 were gifts from Dr. David Stump of Genentech (South San Francisco, California). Antibodies were tested on control and tumor tissues in concentrations that provided maximum staining intensity with minimum background staining. Controls consisted of omission from the procedure of the primary antibody and the use the antibody developed in the same species but with different or irrelevant specificities. Results of the staining of the laryngeal cancer tissues were compared with respective normal tissues processed simultaneously. Antigen staining was detected by the dark-brown reaction product that appeared with antibody labeling in the ABC immunostaining procedure that contrasted with the pale pink appearance of unstained stroma.

RESULTS

Strong staining for plasminogen was detected on nests of cancer cells (Fig. 1), especially at the tumor periphery, and on tumor-associated macrophages. Staining for plasminogen appeared to be stronger on relatively well-differentiated and mature neoplastic cells and less intense on more undifferentiated, less mature malignant cells.

Cancer cells and tumor infiltrating macrophages also stained for t-PA antigen. As with staining for plasminogen, particularly strong staining for t-PA was demonstrated in mature keratotic cancer cells, whereas less mature, more malignant cells showed less intense staining. Epitopes of t-PA were also present in the cells of normal mucous and serous glands as well as in mature cells of normal stratified epithelium of the respiratory tract.

Weak staining using pAb to HMW u-PA was present on differentiated cancer cells localized in the center of cancer foci and in the tumor infiltrating macrophages. HMW u-PA was also present on normal epithelial cells (keratotic and spinous). Staining for LMW u-PA was observed in immature cancer cells, particu-

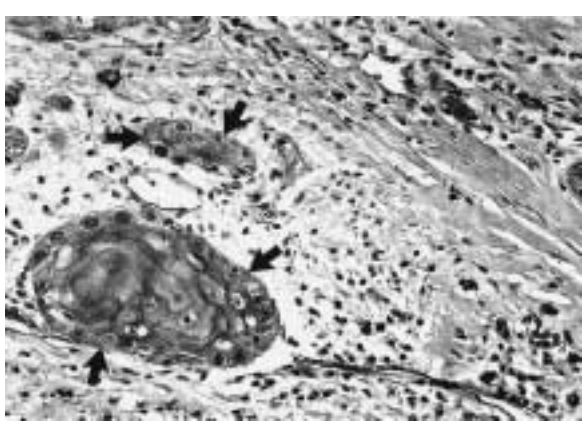

Figure 1 Specific staining of well-differentiated laryngeal carcinoma tumor cells using antibody against plasminogen. Peroxidase technique; hematoxylin counterstain. Original magnification × 400.

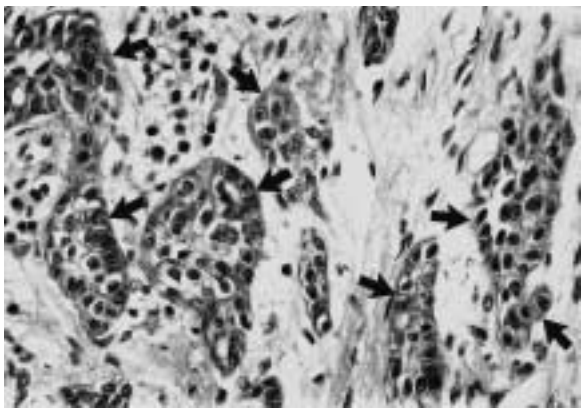

Figure 2 Specific staining of undifferentiated, relatively immature laryngeal carcinoma tumor cells using antibody against LMW u-PA. Peroxidase technique; hematoxylin counterstain. Original magnification × 400.

larly those localized at the invasion front of the tumor (Fig. 2). Relatively weak staining of tumor cells was also observed using antibodies to PAI-1, -2, and -3. PAI-1 and -3 were present, particularly on more mature, well-differentiated tumor cells, whereas PAI-2 antigen was detected in all cancer cells, especially on the periphery of small neoplastic lesions at the invasion front. Staining for u-PAR was slight and restricted to more differentiated keratotic cancer cells.

DISCUSSION

Studies of the occurrence of components of coagulation reaction pathways in situ within tumor tissues are designed to elucidate the role of cells (tumor cells, macrophages, endothelial cells, and so on) in the solid phase coagulation activation that commonly accompanies malignancy.[9] Such studies have shown that tumor types vary in the details of the coagulation-cancer interaction within tissues. We have shown previously that laryngeal carcinoma tissues exhibit tumor cell–associated TF, F VII, and F X.[7] Fibrinogen and F XIIIa exist in the tumor connective tissue. TFPI also exists in the tumor connective tissue and on microvascular endothelial cells and normal squamous epithelial cells but not on the tumor cells. Fibrin (thrombin-cleaved fibrinogen) is present at the host-tumor interface, suggesting that tumor cell–associated clotting factors are enzymatically active in this tumor type.[7]

This report documents the occurrence of components of the fibrinolytic pathways in laryngeal cancer tissues. In general, plasminogen, plasminogen activator, and PAIs were present on tumor cells, especially the more differentiated varieties (see Fig. 1). The exception was LMW u-PA, which was more conspicuously present on relatively immature, less well-differentiated tumor cells (see Fig. 2).

The importance of studies of this kind rests in the fact that these reactions may explain mechanisms of coagulation activation in cancer and may promote tumor growth and dissemination. Evidence supporting these concepts has been reviewed in detail elsewhere.[4–14,17–19] Obviously, our current database on mapping molecular participants in pathways of coagulation and growth control in specific tumor types is incomplete, but further investigation is theoretically capable of producing a more satisfactory picture of tumor pathophysiology. An understanding of these regulatory algorithms is needed for the generation and testing of novel therapeutic hypotheses because administering drugs that interfere with the coagulation and fibrinolytic pathways can alter the course of malignancy in experimental animals.[1,2] Limited studies suggest that similar effects might be observed in human disease, but rational clinical trial design requires connecting drug mechanism with mechanisms relevant to growth of individual tumor types, and these mechanisms are already known to be heterogeneous.[9] For example, warfarin has been shown to improve the course of small cell carcinoma of the lung, a tumor type characterized by tumor cell–associated thrombin generation in situ, but this drug had no effect on the course of various other malignancies, including laryngeal cancer.[9] Aprotinin (an inhibitor of the u-PA–plasmin system) has been shown to have a striking effect in metastatic colorectal cancer, which is a u-PA–expressing tumor type.[12,20] Interest has been directed recently to favorable effects of heparin (LMWH) in human malignancy.[21] This drug inhibits not only coagulation but also angiogenesis needed for tumor growth, heparinase required for tumor cell invasion, and the activity of tumor and angiogenic growth factors.

Existing data suggest a starting point for possible experimental treatment strategies in laryngeal cancer. For example, studies in model systems have shown that u-PA stimulates proliferation, migration, and adhesion of malignant and other cell types.[19] We demonstrated relatively weak expression of LMW u-PA in well-differentiated laryngeal cancer cells but stronger expression in cases of less differentiated laryngeal cancer (see Fig. 2). The presence of this plasminogen activator together with plasminogen (see Fig. 1) suggests the possibility that plasmin may be formed in situ that is relatively unopposed by PAIs and that might contribute to tumor invasion and dissemination. Recently, statistically significant differences in the expression of u-PA and PAI-1 messenger RNA (mRNA) compared with the normal tissue have been reported in laryngeal cancer tissue.[22] Another study revealed that the intensity of u-PA expression in laryngeal cancer cells is independent of the degree of malignancy but correlates with nodal involvement.[23] Many tumor types are known to express u-PAR, and concentrations of this receptor have prog-

nostic significance.[24–28] In the present study, only weak expression of u-PAR was observed in well-differentiated cancer cells. Although binding of u-PA to its receptor increases enzymatic activity in some systems, such binding is not needed in other settings.[29,30]

In summary, our results suggest that further testing of drugs that interfere with coagulation and fibrinolysis pathways may warrant testing in controlled clinical trials in laryngeal cancer. In this regard, LMWH is a particularly appealing experimental agent because of its ability to block coagulation activation, activation of u-PA, and growth factors believed to be involved in laryngeal carcinoma growth.[21,31]

REFERENCES

1. Francis JL, Biggerstaff J, Amirkhosravi A. Hemostasis and malignancy. Semin Thromb Hemost 1998;24:93–109
2. Bromberg ME, Capello M. Cancer and blood coagulation: molecular aspects. Cancer J 1999;5:132–138
3. Wojtukiewicz MZ, Rucinska M, Zimnoch L, et al. Expression of prothrombin fragment 1+2 in cancer tissue as an indicator of local activation of blood coagulation. Thromb Res 2000;97:335–342
4. Wojtukiewicz MZ, Zacharski LR, Memoli VA, et al. Abnormal regulation of coagulation/fibrinolysis in small cell carcinoma of the lung. Cancer 1990;65:481–485
5. Wojtukiewicz MZ, Zacharski LR, Memoli VA, et al. Malignant melanoma. Interaction with coagulation and fibrinolysis pathways in situ. Am J Clin Pathol 1990;93:516–521
6. Wojtukiewicz MZ, Zacharski LR, Memoli VA, et al. Fibrinogen-fibrin transformation in situ in renal cell carcinoma. Anticancer Res 1990;10:579–582
7. Wojtukiewicz MZ, Zacharski LR, Rucinska M, et al. Expression of tissue factor and tissue factor pathway inhibitor in situ in laryngeal carcinoma. Thromb Haemost 1999;82:1659–1662
8. Wojtukiewicz MZ, Rucinska M, Zacharski LR, et al. Localization of blood coagulation factors in situ in pancreatic carcinoma. Thromb Haemost 2001;86:1416–1420
9. Zacharski LR, Wojtukiewicz MZ, Costantini V, Ornstein DL, Memoli VA. Pathways of coagulation/fibrinolysis activation in malignancy. Semin Thromb Hemost 1992;18:104–116
10. Constantini V, Zacharski LR, Memoli VA, et al. Occurence of components of fibrinolysis pathways in situ in neoplasmatic human breast tissue. Cancer Res 1991;51:354–358
11. Ornstein DL, Constantini V, Zacharski LR, et al. Coexistent macrophage procoagulant and tumor cell plasminogen activator in adenocarcinoma and squamous cell carcinoma of the lung. Cancer 1991;68:1061–1067
12. Wojtukiewicz MZ, Zacharski LR, Memoli VA, et al. Indirect activation of blood coagulation in colon cancer. Thromb Haemost 1989;62:1062–1066
13. Wojtukiewicz MZ, Zacharski LR, Memoli VA, et al. Fibrin formation on vessel walls in hyperplastic and malignant prostate tissue. Cancer 1991;67:1377–1383
14. Nasyrov VA, Osmonowa RO, Rachkol AG. State of the hemostasis system in patients with malignant III-stage neoplasms of the maxilla and larynx. Vestn Otorinolaringol 1991; 5:12–15
15. Sato Y, Mukai K, Watanabe S, Goto M, Shimosato Y. The AMeX method. The simplified technique of tissue processing and paraffin embedding with improved preservation of antigens for immunostaining. Am J Pathol 1986;125:431–435
16. Hsu S, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981;29:577–580
17. Collen D. The plasminogen (fibrinolytic) system. Thromb Haemost 1999;82:259–270
18. Wojtukiewicz MZ, Sierko E, Klement P, Rak J. The hemostatic system and angiogenesis in neoplasms. Neoplasia 2001; 51:93–103
19. Carroll VA, Binder BR. The role of the plasminogen activation system in cancer. Semin Thromb Haemost 1999;25:183–197
20. Lentschener C, Li H, Franco D, et al. Intraoperatively-administered aprotinin and survival after elective liver resection for colorectal cancer metastasis. A preliminary study. Fibrinolysis Proteolysis 1999;13:39–45
21. Zacharski LR, Ornstein DL. Heparin and cancer. Thromb Haemost 1998;80:10–23
22. Pasini F, Brentani MM, Kowalski LP, Federico MH. Transforming growth factor-1, urokinase-type plasminogen activator and plasminogen activator inhibitor-1 mRNA expression in head and neck squamous carcinoma and normal adjacent mucosa. Head Neck 2001;23:725–732
23. Parolini S. Flagiello D, Cinquetti A, et al. Up-regulation of urokinase-type plasminogen activator in squamous cell carcinoma of human larynx. Br J Cancer 1996;74:1168–1174
24. Hofmann R, Lehmer A, Buresch M, Hartung R, Ulm K. Clinical relevance of urokinase plasminogen activator, its receptor, and its inhibitor in patients with renal cell carcinoma. Cancer 1996;78:487–492
25. Pyke C, Kristensen P, Ralfkiaer E, et al. Urokinase-type plasminogen activator is expressed in stromal cells and its receptor in cancer cells at invasive foci in human colon adenocarcinomas. Am J Pathol 1991;138:1059–1067
26. Bianchi E, Cohen RL, Thor AT, et al. The urokinase receptor is expressed in invasive breast cancer but not in normal breast tissue. Cancer Res 1994;54:861–866
27. De Vries TJ, Quax PHA, Denijn M, et al. Plasminogen activators, their inhibitors, and urokinase receptor emerge in late stages of melanocytic tumor progression. Am J Pathol 1994; 144:70–81
28. Pedersen H, Grondahl-Hansen J, Francis D, et al. Urokinase and plasminogen activator inhibitor type 1 in pulmonary adenocarcinoma. Cancer Res 1994;54:120–123
29. Cohen RL, Xi X-P, Crowley CW, et al. Effects of urokinase receptor occupancy on plasmin generation and proteolysis of basement membrane by human tumor cells. Blood 1991;78: 479–487
30. Blasi F. Proteolysis, cell adhesion, chemotaxis, and invasiveness are regulated by the u-PA-u-PAR-PAI-1 system. Thromb Haemost 1999;82:298–304
31. Homer JJ, Greenman J, Stafford ND. The expression of vascular endothelial growth factor (VEGF) and VEGF-C in early laryngeal cancer: relationship with radioresistance. Clin Otolaryngol 2001;26:498–504