described in the first case, the nerve tissue must be kept for saving the nervous function postoperatively. A team works with orthopedic surgeon and urologist are emphasized postoperatively for lower leg paralysis and bowel and bladder deficit.

27. Glioma: Its Biology in vivo and in vitro

T. HOSHINO, M. BARKER, R. L. DOWNIE*, and C. B. WILSON

*The Howard C. Naffziger Laboratories, Department of
Neurological Surgery, and the Cancer Research Institute,
University of California, San Francisco, California (USA)*

**Al Holman Undergraduate Fellow (1970-71), United Commercial
Benevolent Foundation, Inc.*

Monolayer cultures of six malignant brain tumors from patients who received ^3H -thymidine at operation were prepared by the trypsinization method.¹ Radioautograms were prepared from the original tumor specimens and from slides of each culture at specified intervals. The percentage of labeled cells in the cultures up to one week after culture preparation varied little and coincided reasonably well with the average labeling indices (LI's) of the parent tumors (5-10%). However, the percentage of labeled cells decreased rather quickly in cultures over one week old. This finding, characteristic of all six cultures, indicated that: (1) the population of cells in primary cultures prepared by trypsinization was similar to the population of viable tumor *in vivo*; (2) the first week of culture constituted a lag phase during which the cells recovered from the effects of trypsinization and adjusted to the *in vitro* environment; and (3) vigorous proliferation commenced during the second week of culture. Additional support for these assumptions was found in the following experiment. A pulse of ^{14}C -thymidine was given to one of the above cultures just before fixation. The LI computed from the cultures fixed during the first week was lower than the LI obtained from the parent tumor, but the LI of the culture increased during the second week. The LI's of transferred cultures contrasted sharply with those of primary cultures. The LI was very high during the first few days after passage, quickly reached a maximum, and then dropped precipitously one week after passage. This pattern was observed with long-term established human glioma cultures as well as with experimental rat glioma cultures. Cultures transferred from the primary explant had a higher proliferation rate, which suggests biological changes occurring in the cells due to the environment. The drop in LI one week after passage could be ascribed to exhaustion of nutrients, since medium added at this time allowed further proliferation until the growth surface was exhausted. Therefore, glioma

Follow-up of Labeled Cells in Tissue Culture

Case	Biopsy Pathology	L. I.	Tissue Culture Labeled cells in per cent/age of culture				
C. P.	Glioblastoma	8.1%	8.0/1	6.2/5	5.6/7	6.1/9	3.1/14 3.7/16 2.8/19 1.7/27
F. C.	Glioblastoma	5.5				2.6/10	1.8/25
L.M.	Glioblastoma	4.4	4.2/2	4.9/4	5.5/6	3.9/8	2.5/10 0.9/16 0.5/19 0.1/21
E. M.	Malig. Astrocytoma	5.4	5.0/2		5.1/7	2.1/9	1.3/12
R. E.	Malig. Astrocytoma	2.2	2.9/3		0.8/6		
H. J.	Metastatic	6.3	0.1/4	6.8/6		3.2/8	3.0/10 1.5/12

L. I.: labeling index

cultures follow a growth pattern of: (1) exponential growth; (2) a plateau or stationary phase due to medium exhaustion; and (3) a plateau due to overcrowding. It is noteworthy that these three growth phases can occur quite rapidly.

Supported in part by Clinical Cancer Research Center Grant 5 PO 2 CA 11067; Academic Senate Grant 07; Cancer Research Funds of the University of California; and Grant 510 from the American Cancer Society, California Division.

Reference

Sato, F., H. Chigasaki, H. Yamada, T. Hoshino, M. Nagai, T. Tsuchida, and K. Sano: Brain Tumors Biochemistry, Immunology and Tissue Culture. Brain and Nerve 18 (3) 245-257, 1966.

28. S-100 Protein in Long-term Cultured Glioblastoma Multiforme Cells

Juji TAKEUCHI, Seishi IGARASHI, Yasufumi UCHIDA
and Hajime HANDA

*Department of Neurosurgery, Kyoto University
Medical School*

S-100 protein was purified from bovine brain following the method of Moore modified by us (1). Pure S-100 protein was obtained easily by this method.

Rabbit antiserum against S-100 protein was obtained following the method of Levin and Moore (2).

Human glioblastoma cells, about 150 days of closed culture, were composed of several morphologically different cells.

1. Bipolar spindle cells.
2. Large round thick cells.
3. Polygonal membranous cells with long cytoplasmic processes.
4. Astrocyte-like cells.
5. Bipolar membranous cells.
6. Kite-shaped cells.
7. Polygonal membranous cells.
8. Cells with polymorphous nuclei.

By immunofluorescent method, we found that the first four groups of cells usually contained S-100 protein, but the last four groups of cells sometimes did not. This shows that fibroblastic transformation in morphology of human glioblastoma cells does exist in cultural condition.

S-100 protein was observed in cytoplasm, but not in nucleus of normal astrocytes and glioblastoma cells. Strong fluorescence was observed in cytoplasm around nucleus and along (glio) fibrils. It was supposed that this protein was produced near nucleus and transported along (glio) fibrils in cytoplasm.

Hyden and McEwen showed that S-100 protein was localized primarily in glial cytoplasm and neuronal nuclei (3). However, S-100 protein was not detectable in nuclei of rat spinal cord neurons in our experiments. These findings suggest that function of S-100 protein is probably the same in glia cells and neurons.