687 bases. Amino acid sequence of the tomD gene product exhibited 71.1% identity with that of 4-*oxalocrotonate decarboxylase from C. testosteroni* TA441.

[PC1-26] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

PI3K-PKCε signaling pathway is essential for the p21WAF1/Cip1 expression by apicidin

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We previously reported that PKC signaling event was closely involved in the expression of $p21^{WAF1/Cip1}$ by apicidin, a novel histone deacetylase inhibitor. In this study, we extended our study to elucidate the upstream signaling pathway for the expression of $p21^{WAF1/Cip1}$ by apicidin. The expression of $p21^{WAF1/Cip1}$ by apicidin appears to be mediated by PKCε, because the expression of dominant negative PKCε significantly attenuated the activation of $p21^{WAF1/Cip1}$ promoter via Sp1 sites and also inhibited the protein level of $p21^{WAF1/Cip1}$. And antisense oligonucleotide against PKCε decreased PKCε expression and inhibited the expression of $p21^{WAF1/Cip1}$, indicating that PKCε signaling event is essential for the expression of $p21^{WAF1/Cip1}$ by apicidin. Next, we examined the involvement of PI3K signaling pathway, a possible candidate upstream molecule of PKCε. LY294002 and wortmannin, a well known PI3K inhibitors, attenuated the activation of $p21^{WAF1/Cip1}$ promoter via Sp1 sites and also inhibited the protein level of $p21^{WAF1/Cip1}$. The expression of dominant negative PI3K abrogated the activation of $p21^{WAF1/Cip1}$ promoter, suggesting that the PI3K signaling event was deeply involved in the apicidin-induced $p21^{WAF1/Cip1}$ expression. And apicidin-mediated PKCε signaling event might be regulated by PI3K signaling pathway, since the expression of $p21^{WAF1/Cip1}$ by PDBu, a PKC activator, was not inhibited by the PI3K inhibitors and membrane translocation of PKCε in response to apicidin was blocked by the PI3K inhibitor. However, the $p21^{WAF1/Cip1}$ expression by apicidin appears to be independent of the histone hyperacetylation, since apicidin-induced histone hyperacetylation was not affected by PI3K inhibitors, suggesting that the expression of $p21^{WAF1/Cip1}$ by apicidin might have been mediated by a mechanism other than chromatin remodeling through the histone hyperacetylation. Taken together, these results suggest that the PI3K-PKCε signaling pathway plays a pivotal role in the expression of the $p21^{WAF1/Cip1}$ by apicidin.

[PC1-27] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

MALDI-TOF MS Approach to Identify the E6AP-interacting factors in HeLa cervical cancer cells

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Cervical cancer is one of the leading causes of female death. Human papillomaviruses have been recognized as the primary cause of cervical cancer. Viral oncoproteins are selectively retained and expressed in carcinoma cells infected with human papillomavirus and cooperated in immortalization and transformation of primary keratinocytes. E6 associated protein (E6AP) is a 100 kDa cellular protein which mediates the stable association of the high-risk HPV E6 oncoprotein with tumor suppressor protein p53, resulting in the degradation of p53. E6AP was known as E3 ubiquitin-protein ligase, which has been proposed to play a role in defining the substrate specificity of the ubiquitin-proteasome degradation. In order to identify the E6AP-interacting molecules, HeLa cervical carcinoma cells having HPV type 18 genome, was used. We have produced his tagged E6AP and E6AP-Ni2+-NTA-affinity column was prepared to obtain E6AP-interacting proteins. The E6AP-interacting proteins were resolved in 2D-gel and analysed by matrix-assisted laser desorption/ionization (MALDI/TOF). Among 17 proteins identified in 2D patterns of

HeLa cell lysates bound to E6AP protein, there is one protein not yet identified. Heat shock cognate 71 kDa protein, CTCL tumor antigen se2-5, HLA-A24, Ku70-binding protein, amphiphysin isoform 2, GR AF-1 coactivator-1, eukaryotic translation initiation factor 4A, and BTB domain protein, were bound to E6AP. These results suggest that E6AP can have several functions by interacting with several proteins related to transcription/translation and cytoskeleton, and heat shock cognate protein in cervical carcinoma cells (This work was supported from the Molecular Medicine Program M1-0106-00-0078, MOST).

[PC1-28] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Study of Metastatic Potential of Gastric Cancer Cell Lines by Comprehensive Proteomic Analysis

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Several methods have been developed for the comprehensive analysis of gene expression in cancer cells. Usually these approaches in the postgenome era begin with either a portion of the cellular transcriptome using cDNA microarray or a portion of the cellular proteome using a 2-D gel and MALDI-TOF analysis. Each approaches has distinct conceptual and methodological advantages and disadvantages. Whilst many detailed biochemical studies have been performed about them there are few clinically relevant studies using genomic and proteomic methods. Gastric cancer is very popular in Korea and metastasis of it is a main obstacle for the treatment of it. Like as many other cancers human gastric carcinoma often metastasizes to lymph nodes, but the mechanisms responsible for lymph node metastasis are not clearly understood. To investigate them, the factors associated with metastasis were identified using proteomic method. We have studied the protein expression profiles of gastric cancer cell line, OCUM-2M LN, with a high rate of lymph node metastasis and its parental cell line, OCUM-2M, which exhibited a low rate of lymph node metastasis by two-dimensional (2D) gel electrophoresis and MALDI-TOF. Protein expression profiles of OCUM-2MLN and OCUM-2M cell lines were generated by two-dimensional electrophoresis (2-DE). Twenty proteins in these cell lines were identified as differentially expressed ones. Mass spectrometric analysis of these spots revealed the metastasis-stimulatory or inhibitory proteins. This finding may explain a marked acceleration in metastatic potential of OCUM-2LN and we will discuss our findings based on known discovery.

[PC1-29] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

The Investigation of joint diseases in Equine with Biochemical Factors: Analysis of synovial fluid and serum

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A modification of a colorimetric assay was used to determine synovial fluid total and individual sulfated-glycosaminoglycan concentration of joint disease in equine. For the identification of enzymatic digestion products of the equine synovial fluid , strong anion exchange-high performance liqid chromatography (SAX-HPLC) was performed. By the action of chondroitin ABC lyase, Three unsaturated disaccharides 2-acetamide-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-D-galactose(△Di-COS), 2-acetamide-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-6-O-sulfo-D-galactose(△Di-C6S) and 2-acetamide-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-4-O-sulfo-D-galactose(△Di-C4S) were produced from the equine synovial fluid.The synovial fluid low concentration of sulfated-GAG in abnormal samples. Total sulfated-GAG concentrations (mean±SD) were decreased in horses with joint disease (0.17 ㎎/㎖ ± 0.12 ㎎/㎖), but synovial fluid total sulfated-GAG concentrations of normal (0.82 ㎎/㎖ ± 0.24 ㎎/㎖). The mean value of HA on diluted normal and joint diseases serum were found to be 77.00 ± 66.14 ㎍/㎖ and 168.50 ± 147.50 ㎍/㎖ by labeled B-HABP. there appears to be some correlation between joint inflammation and circulating HA levels as determined by experimental studies of animals.