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P9-6 **Cancer epigenetics**
がんエピジェネティクス

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P-2357 Inhibition of LIT1 Gene Transcription by PI Polyamide(PIP) in Beckwith-Wiedemann syndrome(BWS) fibroblast cell lines

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S線維芽細胞株における PIポリアミドによる LIT1遺伝子の発現抑制の検討

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ethylation of *LIT1* in maternal allele, called loss of imprinting, occurs everal adult tumors and about half of BWS. *LIT1* inhibits expression ircumjacent genes including *KIP2*, a tumor suppressor gene. We othesized that suppression of *LIT1* gene could induce *KIP2* and result nti-tumor effect. Since PIP can recognize DNA in a sequence specific ner, PIP designed against the transcription factor binding site can inhibit ression of the target genes. In the present study, we generated a PIP for AT box in *LIT1* promoter region and investigated its effect. At first, the hylation status of *LIT1* promoter region in BWS cells(BWS 6, 7, 8, 9) were yzed by MassARRAY EPITYPER. Second, BWS cells were cultured with ithout 1 μ M PIP. Then, the expression levels of *LIT1* and *KIP2* mRNA e analyzed by real time RT-PCR. BWS 6 showed demethylation of *LIT1* moter regions and high expression levels of *LIT1* mRNA. After 72 hours ure, BWS6 showed down-regulation of *LIT1* expression ($p<0.05$) and up-lation of *KIP2* expression ($p<0.05$). Those results indicate that this PIP ld be a therapeutic agent in BWS patients with tumors showing high ression level of *LIT1*.

ywords: imprinting, Methylation

P-2358 PARP inhibitor induces DNA hypomethylation of particular loci in mouse embryonic stem cells

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RP阻害剤によって ES細胞で誘導される DNA脱メチル化変化

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(ADP-ribose) polymerase (Parp) inhibitors are in clinical trials for cancer apy as a DNA repair inhibitor. However, although Parp-1 is recently ested as an epigenome regulator, the action of Parp inhibitors on enome are not well studied. It has been reported that PARP inhibition ed DNA hypermethylation in several cell lines. Interestingly, we previouly rted the enhanced trophoblast differentiation of *Parp-1*$^{-/-}$ embryonic stem (ESCs) as reported in *Dnmt1*$^{-/-}$ ESCs. This led us to examine the role ARP as anti-hypomethylation barrier in ESCs. Here, by analyzing DNA hylation in PARP inhibitor treated and *Parp-1*$^{-/-}$ ESCs in a genome-wide ner, we showed particular loci are hypomethylated. Hypomethylated loci buted to all chromosomes in a similar frequency, whereas the frequency permethylated changes were variable. Comparing with the expression data, hypomethylation seems to contribute to enhance gene expression rious loci. Our study suggests that induction of DNA hypomethylation is ion of PARP inhibitor besides DNA repair inhibition, at least in ESCs.

ords: demethylation, Parp

P-2359 Development of epigenetic treatment using neuroblastoma xenograft

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腫瘍移植腫瘍を用いたエピジェネティック治療の開発

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CpG island methylator phenotype (CIMP) is closely associated with survival in neuroblastomas (NBLs) (HR=22 in 140 Japanese cases; 5 in 145 German cases) [Cancer Res, 65:828, 2005; Cancer Lett, 247:253,]. This fact suggests that a demethylating agent could be an effective eatment of NBLs. Here, we examined the dose effect and toxicity of 5-azacytidine (5-azaC), using a xenograft model of a CIMP (+) NBL cell line. Three to six mice in a group were inoculated subcutaneously with NB-39nu cells (3 x10^6 cells) on day 0. 5-azaC was administered intraperitoneally at a dose (0, 0.2, 0.4, or 0.8 mg/kg) once a day from day 4 to 9. The average volume ± SD (mm^3) of tumors on day 20 were 2983 ± 935, 1893 ± 1728 (P=0.29), 1266 ± 491 (P<0.01), 1709 ± 153 (P=0.07) for the groups of 0, 0.2, 0.4, 0.8 mg/kg,respectively. Adverse effects; body weight loss and leukocytopenia were monitored. Body weight loss was not observed during and after treatment, while leukocytopenia was not observed on day 20, but not examined during treatment. These data suggested that 5-azaC could be effective for neuroblastomas *in vivo*.

Keywords: Epigenetics, DNA methylation

P-2360 CSC-3436, a Novel Compound, Synergizes with Tamoxifen against Estrogen-Negative Breast Cancer Cells

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Tamoxifen is one of the most widely used chemotherapeutic agent for the treatment of estrogen receptor (ER)-positive breast cancer patients. The efficiency of tamoxifen has been demonstrated to induce apoptosis and reduce cell proliferation in tumor cells via inhibition of ER signaling.However, recent study indicated that tamoxifen at high concentration possesses antitumor activities in ER-negative cancer cells. Here, we use CSC-3436, a derivatives of 2-phenylnaphthyridin-4-ones (2-PN) to address the hypothesis that the efficiency of CSC-3436 sensitizes ER-negative breast cancer cells to tamoxifen.Two breast cancer cell lines (MDA-MB-231 and BT-20) lacking expression of ER a were used in this study. CSC-3436 restored the expression of ER a and sensitized to the anti-proliferative effects of tamoxifen both in MDA-MB-231 and BT-20 celllines. Mechanistically, this CSC-3436-mediated sensitization was correlated to switch tamoxifen-induced autophagy to apoptosis. The results showed that CSC-3436 could be used as a potential compound to improve tamoxifen sensitivity inER-negative breast cancer cells. This combinatorial approach is worthy of continuing investigation.

Keywords: Estrogen receptor, Tamoxifen

P-2361 The significance of DNA methylome for tumor progression in multiple myeloma: An MBD-sequencing-based approach

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多発性骨髄腫の発生における DNAメチロームの意義：MBDシーケンシング法によるアプローチ

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[Aim] To assess the significance of DNA methylome in tumorigenesis of multiple myeloma (MM) by integrated analysis including MBD-sequencing. [Methods] After DNA extraction from MM cell lines, and plasma cells of MM, MGUS, and normal plasma cells, we enriched sheared fragments of methylated DNA using Methylminer™ Methylated DNA Enrichment Kit (invitrogen) followed by sequencing analysis using SOLiD™ system (life technologies). We then counted read-tag numbers, which quantitatively reflect the methylation levels. [Results and comments] Referring to the results of mRNA expression analysis using cDNA microarray (Agilent), we found a significant reduction of expression level with tag number increase in the genes with promoter region in which CpG islands located (Rs=-0.562, P<0.001). On the other hand, gene-body methylation level was positively correlated with expression level (Rs=0.351, P<0.001), and this tendency was observed more clearly in non-CpG island genes. Interestingly, genetic deletions in MM were frequently observed in genes with commonly lower methylated gene-body. We will also present the significance of the changes in DNA methylome during multistep tumorigenesis in MM.

Keywords: Multiple myeloma, DNA methylation