S3-02

Application of immunohistochemistry for study of ASC-related molecules in diseases

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An caspase-activating adaptor molecule, apoptosis-associated speck-like protein containing a CARD (ASC), which is involved in inflammation and apoptosis, was cloned and characterized (Masumoto et al, J Biol Chem 274:33835-33838, 1999). ASC possesses two protein-protein interacting domains, which are N-terminal PYRIN domain (PYD) and C-terminal caspase recruitment domain (CARD). The upstream sensor molecules of ASC are nod-like receptor family proteins, which act as intracellular pathogen recognition receptor. By contrast, the down-stream effecter molecules of ASC are caspases, which is involved in apoptosis and IL-1β processing. Using immunohistochemsitry, we previously demonstrated that ASC ubiquitously distributed in various types of epithelial cells and leukocytes. Since a knockdown experiments of downstream caspase of ASC in a zebrafish model showed open-mouth phenotype without relation of apoptosis, we speculate that the ASC-related signaling involves differentiation and development rather than apoptosis. Actually, we recently demonstrated that caspase activation without apoptosis in glioma cells revealed by immunohistochemistry was significantly associated to the patients' prognosis. In this symposium, the significance of ASC expression and consequent caspase activation will be discussed. (Supported by a Grant-in-Aid for Scientific Research C-18590524 from JSPS to J.M.)

S3-03

Recent progress in laser capture microdissection for molecular histology

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Laser capture microdissection (LCM) is providing the next revolution for molecular histology. In this talk, the usefulness of LCM for DNA mutation analysis and gene expression analysis in pathological lesions is described. The mutation of the p53 is one of the most frequent genetic alterations observed in human cancer cells. LCM-selected tissues procured from colorectal adenocarcinoma and breast tumors with LCM were analyzed by SSCP and direct sequencing after PCR amplification. In the LCM- microdissected samples, the mutation of p53 gene could be confirmed in the tissue-cell specific lesions. Carbon tetrachloride (CCl4) induces fatty change through the generation of oxygen radicals by cytochrome P-450. CCl4- induced fatty changes predominantly occur at the centri-lobular regions of the liver. The unfixed frozen cryosections were prepared and the capturing from the central and peripheral areas of the liver was performed. Using amplified-RNAs from the captured cells, DNA microarray analysis was carried out. As results, the expression of 46 genes, including cytochrome P-450, were increased in CCl4-injured centri-lobular region. In contrast, the administration of CCl4 decreased the expression of 24 genes including anti-oxidative enzyme such as glutathione S-transferase. These results suggest that the alterations of gene expression by CCl4 stimulate the radical generation and lipid peroxidation, resulting in the exacerbated fatty change in the liver. In conclusion, LCM technique is useful for the analysis of DNA mutation and gene expression in specific pathological lesions.