In situ hybridization using a fluorescent avidin-biotin technique for detection of c-myc mRNA.

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Hybridization cytochemistry is a unique technique for detecting the localization of gene expression and the viral replication. To detect the oncogene, the radioisotope labeled or hapten labeled probes have been ordinarily used. The fluorescence method seems to have an advantage that the gene amplification is quantitated on hybridization cytochemistry, but it has been extremely difficult. We attempted to apply the fluorescent avidin-biotin technique to detecting c-myc mRNA.

We studied HL-60 cell line and NIH-3T3 cell line. The latter was used in the serum stimulated stage after 24 hrs of serum deprivation. The Northern blotting showed that c-myc mRNA was amplified in both cell lines. The method performed for in situ hybridization was as follows; 1) Harvest the cultured cells and smear them onto the albumin-coated slideglass. 2) Fix in 2% glutaraldehyde (30 min) and Carnoy's solution (30 min). 3) Incubate in a proteinase K solution after 0.2N HCl treatment. 4) Post-fix in 4% paraformaldehyde. 5) Hybridize in situ c-myc mRNA with the biotinylated cDNA probe. 6) Wash out the excess probe in formamide and SSC. 7) React with FITC-avidin D.

The fine, brilliant fluorescent granules were clearly produced mainly in the cytoplasm of the both cells. The HL-60 cells produced more granules than the NIH-3T3 cells. The control samples showed no fluorescent granules.

The present method appears to offer wide applications in the analysis of the localization and amplification of the oncogenes.

Immunohistochemical demonstration of lymphocyte markers in formalin-fixed and paraffin-embedded specimens. Y. Tsutsumi T. Ogata & K. Kawai; Dept. of Pathol., Tokai Univ. Sch. of Med. & Div. of Diag. Pathol., Tokai Univ. Hosp., Isehara 259-11 JAPAN

The present study describes immunohistochemical localization of lymphocyte markers in routinely prepared sections with commercial mouse monoclonal antibodies to leucocyte common antigen (LCA, DAKO, x10), T or B cell (Bioscience, x10) and LeuM1 (Becton-Dickinson, x100). In all, 196 samples were examined by indirect immunoperoxidase method: These include 155 non-Hodgkin lymphomas (NHLs), 13 Hodgkin lymphomas (HLs), 26 myelomas and 2 myeloid leukemias (MLs) as well as reactive lymph nodes and thymuses. In nontumorous tissues, LCA was positive in most lymphocytes while plasma cells and granulocytes were negative. T cell antigen was localized in paracortical lymphocytes, thymocytes, granulocytes and epithelioid cells. B cell antigen was mainly seen in lymphoid follicles while plasma cells were unreactive. LeuM1 was present in granulocytes and some epithelial cells. Results of NHLs were as follows: LCA+T+B+ 6 cases, LCA+T+B- 40 cases, LCA+T-B+ 44 cases, LCA+T-B- 37 cases, LCA-T+B- 8 cases, LCA-T-B- 20 cases and LeuM1+ 6 cases. Admixture of small T and B cells was characteristic of HLs and 6 cases were LeuM1+ in Hodgkin cells. Myelomas were B-LeuM1- and 5 T+ and 3 LCA+ cases were noted. MLs were LCA+T+B-LeuM1+. The following problems are pointed out: 1) LCA negative NHLs are not rarely encountered. 2) Some B cell tumors are unreactive to anti-B cell. 3) Anti-T cell recognizes non-T cells such as granulocytes and some B cell tumors. 4) LeuM1 negative Hodkin cells and LeuM1 positive NHL cells are occasionally seen.

Immunohistochemical localization of human epidermal growth factor in submandibular gland. K.Tsukitani,Y.Tatemoto,M.Mori &*K.Kato Dept.of Oral Surg. Asahi Univ. Gifu 501-02 *Wakunaga Pharm.Co.Ltd. Hiroshima 729-64 Japan

In 1975, Gregory purified β-urogastron from human urine which inhibit gastric secretion. It has been termed as human epidermal growth factor (h-EGF) in respect to it's resembled primary structure and biological effect with mouse EGF. Immunohistochemical localization of h-EGF has already been repoted by using polyclonal human EGF antiserum(poly-h-EGF).

In present study, localization of h-EGF in submandibular gland (SMG) by monoclonal human EGF antibody(mono-h-EGF) was examined and compared with those by poly-h-EGF. Immunological properties were examined by enzyme linked immunosoruvent assay(ELISA) for mono-h-EGF and radioimmunoassay(RIA) for poly-h-EGF. It was confirmed to no cross-react to mouse EGF. Absorption test was carried out on the paraffin embedded section fixed with various fixatives (formalin, Bouin's, Carnoy's). The stainings were performed by indirect method for mono-h-EGF, and PAP method for poly-h-EGF. On the formalin fixed section, positive reactions of m-h-EGF were present at striated and intercalated duct system. Such localization was similar to that on Bouin's fixed section. On the contrary, localization on the Carnoy fixed section was confined to luminal aspects or intercelluler border of acinar cells. Positive reactions by poly-h-EGF localized in similar part of the gland except for endothelial cells. Positive staining of endothelial cells seemed to be false reaction related to non-specific antigen. Precise localization should be obtained by using mono-h-EGF.

Ultracytochemical demonstration of the surfactants in mouse lung alveoli.[1]

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Technical improvements for the ultracytochemical observation on lining layer of the lung alveoli in adult mice were as follows: Vascular perfusion of a twice diluted Karnovsky's fixative was performed on the lungs after air instillation to the airway. The perfused one was fixed in the negatively pressurized, twice diluted Karnovsky's fixative containing 0.04% $CaCl_2$ for 1 hr, and then approximately $1mm^3$-sized tissue blocks were prepared by quick freezing and trimming, which were washed in a cacodylate buffer with sucrose and applied for the ultracytochemical methods. Another perfused lung was treated with conventional techniques. Enzymic digestive method using either a purified phospholipase A_2 (Sigma) or C (Boerhinger-Mannheim) was performed on the floating prefixed minute tissue blocks. After the postfixation in cold osmium buffer, acetone was used for dehydration of the digested blocks to preserve phospholipids. Reaction products after phospholipase-digestive methods, probably indicating the site of phospholipids, are clearly demonstrated on the surface film of the alveolar lining layers and in some lamellar structures within the alveolar lining layers and type 2 epithelial cells. Satulated phospholipids of surfactants could be visualized distinctly under an electron microscope only with the above-mentioned techniques.

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