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Studies on the Cytoplasmic Granules in the Tumor Cells of the MTK-Sarcoma, III. Observations on the Azur Granules

By Tadashi A. Okada
(Zoological Institute, Hokkaido, University)
(With 6 Text-figures)

The morphological features of cytoplasmic granules occurring in tumor cells is of significant importance in relation to their physiological phases. In the papers published by Okada 1954, Tonomura 1955, Okada & Nakahara 1956, Yuize 1956, remarkable evidence has been presented that in rat ascites tumors the morphological changes of cytoplasmic inclusions take place in close association with the physiological functions of tumor cells. These observations were carried out with living material following the supravital staining method with the aid of the phase contrast microscope. In the present study, the morphological features of the cytoplasmic granules were studied in both fixed and stained material, for comparison with the corresponding features in the living material.

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Material and method

Pure bred Wistar albinos (Rattus norvegicus) were used to transmit rat ascites tumors used for study; they are Yoshida sarcoma, MTK-sarcoma II and Hirosaki sarcoma.

Morphological observations on the azur granules occurring in tumor cells of the Yoshida sarcoma and MTK-sarcoma III both being subdiploid tumors were made with daily material through the whole life span of certain tumor-bearing rats, using smear preparations stained by Giemsa’s azuroeosin-methylene-blue solution. Furthermore, the Hirosaki sarcoma (Usubuchi et al. 1955), a hypotetraploid tumor, was studied for contrast with the data obtained in the diploid tumor.

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In order to learn the morphological relation of the azur granules in tumor cells to their metabolic activity, irradiation experiments were undertaken. The experimental procedure is as follows: tumor-bearing rats were irradiated on the 3rd or 4th day after transplantation with X-rays at 200 r and 500 r in the total body exposure. At every one hour after irradiation, bits of the ascites were taken from the treated animals for the observation of the changes occurring in the azur granules in tumor cells.

Results

1. Morphological features of azur granules in the tumor cells and their behavior in a transfer generation. The observations with Giemsa's preparation showed that the azur granules appeared as spherical, purple-red minute bodies of varying sizes existing in the cytoplasm of the tumor cell (Fig. 1). The number

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The results of the foregoing observations have revealed that the data derived from the observations of the azur granule cells and those from the tumor stem-cells run parallel; that is, both showed an increase in frequency from the early part of the transplant generation through the middle part and decreased towards the end of the generation. This evidence seems to imply that the azur granule cells are to be regarded as the tumor stem-cells showing a high metabolic activity in a resting condition. This is an item of evidence just similar to that obtained in the observations of the neutral red granules (Okada 1954), and indicates that the azur granules of tumor cells occur in close association with the metabolic activity of the cells.

2. Behavior of the azur granules as observed after irradiation. In order to
obtain further data on the relation of tumor cells to the metabolic activity, the behavior of the azur granules in response to irradiation was investigated next. X-rays inhibit cell division but do not damage the cytoplasm. The whole body irradiation at 200 r and 500 r was made in tumor-bearing animals on the 4th day after transplantation of the MTK-sarcoma III. Samples of the ascites were taken from the treated animals every 1 hour after exposure and examined. The results of these experiments are illustrated in Figure 6.

Before irradiation, the tumor ascites contained azur granule cells in 70 to 80 per cent of the cells observed. At 1 hour after X-irradiation, mitotic tumor cells were nearly invisible in the ascites. This condition continued for 4 hours after treatment at which time the azur granule cells in the irradiated rats corresponded to 20 per cent of those occurring in untreated rats. At about 9 hours after irradiation, a few mitotic tumor cells were visible in the ascites. Thereafter, a gradual increase of mitotic cells occurred. At the same time, the tumor ascites showed many cells with azur granules in the cytoplasm in a rosette arrangement. Their frequency increased again in 70 to 80 per cent of cells observed. The evidence presented seems to show that the azur granules of the tumor cell occur in close association with the cellular functions.

Concluding remarks

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Okada (1954) and Tonomura (1955) have investigated the corresponding relationship between the cytoplasmic granules of tumor cells and the cellular functions in the MTK-sarcoma II, and III by means of supravital staining methods. They found that the granules stained with neutral red and toluidine blue appeared most markedly in the tumor cells sampled 2 to 3 days after transplantation, while as the end of life of the tumor-bearing rat approached, they were replaced by tumor cells bearing vacuoles. A similar relationship was established in the present study, showing that the metabolic activity of tumor cells is closely associated with the occurrence of the azur granules. It seems probable that these granules occurring in tumor cells are of the same nature as the neutral red granules or toluidine blue granules. The chemical nature of these cytoplasmic granules remains unknown at present, though the neutral red or toluidine blue granules are regarded as a polysaccharide ester sulfate of unknown composition.

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![Graphical representations showing the frequency of tumor cells after X-irradiation in the MTK-sarcoma III. A, tumor cells with azur granules, and B, mitotic frequency.](image)

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References