Coagulation-Cancer Interaction In Situ in Renal Cell Carcinoma

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Fibrin was detected by immunospecific techniques associated with both intravascular and extravascular tumor deposits in renal cell carcinoma. In addition, coagulation factors VII and X were demonstrated in intercellular spaces within tumor tissue adjacent to the surface of tumor cells. Scant accumulation of platelets on intravascular tumor masses was observed. These data suggest that tumor cells in renal cell carcinoma may induce fibrin formation locally by a factor VII–mediated (and tissue factor-initiated) pathway of blood coagulation. This mechanism may also account for the hypercoagulable state that exists with this tumor type. We postulate that local fibrin formation may contribute to the growth and spread of this particular type of cancer and that the course of renal cell carcinoma may be ameliorated by anticoagulant therapy.

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MATERIALS AND METHODS

The avidin-biotin complex (ABC) technique was adopted from the method of Hsu et al using reagents from Vector Laboratories (Burlingame, Calif). Immunofluorescence methods using a specific, polyclonal antibody to human fibrinogen were those described previously for detection of fibrin in SCCL. Antithrombin III was obtained from Behring Diagnostics (American Hoechst Corp, Somerville, NJ). Monoclonal antibodies to human factors VII and X were chosen for optimal end point.

Information on the mechanisms of tumor cell interaction with the host coagulation mechanism has been sought by attempting to identify the end product of coagulation activation, fibrin, in association with tumor masses. Detailed studies of breast cancer,7 lymphomas,8 and SCCL9 have shown that fibrin is deposited in different patterns associated with viable tumor cells in these particular tumor types. In addition, evidence has been presented for the existence of the putative initiator of coagulation, tissue factor, on SCCL cells themselves.9

Although some progress has thus been made in defining the coagulation–cancer cell interaction in human malignancy, information on the occurrence of fibrin in other human tumor types is lacking. Further uncertainties surround the precise mechanism by which fibrinogen is converted to fibrin and the possible role of platelets as well as fibrinolytic enzymes. The purpose of this paper is to describe observations on the occurrence of certain coagulation reaction intermediates and fibrin in renal cell carcinoma (RCC). This particular tumor type was chosen for investigation because it is commonly accompanied by activation of the coagulation mechanism and because of its propensity to flourish in thrombi within vascular channels (see Discussion). Emphasis is placed on establishing methods for detection of coagulation factors VII and X and for test for the existence of these coagulation intermediates of the extrinsic pathway in association with tumor cells.

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antibody tested. The positive control for the antibody to fibrinogen was term placenta; for antibody to factors VII and X, normal liver; for antibody to factor VIII antigen, vascular endothelium; and for antibody to platelet IIb-IIIa glycoprotein, peripheral blood platelets and bone marrow megakaryocytes.

RESULTS

Dense specific fluorescence was observed in sections of fresh-frozen tissue with antifibrinogen antibody that revealed fibrin encasing RCC tumor cells within thin-walled vascular channels (Fig 1) and in the fibrous connective tissue stroma surrounding viable tumor cells in extravascular sites (Fig 2). Phase-contrast–fluorescence photographic pairs of the same microscopic field demonstrated relationships between tumor cells and fibrin. Individual tumor cells in a tumor mass within vascular channels were encased in dense fibrin-specific fluorescence, but connective tissue was lacking (Fig 1). By contrast, fibrin-containing connective tissue surrounded nests of tumor cells within the tissue (Fig 2). The adjacent normal kidney did not contain fibrin, indicating that the fibrin was specifically related to the tumor itself.

The endothelial lining of the vascular channels stained with antibody to factor VIII antigen. Little staining of intravascular tumor masses was observed with the antibody to either factor VIII antigen or the platelet IIb-IIIa glycoprotein. Staining that did exist manifested a sparse, nodular pattern. Neither antibody stained structures in extravascular...
sites. The antibody to platelet IIb-IIIa glycoprotein stained occasional areas in a nodular pattern on the linings of vascular channels as well as peripheral blood platelets.

Antibody to factor VII failed to stain either normal liver or tumor tissue using the fluorescence procedure, but stained both when observed by the ABC technique in sections of fresh-frozen and also formalin-fixed tissue. Factor VII in liver cells was observed in the cytoplasm and also in association with the membranes of hepatic parenchymal cells. Staining in tumor tissue was observed adjacent to the surfaces of tumor cells and also in the intercellular spaces (Fig 3). Using antibody to factor X, we demonstrated factor X in normal liver and in tumor tissue by both fluorescence and peroxidase procedures and in formalin-fixed tissue using the peroxidase procedure. A cytoplasmic pattern was observed in hepatic parenchymal cells, but staining was restricted to the intercellular spaces in tumor tissue (Fig 4). Thus, localization of both factors VII and X was restricted to the pericellular areas around tumor cells. It was not present in perivasculan connective tissue or in areas occupied by inflammatory cells.

Staining with antibodies to fibrinogen, factor VIII antigen, platelet IIb-IIIa glycoprotein, factor VII, and factor X was retained in fresh-frozen (unfixed) tissue despite the repeated washings required for these procedures. Staining was not observed when the primary antibody was omitted or when antibodies of other specificities were used. These procedures, together with preincubation with normal blocking serum, which is part of the methodology, preclude nonspecific binding to Fc receptors. Staining with antibodies to factors VII and X, but not with antibody to fibrinogen, was retained in paraffin-embedded tissue sections. Staining was not observed in normal kidney tissue and was not a consequence of tissue processing. There was no nonspecific tissue staining.

**DISCUSSION**

RCC is a relatively uncommon tumor\textsuperscript{14,15} that is thought to arise from the cells of the proximal convoluted tubule of the nephron.\textsuperscript{16} RCC has a striking propensity to thrive in the form of a tumor thrombus within the renal vein and inferior vena cava.\textsuperscript{14,15} Venous invasion occurs grossly in 46.2% and histologically in an additional 31% of cases and signals more aggressive behavior of the tumor and a poorer prognosis.\textsuperscript{17} RCC tumor cells apparently find conditions within a venous thrombus to their liking since such tumors may achieve considerable proportions, even reaching the right atrium, and may become detached and continue an existence within the vein free of the primary tumor.\textsuperscript{18}

Patients with RCC may manifest peripheral blood changes of disseminated intravascular coagulation.\textsuperscript{3,19,20} Thromboembolic disease may signal the existence of occult RCC or complicate the course of previously identified disease.\textsuperscript{21-24} Sufrin et al\textsuperscript{25} found that fibrinogen and fibrin degradation products (FDP) were elevated in RCC, that fibrinogen elevation (but not the FDP or platelet levels) correlated with more advanced disease and a more rapidly progressive course, and that fibrinogen levels declined following nephrectomy. FDP were more commonly increased in patients with metastatic rather than localized disease.\textsuperscript{26,27} A functionally abnormal fibrinogen with an increased content of sialic acid has been described in RCC.\textsuperscript{28}

A number of coagulation-related studies have been performed on RCC tissue. Cliffon and Grossi\textsuperscript{29} described a plasminogen activator in explants of RCC tumor tissue. By contrast, Yuen and Kwaan\textsuperscript{30} found, in studies of sections of fresh RCC tissue placed on a fibrin plate, that fibrinolysis usually observed about blood vessels in normal tissues was inhibited about vessels in the vicinity of the tumor. This implied that a diffusable inhibitor of fibrinolysis existed within the tumor. O’Meara\textsuperscript{31} observed that extracts of normal kidney inserted into blood induced formation of a clot that subsequently lysed. By contrast, introduction of extracts of RCC tissue into blood produced a clot that did not subsequently lyse. Gordon and associates\textsuperscript{32} identified a coagulant in RCC tissue that differed from the coagulant of normal tissue in that it did not require factor VII for expression of coagulant activity and it was inhibited by
diisopropylfluorophosphate. For these reasons they claimed that this coagulant was unlike tissue factor (tissue thromboplastin).

Studies by Long et al. suggested that RCC tumor cells may manifest coagulative properties in vivo. These authors described a patient with an unresectable RCC involving the renal vein in whom the number of tumor cells within the venous drainage transiently increased during surgery. A subsequent increase in circulating tumor cells was discovered several hours before development of a pulmonary embolism. Two months later the lungs were found by chest x-ray to be studded with metastases.

Naito and colleagues studied the coagulant and fibrinolytic properties of continuous cell lines derived from one patient with rapidly progressive and one patient with slowly progressive RCC. The coagulant corrected the defect in factor IX-deficient plasma and also in factor VII-deficient plasma, but to a lesser extent. The inducer of fibrinolysis produced by these cells was urokinase. Cells from the patient with slowly progressive disease manifested low levels of coagulant and fibrinolytic activity. Upon inoculation of these cells into nude mice, tumors appeared but subsequently regressed. In contrast, cells from the patient with rapidly progressive disease exhibited high levels of coagulant and fibrinolytic activity. Upon inoculation into nude mice, this second cell line demonstrated greater tumorigenicity and more rapid tumor growth than the first cell line.

Steiner found that mouse renal adenocarcinoma cells were potent in vitro inducers of platelet aggregation and release and that platelet lysates promoted mouse renal adenocarcinoma cell growth in culture. Possible platelet aggregating properties of human RCC cells have not been described. Sequestration of fibrinogen (tagged with a radiolabeled antibody to fibrinogen) into RCC tumor masses in vivo has been reported in six cases. The effects of coagulation-reactive drugs in RCC have rarely been studied. However, Larsen et al. observed no change in the condition of a single patient with RCC treated with a fibrinolytic enzyme, heparin, and aspirin.

In the present study we have documented the occurrence of fibrin associated with extravascular deposits of viable tumor in RCC and also surrounding tumor masses within vascular channels. It is likely that our findings are, in fact, indicative of fibrin. However, the possibility remains that at least a portion of the fluorescence observed with the polyclonal antifibrinogen antibody used was due to the presence of fibrinogen that had leaked from blood vessels or of polymerized but non–cross-linked fibrin. Existing literature suggests that tumor cell–associated coagulants may be responsible for initiating the sequence of enzymatic reactions that lead to local fibrin formation through factor VII–independent (cancer procoagulant A–initiated) coagulation pathways. By contrast, the present observations support the possibility that a factor VII–dependent, tissue factor–initiated reaction sequence may be responsible for the fibrin deposited in RCC. Thus, factors VII and X were present extravascularly in the pericellular spaces around tumor cells and adjacent to tumor cell surfaces. These factors were not eliminated from fresh-frozen tissue sections by the repeated washings required for the immunologic techniques used. This suggests that these factors were bound in place and, therefore, possibly in active form. Neither factor VII nor X were present in normal tissue, perivascular connective tissue, or areas occupied by inflammatory cells in tumor tissue. A role for the factor VII–dependent, tissue factor–initiated pathway of fibrin formation would be supported by demonstration of tissue factor antigen on RCC tumor cells. Although such evidence is lacking for RCC, we have previously demonstrated tissue factor on SCCL tumor cells using an antibody to bovine tissue factor that cross-reacted with human tissue factor. Evidence was obtained for platelet deposition on intravascular tumor, but such deposits were smaller than might have been expected from certain experimental studies that have shown platelet masses of substantial proportions associated with tumor cells within blood vessels. The extent and significance of platelet involvement in RCC remains undefined.

In summary, evidence presented suggests that activation
of the host coagulation mechanism by tumor cells in RCC results in local fibrin formation. Since RCC appears to thrive within a fibrin clot in blood vessels, such clot formation may accommodate tumor growth. Previous studies have shown an abundance of fibrin associated with extravascular tumor deposits in SCCL and that the clinical course of SCCL is ameliorated by warfarin anticoagulation. We postulate that tumor progression in RCC may also be interrupted by agents that are capable of limiting local coagulation reactions.

REFERENCES

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