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Review

Molecular Basis of Resistance to Thyroid Hormone (RTH)

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Introduction

THYROID hormone exerts profound effects on normal growth and development as well as several metabolic pathways [1]. These actions are mediated through nuclear thyroid hormone receptors (TRs) [2], which belong to a member of steroid/thyroid hormone receptor superfamily. TRs directly bind to a target binding site of thyroid hormone responsive genes and regulate transcriptions of the genes in a ligand-dependent manner. Several disorders ascribed to the abnormality of the steroid/thyroid hormone receptor superfamily. Among them, a syndrome named as resistance to thyroid hormone (RTH) is mainly caused by the mutations in TRβ gene. The clinical features and laboratory findings of this syndrome have been well documented [3]. In this review, the molecular aspect of TR action and the pathogenesis of RTH will be presented.

Thyroid Hormone Receptors (TRs) and Their Action

TRs are encoded by two different genes, TRα and TRβ, which are located on chromosome 17q and 3p, respectively. TRα is highly homologous to viral oncogene v-erbA, which causes erythroleukemia in chicken [4]. Thus, TRs are considered as a cellular proto-oncogene. Several isoforms are generated by alternative splicings of the TR genes (Fig. 1). TRα1, α2 and α3 are C-terminal splicing variants generated from the transcript of TRα gene. The carboxyterminal 40 amino acid (a.a.) of TRα1 is replaced with 120 a.a. (human) or 122 a.a. (mouse, rat) of TRα2 specific sequence [5, 6], and the N-terminal 39 a.a. of the α2 specific sequence is deleted in TRα3 [7]. TRα1 binds to T_3, and induces or represses transcription of T_3-responsive genes. On the other hand, TRα2 and α3 do not bind to T_3 and do not mediate T_3 action, indicating that TRα2 and α3 are not genuine TRs. Rather TRα2 is reported to inhibit normal TR action in a dominant negative manner [8, 9], suggesting that it may act as an endogenous

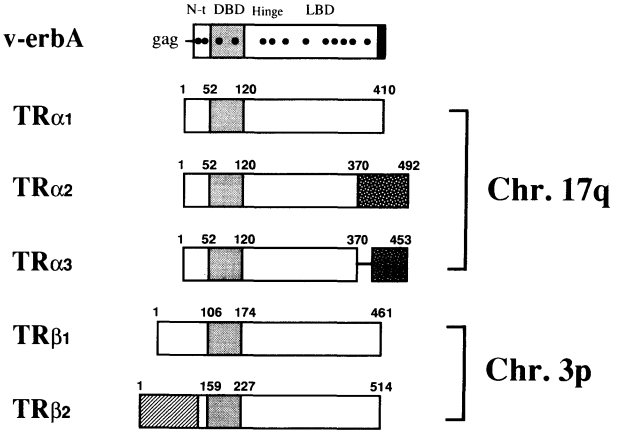

Fig. 1. TR isoforms. TRs are located on two different genes, TRα and TRβ. TRs are proto-oncogene of erythroleukemia virus, v-erbA. Several TR isoforms are created by alternative splicings of these genes. N-t, N-terminal domain; DBD, DNA binding domain; LBD, ligand binding domain. DBD is conserved among the TRs. Difference in the amino acid sequence among TRα and TRβ are also indicated.

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inhibitory protein of thyroid hormone action. Although the functional role of TRα2 remains to be clarified, its expression is more abundant than TRα1 in certain tissues including brain, testis and the immune system [10]. The role of TRα3 is also unclear.

The TRβ gene generates two TRβ isoforms, TRβ1 and β2, in which N-terminal 93 a.a. of TRβ1 is replaced with 146 a.a. of β2 specific sequence [11]. TRβ2 is only expressed in the pituitary and hypothalamus and is involved in the regulation of the genes specific in these organs, whereas TRβ1 is widely expressed.

The structure of TRs is basically conserved in the members of steroid/thyroid receptor superfamily. It is divided into A to E domains depending on their specific functional roles (Fig. 2). The aminoterminal A/B domain of certain receptors including glucocorticoid receptor (GR) and estrogen receptor (ER) plays a role in a ligand-independent transcriptional activation [12]. This domain is also called as activation function-1 (AF-1). However, the presence of this function in TRs is still controversial. The central C domain is the DNA binding domain, which is composed of two zinc finger motifs to confer DNA binding and dimerization [13]. The D domain (hinge region) mainly includes two functional regions; one is for nuclear localization of receptor and another is for the repression of transcription in the absence of ligand (named as basal repression). Recently, co-repressor molecules such as N-CoR [14] and SMRT [15] bind to this region to exert basal repression (see below). The C-terminal E domain confers several functions of TRs such as ligand binding, dimerization and ligand-dependent transcriptional activation. The region responsible for the ligand binding is relatively broad. The dimerization region is composed of several repeated heptads of leucine residues. The mutational studies indicated that the ninth heptad repeat region is most critical for receptor dimerization. The extreme C-terminal region is important for the ligand-dependent transcriptional activation, named as AF2 domain [16].

TRs bind to TRE as a monomer, a homodimer or a heterodimer with retinoid X receptor (RXR) [17]. The association of these complexes with DNA activates or represses transcriptions in a ligand-dependent manner [18]. However, the

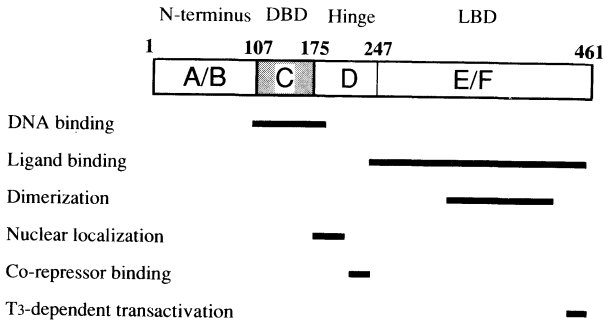

Fig. 2. TR structure and functions. Structural domains of TRs (named A to E) are conserved in the steroid/thyroid hormone receptor superfamily. The functional mapping of the TR structure are indicated by lines.

transcriptional regulation by TRs is not as simple as previously considered. The TR complex interacts with other co-factors which intermediate the T_3 signaling to the basal transcriptional machinery. Co-factors are divided into two categories; co-repressor and co-activator. As co-repressor, N-CoR and SMRT were cloned. Instead of two co-repressors, several co-activators were identified such as SRC-1 [19], TIF-2 [20]/ GRIP-1 [21] and AIB1 [22]/ ACTR [23]/ pCIP [24]. Co-repressor binds to the hinge region of TR and represses transcription in the absence of ligand (basal repression). On the other hand, the binding of ligand with TR dissociates co-repressor from TR, and co-activator, in turn, associates with TR at its C-terminal AF2 domain. The interaction enhances transcriptional activation by TR in the presence of ligand.

Resistance to Thyroid Hormone (RTH)

Resistance to thyroid hormone (RTH) is a disorder characterized by the refractoriness to thyroid hormone in target organs. The patients exhibit elevated serum thyroid hormone levels, and an inappropriately normal or slightly elevated TSH levels [3]. These endocrinological findings reflect a compensated state in which diminished feedback inhibition of the hypothalamic-pituitary axis by thyroid hormone results in TSH stimulation of the thyroid gland to produce a higher steady state level of thyroid hormones (Fig. 3). The clinical features vary due to a balance between the tissue

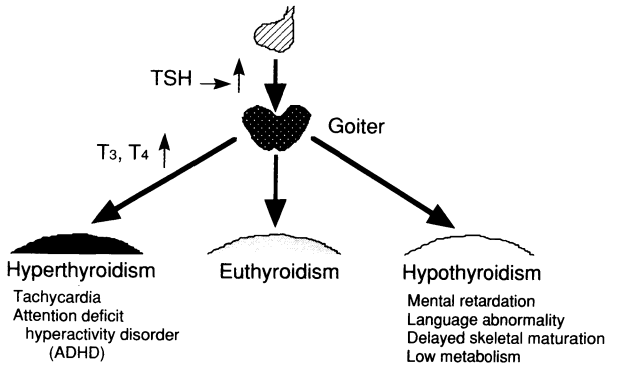

Fig. 3. Clinical features of RTH. RTH is characterized by the elevation of serum thyroid hormone levels, and the inappropriately normal or elevated TSH levels. Almost all patients have goiter. The clinical symptoms of RTH depend on a balance between the tissue refractoriness to thyroid hormone and the increased levels of serum thyroid hormone. Usually, the symptoms of hyper-, eu- and hypo-thyroidism are mixed in an individual.

refractoriness to thyroid hormone and the availability of thyroid hormone. In the tissues refractoriness to thyroid hormone, the symptoms of hypothyroidism (mental retardation, language abnormalities, delayed skeletal maturation and low metabolic rate) are exhibited, when elevated thyroid hormone cannot compensate. When the increased levels of thyroid hormone compensate tissue hypothyroidism, no impairment of thyroid hormone action (euthyroidism) is manifested. In the tissues normally responsive to thyroid hormone, elevated serum levels of thyroid hormone cause hyperthyroidism such as tachycardia and attention deficit hyperactivity disorder (ADHD). These hypo-, eu- and hyperthyroid states are mixed in an individual to show a mosaic state. In addition, most of RTH patients have goiter, reflecting increased production of thyroid hormones.

The inheritance of the first RTH case reported in 1967 is an autosomal recessive [25]. However, in other cases, this syndrome was inherited as an autosomal dominant manner, or found as a sporadic case [3, 26]. Since the first description of the syndrome, it has been speculated that mutation of TR genes may be responsible for this syndrome. The cloning of human TRβ [27] and TRα1 cDNAs [28] from 1986 to 1987 enabled to identify the mutation in the TRs. At first, RFLP (Restriction Fragment Length Polymorphism) study revealed that this syndrome is linked to TRβ gene, but not to TRα gene [29]. Subsequently, the sequence analysis of TRβ gene of an affected patient identified a point mutation in the ligand binding domain of TRβ [30]. Until now, more than 60 different mutations were identified in patients with autosomal dominant trait (Fig. 4). These mutations are clustered mainly in three regions of the ligand binding domain. No mutation was found in the aminoterminus, the DNA binding domain and several specific regions of the ligand binding domain. In contrast, a large deletion of TRβ gene was identified in an autosomal recessive case [31]. These results provide an important clue to decipher the molecular aspect of the pathogenesis to RTH. The individuals homozygous for this TRβ deletion exhibited hormone resistance, whereas the heterozygotes were normal. This observation supports a concept that the more common autosomal dominant form of RTH is caused by mutant receptor which inhibit normal receptor function in a dominant negative manner [32].

Molecular Mechanism of RTH

Since the dominant negative inhibition is the key concept of the pathogenesis of RTH, the functional analysis of the mutant receptors will lead to the molecular mechanism for the pathogenesis of RTH.

T_3-binding assays revealed that most of TR mutations associated with RTH exhibits the impairment of the T_3-binding activity [3, 26]. The decrease in T_3-binding affinity varies from a half of normal values to less than 100-fold, although normal T_3-binding affinity was reported in a few cases. However, the defective binding activity to T_3 does not correlate to the potency of dominant negative inhibition [33]. In addition, the clinical severity of the disorder does not relate to T_3-binding affinity of the mutations [34]. Transfection assays demonstrated that the mutant TR inhibits normal TR function in a dominant negative manner [32], supporting the concept of dominant negative inhibition by RTH mutation. However, a mutant TR with no T_3-binding activity did not necessarily inhibit normal TR function [35], suggesting that the impaired T_3-binding activity is not only the determinant for dominant negative action.

As for the domains critical for dominant negative inhibition, it is important to consider the sites of

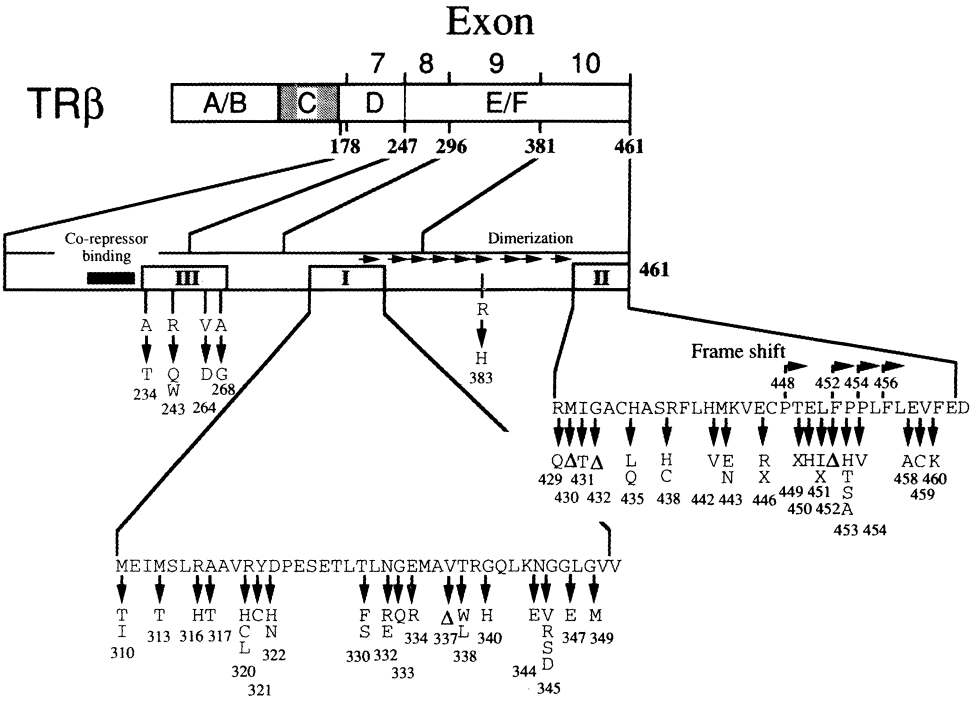

Fig. 4. Distribution of RTH mutations in TRβ. Most of RTH mutations are clustered in the three region of the ligand binding domain of TRβ. These hot spots are distributed in the regions except for the critical functional domains such as DNA binding, dimerization and co-repressor binding.

RTH mutations, which are clustered in the three regions of the ligand binding domain. No RTH mutations were identified in the aminoterminus, the DNA binding domain, the dimerization region and the hinge region of the ligand binding domain [3]. Based on these findings, it is speculated that the regions without RTH mutations might be functionally silent and does not cause autosomal dominant form of RTH. Loss of DNA binding activity by introducing the artificial mutation into the RTH mutant did not exhibit dominant negative action, suggesting that the DNA binding activity is crucial for dominant negative inhibition [36].

For receptor dimerization, two regions are reported to be responsible; one is in the DNA binding domain [37–39] and another in the ligand binding domain. The DNA binding domain for dimerization is important for the recognition of specific arrangement and spacings of TRE half sites [37, 38]. The ligand binding domain is absolutely required for receptor dimerization. In the ligand binding domain, the repeated heptads ranged from a.a. 334 to 428 of TRβ are formed for protein-protein interfaces important for dimerization [40]. Several mutational studies indicated that the last heptad structure named as the ninth heptad repeat is most important for receptor dimerization [41-43], especially for heterodimerization with RXR, but not homodimerization [44, 45]. No RTH mutations in this region also indicate that the dimerization region might be important for dominant negative activity. An amino acid substitution in this heptad (L428R) eliminated the heterodimerization of TR with RXR, but not for homodimer formation [35, 46]. An artificial introduction of this dimerization defective mutation into RTH mutant eliminated dominant negative activity [35], indicating that the dimerization activity, especially heterodimerization, is important for dominant negative action. In one report [47], several point mutations were created in the dimerization region, depending on the hot spot rule in that CpG dinucleotides are changed to TG or CA as a common nucleotide substitution. These mutants showed almost normal T_3-binding activities, transactivation and no dominant negative inhibition, suggesting that mutations in this region fail to manifest clinical symptoms and escape to be identified as RTH.

It is important to consider the function of the hinge region for dominant negative activity, because RTH mutations were neither identified in this region. Recently, co-repressors such as N-CoR and SMRT were reported to associate TRs in the absence of ligand and to repress transcriptions [14, 15]. No RTH mutations in the hinge region will raise the possibility that the defective association with co-repressor might interfere with the dominant negative action of TRs. A mutation (P214R) in the hinge region was shown to impair its binding activity to nuclear co-repressor without altering its DNA binding and dimerization activity [48]. The introduction of this mutation into RTH mutant abolished the dominant negative activity of RTH mutations [49, 50]. These results indicate that the association with co-repressor is also critical for dominant negative inhibition of RTH mutants [49–51]. The mutations associated with RTH are mainly impaired to bind to T_3, but are required to preserve the DNA binding, dimerization and co-repressor binding activities. When RTH mutation sites of TRβ are plotted on the corresponding sites in the crystallography of TRα1 [52], RTH mutations in the three hot spots are closely located surrounding the T_3 binding pocket. The three-dimensional structures for dimerization and co-repressor binding are not disturbed by these mutations, supporting the functional requirement of these regions for dominant negative inhibition. These findings will propose a model that the RTH mutant forming a heterodimer with RXR stably associates with co-repressor, binds to DNA and represses transcriptions even in the presence of ligands (Fig. 5).

Recently, new types of RTH mutations were identified to cause RTH. As mentioned above, the common feature of RTH mutations is impaired T_3-binding activity with preservation of the DNA binding, dimerization and co-repressor binding activity. However, a point mutation in the extreme C-terminus of TRβ (L454V) has a specific feature for TR functions [53]. In this mutation, three critical TR functions for dominant negative inhibition were also preserved. Although this mutant has normal T_3-binding affinity, the association with co-activator is impaired (Fig. 6). Co-activator is an important molecule to mediate T_3 signaling from TR to the basal transcriptional machinery. The impairment of the mutant TR to associate with co-activator implies that the defective association with co-factor can cause autosomal dominant form of RTH.

A few mutations associated with RTH have been reported to be normal in their T_3 binding affinities. Since the association with either co-repressor or co-activator might influence dominant negative activity of RTH mutants, it is intriguing that some functional differences in the association with co-factors could explain the special features of RTH mutants with normal T_3 binding activity. One

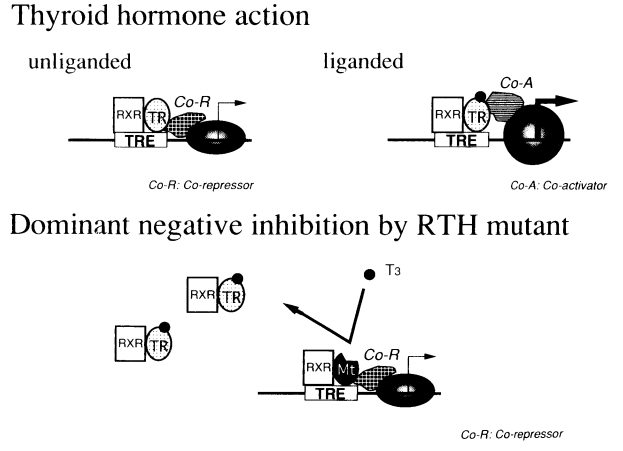

Fig. 5. Molecular mechanism of dominant negative inhibition by RTH mutation. Requirement of DNA binding, dimerization and co-repressor binding activities for dominant negative inhibition will propose the model that the RTH mutant with impaired T_3 binding activity forms stable complex with RXR and co-repressor, and competes the wild type TR complex on TRE site.

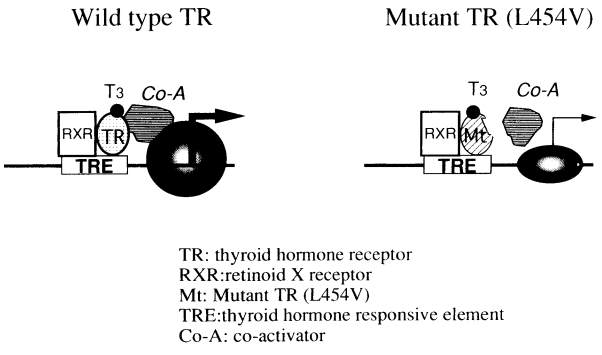

Fig. 6. Defective association of RTH mutation (L454V) with co-activator. Even with normal T_3 binding affinity, RTH mutant (L454V) does not associate with co-activator. The defective association with co-activator could also be the cause of RTH.

mutation (R383H) was reported in a family with RTH, and the T_3-binding affinity of this mutation was normal [54]. The same mutation was created by CpG dinucleotide hot spot rule, and the functional analyses revealed that this mutant had normal T_3-binding affinity and no apparent dominant negative inhibition on positive TRE *in vitro* [47]. However, the presence of this mutation in RTH patient indicates that other functional abnormality of the mutant receptor. It was reported that the dissociation of co-repressor from R383H mutant TR in the presence of T_3 was relatively slow in comparison with wild type TR (Fig. 7). Furthermore, the dominant negative activity of this mutation is evident for TSHβ and TRH gene which are negatively regulated by T_3, but not for the genes positively regulated by T_3. This difference of kinetics to dissociate co-repressor from TR might be another cause of RTH. Crystallographic study indicated that the position of R383 forms a charged bond with two other amino acids (E311 and R429 of TRβ). One RTH mutation was found at one of these amino acids (R429H) [55]. Interestingly, the functional features of this mutation were quite similar to that of R383H [54], providing an evidence for the new functional domain to regulate co-repressor release.

Animal Models to Study the Pathogenesis of RTH

Although the extensive functional analyses of RTH mutations *in vitro* delineate the molecular mechanism of RTH, the physiological significance of these mutations could not be ascertained before the development of animal models. The functional roles of TRs were established by the knockout mice of either TRα1 or TRβ. TRβ knockout mice could serve as a model of the recessive case of RTH, in which the deletion of TRβ gene was identified [31]. Concordant with the human RTH, the TRβ knockout mice showed deafness, which was caused by the abnormality of neuronal maturation in the auditory tract [56]. The presence of TRα did not compensate this function, supporting the isoform-specific role of TRβ for auditory pathway [57]. The hormonal levels in TRβ knockout mice also resembled to RTH patients [58]. Serum T_3 and T_4 levels were relatively high, but TSH levels were not suppressed. It is implicated that the negative feedback regulation of TSH could be mainly medicated by TRβ but not by TRα1. In contrast, the TRα1 knockout mice were different from those of TRβ knockout mice. The mice showed the decreased heart rates and low body temperature

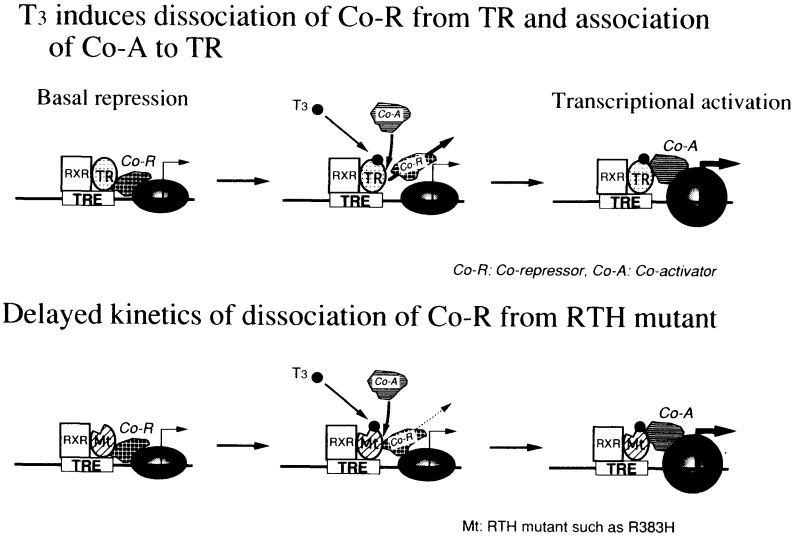

Fig. 7. Impaired kinetics of co-repressor dissociation from RTH mutation (R383H). One RTH mutant (R383H) can dissociate co-repressor in the presence of T_3, and associate with co-activator. However, the dissociation of co-repressor from this mutant is relatively slow compared to that from the wild type receptor. The different kinetics between the mutant and the wild type TR may be another cause of RTH.

with mild hypothyroidism [59]. The TSH levels of the mice were shown to be relatively low with high free T_4 and normal free T_3. The decreased heart rates were increased with the treatment of T_3, but it did not reach to the rates of wild type mice, even with high dose of T_3. One can speculate that some functional disturbance in cardiac system might due to functional abnormality of TRα1. If the patients with TRα1 mutation are present, the clinical characteristics different from the patients with TRβ mutation could be anticipated.

Although the knockout mice demonstrate the functional role of TR isoforms, the pathophysiological significance of RTH mutants can not be studied. Using adenovirus-mediated gene transfer technique, it was demonstrated that the expression of RTH mutant (G345R) in mouse liver inhibited T_3-dependent induction of 5'-DI gene expression [60]. The serum cholesterol levels in the mice were not decreased by high dose of T_3 as wild type animals, reflecting peripheral hypothyroidism by the expression of RTH mutation. Since RTH is based on the central and peripheral refractoriness to thyroid hormone, transgenic expression of RTH mutant in the pituitary and/or peripheral tissues may produce useful animal models to study the molecular mechanism of RTH *in vivo*. Indeed, selective pituitary resistance to T_3 was demonstrated in transgenic mice by targeting TR mutant (G345R) to the pituitary thyrotroph by placing the mutant cDNA downstream of the mouse TSHβ promoter [61].

Future Direction

The extensive studies on the functions of TR contributed to the understanding of the pathogenesis of RTH. It is of note that, even with the same mutation, the clinical phenotype of affected patients varies. Detailed analysis of the cofactors affecting TR function in each RTH patient may clarify the difference of the clinical phenotype.

Furthermore, the recent development such as the discovery of cofactors led us to propose that RTH could be caused by mutation of the genes other than that for TRβ. Supporting this concept, one family associated with autosomal dominant form of RTH was shown not to have any mutations in either TRα or TRβ gene. Using gel shift assay or far-Western analysis, it was revealed that factor(s) associated with TR might be different in the nuclear extract of the cultured fibroblasts obtained from the patients [62]. The identification of abnormality in cofactors would delineate a new insight of the pathogenesis of RTH. In addition, the establishment of the knockout mice of cofactors will provide an important clue to understand the thyroid hormone action.

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