P-13

Actin Filament Organization in Multidrug Drug Resistant Osteosarcoma Cells

Hideyuki Takeshita, Katsuyuki Kusuzaki, Yoshiro Tsuji, Masazumi Hirata, Shin Hashiguchi, Yoji Urata, Yasusuke Hirasawa and Tsukasa Ashihara

Departments of Orthopaedic Surgery and Pathology, Kyoto Prefectural University of Medicine, Kyoto; Department of Orthopaedic Surgery, Otsu Municipal Hospital, Otsu

P-glycoprotein (Pgp)-positive, multidrug resistant (MDR) murine osteosarcoma cell lines demonstrate higher alkaline phosphatase activity than the parent cells, and produce more differentiated, osteoblastic sarcomas in mice. In the present study, we investigated *in vitro* morphological differentiation by staining cellular actin, and the effect of actin depolymerization agent cytochalasin B (CB) on ADR resistance. We found that in the parental cells, the actin filaments were sparsely distributed or were diffusely spread throughout the cytoplasm. In contrast, the MDR osteosarcoma cells exhibited a number of well-organized actin stress fibers. Furthermore, CB dramatically disrupted this network of stress fibers, increased the intracellular accumulation of ADR, and modified the resistance against ADR in the MDR osteosarcoma cells. These effects were much less dramatic in the parent cells. These results suggest that the overexpression of Pgp in MDR osteosarcoma cells was associated with morphological and functional differentiation. In addition, the actin organization may be important for the expression of Pgp function.

P-14

Enzyme-and Immuno-histochemical Analysis of Human Hematopoietic Cell Lines with Endocytotic Activity

Shigeru MORIKAWA, Ikuko TORII, Makoto NAGASAKI, and Satoko MISHIMA

Department of Pathology, 1st Unit, Shimane Medical University, Izumo

Capture and endocytosis of foreign bodies are the first step of defence against pathogenical microbes. Endocytosis is subdivided into two categories, phagocytosis and pinocytosis, based on the size of ingested molecules.

Recently we have established a cell line (HBM-Noda) from the bone marrow cell cultures of 68 year-old male. Noda cells show dendritic morphology and locomote in vitro. Nature of Noda cells are considered to be dendritic cells, a group of 'professional' antigen presenting cells (APC). Noda cells show pinocytotic activity with Lucifer yellow dye and phagocytotic activity with FITC-labelled latex beads.

In this study, to clarify the difference of endocytotic activities of APC and 'professional' phagocytotic cells, enzyme-and immunohistochemical properties of Noda cells are investigated in comparison with human monocyte/macrophage cell lines (U937 and HPL-Hod-1).

Each cell line possesses its characteristic profiles of enzymatic activities, as well as immunological markers. Hod-1 and U937 cells show rather common properties which suggest their monocyte macrophage origin.

On the other hand, Noda cells show unique enzymatic activities, such as membrane ATPase and thiamine pyrophosphatase activity. These activities are thought to function at antigen processing and presentation.

In conclusion, it is suggested that dendritic cells belong to different cell lineage with high endocytotic activity from monocyte/macrophage lineage in haematopoiesis.

P-15

Demonstration of two antigens on cell-cell junctions by the immunoelectronmicroscopic double-staining method

Akira Mizoguchi and Chizuka Ide

Department of Anatomy and Neurobiology, Graduate School, Kyoto University

In the cultured epithelial cells (MDCK), we have demonstrated the localization of two antigens involved in the cell-cell adhesion such as cadherin/catenin and ZO-1 by the immunoelectronmicroscopic double-staining method. For the double-staining method, rat anti-cadherin antibody was mixed with mouse anti-catenin or anti-ZO-1 antibody and used. The tissue-bound rat IgG and mouse IgG were separately shown in the same cryostat section using the silver-enhanced immunogold method and the HRP/DAB method. We have found that cadherin and catenin were located diffusely on the lateral plasma membranes along their entire length without intense accumulation on the adherens junction. ZO-1 was shown to be localized at the apical-most region of the lateral plasma membranes with length from 0.2 to 1.5 μm.

P-16

Immunohistochemistry of type IV collagen alpha chains in human lung basement membrane

Ichiro NAITO, Satoko INOUE and Satimaru SENO

Division of Ultrastructural Biology,
Shigei Medical Research Institute, Okayama

Type IV collagen is composed of 6 kinds of α chains, and chain-specific localization has been recognized in human kidneys. Anti-glomerular basement membrane (GBM) antibody-induced glomerulonephritis is known to be caused by autoantibody against α3(IV) chain, and is accompanied with lung hemorrhage. This indicates a similarity of α(IV) chain composition between GBM and alveolar basement membrane (ABM) of lung.

α (IV) chain composition of human lung was studied by immunohistochemistry using chain-specific monoclonal antibodies raised by us. α1(IV) and α2(IV) chains was demonstrated in all basement membranes, while α3(IV) and α4(IV) chains were limited to the ABM. α5(IV) is localized in ABM together with α3(IV) and α4(IV), and in submucosal basement membrane of bronchi with α6(IV) chain.

The results demonstrated the similarity of α(IV) chain composition between ABM and GBM.