Distribution of GFAP and Vimentin in the Subcommissural Organ of the Mouse

Shigeichi KOBAYASHI

Department of Pediatrics,
Jichi Medical School, Tochigi

The subcommissural organ (SCO) is one of the circumventricular organs. The distribution of the two intermediate filaments -- glial fibrillary acidic protein (GFAP) and vimentin -- was immunohistochemically studied in the SCO of the mouse.

Male CD-1 mice of 60 days of age were used. Polyclonal antisera against GFAP and vimentin were gifts from Dr. L.F. Eng and R.O. Hynes respectively. GFAP- and vimentin-immunohistochemistry at the light microscopic level and GFAP-immunoelectronmicroscopy were performed.

Most of the cells in the SCO (SCO epithelial cells) and most of the nonspecialized ependymal cells of the aqueduct were vimentin-positive and GFAP-negative. GFAP-positive cell processes were seen to surround the SCO and interpose between the SCO and nonspecialized ependymal cells. There were also some elongated GFAP-positive cells in the SCO.

In conclusion there were two cell types in the SCO. One was vimentin-positive and GFAP-negative. The other was GFAP-positive. The SCO epithelial cells were of the same charactar as nonspecialized ependymal cells in expressing vimentin and not GFAP.

Analysis of Carcinoembryonic Antigen on Human Gastric Cancer Cell Lines by Flow-cytometry.

Tatsuo KODAMA*, Kenichi INADA, Yasuo UTSUMI**
Takayuki HARADA and Shigeru MORIKAWA

Department of Pathology, Ophthalmology* and Surgery**, Shimane Medical University

It was revealed by immunohistoogical study that the distribution of Carcinoembryonic antigen (CEA) in human gastric cancer cell lines (HPE-GAC-T,-3) changed upon stimulation by a tumor promotor (TPA) or costimulation of TPA and Ca ionophore (A23187). A quantitative analysis of CEA expression changed in each cell was not sufficient by immunohistological methods. By a western blotting analysis on cell lysates, we comfirmed increase of CEA production by stimulation with TPA or A23187. Intending to analyze CEA contents in each cell, we examined GAC-T, -3 cells by a frowcytometry assay. We had compared the intensity of specific fluorescence for CEA in viable cells and variously fixed cells, which were pre-stimulated with TPA, A23187, or combination of both. It was suggested that frowcytometry enabled to analyze the CEA production and kinetics on the cell membrane.

Tubular Lysosomes with TMPase Activity

Toshihiro KOBAYASHI, Teruhiko OKADA,
Shoji NISHIYAMA* and Harumichi SEGUCHI

Departments of Anatomy and *Otorhinolaryngology, Kochi Medical School, Nankoku, Kochi

The localization of trimetaphosphatase(TMPase) activity in the epithelium of the cochlear duct of guinea pigs was investigated and compared with that of ACPase activity and with the uptake pattern of HRP injected in the subarachnoidal space(100μl of 5% HRP). TMPase was elucidated by the lead-based medium of Doty et al(1977), or by the cerium-based method of Kobayashi et al(1988). ACPase activity was elucidated by the method of Robinson and Karnovsky(1983). TMPase positive tubular lysosomes were found in the epithelial cells of the cochlear ducts except the epithelial cells of the spiral prominence, Claudius' and hair cells. These structures were most abundant in the root cells. TMPase activity was found in globular and tubular structures in the cytoplasm of Hensen's cells near the outer tunnel, and also in similar structures situated at the supranuclear area of the interdental cells. ACPase activity was found mainly in globular and in a small number of tubular lysosomes. The localizing pattern of TMPase positive structures corresponded with that of HRP. TMPase positive tubular structures seem to be related to pinocytosis, and might be different from those possessing ACPase activity.

In situ localization of specific mRNA using T-T dimerized single-stranded DNA probes.

Takehiko Koji*, Isamu Sugawara** and Paul K. Nakane*

*Dept. of Cell Biol., Tokai Univ. Sch. of Med., Kanagawa. **Dept. of Pathol., Inst. of Med. Sci., Univ. of Tokyo, Tokyo.

In situ hybridization is usually done using either double-stranded (ds) or single-stranded (ss) DNA probes. We reported the usefulness of T-T dimerized ds DNA as non-radioactive probe. In this study, we examined whether T-T dimerized ss DNA probes also can be used. Antisense ss DNA (2.2 kb) complementary to multi-drug resistance gene (MDR) mRNA prepared from recombinant M13 DNA was T-T dimerized by UV-irradiation (7,000 J/m^2), fragmented by S1 nuclease to the size of 300-450 bases and used as the probe. Under the tissue processing conditions optimized previously (A.H.C. 21:187, 1988), it was found that the antisense ss DNA was useful for the localization of MDR mRNA in 4% formaldehyde (PFA)/PBS fixed K562/ADM cells and in frozen sections of 4% PFA/PBS fixed gastric carcinoma (SC6). To extend this approach, oligo DNA (57-65 mer) complementary to the region of the human c-myc mRNA was synthesized and UV-irradiated. Using this oligo DNA, specific mRNA was detected on nitrocellulose filters and in HL-60 cells. The ss DNA probes could be used at a much higher concentration than ds DNA probes and speeded up the rate of annealing with mRNA, and the use of ss DNA probes avoids the reannealing between strands of melted ds probes.