SUBCELLULAR LOCALIZATION OF NEUROTROPIN RECEPTORS IN PC12 CELLS:TOMOGRAPHY BY HIGH-VOLTAGE ELECTRON MICOSCOPE

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Recently, it has been suggested that the nerve growth factor (NGF) receptor TrkAs are associated with caveolae which are small invaginated pits on cell membrane. To clarify the ultrastructual localization of TrkA receptors in neurons, we did immunohistochemistry of TrkA and Cav-1, caveola-specific protein, in PC12 cells and analyzed by conventional electon microscope and high-voltage electron microsopic tomography. Our results indicated that TrkA and Cav-1 immunoreactivities were shown in some of vesicle membrane, but both immunoreactivities were not always co-localized. TrkA immunoreactivity was localized mainly in many clusters of invaginated pits of cell membrane, that are different from caveolae.

THE EFFECTS OF OUTGROWTH OF NEURITES AND ADHESIVE REPULSION BY CULTURED VASCULAR SOOTH MUSCLE CELLS

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In an attempt to investigate the role of target cells for the differentiation of nerve cells, we have co-cultured NG108-15 cells with vascular smooth muscle cells (SM-3). The outgrowth of nerve fibers was promoted, but their growth cones were repelled by SM-3 cells. DNA microarray, to analyze factors involved in these phenomena, indicated that NG108-15 cells up-regulated several characteristic genes, related in cell survival and growth, but also cytoskeletons, axonal transports and repulsive receptors for adhesion.

IDENTIFICATION BY mRNA DIFFERENTIAL DISPLAY OF GENES ASSOCIATED WITH SERUM-INDUCED APOPTOSIS IN B16 MURINE MELANOMA SUBLINES

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High concentration of fetal bovine serum (FBS) is known to induce apoptosis of cells in culture. However, this mechanism is unclear. Since tumor cells can break away from a primary tumor and pass through the blood vessels or lymphatic vessels to other parts of body for metastasis, we hypothesized that they must express genes that protect them from serum-induced apoptosis. Therefore we used well-known B16 melanoma sublines, low-metastatic subline B16 and high-metastatic subline B16F10, established from C57BL/6 mouse melanoma to confirm it because they had the variety of metastatic potentials and behaviors, despite the same origin.

When B16 and B16F10 cells were incubated in 100% FBS, unexpectedly apoptosis was induced in B16F10 cells while B16 cells were alive and continued to proliferate. We thought these differences were regulated by transient or permanent changes at DNA, mRNA and/or protein levels in different genes. Consequently we isolated 41 cDNA fragments that changed their expression levels induced by 100% FBS with mRNA Differential Display method. Sequence analysis revealed that apoptosis-related genes, such as GST-homolog and TNF alpha-induced protein 8, were included.

IN VITRO MUSCLE FIBER FORMATION ON PATTERNED GELATIN STRIPES PRINTED BY A COLOR INK JET PRINTER

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The skeletal muscle fibers align to the direction of muscle tension. However, this phenomenon isn't observed in vitro, and the direction of the muscle cells are formed at random. In this study, we used a color ink jet printer to print the gelatin stripes on the polystyrene sheet and the mouse myoblast cell line C2C12 was cultured on it. As in vivo, multinucleated myotubes were formed on the gelatin stripes. I examined the differences in proliferation, differentiation, maturation, and the appearance of the muscle satellite cells between the culture on gelatin stripes and that on conventional gelatin-coated dishes.

GLUCOSE INHIBITS ENDOTHELIAL CELL DEATH INDUCED BY ASCORBIC ACID

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Ascorbic acid is coenzyme of collagen generation, therefore ascorbic acid is essential for vein maintenance. However, high concentration of ascorbic acid induced cell death had been observed in several cell lines *in vitro*. We previously reported that high concentration (under 3mM) of ascorbic acid induces bovine aortic vascular endothelial cell death. In the present research, lower volume of glucose inhibits cell death induced by ascorbic acid (under 3mM), but not inhibits cell death by ascorbic acid (over 3mM). These results suggest that glucose transporter is associated with cell death by ascorbic acid.

A NEW METHOD OF SPECIFIC INTRODUCTION OF SUBSTANCES INTO MYOTUBES USING MARCAINE

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It is well known that bupivacaine-hydrochloride (marcaine), a local anesthetic, has a specific toxicity to muscle. The purpose of this study is to investigate whether it is possible to introduce substances specifically into myotubes using marcaine. We used Hoechst 33258, a DNA binding fluorescent dye, to evaluate an introduction efficiency. At a lower concentration(0.1μg/ml) marcaine enhanced Hoechst 33258 introduction into myotubes specifically. And what's more, We observed low toxicity to cells compared with a higher concentration(1μg/mg). Our data also suggest that introduction efficiency varies for various fluorescent dyes mainly due to water solubility.

FUNCTIONAL ANALYSIS OF A NEURON-SPECIFIC MAP4 ISOFORM

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We identified a novel variant of MAP4 lacking 72 consecutive amino acid residues within the proline-rich region, as compared with the full-length MAP4 (The 74th ZSJ annual meeting in Hakodate). A MAP4 variant cDNA homologous to the bovine form was also detected in rat brain and rat cultured cells (PC12 cells), suggesting that the new variant can be generated not only in bovine but also in other mammalian species. The mRNA expression of the rat version isoform was elevated by NGF treatment of the PC12 cells, suggesting that this isoform is important for neuronal functions. Although the novel isoform retained the same microtubule assembly and binding activities as the intact MAP4, the microtubules assembled in the presence of the novel isoform failed to be bundled. Instead, a constant spacing between neighboring microtubules was observed. This suggested that N-terminal of the isoform protruded from themicrotubules and acted as a spacer. We hypothesized that the deletion in the Pro-rich region affected the conformation, rather than the activity, of MAP4.

DESMIN SPECIFIC PROTEOLYTIC ENZYME EXIST IN SOLUBLE FRACTION OF SKELETAL MUSCLE OF CHICK EMBRYO

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As reported previously, chick embryonic skeletal muscle contained ten time more desmin than adult muscle. When desmin is detected by immuoblot analysis in homogenate of 18D-chick embryos, protein bands with MW of 48k, 43.5k, 38.8k, 37.7k, and 35.8k were stained by anti-desmin beside 53k-desmin. These cleavage products were not found in chymotrypsin- or trypsin-digests of desmin. Then, it is suggested that there exist a desmin-specific protease in soluble fraction of embryonic muscle. Judged by the specificity of antibody used and MWs of the products, this enzyme would selectively hydrolyze head or tail domain of desmin but not central rod domain. The activity of the protease in vitro was examined using desmin band cut out from SDS-gel as a substrate. The gel was immersed in the embryonic extract and cleavage products were analyzed by SDS-PAGE of the substrate-gel. The cleavage products obtained by this in vitro assay was the same as obtained in the endogenous products, supporting that the embryonic extract contained characteristic protease against desmin. It is to be noted that the activity was not affected by protease inhibitors as EGTA, leupeptin, and E64.

ANALYSIS OF DESMIN DOUBLETS IN A MUSCLE TISSUE FROM A BOVINE UTERUS

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Desmins are type III subunits of intermediate filaments expressed specifically in muscle cells. Although mammals are reported to have a single copy of desmin gene, we detected two desmin bands in crude and purified preparations of bovine uterus muscle. The MW of two desmin bands were 53K (Des I) and 52K (Des II) respectively