—Preliminary Note—

Usefulness of transplanted glioma cells in the subcutaneous tissue of the nude mice, for assessment of therapeutic effect of the anti-tumor chemotherapy

Toshiro Shimura, Shozo Nakazawa,
Kaoru Aihara*, and Goro Asano**Department of Neurosurgery, The First Department of Pathology*,
The Second Department of Pathology**, Nippon Medical School

Since the brain tumor of the nude mice is very tiny, difficulty is frequently observed in evaluating the effectiveness of the experimental chemotherapy on the brain tumor cell of the aforementioned mice. If we could transplant the tumor of the identical property to the different portion of the subject of easy access this could offer better opportunity of evaluating the effectiveness of the local chemotherapy. This investigation has been undertaken to seek for this possibility and experimental glial tumors are transplanted in the brain and subcutaneous tissue and a comparison of the ultrastructural and cytochemical features of them has been made. The cultures cells derived from a primary brain tumor which was experimentally induced by installation of the N-nitrosomethylurea. Histologically the cells are mainly composed of the astrocyte-like cells. It has already been reported that these cells contain considerable amounts of s-100 protein and aldolase C¹⁾. This cell strain is assumed to have a character of the neuroectodermal origin. An emphasis is placed on clarifying the ultrastructural and enzymatic histochemical properties of cells of RGC 6 which are transplanted in the brain as well as the subcutaneous tissue in this study.

We have used eight nude mice of 3 months old. RGC cells of 1×10^6 population were transplanted in the brain as well as subcutaneous tissue to the nude mice.

The method was also described in full detail by Takeshita²⁾. The mice were sacrificed 3 weeks later after RGC inoculation. The grown tumor was excised and fixed in 2.5% glutaraldehyde and then post-osmificated, dehydrated in graded alcohols and embedded in Epon 812 for transmission electron microscopic study. Moreover, the acid phosphatase activity was studied by the method of Barka and Anderson³⁾ and Adenosine Triphosphatase was also studied by the method of Wachstein and Meisel^{4,5)}.

The light microscopic study on the transplanted tumor cells in subcutaneous tissue and brain disclosed hypercellularity which was composed mainly of elliptical and spindle-shaped cells and tend to be arranged in bundles. Individual tumor cell has slightly atypical nuclei and cytoplasmic processus. Using the PTAH stain of both brain and subcutaneous specimens, the tumor cells show rather well differentiated cytoplasmic processus. The tumor which was implanted in the subcutaneous lesion disclosed identical morphological features with those of the lesion in the brain. Simultaneously, the ultrastructural study revealed

identical features of the intracytoplasmic organelles and nucleus in both brain and subcutaneous tumors. The desmosomes are also well developed (**Fig. 1 B**). Numerous microvilli were observed on the free surface of intercellular space (**Fig. 1 C**). The cells in brain and subcutaneous tissues disclose round or spindle-shaped configuration. The cytoplasm also contains abundant Golgi complexes, rough-surfaced endoplasmic reticulum, free RNA granules and canalicular structures. It is of interesting to note that the fine filamentous materials, measuring approximately $80 \text{ \AA}$ in width are dispersed in the cytoplasm of both brain and subcutaneous tumors (**Fig. 1 A**). The fibrils are clearly demonstrated

Fig. 1 A Subcutaneous tumor
Glial Filaments are well developed. ($\times 10,000$, bar 0.5μ)
1 B Subcutaneous tumor
Desmosomes are well developed. ($\times 20,000$, bar 0.5μ)
1 C Brain tumor
Numerous microvilli are well developed. ($\times 7,000$, bar 0.5μ)
1 A, 1 B and 1 C stained with uranyl acetate and lead hydroxide

between the junctional portions of the tumor cells. This junctional complexes have cytoplasmic microfilaments which merged with cell membrane (**Fig. 1 B**). In the marginal portion of the subcutaneous tumor, excessive growth of the collagen fibrils are encountered. Moreover, in both the brain and subcutaneous tumors, intratumorous vasculature, containing non-fenestrated capillary channels as seen in human glioma are confirmed. Enzyme histochemical study by light and electron microscopy revealed identical enzyme activities in both brain and subcutaneous tumors. The electron histochemistry disclosed acid phosphatase and adenosine triphosphatase activities in the cisterna of the endoplasmic reticulum, lamellae of Golgi complexes, lysosomes and cell membrane per se (**Fig. 2**).

In conclusion both brain and subcutaneous tumors of nude mice showed identical morphological features which were substantiated by light and electron microscopy as well as cytochemical study. It may be possible to utilize the subcutaneously implanted tumor insted of the brain tumor in nude mice for the assessment of the effectiveness of the chemotherapy as it is very easy to manipulate.

Fig. 2 Subcutaneous tumor
Acid phosphatase activity is intensely observed in the cisterna of the rough-surfaced endoplasmic reticulum. ($\times 25,000$, bar 1μ)
Barka and Anderson's method

References

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