Aberrant Histone Modification at the Thyrotropin-Releasing Hormone Gene in Resistance of Thyroid Hormone

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Histone modification such as acetylation and methylation status influences transcriptional activity, and the mechanism of negative gene regulation by thyroid hormone remains unclear although its impairment by a mutant thyroid hormone receptor (TR) is critical for resistance to thyroid hormone. To analyze the dynamics of histone status *in vivo*, we established cell lines stably expressing a typical negatively regulated gene by thyroid hormone, TRH promoter, and wild-type or a novel mutant TR, F455S. The F455S mutant exhibited impaired repression of the thyrotropin-releasing hormone (TRH) gene and had a strong dominant negative effect on the gene. F455S strongly interacted with NCoR, and was hard to dissociate from it by addition of thyroid hormone. Treatment with a histone deacetylase inhibitor, Trichostatin-A, completely abolished the repression of the gene, suggesting that histone deacetylation is essential

for the repression of the gene by thyroid hormone. Chromatin immunoprecipitation (ChIP) assay demonstrated that the lysine residues of the histone H3 and histone H4 at the TRH promoter were acetylated in the absence of thyroid hormone, and the addition of thyroid hormone caused rapid recruitment of histone deacetylase (HDAC) 2 and HDAC 3 within 15 mins, resulting in a transient deacetylation of the histone tails. TR and NCoR without HDAC3 were located on the promoter in the absence of thyroid hormone, and thyroid hormone caused NCoR dissociation and SRC-1 recruitment sequentially. Furthermore, ChIP assay demonstrated that the ratio of dimethylated lysine 4 residue of histone 3 did not change in 48 hours after the addition of T3. Treatment of MTA, an inhibitor specific for methylation of lysine 4 residue of histone 3,significanrtly decreased the ligand independent activity. In the presence of F455S, both histone 3 and histone 4 became hyperacetylated in the absence of T3, and HDACs recruitment and histone deacetylation were significantly impaired. These findings demonstrated the dynamics of histone modification induced by thyroid hormone on the TRH gene and changes in the histone status in resistance to thyroid hormone.

2005 SFN abstract

Ultrastructural analysis of spine density and distribution of drebrin A within spines of drebrin A-transgenic mice.

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Drebrin A is an actin-filament binding protein expressed in neurons. It is known to be involved in neuronal morphological changes. We have previously reported that LTP of drebrin A transgenic mice, which over-express drebrin A in postnatal forebrain, is enhanced in hippocampal CA1. This suggests drebrin A's regulatory involvement in synaptic plasticity via reorganization of actin cytoskeleton. In this study, we analyzed spine morphology of layer 1 rhinal cortex of drebrin A transgenic mice by immuno-electron microscopy.

Over-expression of drebrin A did not change the number of axo-spinous synapses encountered. We found that not all spines forming asymmetric synapses have drebrin A, and the ratio of drebrin-positive to drebrin-negative spines was not changed by drebrin A over-expression. We also analyzed the distribution of drebrin A within spines. Within spines of wild-type mice, drebrin A occurred more in the cytoplasm relative to the zone near or at the PSD or along the non-synaptic plasma membrane. For spines of transgenic mice, the overall level of drebrin A per spine remained unchanged, yet the balance shifted to the cytoplasm relative to the plasma membrane + PSD, when compared to the wild-type spines.

These results suggest that drebrin A expression does not promote spinogenesis in mature brains, but that some kind of a regulatory mechanism exists to maintain certain proportion of spines drebrin-positive which may enhance trafficking of synaptic molecules. Also, the shift in drebrin A's distribution may change the site of interaction of drebrin A with actin filament within spines, which in turn, may account for the enhancement of LTP.

Key words: ACTIN, ELECTRON MICROSCOPY, TRANSGENIC, SPINE

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Drebrin E expression in migrating neuronal precursors of adult brain

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Abstract

In order to clarify drebrin isoform expression in the migrating neuronal precursors in the adult rat brain, we carried out immunohistochemistry with the brain sections of the adult male SD rats. Using two kinds of drebrin antibodies, M2F6 which can recognize A and E isoforms of drebrin and DAS2 which can specifically recognize A isoform of drebrin, we double stained the brain sections to analyze drebrin isoform expression. Anti-Ki67, anti-PSA-NCAM and anti-GFAP antibodies were used for double immunostaining with M2F6. The packed cells in subventricular zone (SVZ) and rostral migratory stream (RMS) were strongly immunostained with M2F6 but not DAS2. This shows that these cells expressed drebrin E but not drebrin A. They were GFAP-negative and PSA-NCAM-positive. Subsets of them were Ki-67-positive. The cell shape of drebrin E-positive cells was bi-polar shape, and drebrin E immunostaining was located in soma and their two major cell processes. Therefore they had morphological and immunostaining characters of the tangential migrating neuronal precursors in the adult rat brain. It has been reported that removal of the olfactory bulb increase the number of migrating cells in RMS. Therefore, we removed the right olfactory bulb, and double-immunostained the brain section with M2F6 and DAS2. Our preliminary data shows that, after the olfactory bulb was removed, the number of cells that express drebrin E but not drebrin A was increased in the operation side comparing with the control side. Together, we concluded that the migrating neuronal precursors in the adult rat brain expresses drebrin E but not drebrin A, and this double-staining method using M2F6 and DAS2 is a new useful tool to analyze neurogenesis and neuronal migration in adult brain.

Keywords: ADULT NEUROGENESIS; SUBVENTRICULAR ZONE; ACTIN; BULBECTOMY

ANTITUMOR EFFECT OF CHEMICALLY SYNTHESIZED NOVEL GLYCOCONJUGATES

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Tumor-related expression of fucosylated antigens and its suppression by cell-mediated priming using sugar acceptors for fucosyltransferases were previously demonstrated in colorectal cancer cells (1). Treatment of these cells with GlcNAcβ1,3Galβcholestanol at low concentrations caused a significant suppression of sialylated Le^X and YB-2 (1) antigens on the cell surface, while at higher concentrations, the cytotoxicity of the sugar-cholestanol was also observed in a variety of cancer cells. In this study, newly synthesized glycoconjugates with cholestanol aglycons were evaluated for their antitumor actions both *in vitro* by a cell proliferation inhibition assay and *in vivo* in a mouse model of peritoneal dissemination. Liposomes encapsulated or cyclodextrin included sugar-cholestanols could be used for both *in vitro* and *in vivo* investigations even though sugar-cholestanols *per se* were insoluble in water. GlcNAcβ1,3Galβcholestanol added to the cell medium was found to be taken up by the cells, and then glycosylated through intercellular glycosylation pathways. Then the glycosylated, primed sugar-cholestanol was secreted. By increasing the concentration of the sugar-cholestanol, the viability of the cells was found to decrease sharply together with apoptotic changes induced in a short period of time. Further, intraperitoneal treatment of liposomes encapsulated GlcNAcβ1,3Galβcholestanol in the mouse model of peritoneal dissemination showed that tumor growth was suppressed and the survival rate was significantly improved. These observations led us to conclude that GlcNAcβ1,3Galβcholestanol might be effective for the prevention and treatment of peritoneal metastasis. (1) S. Yazawa, *et al.*, Glycobiology, 12:545-553, 2002.

Abstract Title

Prognostic analysis of WHO grade 3 gliomas treated with post-operative radiation therapy.

Authore

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Objectives:

WHO grade 3 gliomas show relatively better prognosis compared to glioblastoma. However, only a little has been investigated about the prognostic impact of histological subtype in the grade 3 gliomas. Hence, we investigated the prognostic impacts of various clinical factors including histological subtypes.

Methods:

Sixty-three patients consisted with 41 anaplastic astrocytomas (AA), 11 anaplastic oligodendrogliomas (AO) and 11 anaplastic oligoastrocytomas (AOA). They consecutively received biopsy or operation followed by external radiation therapy between 1984 and 2001. The relationship was evaluated between prognosis and clinical factors including age, sex, histological subtypes, radiation dose and extent of surgery.

Abstract

Results:

For all grade 3 glioma patients, 2- and 5-year survival rates were 55 % and 38 %. Patients younger than 65 years old had significantly better prognosis than older (P=0.019). Gender and radiation dose were not significant factors. Regarding histological subtypes, the 2- and 5- year survival rates of AA, AO, and AOA were 35% and 27%, 100% and 76%, and 81% and 48%. The patients with AA had significantly worse survival curve than the patients with AO or AOA (P=0.001). The 5- year survival rates by extent of surgery for biopsy, subtotal resection and gross total resection were 7.4%, 47.7% and 48.6% (P=0.001). On multivariate analysis, extent of surgery was the strongest clinical prognostic factor, whereas histological subtypes was somewhat a prognostic factor.

Conclusions:

This present study suggested that the extent of surgery, age and histological subtypes were significant prognostic factors. On multivariate analysis, extent of surgery was the strongest prognostic factor followed by histological subtype and age.

Reply Confirmation Letter

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Title: Angiotensin II blockade completely inhibits the development of mice model of HIV-associated nephropathy

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Recently, we have developed a new transgenic mice model of HIV-associated nephropathy (HIVAN). In this model, aggressive collapsing focal segmental glomerular sclerosis with massive proteinuria and deterioration of renal function can be induced only within one month. In the current study, we aimed to determine whether an angiotensin II receptor blocker (ARB) could attenuate the development of HIVAN. We used conditional transgenic mice (rtTA-Vpr) that express the HIV Vpr gene selectively in podocytes using the murine podocin promoter and tet-on system in response to doxycycline administration. One week before the administration of doxycycline, the rtTA-Vpr mice were heminephrectomized. An ARB, olmesartan (10mg/kg/day), was administered together with doxycycline. At 4 weeks after the initiation of doxycycline, the control mice without olmesartan (Cont) showed severe renal injuries. In contrast, olmesartan almost completely inhibited the development of the disease: urinary albumin (mg/day), 87.1±44.4 vs. 0.65±0.42, $p<0.01$; serum creatinine (mg/dl), 0.36±0.20 vs. 0.14±0.09, $p<0.01$; glomerular sclerosis score (0 - 4), 2.5±0.6 vs. 0.05±0.04, $p<0.01$; Cont vs. olmesartan treated mice (ARB-treated), respectively. Immunohistochemical examination showed that the expression of WT1 and synaptopodin, markers of differentiated podocytes, was reduced or disappeared in Cont mice, but remained in ARB-treated mice. The expression of PCNA, a marker for proliferation, was increased in podocytes in Cont mice, but not in ARB-treated mice. These data suggest that an ARB could be a useful therapeutic strategy for the treatment of HIVAN. Moreover, angiotensin II may play a critical role in the progression of podocyte injuries in HIVAN.

Leptin and leptin receptor long isoform (ob-Rb) are upregulated in cardiac myocytes by hypertensive stress.

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Abstract

Obesity is a major risk factor for the development of hypertension and cardiac hypertrophy. Leptin, adipocyte derived hormone, mediates energy intake and expenditure, and circulating level is elevated in patients with massive obesity. Recent studies suggest that leptin participates in progression of hypertension and cardiac hypertrophy. Leptin receptors are also expressed in various tissues including heart, however, the role of leptin receptor in cardiac hypertrophy is poorly understood. We therefore examined the expression of leptin (ob) and leptin receptor isoforms (ob-Ra, Rb) in pressure-overloaded rat heart. Pressure overload was produced by ligation of the abdominal aorta of Wistar rats at 1 day to 28 day. Using Real-time PCR, ob-Ra mRNAs expression was not changed in pressure overloaded rat, while ob and ob-Rb mRNA was significantly increased after aortic banding (ob; 2w: 2.2±2.3, 4w: 2.7±1.6*. ob-Rb; 2w: 1.6±0.5*, 4w: 1.9±0.7*. relative to sham. *$p<0.05$). We also examined protein expression of ob-Rb by immunohistochemistry, in consequence, ob-Rb was particularly detected in hypertrophic cardiomyocytes (2w: 0.057±0.094 vs 0.181±0.113*, 4w: 0.024±0.028 vs 0.101±0.054*, % positive area, sham vs banding; *$p<0.05$). Plasma leptin levels were not different between sham and banding groups (2w: 3.62±2.11 vs 3.40±2.54, 4w: 3.89±1.82 vs 3.22±1.41, ng/ml, sham vs banding). To clarify which stress activates ob and ob-Rb expressions, we examined these mRNA expressions in neonatal rat cardiac myocytes treated with angiotensin II (AII: 1-10 μM), endothelin-1 (ET-1: 0.01-0.1 μM) or cyclic mechanical stretch. AII, ET-1, and stretch stimulated ob mRNA expression (1.6 - 2.2 fold), but ob-Rb was activated only stretch (2.2 fold). ob-Ra was unaffected by any stress. In conclusions, our results demonstrated for the first time that leptin and leptin receptor were upregulated in pressure-overloaded rat heart. Furthermore, these activations were confirmed in cardiac myocytes treated with AII, ET-1, and mechanical stretch. Hyperleptinemia in obesity is known to participate in cardiac hypertrophy and hypertension, thus, this study has a significant contribution to the elucidation of novel link between obesity, hypertension, and cardiac hypertrophy.

Key Words: leptin, obesity, hypertension, cardiac hypertrophy