Fibrin Precursors in Early Stages of Metastases

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The functional role of fibrin and/or fibrin precursors in association with tumor metastases has long been a matter of dispute. Some investigators (Warren and Gates 1936, Baserga and Saffiotti 1955, Wood 1964) have demonstrated fibrin in the early stages of metastases, while others (Ludatscher 1967, Cotmore and Carter 1973, Locker Goldblatt and Leighton 1970, Sindelar, Tralka and Ketcham 1975) have not been able to locate any fibrin in association with the early stages of tumor cell arrest. Jones, Wallace and Fraser (1971) were able to detect fibrin precursors by fluorescein-tagged antifibrin antibodies although no polymerized fibrin was seen by light and electron microscopy for about 8 hours after tumor cell injection.

In our previous studies, using immuno-electron microscopic technique with peroxidase-labelled goat anti-rabbit gamma globulin, we have established a method to demonstrate fibrin and fibrin precursors in connection with tumor cell emboli as early as 30 seconds after inoculation (Chew, Wallace and Hunter 1974, Chew and Wallace 1976).

The present communication reports the study of artificial induced metastases in Chang’s hepatoma in rats by using the immuno-peroxidase techniques established by two of us (Chew and Wallace 1976).

Materials and methods

The Chang’s hepatoma was kindly provided by Professor J. P. Chang of the University of Texas Medical Branch, Galveston, Texas. The tumor line was carried in our laboratory by intraperitoneal and intramuscular injections.

The method of tumor cell inoculation and tissue processing for immuno-electron microscopy have been described in detail in one of our previous publications (Chew and Wallace 1976). Rabbit anti-rat fibrinogen antibody and peroxidase-labelled antibody were purchased from Cappel Laboratory, Downingtown, Pa. We have previously found that the peroxidase-labelled antibody purchased from Cappel was as pure as our own preparation (unpublished result).

Results

The ultrastructural aspects of Chang’s hepatoma have been described by Chang

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in 1976 and by us (Chew, Sun and White 1976). Tumor cells were located in pulmonary vessels as early as 30 seconds after intravenous injection. A platelet-tail was usually associated with one pole of the tumor cell. At 15 minutes after the tumor cell inoculation, peroxidase-positive material was always sandwiched

Figs. 1-4. 1, a tumor cell (T) in a pulmonary vessel 15 minutes after intravenous injection. Peroxidase-positive material (arrow) is present between the tumor cell and endothelial lining (E). ×10,000. 2, a tumor cell (T) in a capillary 15 minutes after I.V. injection. A 'platelet-tail' is associated with the tumor cell. Peroxidase-positive material (arrows) is located at the center of the platelet aggregate. ×6,200. 3, a tumor cell (T) in a pulmonary vessel 30 minutes after I.V. inoculation. More peroxidase-positive material (arrow) is present at this stage. ×10,920. 4, higher power view of part of the peroxidase-positive material shown in figure 3, at a different level. The periodicity of fibrin fiber measures about 195 Å. ×38,000.
between the tumor cell and endothelial lining and at the center of the platelet tail (Fig. 1). The tumor cell always occluded the capillary and remained in the intra-vascular position (Fig. 2). At 30 minutes after tumor cell injection, the platelet tail was still present at one pole of the tumor cell. However, the peroxidase-positive material seemed to increase in amount (Fig. 3). Besides, more polymerized fibrin fibers were observed at this time interval. The fiber has a periodicity measured at about 195 Å (Fig. 4).

Discussion

Our results clearly indicate that fibrin and/or fibrin precursors are associated with the early stages of tumor cell arrest in the tumor line used in this study. In the very early stages, only a trace amount of fibrin precursors are detected. The fibrin precursors are either located at the center of the platelet-tail or sandwiched between the tumor cell and the endothelial lining (Figs. 1 and 2). Polymerized fibrin, i.e. fibrin with a distinct periodicity, usually appears at 15 to 30 minutes after tumor cell inoculation (Fig. 4), while the fibrin precursors that appear in the earlier stages are usually amorphous (Figs. 1 and 2). No damage of the endothelium is observed in the sequential study and the platelet-fibrin complexes disappear about 9 hours after tumor cell inoculation (Chew, Sun and White 1977). This is in accordace with one of our previous studies using Walker 256 carcinoma as a model (Chew et al. 1974, 1976, Chew and Wallace 1976). Thus it is likely that the fibrin and/or fibrin precursors facilitate the adherence of tumor cells to the endothelium. The uniform presence of fibrin precursors lying between the tumor cells and endothelium in the early stages of metastases, as well as the presence of platelet-fibrin complexes appearing later, suggest that these materials act as adhesives to secure the tumor cells to vessel walls. However, the exact mechanism of the tumor cell arrest needs further investigation.

In this and our previous studies, the appearance of fibrin and fibrin precursors is only a transient event that lasts 6 to 9 hours after the inoculation of tumor cells. This may be the reason why other investigators have not been able to locate fibrin in their studies. However, it is also possible that difference processes may occur with different tumor lines. Hence further study on a variety of tumor lines is a necessity.

Abstract

A suspension of Chang's hepatoma cells was inoculated into the tail vein of rats. Rats were killed at different intervals ranging from 30 seconds to 3 hours. Peroxidase-labelled goat anti-rabbit gamma globulin was applied to the tissue by an indirect method. Peroxidase-positive material was always found between the tumor cell and the endothelial lining and at the center of the 'platelet tail' as early as 30 seconds. Polymerized fibrin fibers first appeared at 15 minutes and more at 30 minutes. It is suggested that fibrin precursors act as a 'glue' for the tumor cell arrest.
References


