P-1099 B Loss of proapoptotic activity of Ret tyrosine kinase leads hyperplasia in Ret expressing tissues

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Ret チロシンキナーゼのアポトーシス活性を抑制した変異マウスには組織の過形成病変が生じる。

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The ret proto-oncogene encodes a receptor tyrosine kinase and targeted disruption of ret in mice causes intestional agangleonosis and renal agenesis. Ret is activated by the GDNF family of ligands and Ret signaling is important for survival, proliferation and differentiation of the enteric sympathetic, parasympathetic and dopaminergic neurons. Recently, Ret was reported to be a "dependence receptor" which induces apoptosis in the absence of its ligands. Ret was cleaved at D707 and D1017 by caspase, and both D707N and D1017N mutation fails to induce cell death in cultured cell line.

To determine the importance of Ret proapoptotic activity in vivo, we generated Ret D707N mutant mice (collaboration with Dr. F. Costantini, Columbia Uni., USA). D707N Homozygote showed hyperplasia of neural cells in enteric, sympathetic and parapympathetic neural systems. In addition, adenopituitary lobe in pituitary gland was hyperplastic in D707N mice. As D707N mutation would inhibit apoptosis, hyperplasia in mutant mice might be reasonable. These data may suggest the possibility that loss of Ret proapoptotic activity relates to the tumorigenesis in Ret expressing tissues.

Keywords: Ret, Apoptosis

P-1100 B *O*-GlcNAcase cleavage by caspase-3/7 links to executing camptothecin-dependent apoptosis in HL-60 cells

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カンプトテシンによるHL-60細胞アポトーシス誘導時のカスパーゼ3/7依存的*O*-GlcNAcase切断の意義

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Dynamic glycosylation of nucleocytoplasmic proteins by *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) is regulated by two nucleocytoplasmic enzymes, *O*-GlcNAc transferase and *O*-GlcNAcase. *O*-GlcNAcase has been characterized as a substrate for caspase-3 *in vitro*. Here we report that *O*-GlcNAcase is cleaved in HL-60 cells by caspase-3/7-dependent manner. Camptothecin, which is known to induce caspase-3/7-dependent apoptosis to HL-60 cells, caused *O*-GlcNAcase fragmentation. A caspase-3/7-specific inhibitor diminished the fragmentation. To gain insight into the relevance of *O*-GlcNAcase in apoptosis, we analyzed the role of *O*-GlcNAcase in camptothecin-dependent apoptosis with an *O*-GlcNAcase inhibitor. Quantification of apoptotic cells by staining nuclei with propidium iodide and phosphatidylserine with annexin V showed that camptothecin-dependent apoptosis is suppressed by inhibition of *O*-GlcNAcase. Our results show that cleavage of *O*-GlcNAcase by caspase-3/7 may play a key role in executing apoptosis. Collaborators: Hisayuki Okuno, Reiko Ohmori, Hideaki Nakamura, Shoji Ohnishi (Dept. of Bio-Sci., NIBST), & Gerald W. Hart (Dept. of Biol. Chem., Johns Hopkins Univ. Sch. of Med., USA).

Keywords: Apoptosis, glycosylation

P-1101 B Galanin Receptor subtype 2 Induces p53-independent Apoptosis in Head and Neck Cancer Cells.

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ギャラニン受容体2型は頭頸部癌細胞でp53非依存性にアポトーシスを誘導する.

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Galanin and its receptors are potential therapeutic targets for cancer therapy but their function in cancer is poorly understood. Galanin receptor 2 (GALR2) was reported to induce apoptosis in neuroblastoma cells with wild-type p53 but was also reported to stimulate cell proliferation in small cell lung cancer. We investigated the role of GALR2 in head and neck cancer, in which p53 is frequently mutated. HA-tagged GALR2 was stably expressed in a human oral carcinoma cell line (UM-SCC-1-GALR2) that has a 46-bp p53 deletion affecting exon 5. Galanin treatment of UM-SCC-1-GALR2 caused a dramatic decrease in cell number with morphological changes indicative of apoptosis that were not seen in mock-transfected cells. Induction of cell death by an apoptotic process was confirmed by annexin-V staining and DNA fragmentation analysis. Galanin also induced expression of the cyclin-dependent kinase inhibitors p27Kip1 and p57Kip2 and reduced the expression of cyclin D1. This study demonstrated that exogenous GALR2 expression can regulate cell cycle genes and induce apoptosis in head and neck cancer cells with mutant p53, and therefore identified GALR2 as a feasible target for HNSCC therapy.

Keywords: G protein, Squamous cell carcinoma

P-1102 B Enzyme-digested Fucoidan Extract from Seaweed *Mozuku* enhances sensitivity of lectin-induced apoptosis of tumor cells

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酵素消化低分子化フコイダン抽出物によるガン細胞のアポトーシス感受性増強効果

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Fucoidan is sulfated polysaccharides derived from brown algae. Recently, the abalone glycosidase-digested fucoidan extract (fucoidan extract) derived from seaweed *Cladosiphon novae-caledoniae* Kylin (*Mozuku*) draws much attention because of its clinical anti-cancer effect in Japan. Here, we report the cancer cells-specific apoptosis inducing effects of the fucoidan extract. The fucoidan extract suppressed the growth of various anchorage-dependent and -independent cancer cells. It has been known that sugar chain expression on the surface of cancer cell membrane changes dependent on their malignancy. The analysis on sugar chain expression profiling using FITC-labeled lectins revealed that the expression of concanavalin A (Con A) binding sugar chain was enhanced by the treatment of human lung adenocarcinoma A549, human uterine carcinoma HeLa and human fibrosarcoma HT1080 cells with the fucoidan extract. Con A-induced apoptosis of cancer cells was stimulated in a dose- and time-dependent manner by the treatment with the fucoidan extract but not of human normal fibroblast TIG-1 cells.

Keywords: Apoptosis, Fucoidan

Room 17(II) Oct. 28 (Tue.) 17:00-17:40

8-5 Apoptosis(5) アポトーシス(5)

Chairperson: Akemi Hayakawa (Tokyo Univ. of Sci., Yamaguchi)
座長：早川 あけみ（山口東京理科大学・基礎工学部）

P-1103 B Identification of novel interacting proteins in Fas-mediated apoptosis signaling using proteomic approach

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Fas細胞死シグナルにおける新規相互作用分子のプロテオーム解析

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Apoptosis signaling is a very important mechanism in cancer biology. Fas receptor-mediated apoptosis signaling is particularly well studied, and many functional molecules have been identified. However, while past studies have only focused on interaction of a number of key molecules, it should involve more and perhaps novel components in the Fas-associated death-inducing signaling complex (DISC), allowing for the functional complexity of the signaling mechanism.

Here, we have developed a direct and comprehensive method to identify the components of Fas-binding proteins. Two-step-purified recombinant GST-Fas death domain was conjugated to NHS-activated beads, and Fas-binding proteins were concentrated from Jurkat cell lysate by affinity chromatography, and identified by mass spectrometry.

Using this method, we have observed that the elution profile significantly changed upon Fas ligand (CH-11) stimulation in a time-dependent fashion. No significant change was observed by control IgM addition. Our data suggested that the signaling proteins sequentially bind to Fas death-domain upon ligand stimulation and are continuously degraded.

Keywords: Fas, Proteomics

P-1104 B Apaf-1-independent mitochondrial *in situ* caspase-9 activation

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Apaf-1非依存性ミトコンドリア内カスパーゼ9活性化

倉田 俊一[1]、福西 菜穂子[1]、加藤 伊陽子[2]（[1]東京医歯大・難治研・レドックス応答、[2]山梨大・院医工・医・微生物学）