Session 3 Thyroid Hormone

Endocrine Disrupters and Thyroid Function

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Thyroid hormone (TH) plays important role on development and metabolism. Of particular importance, some of the most important effects of TH occur during fetal development and in early childhood. Congenital hypothyroidism caused by impaired TH production is characterized by delayed growth and mental retardation. Severe cases with resistance to thyroid hormone caused by the mutation of thyroid hormone receptor (TR) β gene also manifest similar findings. Maternal hypothyroidism also affects the maturation of her child as manifested by lower IQ than the child from normal mother.

Recently, epidemiological studies on wild life and humans are providing evidences that endocrine disrupters interfere with thyroid functions. Especially, dioxins and polychrolinated biphenyls have received special attention due to their structural similarities with TH. Low-level perinatal exposure to dioxins and polychlorinated biphenyls (PCB) affect cognitive and motor development of children

Cheek et al. (Environmental Health Perspectives 107, 273-278, 1999) demonstrated that some of the hydroxylated PCBs binds to serum TH-binding proteins, especially to tranthyretin with higher affinity than thyroxine. We have recently shown that one of the T4 transporter (L-amino acid transporter) expression is increased by dioxin at the transcriptional level. It also increases the expression of UDP-glucuronyl transferase which accelerates biliary excretion of T4 by glucuronylation. These findings suggest that dioxins and PCBs accelerate the metabolism of TH, thereby inducing hypothyroidism. It is notable that dioxin intake from maternal milk negatively correlate with the levels of serum thyroxine and positively correlate with those of serum TSH. A finding that administration of PCB to pregnant rats causes an increased expression of thyroid hormone responsive genes, such as RC3/neurogranin in the offspring suggest PCBs could exert thyroid hormone-like effect mediated through TR.

TH is considered to exert its action through interaction with nuclear receptors, altering the expression of target genes. TRs are encoded by two different genes, TR α gene on chromosome 17 and TR β on chromosome 3. TR α gene endcode 2 isoforms, α1 and α2 and TR β, β1 and β2. The structure of TR is conserved among nuclear receptor families and consists of N-terminal domain, DNA-binding domain, hinge region and ligand-binding domain. TR α1, TR β1 and TR β2 isoforms share high homology except for N-terminal region. They bind with TH and thus functions as TR. TR α2, however, does not bind with TH because of altered C-terminal amino acid sequence. It is believed that TR α2 inhibits the function of other TR isoforms. Ubiquitous expression of TR α1 is observed from early embryonic life. On the other hand, TR β is expressed in late embryonic life in a tissue specific manner. For example TR β2 is only expressed in hypothalamus and pituitary. Temporal and spatial specific expression of TR isoforms defines critical period for thyroid hormone actions on different organs.

TH is indispensable for the growth, development and metamorphosis in frogs. Xenopus laevis like other vertebrate, has two TR isoforms, TR α and TR β. TR α is distributed widely in tissues even before the formation of thyroid gland. During premetamorphosis the TH concentration and the TR β levels are very low when the early events of tadpole development such as limb growth and DNA replication in the brain occur. TR β, a TH response gene by itself increases along with the rise in endogenous TH during metamorphosis when the final changes such as gill and tail resorption and intestinal remodeling occur. Thus, it is likely that TR is involved in the development of metamorphosis. Indeed, transgenic frogs expressing dominant negative TR α results in complete inhibition of TH-mediated metamorphosis. Furthermore, it has been shown that injection of mRNA encoding TR into fertilized eggs and subsequent incubation with TH results in teratogenesis in tadpoles. These findings suggest that frog is suitable for the screening of environmental chemicals affecting thyroid function.

Statistical analysis in Japan demonstrated that incidence of cretinism tripled from 1981 to 1995 despite of the steady decline in birth rate. Is this increased incidence of cretinism due to endocrine disrupters? Collaborative studies with thyroid specialists and biologists studying frog development will give us the answer.

Steroid Receptor Coactivator, SRC-3, and Prostate Cancer

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Steroid hormone receptors require coactivators to mediate transcriptional activation to their target genes. The steroid receptor coactivator (SRC) family consists of three newly identified members (SRC-1, -2 and -3). Among them, SRC-3 (PCIP/ACTR/RAC3/AIB1/TRAM) has been shown to be amplified and/or be overexpressed in many types of tumors such as breast cancers, ovarian cancers and gastric cancers, which suggests that SRC-3 may play vital role in controlling cell proliferation and tumorigenesis. Here, we report that SRC-3 is also overexpressed in prostate cancers. We found that 46% of human prostate cancer samples overexpress SRC-3 in the tumor area but not in the adjacent normal area as assayed by *in situ* hybridization. Furthermore, the overexpression of SRC-3 is correlated with the severity of prostate cancer samples. In addition, our results show that levels of endogenous SRC-3 expression also correlate with cell growth rate in different prostate cancer cell lines. By analyzing stable-transfected prostate cells expressing SRC-3, we showed that SRC-3 is indeed able to promote cell growth, cell cycle progression and cell survival. In addition we demonstrated that SRC-3 activates PI(3) kinase/Akt signaling pathways through the induction of Akt expression and phosphorylation in LNCaP cells, which is consistent with its oncogenic roles in promoting cell proliferation, cell growth, cell survival. Together, these findings suggest that SRC-3 may play an important role in prostate cancer development and that the expression levels of SRC-3 may sever as an additional biomarker for prostate tumor progression.

Thyroid Hormones: Multiple Roles through Multiple Receptors

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Thyroid hormones and mainly the triiodothyronine, T3, control many functions in Vertebrates. During development they activate growth of the body, maturation of the central nervous system. In the adult they control physiological homeostasis of carbohydrate and lipid metabolism. They also control their own production through the hypothalamo-pituitary-thyroid axis.

The effects of T3 are mediated by specific nuclear receptors which are transcription factors. Encoded by two different genes, respectively TR α and TR β. The TR α gene encodes the TR α 1 receptors and isoforms like TR α 2, TRΔ α 1 and TRΔ α 2 which do not bind T3 and behave as dominant negative inhibitors against T3 receptors.

The TR β gene encodes three isoforms of receptors, namely TR β 1, TR β 2 and TR β 3, and one inhibitory isoform TRΔ β 3.

The receptors bind to the DNA on specific sequences usually located in the promoter region of target genes. In the absence of hormone, these receptors behave as transcription repressors. Upon T3 binding, the receptors bind activators and induce transcription of the downstream gene. On some very specific genes, mainly in the central nervous system, the receptors behave as ligand dependant transcriptional repressors.

By using experimental genetic approaches in the mouse, we have investigated the respective functions of various TR isoforms in the development and physiological regulation by T3. Several mouse mutants were created by knocking out specific regions of the TR α and TR β genes.

From this whole work, we conclude that TR α is the master receptor that controls postnatal development of major tissues like bone, intestine, hematopoietic tissue. TR β is mostly involved in developing sensory functions and controlling thyroid hormone production. In some tissues like brain, both receptors cooperate for maturation. In the adult, various physiological functions seem to be controlled independently by the TR α and TR β isoforms.

These data suggest that in Mammals TR α and TR β receptors have acquired specific respective functions and open new perspectives for understanding thyroid hormone pathologies.

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Thyroid Hormone Receptor Mutations and the Development of Thyroid Cancer

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The molecular genetic basis of thyroid carcinogenesis is not well understood. Most of the existing models of thyroid cancer only rarely show metastatic spread, and this has limited progress in the understanding of the molecular events in thyroid cancer invasion and metastasis. We have recently generated a mutant mouse by introducing a dominant negative mutant thyroid hormone nuclear receptor gene, *TR*β*PV*, into the *TR*β gene locus. In this *TR*β*PV* mouse, the regulation of the thyroid-pituitary axis is disrupted, leading to a mouse with high levels of circulating thyroid stimulating hormone and extensive hyperplasia of follicular epithelium within the thyroid. The weights of thyroid increased with age, with a12-,18- and 36-fold increase at the ages of 3-4, 5-7 and >12 months, respectively, as compared with wild-type mice. Importantly, as *TR*β$^{PV/PV}$ mice, but not *TR*β$^{PV/+}$ mice, aged, metastatic thyroid carcinoma developed. Histological evaluation of thyroids of 5-14 month-old mice showed capsular invasion (91%), vascular invasion (74%), anaplasia (35%) and metastasis to the lung and heart (30%). These findings suggest that thyroid carcinogenesis progresses with sequential capsular invasion, vascular invasion, anaplasia and eventually metastasis. cDNA microarray analyses of RNAs prepared from the thyroids of 5-month old *TR*β$^{PV/PV}$ and wild-type mice indicate that the expression of *TR*β*PV* gene led to an up-regulation of 200 genes and down-regulation of 95 genes. One gene, cyclin D1, was activated ~8-fold, which was confirmed by Northern blot analysis. These results are consistent with the findings in human thyroid carcinoma that show a high frequency of over-expression of cyclin D1. These mice provide a unique model for clinically important parameters in human thyroid cancer, namely, invasion and metastasis. Importantly, this model provides an unprecedented opportunity to study the alterations in gene regulation that occur during progression and metastasis in a predictable fashion.