HEMORHEOLOGY STUDY DURING INVASION AND METASTASIS AFTER THE SUBCUTANEOUS TRANSPLANTATION OF MOUSE UTERINE CERVIX CARCINOMA U14

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The hemorheological changes during invasion and metastasis in mice injected with uterine cervix carcinoma U14 were studied. The tumor cells were transplanted subcutaneously into 615-strain inbred mice. Animals were sacrificed at days 5, 9, 15 and 19. Every mouse was examined histologically and viscosity tests were performed, including determination of plasma viscosity, blood viscosity, hematocrit and aggregation of red cells. The results of the histological examination showed that tumor invasion is slightly apparent 5 days after transplantation (grades I-III). The invasive behavior of the tumor cells increased 9 days after inoculation, when interstitial edema and new capillary formation occurred adjacent to the solid tumor. At 15 days, invasive tumor behavior was exhibited, with extensive interstitial edema, new capillary formation (10090 cases) adjacent to the solid tumor, and lymphatic metastasis (71% cases). Nineteen days after transplantation the invasive behavior of the tumor cells was of grade III and lymphatic metastasis was present in 100% of cases. The hemorheological investigation showed no change after 5 days. After 9 days, however, plasma viscosity, aggregation of red cells and blood viscosity increased significantly. At 15 and 19 days after transplantation, all four hemorheological values showed a significant decrease. The results are discussed.

It is well known that cancer affects the circulatory system of man. Many researchers have studied the relationship between blood viscosity and cancer metastasis. Liang, Zi-Jun reported that cancer of the lung, uterine cervix and stomach resulted in significant changes in the hemorheology of these patients. For example, he found that early stage patients with tumor metastasis exhibited a definite increase in blood viscosity which decreases at a later stage of metastasis. The purpose of this work was to define abnormalities in the viscosity of blood and plasma, the aggregation of red cells and the hematocrit during different stages of tumor development. Abnormal hemorheological factors can hopefully be used one day as diagnostic or prognostic tools. Transplantable mouse uterine cervix carcinoma U14 was used in this experiment since it metastasizes well. The process of tumor development can be divided into four stages: a latent stage or early invasion stage; an invasive stage and an early and middle stage of metastases.

MATERIALS AND METHODS

Inbred 615-strain female mice weighing 24-26g each (8-10 weeks old) were used. The mice were raised at the Institute of Experimental Animal Research, CAMS, Beijing, China. Transplantable uterine cervix carcinoma U14 was used to induce ascites by introduction every 6-7 days. The viability of the tumor cells was determined by the 0.1% trypan blue exclusion test. The tumor cells were diluted (5×10^6 cells, 0.2ml) by phosphate-buffered saline (PBS) and transplanted subcutaneously into the right costal region of the mouse.
Mice were killed by bloodletting after excision of the eyeball. The mice were divided into four groups: group A: 14 mice, killed 5 days after tumor transplantation (during the latent period or early stage of invasion); group B: 15 mice, killed after 9 days (the invasion period); group C: 14 mice, killed after 15 days (early metastatic period); group D: 14 mice, killed after 19 days (middle period of metastasis).

For the light microscopic examination, lymph nodes, tumor tissue and lung tissue were fixed in Bouin's solution for 2 days and processed for embedding in paraffin. They were serially sectioned into 5-8 um thick sections, each stained with hematoxin and eosin.

Blood was obtained from the area of the excised eyeball between 9 and 10 AM. The blood was anticoagulated with heparin and used immediately for determining the hematocrit, blood viscosity, plasma viscosity and aggregation of red cells.

For determining the hematocrit, blood in Winthrobe tubes was centrifuged at 1000 g for 30 minutes and the hematocrit observed. For plasma viscosity, blood was centrifuged at 1000g for 30 minutes and the plasma removed for determination by the NZU type cone-plate viscometer (Tianjin Analytic Instrument Factory, China) at 25°C with a 100s⁻¹ shear rate. Blood viscosity was also measured at 200s⁻¹, 100s⁻¹, 40s⁻¹, 20s⁻¹, 10s⁻¹, 4s⁻¹, 2s⁻¹ and 1s⁻¹ shear rates. The aggregation index of red cells was estimated by the following formula:

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\text{Aggregation index of red cells} = \frac{\text{Blood viscosity at 1s}^{-1} \text{ sheart rate}}{\text{Blood viscosity at 200s}^{-1} \text{ sheart rate}}
\]

The extent of lymph-node metastasis and lung metastasis are described in a previous work.7

The extent of tumor invasion to neighboring host tissue after subcutaneous transplantation was divided into 4 grades: at grade I, tumor cells began to invade muscle or fat tissue; at grade II, the muscular tissue and derma were invaded and some invaded muscle tissue began to atrophy and degenerate; grade III tumor cells invaded the epidermis and muscle tissue of the thoracic wall, edema and formation of new blood vessels (neovascularization) were observed around the growing tumor (Figures 1 and 2); grade IV tumor cells invaded the muscle tissue of the thoracic wall and costa, and extensive edema and neovascularization were observed.

**RESULTS**

The invasive grades of the tumors at different days follow. Of group A, 8 cases were invasive grade (Table 1), 5 were grade II and one was grade III. Edema and neovascularization around the tumor nodule was not observed. This slow period of tumor invasion is called the latent period or early stage of invasion. In group B, 3 cases were grade I, 6 were grade II and 4 were grade III. Edema and neovascularization were present but no metastasis was found (Tables 1 and 2). This period is called the invasive stage. In group C, tumor invasion was of grade II or