Occurrence of Fibrin and Tissue Factor Antigen in Human Small Cell Carcinoma of the Lung

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ABSTRACT

Fibrin was detected by specific immunofluorescence in tissue obtained from five of six cases of small cell carcinoma of the lung. Dense specific fluorescence was observed in the connective tissue stroma surrounding metastatic tumor nodules and frequently in the scant extracellular stroma surrounding individual viable tumor cells and small tumor cell clusters. When observed by electron microscopy, the fibrin hugged tumor cell plasma membranes and, in some areas, seemed to envelop the cells. Fluorescent staining of tumor cells, but not the stroma, was observed with an antibody to tissue factor. These findings suggest that local activation of coagulation occurs in small cell carcinoma of the lung. Deposited fibrin may contribute to the growth and spread of this particular type of cancer.

INTRODUCTION

A role for blood coagulation reactions in tumor dissemination has been proposed based on studies conducted in experimental tumor systems (15, 26, 27). In these studies, it has been shown (for certain experimental cancers) that treatment with either anticoagulant or antiplatelet drugs reduces metastatic dissemination. The applicability of these concepts to human cancer is uncertain, since controlled clinical trials of antithrombotic agents have been initiated only recently (26, 27). However, preliminary evidence of a beneficial effect of warfarin in SCCL (28) provides evidence of a beneficial effect of warfarin in SCCL (28) provides an incentive to investigate activation of coagulation, both local and systemic, in patients with such tumors.

Neoplastic cells are known to interact with the host coagulation system in several ways. Thus, certain tumors have been shown to manifest procoagulant (11, 21), platelet-aggregating (15, 27), and fibrinolytic (3) activities. The procoagulant properties of tumor cells may account for the occurrence of fibrin clots or platelet aggregates about circulating tumor cells (15, 26, 27) and for the fibrin deposition which commonly is associated with extravascular tumor deposits (4, 5, 13). Although such extravascular deposition of fibrin is thought to be a feature of a variety of forms of cancer (5, 26), detailed morphological descriptions of this association have been forthcoming only recently. Such studies have shown that differences exist in the patterns of fibrin involvement in breast cancer (4) and in lymphomas (13). In breast cancer, the tumor-related fibrin is found in the fibrous stroma that surrounds tumor nodules (4). In lymphomas, and particularly in Hodgkin's disease, the fibrin is found laced between the cells of the tumor (13). These divergent patterns may signal differences in the manner of interaction of these 2 tumors with coagulation reactions that could provide the basis for therapeutic intervention with antithrombotic drugs. Because of this possibility and because of preliminary evidence for efficacy of warfarin in SCCL (28), we investigated SCCL for the local occurrence of fibrin and the putative initiator of fibrin formation, tissue factor.

MATERIALS AND METHODS

Tissue was obtained from 6 cases of SCCL. In one of these, the specimen consisted of a circumscribed lesion 2 cm in diameter that appeared on chest X-ray in the left upper lung field and that, upon surgical excision and histological examination, was found to be SCCL. Tissue was obtained from the remaining cases at postmortem examination. Blocks prepared from the peripheral regions of tumor masses were placed in plastic vials, covered with ornithine carbamyl transferase (Tissue Tek II; Miles Laboratories, Naperville, Ill.) and tightly capped to prevent dehydration, and stored frozen at -70°. Frozen sections were cut on a cryostat, mounted on gelatin-coated slides, air dried at room temperature, and washed with PBS, pH 7.2. For this purpose, Dulbecco's PBS (Grand Island Biological Co., Grand Island, N. Y.) was used. This solution contained CaCl2 (0.1 g/liter), KCl (0.20 g/liter), KH2PO4 (0.20 g/liter), MgCl2 (0.047 g/liter), NaCl (8 g/liter), and Na2HPO4 (1.15 g/liter).

Sections were stained by the direct immunofluorescence technique using a fluorescein-conjugated rabbit anti-human fibrinogen antibody or by the indirect immunofluorescence technique using a rabbit anti-human fibrinogen antibody followed by a fluorescein-conjugated goat antibody to rabbit IgG (Cappel Laboratories, West Chester, Pa.). These antibodies to fibrinogen formed a single precipitin line with normal plasma on immunodiffusion plates. In certain experiments, antifibrinogen antibodies were used following adsorption with Sepharose-conjugated human fibronectin (13). Antibody incubations were carried out for 20 min, and PBS washes between steps were carried out for 5 min. In experiments performed on frozen sections of term placenta, a selective, nodular pattern of brilliant fluorescence was observed on the peripheral villi. Since fibrin is known to exist in this location (7), placental tissue provided a positive control for test reagents. The antifibrinogen antibodies are capable of reacting with fibrinogen, fibrin, or certain of their degradation products. For present purposes, the antibody is considered to be reactive with fibrin, since the antigen is immobilized within the substance of the tumor tissue where it sometimes manifests a fine-stranded appearance (see below). However, it is understood that the precise state of this antigen in the tissue is currently undefined. Stained slides were mounted in polyvinyl alcohol (Gelvitol 20/30; Monsanto, Indian Orchard, Mass.) at pH 8.0. When thus mounted, the fluorescence was stable for several weeks.

In other studies, rabbit antibodies to human platelet factor 4 (obtained from Dr. Robert Handin or Dr. Peter Levine), to bovine tissue factor (obtained from Dr. Ronald Bach and Dr. Yale Nemerson), and to human factor VIII antigen obtained from Behring Diagnostic (American Hoechst Cooperation, Somerville, N. J.) were used as immunofluorescent probes. Interpretation of immunofluorescence studies was facilitated by examination of the same field by both phase-contrast and immunofluorescence microscopy, by staining of adjacent tissue sections for immunofluores-
RESULTS

In studies using an antifibrinogen antibody, copious quantities of fibrin were observed within metastatic foci of SCCL. Fluorescence was observed in the dense fibrous stroma surrounding tumor nodules and also in the scant connective tissue adjacent to individual cells or small clusters of cells within tumor nodules. This appearance is illustrated in Fig. 1. The fine, stranded appearance of fibrin could frequently be observed in the capsular stroma of the tumor. Fluorescence with the distribution characteristic of fibrin was not observed in the walls of blood vessels or when antibodies of other specificities were used, and it was eliminated when the primary (antifibrinogen) antibody was omitted or when antibody was absorbed on a fibrin clot. However, fluorescence persisted following absorption on fibronectin-Sepharose. The cytoplasm of hepatic parenchymal cells adjacent to tumor nodules manifested fluorescence indicative of the existence of fibrinogen which is synthesized within these cells (8).

The pattern of fluorescence described above was observed in 5 of the 6 specimens examined. The exception was the small primary tumor that was excised surgically. This specimen lacked fibrin as judged by the techniques used.

Review of electron micrographs on 9 available cases revealed evidence of fibrin in 3 cases. In one case, the fibrin appeared as a dense meshwork that frequently filled the spaces between adjacent viable tumor cells and hugged the cytoplasmic membrane to virtually encase individual cells or clusters of cells in certain areas. This appearance is depicted in Fig. 2. In the other 2 cases, small wisps of fibrin were seen focally in the intercellular stroma between tumor cells. In other areas, fibrin was not evident between cells.

Antibody to factor VIII showed selective uptake into the endothelium of blood vessels present within tissue sections. No staining was evident in either the tumor cells or the tumor stroma in which fibrin was demonstrated. The anti-bovine tissue factor antibody showed cross-reactivity with human tissue factor, since it was capable of neutralizing the coagulant activity of a preparation of human brain microsomes. Using immunofluorescence techniques, this antibody produced staining of tumor cells (Fig. 3) and of the full thickness of blood vessel walls but not of the tumor stroma. No staining was evident in studies using the antibodies to human platelet factor 4.

DISCUSSION

Accumulation of fibrinogen at tumor sites has been demonstrated by scanning procedures following injection of radioisotopically tagged fibrinogen (26) and by a variety of morphological techniques that include autoradiography, electron microscopy, immunofluorescence, and standard histochemical staining (4, 5, 13, 26). Scanning procedures have the advantage of being applicable to studies in vivo but provide no information as to the pattern of fibrinogen distribution or the nature of the fibrinogen-fibrin deposition. However, morphological techniques allow distinctions to be made between fibrin that might form nonspecifically, for example, in thrombosed blood vessels in the vicinity of the tumor or in areas of tumor necrosis, and that which occurs in proximity to viable tumor cells. Moreover, since fibrinogen and certain fibrinogen-fibrin degradation products are soluble in aqueous buffers such as PBS, they will be washed away in the course of preparations for fluorescence microscopy, leaving behind only insoluble fibrin. Immunological methods have the further advantage of specificity for fibrin and rapid availability of results. They also avoid the sampling problems inherent in electron microscopy. Immune-specific methods available for fibrin detection include immunoperoxidase and immunofluorescence procedures. The immunoperoxidase technique has not been useful in our experiments for fibrin detection in tissues. We have found that procedures required for preparation of tissues for examination by this technique apparently alter the fibrin such that it is much less readily recognized by antibody. In this respect, fibrin resembles certain other antigens that are altered by these procedures (9, 10, 22). Thus, immunofluorescence together with electron microscopic techniques were chosen for the present studies.

These methods have permitted demonstration of copious quantities of fibrin in association with metastatic deposits of SCCL. This fibrin was evident in the relatively acellular fibrous stroma surrounding tumor nodules as well as in the traces of connective tissue within tumor nodules intimately associated with certain individual or small clusters of tumor cells. The exception was a small primary tumor which was the only specimen in this series that was not obtained at autopsy that lacked fluorescent staining. The significance of the latter observation remains to be determined, since it is unusual to have opportunity to examine small tumors early in the course of SCCL obtained by surgical excision, and it is hazardous to draw conclusions based on a single case. It is possible that investment of SCCL with fibrin is a feature of the invasive and metastatic properties of the tumor and may not be involved with the tumor at its inception. Alternatively, the absence of fibrin might be due to exaggeration of fibrinolysis during surgery or in the postexcision period. All of the remaining specimens were obtained at autopsy. While we cannot exclude the possibility that the time interval from death to the procurement of the specimen might have influenced the results, this seems unlikely.

Our understanding of the nature and significance of fibrin associated with tumor deposits in SCCL as well as other cancers is, at present, incomplete. However, based upon evidence obtained from a variety of studies in human as well as experimental cancers, a coherent hypothesis may be constructed. Thus, neoplastic cells are known to manifest coagulant activity (11, 21). They may, therefore, be responsible for the generation of increased quantities of FPA in cancer patients (17, 23). Studies of the kinetics of FPA in plasma (23) together with the observation that FPA levels are not readily reduced by infusion of heparin (17) have suggested that the increased levels of FPA are attributable to formation of thrombin in extravascular sites. Thrombin generated extravascularly is assumed to be responsible for local fibrin formation. The fibrin that forms may be related in some
way to the success of the invasive, metastatic behavior of the tumor cells, since administration of anticoagulants retards the spread of tumors in animals (26). Warfarin has been shown to both reduce the coagulant properties of tumor cells (18) and limit local fibrin deposition (20) in certain experimental tumors. The antimitastatic effect of this particular anticoagulant might be exerted by this mechanism (26).

Unfortunately, systematic studies that would provide a complete profile of the coagulation-cancer interaction are available for few experimental tumors, and the information available for human tumor types, among which is SCCL, is little more than fragmentary. However, the clotting mechanism is known to be activated in SCCL as manifested by the occurrence of the coagulation changes indicative of disseminated intravascular coagulation (1, 12) and the occurrence of localized thromboembolic events (2) in this particular tumor type. Markus et al. (16) found that fragments of small-cell lung cancer tissue, obtained following surgical excision in 3 cases, liberated a urokinase-like activator of plasminogen when incubated in short-term culture. The amount of activator produced was in excess of that produced by normal lung tissue. These observations must be interpreted with caution, however, since sections of fresh small-cell lung cancer tissue were found by Kwaan5 to lack fibrinolytic activity when studied by the fibrin slide lysis method as has been observed in tissues from other types of tumors (24). Based on our immunofluorescence studies, local fibrin formation could result from the tissue factor activity of the tumor cells themselves. While the status of fibrinolytic reactions that may be occurring in vivo is uncertain, they are evidently insufficient to completely forestall the local deposition of fibrin in metastatic tumor sites in small-cell lung cancer.

Preliminary (unconfirmed) evidence indicates that warfarin anticoagulation may delay progression and thus prolong survival in SCCL (28). Conceivably, this could be related to the ability of warfarin to anticoagulate the extravascular space by reducing either the activity of vitamin K-dependent clotting factors in the vicinity of the tumor or tumor cell coagulant activity (18, 26). Such an effect could result in limitation of fibrin formation about existing tumor masses and in association with nascent metastases. This concept is supported by evidence that warfarin anticoagulation results in a reduction of both plasma fibrinopeptide A levels and peripheral blood monocyte factor activity in cancer patients (6). However, while SCCL cells in situ appear to possess tissue factor antigen, it is not known whether these cells manifest tissue factor coagulant activity, whether this coagulant activity (if it exists) is reduced by warfarin anticoagulation (18, 26), or whether such anticoagulation interferes with tumor-related fibrin deposition. It seems unlikely that local coagulation is induced by mononuclear cells invading the tumor, since SCCL is notably lacking in such an inflammatory cell infiltrate (14). Obviously, there is a pressing need to compare the appearance of fibrin in SCCL observed in the present study with that observed in other tumor types and to attempt to correlate these findings with warfarin responsiveness versus unresponsiveness. Unfortunately, such studies are, at this time, lacking.

The results reported here appear to be beyond what could be accounted for on the basis of nonspecific fibrin deposition associated with tumor cell necrosis. Rather, they suggest that fibrin may be present in association with apparently viable tumor cells in SCCL. Furthermore, the antigenic form of the putative initiator of coagulation, tissue factor, is present on the tumor cells themselves. We postulate that the existence of fibrin reflects the ability of SCCL tumor cells to induce local fibrin formation. Conceivably, the occurrence of fibrin with tumor masses in SCCL could serve the interests of the tumor to the detriment of the host by protecting the tumor from host defenses (4, 5) or by providing a lattice upon which tumor cells can proliferate (26). These possibilities are consistent with the observation of deposits of proliferating tumor cells embedded in the dense connective tissue matrix characteristic of SCCL (19).

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REFERENCES

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Fig. 1. Appearance of fibrin observed by immunofluorescence in the fibrous stroma surrounding a nodule (a) and around individual cells and small clusters of cells (b) in a metastatic deposit of SCCL within the liver. × 160.

Fig. 2. Appearance of fibrin (arrows) observed by electron microscopy between tumor cells in SCCL. a, × 5000; b, × 7500.

Fig. 3. Observation of SCCL tumor cells by immunofluorescence using an antibody to tissue factor. Note lack of staining in the fibrous stroma surrounding the tumor cells (arrow). × 160.
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