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Gene Section

Review

THRB (Thyroid Hormone Receptor, Beta)

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Abstract

Review on THRB, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

Identity

Other names: C-ERBA-2, C-ERBA-BETA, ERBA2, GRTH, NR1A2, PRTH, THR1, THRB1, THRB2

HGNC (Hugo): THRB

Location: 3p24.2

Local order: According to the NCBI map viewer genes flanking THRB (3p24.2) from telomere to centromere are: UBE2E2-AS1 (UBE2E2-AS1 UBE2E2 antisense RNA 1 (head to head)), UBE2E2 (ubiquitin-protein ligase E2), LOC100420471 (ADP-ribosylation factor-like 4A pseudogene), UBE2E1 (ubiquitin carrier protein E1), NKIRAS1 (NFKB inhibitor interacting Ras-like 1), RPL15 (ribosomal protein L15), NR1D2 (nuclear receptor subfamily 1, group D, member 2), NPM1P23 (LOC100422256, nucleophosmin 1 (nucleolar phosphoprotein B23, numatrin) pseudogene 23), LINC00691 (LOC152024, long intergenic non-protein coding RNA 691), and intra-THRB: LOC101927854 (uncharacterized LOC101927854) sharing some exons with two transcript variants (GeneBank: CB994391.1, AW950510.1) present in ACE View description of NR1D2 gene locus, RPL31P20 (ribosomal protein L31 pseudogene 20), THRB-IT1 (THRB intronic transcript 1), THRB-AS1 (LOC644990, THRB antisense RNA 1), at 5' side of THRB: MIR4792 (microRNA 4792), EIF3KP2 (eukaryotic translation initiation factor 3, subunit K pseudogene 2), LOC101927874 (uncharacterized LOC101927874), RNA5SP125 (RNA, 5S ribosomal pseudogene 125), RARB (retinoic acid receptor, beta), CFL1P7 (cofilin 1 (non-muscle) pseudogene 7), RNA5SP126 (RNA, 5S ribosomal pseudogene 126), LOC100505947 (uncharacterized LOC100505947), TOP2B (topoisomerase (DNA) II beta 180kDa), MIR4442 (microRNA 4442), CRIP1P2 (cysteine-rich protein 1 (intestinal) pseudogene 2), NGLY1 (N-glycanase 1), RPL32P11 (ribosomal protein L32 pseudogene 11), TAF9BP1 (TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31kDa pseudogene 1), OXSM (3-oxoacyl-ACP synthase, mitochondrial), LINC00692 (long intergenic non-protein coding RNA 692), RPEP2 (ribulose-5-phosphate-3-epimerase pseudogene 2), HMGB3P12 (high mobility group box 3 pseudogene 12), VENTXP4 (VENT homeobox pseudogene 4), see diagram 1.

Note

The human thyroid hormone receptor beta (THRB), a member of several nuclear receptors for thyroid hormone, has been shown to mediate the biological activities of triiodothyronine (T3). This gene encodes 3 protein isoforms, the TRβ1, TRβ2, and TRβ4 differentially expressed in developmental and tissue-specific patterns and implicated in regulation of transcription of target genes affecting multiple physiological processes, including cell growth, differentiation, apoptosis, and maintenance of metabolic homeostasis. The gene controls thyroid hormone levels, liver and kidney metabolism and is critical for normal development of auditory and visual systems. The THRB has been also implicated in the pathology of numerous diseases including thyroid hormone resistance syndrome (RTH), obesity, neurodegenerative disorders and cancer.

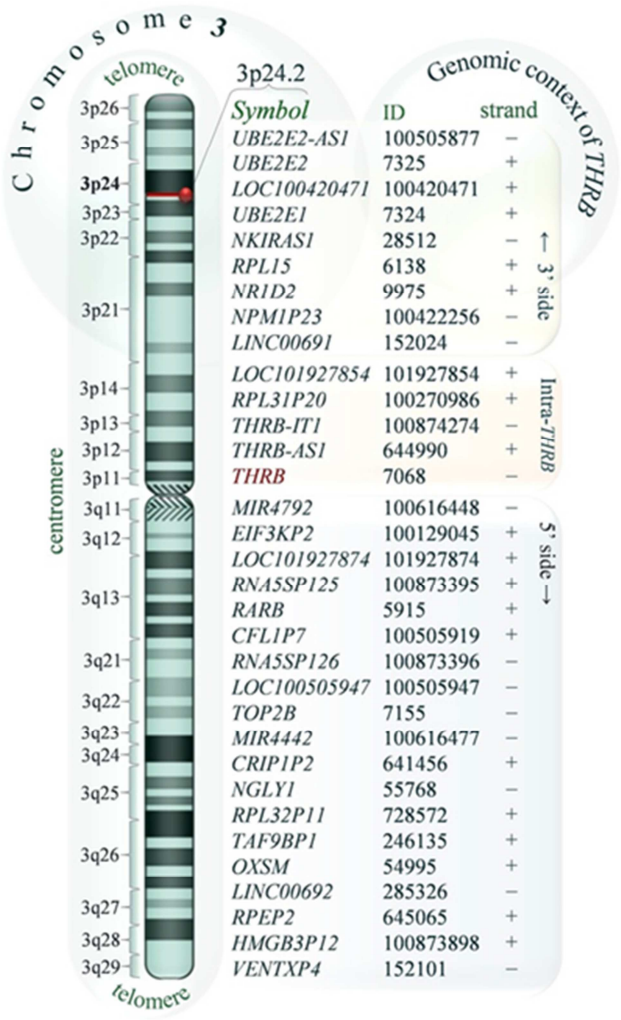

Diagram 1. Schematic representation of human chromosome 3 indicating the position of THRB locus (red bar). The official symbols, NCBI IDs and relative transcriptional orientation of genes in the 3p24.2 locus (+ plus or - minus DNA strand) are shown with respect to the centromere. The genes located at 5'- and 3'-side of the THRB as well as the genes positioned at least in part inside the THRB sequence (intra-THRB genes) are highlighted by three coloured rectangles. The diagram was drawn on the basis of the standard ideogram taken from the NCBI Map Viewer and NCBI Human Genome Resources.

This gene may function as a tumor suppressor and disturbances of the THRB expression are frequent findings in various cancers. However, the genomic actions of the nuclear receptor can interface with nongenomically initiated and TRβ -mediated effects of thyroid hormone on angiogenesis and cancer cell proliferation (Davis et al., 2009; Puzianowska-Kuznicka et al., 2013).

DNA/RNA

The human THRB gene spans a region of 376609 bp and is divided into 20 different exons inlcuding 16 separated by large introns which may give rise to 19 various TRβ1 transcripts mostly differing in 5' untranslated region (5'UTR), 1 truncated variant TRβ4 (TRβ1 isoform) and 1 TRβ2 transcript with differential promoter usage (see diagram 2 and 3). Note that several sense and antisense non-TRβ transcripts were shown to be expressed in the THRB locus, in which may affect the expression of the thyroid hormone receptors (see diagram 2). The THRB gene is one of two thyroid hormone receptor genes in the human genome located on chromosome 3. The other gene, THRA located on chromosome 17, encodes the related thyroid hormone receptors TRα1, TRα2 and TRΔα1 (p28 and p43). These genes were initially identified by their homology to the avian retroviral oncogene v-erbA encoding a mutated variant of chicken TRα1.

Description

According to the NCBI Gene, the THRB maps to NC_000003.11 (24158644..24536772, complement) and spans a region over 376 kilo bases. Within the sequence, some other transcriptionally active genes have been identified (see diagram 1 and 2).

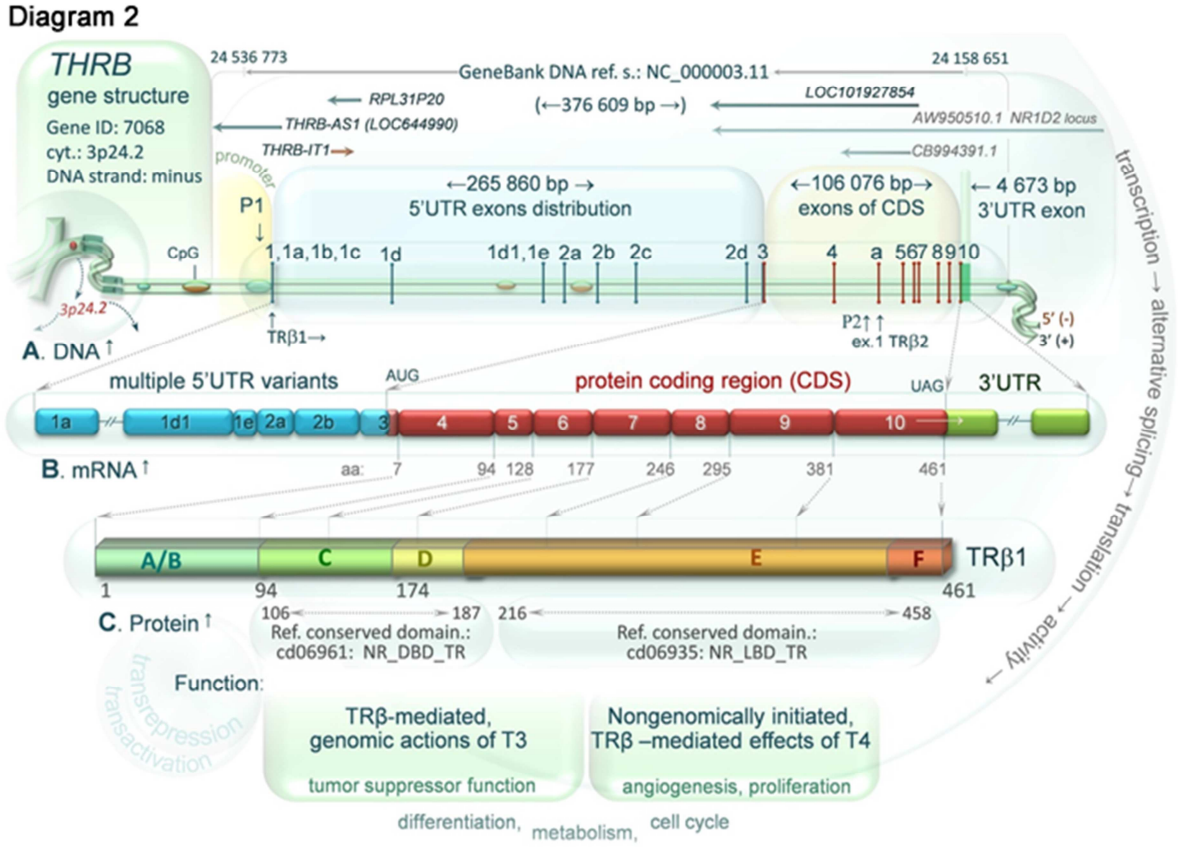

Diagram 2. Human THRB Gene Structure, a transcript and protein. Multiple transcript variants and 3 protein isoforms (TRβ1 TRβ2 and truncated variant TRβ4) are generated by transcription of pre-mRNA using two THRB promoters (P1 and P2 located between exon 4 and 5), alternative splicing of exons marked with numbers 1-10 (horizontal bars) and separated by large introns (shown by distances between exons) as well as protein translation, which is differentially regulated by the 5'UTR variants. **A.** Genes localized within the THRB sequence are marked with orange or blue arrows. The orange one (THRB-IT1, THRB intronic transcript 1) is transcribed in the same orientation (-) to the THRB, whereas blue arrows indicate the genes in opposite transcriptional orientation (+): THRB-AS1, RPL31P20, LOC101927854, and two transcript variants (GeneBank: CB994391.1, AW950510.1) described in NR1D2 gene locus (see local order). Expression of the genes may result in production of long naturally occurring antisense transcripts (long NATs) that may bind complementary target strands of THRB DNA or newly synthesized pre-mRNA. These sense-antisense pairs may affect the expression of the THRB gene (for more details see diagram 1). Large CpG islands identified as methylated within THRB sequence in human cerebellum, sperm and bone marrow cells are marked with small green ovals on double stranded DNA, whereas the green/orange ovals represent CpG islands identified as methylated in human colon mucosa and colorectal tumor (drawn on the basis of the NCBI Epigenomics database). **B.** An example of the multiple mRNA variants, wherein blue boxes represent alternatively spliced exons of 5' untranslated region (5'UTR), red - protein coding sequence, green - TRβ1 3'UTR (exon 10). **C.** One of two TRβ proteins (here TRβ1) and its functional domains: N-terminal AF1 domain (A/B), DNA binding domain (C), hinge region (D), ligand (T3) binding domain (E) and C-terminal AF2 domain (F), all presented in the context of subsequent amino acids (aa) encoded by the TRβ1 mRNA (CDS). Amino acid ranges of the NCBI reference conserved domains: C and E are indicated by gray arrows. For protein function see the text below.

Two precursor mRNA (pre-mRNA) are transcribed using two THRB promoters: P1 (GeneBank: S37458.1), updated according to the sequence of chromosome 3 genomic contig (GenBank: NT_022517.18) allowing for expression of TRβ1 and P2 promoter of TRβ2 isoform (see diagram 3), located between exon 4 and 5 (GeneBank: NG_009159.1; 330058..334363), however the sequence range of P2 has not been exactly established.

The THRB gene consists of 20 different exons including 16 separated by large introns.

The exons are described in details above (see diagram 3).

The alternative TRβ1 splicing results in expression of multiple mRNAs encoding the same protein, however its translation is differentially regulated by the 5'UTR variants.

These transcripts consist of 10 - 14 different exons forming a 5'UTR region (exons 1, 2 and the beginning of exon 3), protein coding region (the end of exon 3, 4-9, first 242nt of exon 10) and long 3'UTR (last part of exon 10) (Frankton et al., 2004).

The exons 5-10 are common to the most of transcripts including TRβ2 mRNA that contains one more, first exon named "a" (see diagram 2 and 3).

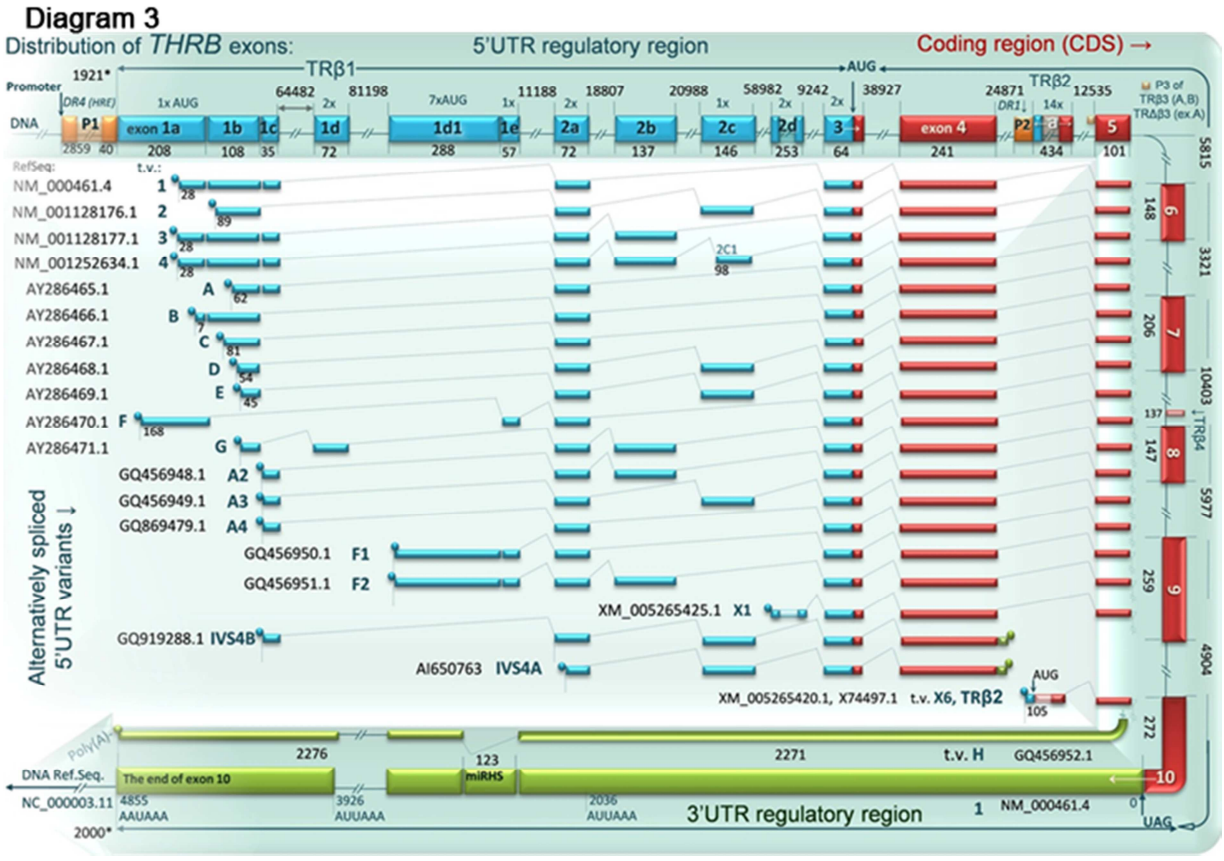

Diagram 3. THRB exons distribution and alternatively spliced 5'UTR variants of TRβ1 TRβ2 and TRβ4 mRNAs. At least 19 mRNA variants of TRβ1 and one of TRβ2 are synthesized using two THRB promoters: P1 and internal promoter P2 located between exon 4 and 5. The human variant TRβ4 is a carboxyl-terminal splicing variant of TRβ1 (using P1 promoter), which contains a stop codon due to the presence of a 137-bp insertion located between exon 7 and 8.The third known, non-functional in humans promoter (P3) is shown as well. This locus is located upstream of the exon 5 but downstream of the P2 promoter and allows for transcription of two additional isoforms: TRβ3 and TRΔβ3, expressed ubiquitously only in rats, however, the regulatory elements present in this region may interfere with transcription of the TRβ2 in humans. The mRNA variants are shown in the context of alternative and constitutive exons and their distribution within the DNA sequence. Blue boxes represent alternatively spliced exons of 5' untranslated region (5'UTR), red - protein coding sequence, green -3' untranslated region (3'UTR, the longest 3' part of exon 10). Most of exons are separated by large introns, which are marked at the beginning and the end by numbers representing their relative position on DNA. The diagram shows a region of 378,609 kb (24133709..24516317; Ref.seq. : NC_000003.11). The exact size (nt) of each exon is given below the exon boxes. The 5'UTR region of TRβ1 may include up to 10 alternatively spliced exons and 44 bp of exon 3, whereas 5'UTR of TRβ2 contains only 105 of 434 nucleotides of exon 1 named "a". The exons 5-10 are mostly constitutive and common with almost all transcripts including TRβ2 mRNA. The most of identified transcript variants (shown at the middle of the diagram) differ only in the 5' untranslated region, which can influence the protein synthesis. 3'UTR variant H lacks 123 nucleotides (miRHS fragment). This region contains a putative binding site for miRNA-204, located between nucleotides 2313-2319 of TRβ 3'UTR. Variants IVS4A and IVS4B contain alternatively spliced exons of 5'UTR, exon 3, 4 and a fragment of intron with a stop codon located downstream of the exon 4 that may result in translation of truncated protein (28 amino acids) of unknown function. The GeneBank accession numbers for each reference sequence are given next to the adjacent transcript variant.

The variant TRβ4 contains additional 137-bp insertion (exon) between exons 7th and 8th shown in diagram 3 (intron 5th according to Tagami et al., 2010) that results in synthesis of a truncated protein lacking the ligand binding domain.

This isoform may modulate T3 action as an endogenous antagonist (according to Tagami et al., 2010).

The multiple 5'UTR splice variants of the TRβ1 can differently regulate translation of the TRβ1 coding sequence. The 5'UTRs vary in length, GC-content, secondary structures, number of upstream open reading frames (uORFs) and internal ribosome entry site (IRES) predicted in the variant A (GeneBank: AY286465.1). These regulatory sequences may be organized in secondary and tertiary RNA structures that are recognized by trans-acting factors such as protein translation factors and naturally occurring small RNAs. Moreover, the most of TRβ mRNA variants contain long 3'UTR (see diagram 3) with multiple microRNA binding sites that may affect the expression of these receptors. Disturbances in the expression of the TRβ mRNA variants have been

reported in various cancers. Some of the disturbances seem to be a cancer specific. For example, the loss of transcript variants F1 (GQ456950) and IVS4B (GQ919288.1) has been observed in clear cell renal cell cancer (ccRCC). In the same tissues, over 70% reduction in TRβ1 mRNA (coding sequence) has been reported. Simultaneously, expression of 5'UTR variants A and F (AY286470.1) has been reduced in ccRCC by 75% and 62%, respectively, compared to control samples (Master et al., 2010).
Note also that several sense and antisense non-TRβ transcripts have been shown to be expressed in the THRB locus, in which may affect the expression of the thyroid hormone receptors (see diagram 2 and 3).

Transcription

Two precursor mRNAs (transcribed from two different promoters of the THRB) undergo extensive co- and post-transcriptional modification in the nucleus that includes tissue specific, alternative splicing of the pre-mRNAs.

Pseudogene

No pseudogene has been reported for the THRB gene. Nevertheless, the THRB may be regulated by pseudogenes identified within the THRB gene sequence or in the same locus (see diagram 1 and 2). The pseudogenes may produce long naturally occurring antisense transcripts (cis-NATs) forming sense-antisense pairs (see Homology). These sense-antisense pairs may activate numerous mechanisms, similar to those observed in a pseudogene-mediated regulation of a target gene via pseudogene-derived small interfering RNAs or on the level of RNA-directed DNA methylation, pre-mRNA transcription, alternative splicing as well as RNA editing, transport and localized protein translation. However, this regulation remains to be established for the THRB gene.

Protein

Note

The human THRB encodes three protein isoforms, the TRβ1 and TRβ2 that are T3-dependent receptors mediating genomic and nongenomic actions of the thyroid hormone, and TRβ4 isoform, which is a carboxyl-terminal splicing variant of TRβ1 that lacks the ligand binding domain and thus, may modulate T3 action as an endogenous antagonist in the tissue or cellular context (Tagami et al., 2011). The TRβ proteins are implicated in regulation of transcription of target genes and control key cellular processes including differentiation, proliferation, apoptosis and metabolism.

Description

There are three human TRβ isoforms TRβ1, TRβ2 and TRβ4, which are differentially expressed in various tissues. The TRβ1 and TRβ2 receptors have the typical domain structure of a nuclear receptor with an N-terminal domain (A/B), central DNA binding domain (C) consisting of a double C4-type zinc fingers, hinge region (D), C-terminal ligand binding domain (E), and AF2 domain (F). These receptors have a variable N-terminal domain (A/B) that differs between TRβ1 and TRβ1 isoforms (see diagram 4). The N-terminus is responsible for cofactor and regulatory protein binding, T3-independent transactivation, receptor dimerization and DNA recognition. Local dimerization sites have been found in domain C, E, corepressor interaction sites in domain D, E and coactivator interaction sites in domain A/B, E and F. The hinge region (D) is also found to be required for TRβ dependent suppression of ras-mediated responses.
The TRβ1 can bind to target DNA sites regardless of T3 binding status as a homodimer or heterodimer with retinoid X receptors (RXR) or as a monomer (more weakly).
TRβ can bind to DNA in the absence of ligand and therefore is thought to have the potential to mediate both T3-dependent and T3-independent regulation of target gene transcription. The protein phosphorylation has been shown to enhance its cytoplasmic-nuclear import (Maruvada et al., 2003). Phosphorylated T3 receptors can exhibit increased TRE binding as a homodimer, but not as heterodimer or monomer. Moreover, integrin-mediated non-genomic action of T4 (Davis et al., 2009) may result in phosphorylation of TRβ1 Ser142 leading to dissociation of the corepressors and transactivation of target genes (Davis et al., 2000). In case of typical positively regulated genes such as DIO1 encoding iodothyronine deiodinase type I and GH1 of human growth hormone 1, T3 binding stimulates a conformational changes in the TRβ allowing for dissociation of corepressors followed by recruitment of coactivators to form an activating complex stimulating the transcription.
There is also identified a group of negatively regulated genes that includes hypothalamic thyrotropin-releasing hormone (TRH) and pituitary thyroid stimulating hormone (TSH) encoded by TSHB and controlling the hypothalamic-pituitary thyroid (HPT) axis. In this regulation liganded nuclear receptors down-regulate target gene transcription, with the cooperative binding of various transcription factors to multiple regulatory elements on DNA (for more see genomic actions of T3 mediated by TRβ receptors).

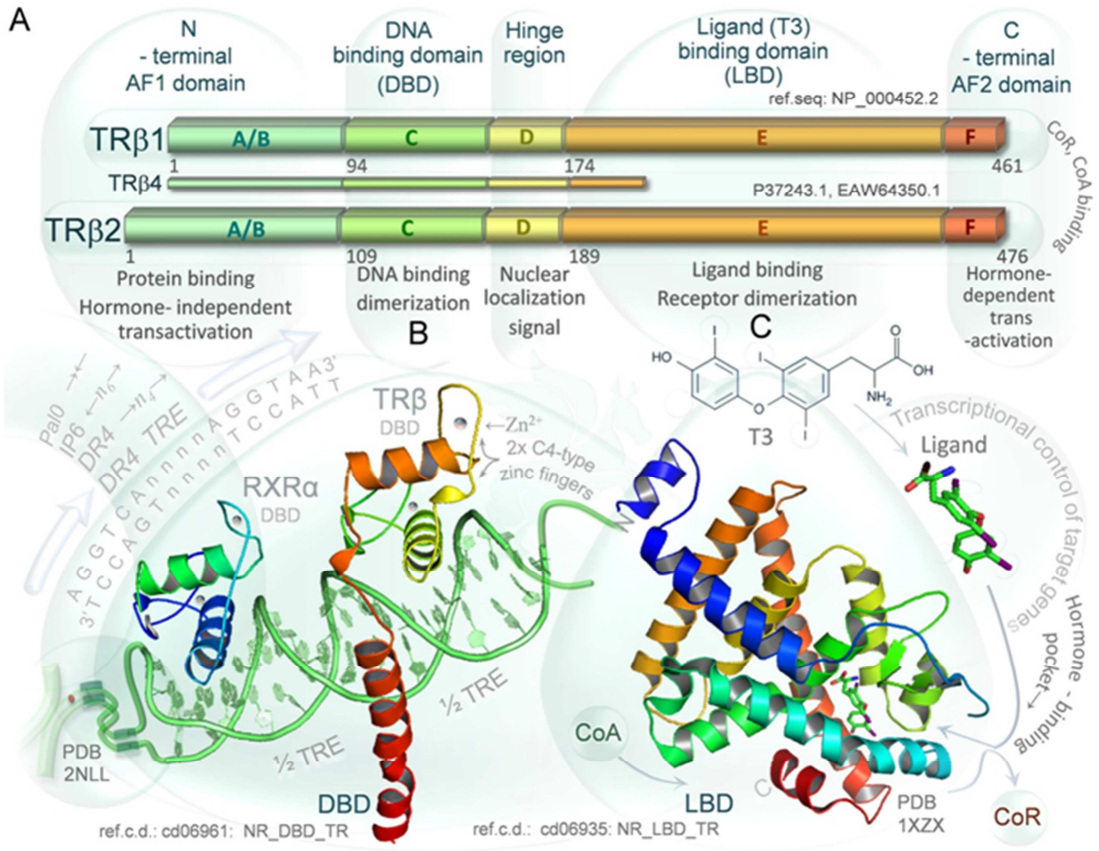

Diagram 4. Structural and functional organization of TRβ proteins. A. Three thyroid hormone receptor beta isoforms: TRβ1 TRβ2 and truncated variant TRβ4 are encoded by the human THRB gene. Functional domains of the transcription factors are divided as follows: N-terminal AF1 domain (A/B) responsible for hormone independent transactivation and regulatory proteins binding; DNA binding domain (C) containing two C4-type zinc fingers; hinge region (D) with nuclear localization signal allowing for nuclear transport (regardless of T3 binding status); ligand (T3) binding domain (E) that allows for dimerization (usually with RXRα receptor) as well; C-terminal AF2 domain responsible for T3-dependent transactivation of target genes (F). The domains E and F bind corepressors or coactivators regulating the activity of the TRβ receptors. The TRβ1 and TRβ2 are functional receptors of T3 differing only in the length of the A/B domain, however their expression is tissue specific. The human variant TRβ4 is a truncated variant of TRβ1, lacks T3 -binding ability and acts as an endogenous dominant-negative isoform. **B.** Crystallographic structure of heterodimer formed by the human thyroid hormone receptor DNA-binding domain and retinoid X receptor DNA-binding domain (rainbow colored) complexed with double strained DNA (green). Zinc atoms are depicted as blue/pink dots. The structure is presented in the context of the thyroid hormone response elements (TREs) that are specific DNA sequences recognized by the receptors. The TRE-DR4 is formed by consensus core recognition motif (AGGTCA) that is positioned as direct repeats separated by 4 nucleotides. The other TREs including palindrome (TRE-P0) or inverted palindrome (TRE-IP6) are shown as well. **C.** Crystallographic structure of the ligand binding domain of the human thyroid hormone (T3) receptor beta (rainbow colored, N-terminus in blue, C-terminus in red) complexed with triiodothyronine (T3). Binding of unliganded receptor alone to DNA usually leads to recruitment of corepressors (CoR) and inhibition of target gene basal transcription, whereas binding of T3 in hormone binding pocket is thought to cause conformational changes leading to dissociation of corepressors followed by recruitment of coactivators (CoA) and activation of the transcription. Mutations in C-terminal α-helix domain (red) that can close the hormone binding pocket and the α-helix shown here in green are frequent findings in the thyroid hormone resistance syndromes (RTHs) and cancers. The conserved domains were visualized using PyMOL 1.3 Molecular Graphics System, on the basis of crystallographic structure files (PDB: 2NLL, 1XZX) of The RCSB Protein Data Bank and The NCBI Conserved Domains Database (CDD, ref.c.d.: cd06961 NR_DBD_TR, cd06935: NR_LBD_TR).

The TRβ4 is a C-terminal splicing variant of TRβ1 which contains a stop codon in an 137-bp insertion (exon) insertion that results in synthesis of a truncated protein lacking the ligand binding domain (see diagram 4). The TRβ4 contains full A/B, C, D domain of TRβ1 and a fragment of domain E (is identical for the first 246 amino acids) but the carboxyl-terminal 215 amino acids of TRβ1 are replaced by an entirely distinct sequence of 13 residues, which results from an insertion (see RNA). Therefore, this truncated protein is unable to bind thyroid hormone, thus may modulate T3 action as an endogenous antagonist in the tissue or cellular context. The TRβ4 cannot mediate T3-dependent gene regulation but may inhibit the negative regulation of TSH mediated by TRβ1 or TRβ2, that was shown in TSA-201 cells, a clone of human embryonic kidney 293 cells (according to Tagami et al., 2010 and Tagami et al., 2011). These findings are consistent with current model for T3-dependent negative regulation of TSHB gene (see genomic actions below).

The levels of TRβ proteins depend on the protein stability and transcription/translation efficiency that is tissue specific and differentially regulated in various mRNA variants. The frequently reported lack of correlation between the mRNA and protein suggests that apart from transcriptional control the expression of TRβ receptors is accurately controlled at the level of translation. In fact, multiple 5'UTR variants of TRβ1 have been shown in vitro to differentially regulate the protein translation. The major renal TRβ1 transcript contains a 5'UTR relevant to variant A (GeneBank: AY286465.1). Analysis of the effects of 5'UTR variants on protein expression in JEG-3 choriocarcinoma cells (Francton et al., 2004) and Caki-2 renal cancer cells (Master et al., 2010) indicated that the weakly folded variant A permitted also the highest level of protein expression. In contrast, the strongly folded 5'UTR variants: F (AY286470.1) or F1 (GQ456950) was identified to be transcribed and translated at the lowest levels. Although, structured 5'UTR such as variant F treated in vitro with a trans-acting factor (an antisense oligonucleotide) significantly up-regulated the translation efficiency of a downstream sequence up to the level of the variant A. This may estimate the potential of the 5'UTR-mediated translational control during expression of TRβ receptors. The translation of the TRβ can be also modulated by multiple regulatory ORFs that exist upstream of the primary ORF. On the other side, the protein synthesis may be controlled by long TRβ 3'UTR through binding of various microRNAs including miR-21 and miR-146a. The microRNA can trigger the RNA interference (RNAi) phenomenon that may lead to translational repression or even degradation of TRβ mRNAs (Jazdzewski et al., 2011). Since UTR-mediated translation initiation is a key rate-limiting phase affecting efficiency of the protein synthesis, the translational control mediated by the multiple UTR variants is emerging to be a major regulator of the final protein levels in cells. The TRβ receptors have been also reported to be affected by aberrant promoter methylation, alternative splicing and impaired cell signaling.

Expression

The thyroid hormone receptor isoforms are products of both the THRB and THRA genes. During development, TRβ and TRα isoforms are differentially expressed in a temporospatial and tissue-specific patterns and in adult tissues are present in distinct ratios (Williams, 2000; Francton et al., 2004).

TRβ1 is widely expressed in all human tissues, but is prominent in brain, thyroid, kidney and liver. TRβ1 controls liver and kidney metabolism and mediates cholesterol lowering effects of thyroid hormones. Both the TRβ1 and TRβ2 are essential for regulation of thyroid hormone levels through the hypothalamic-pituitary-thyroid (HPT) axis, a negative feedback loop, which includes auto-control of hypothalamic Thyrotropin-Releasing Hormone (TRH) and pituitary Thyroid-Stimulating Hormone (TSH) by the thyroid hormones (TH). In this regulation, the TRβ receptors mediate TRH- and TSH- lowering effects of TH.

TRβ2 is restricted to the hypothalamus, anterior pituitary, developing brain, cochlea (inner ear) and retina, wherein TRβ2 alone is crucial for development of mid-wavelength (MW) cones photoreceptors, which play a significant role in circadian clock light entrainment and in phase shifting of the circadian oscillator (Dkhissi-Benyahya et al., 2007). This isoform is therefore important for visual and auditory function. Down regulation of hypothalamic TRH is TRβ2 specific. TRβ2 mRNA has been also identified in situ in human chondrocytes and osteoclasts (Abu et al., 2000).

TRβ4 isoform is expressed in various human tissues but is highly abundant in testis and skeletal muscle. This isoform lacks T3 -binding domain and may act as a dominant-negative protein. It has been identified in a TSH-secreting pituitary adenoma (TSHoma) as well (Tagami et al., 2011).

In contrast, TRα mediates T3 actions during development of heart, bone, intestinal and is responsible for body temperature and basal heart rate in adults. TRα1 and TRα2 isoforms are highly expressed in the brain, with lower abundance in the kidneys, skeletal muscle, lungs, heart, and testes. The TRα1, TRβ1, and TRβ2 isoforms can bind DNA and T3 acting as functional thyroid hormone receptors, whereas TRα2 and TRα3 do not bind T3 due to the presence of the longer AF2 (F) domain, and act as antagonists. There are some truncated isomers of TRα with specific mitochondrial functions (p28, p43) or may act as dominant-negative receptors (TRΔα1, TRΔα2). There are also known two additional TRβ receptors expressing only in rats: functional T3 receptor - TRβ3 and its dominant negative isoform -TRΔβ3 (Williams et al., 2000).

Both, TRβ and TRα isoforms are involved in circadian cycle that was demonstrated in an in vivo mouse model, in different metabolic tissues including white adipose tissue (WAT), brown adipose tissue (BAT), liver, and skeletal muscle (Yang et al., 2007). While TH levels are generally constant, the TRβ and TRα along with their key target genes dramatically cycle in a coordinated manner that is in agreement with known cyclic behaviour of lipid and glucose metabolism. TRβ has been shown to cycle in WAT, whereas TRα in WAT, BAT and liver. Analysis of TRβ mRNA expression revealed a unique rhythmic pattern in which their transcripts spike at ZT4 (Zeitgeber

time, 4h after lights were turned on) followed by a precipitous decline in the next 4h, and remain at low levels through the rest of the day. Interestingly, mRNA levels of heme-liganded nuclear receptor Rev-erbβ encoded by NR1D2 gene, which may interact with THRB expression (see diagram 1 and 2), dramatically cycle in the all examined metabolic tissues.

Although precisely how the circadian clock acts to control metabolic rhythms is not clear, it is known that the ratio of TRα1/TRα2 isoforms is closely determined by co-expression of Rev-erbα (NR1D1) - a key component of the circadian core oscillator complex.

The Rev-erbα represses the expression of BMAL changing the expression of other genes of the cycle including CLOCK, PER1, PER2, PER3 and cryptochrome genes CRY1 and CRY2 responsible for blue light photoreceptor activity (see specific functions of TRβ2).

The cell -autonomous feedback loop allows for cyclic expression of these oscillator genes at various phases with the same period length of approximately 24r (Zhang et al., 2009).

All this findings suggest a role for TRβ, TRα and heme receptors (Rev-erbβ, α) in coupling the peripheral circadian rhythm to divergent metabolic outputs.

Since the expression of TRβ is prominent in brain and metabolic tissues, it is expected that these receptors may serve peripheral clock input pathways that can integrate signals from the light-sensing central clock in the suprachiasmatic nuclei (SCN) and other cues including xenobiotic metabolism pathways.

Interplay between the circadian clock and TH receptors may be a part of a large -scale signaling network that links biological timing to metabolic physiology (Yang et al., 2006).

Localisation

Subcellular localization and changes in expression of TRβ receptors can vary depending on the cell cycle phase, cell density, cellular stress, signaling events, tissue types, metabolic rate or even circadian cycle (Maruvada et al., 2003).

Typically, TRβ1 and TRβ2 isoforms are predominantly localized to the nucleus and retained in the nucleus regardless of the ligand binding status (in the absence and presence of T3). However, T3 can induce a nuclear reorganization of TRβ receptors. The nuclear localization is essential for TRβ-mediated genomic actions of T3. In standard conditions, a minor fraction of TRβ proteins resides in the cytoplasm, wherein the receptors are thought to be mediators of nongenomic actions of thyroid hormone (TH). The cytoplasmic-nuclear shuttling is facilitated by the presence of a nuclear localization signal (NLS) in the TRβ hinge region (D). The TRβ nuclear import is ATP-dependent and can be regulated by nongenomic actions of TH through 1) T4-dependent activation of plasma membrane integrin αvβ3 (Davis et al., 2009) followed by activation of downstream pathways leading to phosphorylation of ERK1/ERK2 and TRβ1 proteins and/or 2) via T3-dependent formation of cytoplasmic TRβ1 complexes with p85 subunit of PI3K that may activate downstream pathways. The TRβ complexes with p85, ERK1/2 or nuclear receptor coactivators may facilitate nuclear import as well. TRβ rapidly shuttles between the nuclear and the cytoplasmic compartments. Energy-dependent blockade (ATP depletion) enhances TRβ nuclear export to cytoplasm. Nevertheless, coexpression of nuclear corepressors (NCoRs) and/or retinoid X receptors (RXRs) can markedly decrease the shuttling by maintaining unliganded TRβ within the nucleus. A TRβ mutant defective in DNA binding has a slightly altered nuclear-cytoplasmic distribution when compared with wild-type TRβ. TRβ mutants that abrogate its interaction with the NCoRs accumulates within the cytoplasm due to an increase in the rate of nuclear export when compared with nuclear import. Nuclear-cytoplasmic shuttling has been proposed as a mechanism for modulating TRβ-mediated regulation of transcription (Maruvada et al., 2003).

Subcellular localization of TRβ4 is unknown; however the lack of T3 binding domain might suggest an altered nuclear/cytoplasmic distribution.

Function

TRβ proteins are high affinity receptors for thyroid hormone (TH) functioning as ligand-dependent (T3) and sequence-specific DNA binding (TRE) transcription factors (see genomic actions below) that regulate expression of target genes affecting cell growth, development, proliferation, differentiation, apoptosis, organ morphogenesis, heart rate, body fat distribution, bone density. These receptors are required for the development of the auditory system and of the cone photoreceptors that mediate colour visual function. TRβ1 isoform is expressed in most tissues, whereas TRβ2 is restricted to the hypothalamus, pituitary, cochlea, and retina that may indicate the functional specificity of the isoforms.

The TRβ1 control the major responses of the liver and kidney to T3 and play a critical role in mediating changes in metabolism and thermogenesis. The T3 receptors are able to increase metabolic rate by accelerating fuel oxidation in most of tissues wherein they may activate lipolysis, glucose metabolism and protein synthesis.

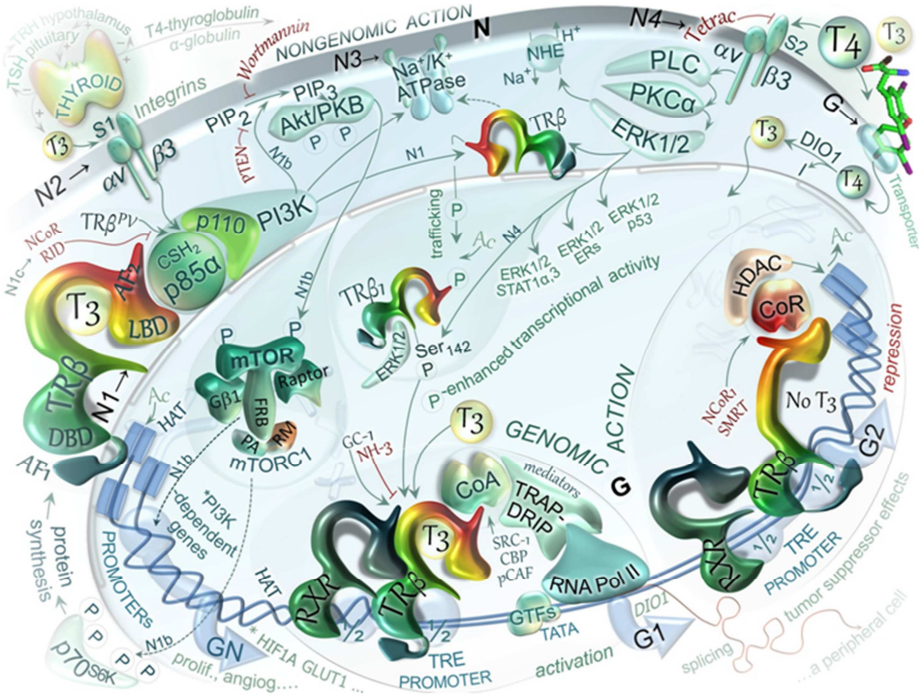

Diagram 5. Schematic representation of selected TRβ-mediated, genomic and non-genomic actions of TH. The thyroid gland, in response to TSH, produces thyroxine (T4) and 3,5,3'-triiodo-L-thyronine (T3), however greater amounts of T4 are produced than T3. The thyroid hormone (TH) in the circulation are bound to protein -transporters that deliver TH to peripheral tissues wherein TH triggers TRβ-mediated effects including negative regulation of the hypothalamic-pituitary thyroid (HPT) axis. For genomic actions, T4 needs to be converted to T3 by DIO1 or DIO2 present in peripheral tissues such as liver, brain or kidney. **G.** Classical model of TH actions in the nucleus. The model is based on the action of triiodothryonine (T3, ligand) on positively regulated genes. The regulation requires: thyroid hormone response elements (TREs) on specific genes, complexes of nuclear TH receptors (TRs) and T3, coactivator (CoA) or corepressor (CoR) nucleoproteins, and histone acetyl transferase (HAT) or deacetylase (HDAC). **G1.** Ligand-bound state. TRβ can bind to DNA as heterodimer with RXR and regardless of T3 binding status. However, T3 binding results in release of corepressor complex, recruitment of a coactivator complex (e.g. SRC-1, CBP, p300, pCAF, TRAP-DRIP, mediator/integrator components) and HAT. The HAT activity allows for reducing chromatin compaction and permitting general transcriptional factors (GTFs) to interact with DNA and activate transcription of a target gene. **G2.** Ligand-free state. In absence of ligand, the unliganded TH receptors interact with a copressor complex that may include NCoR, SMRT and histone deacetylase 1 (HDAC1). The recruitment of this complex may result in reduced histone acetylation (shown as Ac), which in turn compacts chromatin structure and represses the gene transcription. The transactivation domain of the T3-free receptor, as a heterodimer with RXR, assumes a conformation that promotes interaction with a group of transcriptional corepressor molecules. **N.** Nongenomic actions of TH mediated by TRβ1. These actions are fast (within 10-40 min), frequently reported to result in pro-proliferative, pro-angiogenic, anti-apoptotic effects. These actions may be simplified into two main signaling cascades: N4) extracellular-T4/ αvβ3-integrin/ PLC/ PKCα/ ERK1/2/ TRβ1-Ser_{142} phosphorylation that among others can result in specific gene transactivation or transrepression (N4); N1) cytoplasmic-T3/ TRβ1/ CSH_2-p85α-p110(PI3K)/ Akt/ mTOR phosphorylation leading to transcription of PI3K-specific genes such as HIF1A and GLUT1 (SLC2A1) (N1, N1b, N1c). Specific inhibitors of these pathways are shown in red font. **N1.** Nongenomic effects of T3 may be initiated in cytoplasm by TRβ-dependent activation of PI3K that leads to sequential activation of Akt/PKB/mTOR-$p70^{S6K}$ as well as the other mTOR targets including upregulation PI3K-dependent genes. A fraction of TRβ1 present in the cytosol forms a complex with p85α subunit (regulatory subunit of PI3K) in a ligand independent manner that activate PI3K. The kinase generates phosphatidyl inositol-3,4,5-triphosphate (PIP_3) from PtdIns(4,5)P2 (PIP_2) activating downstream pathways via Akt/PKB (N1b) or through phosphorylation of the TRβ1 followed by its nuclear import. Wild-type TRβ1 competes with corepressor NCoR or mutant $TR\beta^{PV}$ for binding to the CSH_2 domain of p85α (N1c). This PI3K activity is blocked by specific inhibitors such as Wortmannin or LY294002. **N2.** Signal transduction via plasma membrane receptor αvβ3 by T3 binding to the extracellular part of the receptor. The binding domain includes a receptor site (S1) exclusively for T3 that activates phosphatidylinositol 3-kinase (PI3K) and leads to shuttling of cytoplasmic TRβ to the nucleus followed by transcription of specific target genes such as HIF1A. **N3.** Cytosolic TRβ1 and PI3K are involved in T3-stimulated activation of Na^+/K^+-ATPase and other features of the sodium pump (gene expression, plasma membrane insertion). Besides, TH is known through αvβ3 to modulate the activity of several other ion transport systems including Na^+/H^+ exchanger NHE (SLC9C1). **N4.** T4-induced activation of ERK1/2 through plasma membrane receptor αvβ3 (site S2). This action is relevant to: intracellular trafficking of proteins, including TRβ1, serine phosphorylation (P) and acetylation (Ac) of this nuclear receptor, assembly within the nucleus of complexes of coactivators or corepressors and transcription of specific genes, including that for TRβ1. The action includes T4 binding to the extracellular part of the receptor, activation of PLC, PKC, ERK1/2 (MAPK) pathway, phosphorylation on TRβ (Ser_{142}), derepression and enhancement of transcription. Among the consequences of ERK1/2 activation are specific serine phosphorylation of the cytoplasmic/nuclear TRβ1 (Ser_{142}) and estrogene receptor α ERα, phosphorylation of signal transducers and activators of transcription STAT1α, STAT3 as well as p53, wich were found to be co-immunoprecipitated with the activated ERK1/2. Cytoplasmic fraction of TRβ are shuttled to the nucleus, wherein the proteins are transcriptionally active and can modulate the actions of certain cytokines and growth factors including those involved in tumor cell proliferation and on angiogenesis. TETRAC is an analog of T4 that can inhibit this nongenomic action of T4, however, showing thyromimetic properties it can also affect gene expression in the cells, regulating transcription of target genes such as THBS1, CASP2 and CBY1. For details see text.

TH receptors are responsible for T3-dependent homeostasis and maintenance of a steady body temperature that is realized via an auto-regulatory negative feedback loop controlling the hypothalamic-pituitary thyroid (HPT) axis. Its regulation is mainly determined by local T3 concentration in the anterior pituitary as well as paraventricular nucleus (PVN) of the hypothalamus wherein low levels of T3 abolish TRβ-mediated transcriptional repression of pituitary thyroid-stimulating hormone (TSH) promoter and hypothalamic thyrotropin-releasing hormone (TRH) promoter. However, the local tissue T3-levels are dependent on the availibility of circulating thyroid prohormone - thyroxine (T4), its intracellular transport and cytoplasmic T3 production, catalyzed by iodothyronine deiodinases. Since TRs can form heteroduplexes with retinoid X receptors, TH is found to modulate the skin response to retinoids. Genomic effects of TRβ1 have been recognized as tumor suppressive and disturbances of the TRβ1 expression have been found in different cancers (Martínez-Iglesias et al., 2009b; Kim at al., 2013).

Description

TRβ is involved in many processes that compose thyroid hormone (TH) actions and gene expression. This T3-dependent receptor act as a transcription factor regulating expression of genes involved in the cell cycle progression, differentiation, apoptosis and cellular metabolic rate. TH exerts a pleiotropic effect on development and homeostasis. This effect results from genomic and nongenomic TH actions mediated by both, the TRβ and TRα receptors (TRs) that regulate hundreds of genes responding to T3-liganded or unliganded receptors. A large number of genes have been identified to respond to T3 stimulation (~10% of all expressed genes) and the divergence between T3-treated and untreated cells can grow rapidly over time. The initial studies of TRs actions revealed near complete overlaps in their effects (TRα and TRβ can regulate similar gene sets). However, gene-specific differential TRα and TRβ actions are reported as well. These differences appear to result from the 1) differences in tissue-specific expression patterns, 2) diurnal rhythm (see expression and specific functions), 3) various time-courses of actions with different kinetics 4) target gene-specific variations in pattern of response to T3 concentration. For instance, TRβ has been shown to exhibit gene-specific requirements for higher T3 levels (compared to TRα) for regulation of HR, MYH6, ALPI and FURIN genes, whereas HIF1A is identified to have lower T3 requirements during TRβ-mediated regulation. ANGPTL4 encoding a PPARG angiopoietin related protein is a verified in parental HepG2 cells direct TRβ target. Prolonged T3 treatment selectively augments TRβ action in the context of the TRβ-dependent genes. Moreover, several T4- and T3- analogues (see ligands) have been also reported to induce TRβ-specific response.

Genomic and nongenomic actions

Genomic actions of TRβ are initiated with nuclear translocation of the newly synthesized receptors (Maruvada et al., 2003) that is facilitated by nuclear localization signal (NLS) found in the hinge region (D) of the receptors (see diagram 2 and 3). The TRβ proteins are classified as type II nuclear receptors, which are retained in the nucleus regardless of the ligand binding status (free or occupied by T3) and bind to TREs (thyroid hormone response elements) as heterodimers with retinoid X receptors (RXR), rarely as homodimers or monomers. The TREs are specific DNA sequences of the consensus core recognition motif AGGTCA, AGGACA or (A/G)GGT(C/A/G)A hexamers (half-site →), in which two or more motifs are positioned as direct repeats separated by 4, 0 or 6-nucleotide: (TRE-DR4, $\rightarrow n_4 \rightarrow$), palindromes (TRE-P0, →←) or inverted palindromes (TRE-IP6, $\rightarrow n_6 \leftarrow$). The analysis of the response elements formed by direct repeats of the half-sites demonstrated that a spacer of 4-nucleotides can provide maximal transactivation by TRβ in TRβ-RXR heterodimers but the transactivation efficiency may depends on sequence context of the TRE, tissue specific trans-acting factors and ligand concentration. It has been also shown that for an efficient genomic action of the heterodimers, the presence of TRβ and RXR ligands: triiodothyronine (T3) and 9-cis retinoic acid (9-cis RA) may be necessary. However synergistic effects of the RXR ligand and T3 on the heterodimers (RXR/TR)-mediated transcription have been reported for specific promoters, the other studies suggest that RXR ligands may inhibit T3-dependent transactivation, possibly by promoting the formation of RXR homodimers. The biological effects of TRE binding by the unoccupied versus the T3-occupied receptor are quite different. In many cases, binding of the unliganded or antagonist-liganded receptor alone to DNA may lead to repression of transcription, whereas binding of the agonist (T3)- liganded receptor complex activates transcription. However, the receptor activity is mainly regulated by ligand-dependent interactions with corepressor (CoR) and coactivator (CoA) proteins. In the absence of ligand (T3), the TRβ receptors are often complexed with corepressor proteins (CoRs, NCoR, SMRT) and histone deacetylases (HDACs) allowing the histones to wrap the DNA more tightly. T3 binding to these nuclear receptors causes conformational changes leading to dissociation of corepressors and recruitment of coactivator proteins (CoAs, SRC3, CBP, p300, pCAF), as well as mediators (TRAP-DRIP multi-subunit complex) that are thought to target the entire complex to a liganded receptor through a single subunit, TRAP220. The thyroid

hormone (TH) receptors, similarly as vitamin D3 receptors (VDRs), and peroxisome proliferator-activated receptors (PPAR), exhibit a strong activation function 2 (AF2)-dependent preference for receptor binding domain 2 (RBD-2) of the TRAP220 protein. It has been also demonstrated that RXR receptor (in TRβ/RXR heterodimer) displays a weak yet specific AF2-dependent preference for another TRAP220 RBD-1 domain. Addition of ligand for the RXR receptor (9-cis RA), in addition to T3 for TRβ partner, might further strengthen the RXR-RBD-1 interaction and presumably stabilize the overall association of TRAP220 with the heterodimer. After formation of the coactivator multi-subunit complex, general transcription factors (TAFs, TFIIA, TBP) and RNA polymerase II are recruited to the complex that initiates transcription DNA into pre-RNA. The pre-mRNA undergoes further modifications including alternative splicing, mRNA editiing and translation into protein that may result in a change in cell function. The nuclear receptors including TRβ proteins can regulate gene expression by binding directly or indirectly (via other proteins) to specific sequences in the promoters of target genes (Puzianowska-Kuznicka, 2013). Apart from the current model of HRE/TRE - dependent transcriptional control, it has been proposed that one transcription factor may repress the activity of a second transcription factor through a protein-protein interaction, without the requirement of two different DNA binding sites. These proteins, notably AP1 and NF-kB, can act by interfering with transcriptional complex formation in a DNA-independent manner and may lead to transrepression of target gene transcription, what has been also shown in TRβ-mediated negative regulation of the hypothalamic-pituitary thyroid (HPT) axis. TH responsive genes can be both positively and negatively regulated by T3-liganded TRβ receptors. Iodothyronine deiodinase type I (DIO1) or GH1 of human growth hormone 1 are up-regulated in the presence of T3 (see positive regulation model - diagram 5). In contrast, pituitary TSH encoded by TSHB gene is down-regulated by T3 and rises during T3 deprivation. In this regulation, liganded nuclear receptors down-regulate target gene transcription, with the cooperative binding of various transcription factors to multiple regulatory elements on DNA. It has been proposed that the negative regulation of the TSHB gene may require at least two response elements on promoter DNA (nTRE-"negative" TRE and GATA-RE) as well as dimerization of liganded TRβ with a GATA transcription factor (e.g. GATA2), that can repress the activity of the GATA. In fact, the nTRE in the promoter of TSHB gene contains a single half site-like sequence (GGGTCA). The GATA2 alone can activate TSHB promoter, and this activation was repressed by Zn-finger region of liganded TRβ. Upon T3 binding, the T3/TRβ complex is thought to be released from nTRE, favoring its translocation and interaction with GATA-2 zinc finger region (GATA2-Zf) on GATA-RE located next to the nTRE. The T3/TRβ/GATA2-Zf/DNA complex formation is the principal complex responsible for the TSHB gene down regulation. This interaction occurs via the highly conserved zinc-finger in DNA binding domains that may resemble the trans-repression-like mechanism in which direct binding of the receptor to another transcription factor occurs in a DNA-independent manner. In the absence of T3 TRβ prefers nTRE element, allowing for the gene transcription. This negative regulation shows that T3 weakens TRβ binding to the regulatory element (nTRE) in the TSHB promoter. The displacement of the liganded TRβ from nTRE to another site (GATA-RE/GATA2-Zf) seems to be critical for the mechanism used by the cell for silencing the TSHB gene transcription (Figueira et al., 2010). Down-regulation of hypothalamic thyrotropin-releasing hormone TRH gene by T3/TRβ complexes involves acetylation and methylation of specific residues of histone tails in the TRH promoter region and relies on changing amount of the TRβ receptors on TRH nTRE after T3 binding (Umezawa et al., 2009). Prolonged administration of T3 causes demethylation of specific histones and subsequently the release of TRβ receptors from the gene to suppress it. Both negative regulations may require T3/TRβ to be removed from its nTRE to down-regulate the genes, although the mechanism used by a ligand-bound TRβ leading to repression of transcription is still a subject of contention. The classical mechanism of TRβ activity suggests that receptors typically complexed with RXR bind more strongly to DR4 in a clearly cooperative binding between the transcription factors and DNA (see diagram 5). Nevertheless, coexpression of RXR and TRβ2 has been also shown to slightly reduce both the transactivation and transrepression by liganded TRβ2 that may act more strongly as homodimer or monomer, depending on the architecture of the TRE. Thus, the mechanism responsible for TRβ-mediated genomic action of T3 is still unclear. Several recent studies indicate that at the genomic level TRβ may act as a tumor suppressor. For instance, the gain-of-function approach by stably expressing TRβ in a human breast cancer cell line MCF-7 (MCF-7-TRβ), which normally lacks the TR expression, has been reported to inhibit the growth of the MCF-7 cell tumors in xenograft models (Park et al., 2013). These estrogen (E2) dependent cells show elevated JAK2-STAT3-cyclin D signal that is repressed by the TRβ expression at the level of transcription. Interestingly, other studies indicate that TH has anti-apoptotic and pro-

proliferative effects in ERα-positive human breast cancer cells (Perri et al., 2013). However, these opposite effects are recognized as a result of the αvβ3 integrin -dependent nongenomic actions of TH (see below).

Nongenomically initiated, TRβ -mediated thyroid hormone (TH) actions occur at the plasma membrane or in cytoplasm and may also culminate in complex, nucleus-mediated cellular events leading to the transcription of specific genes such as HIF1A, MCL1 or GLUT1 (Moeller et al., 2006; Davis et al., 2009). The nongenomic actions of TH can interfere with genomic effects of T3, hence, a clear distinction between nongenomic and genomic effects may no longer be practical. However, initiation events leading to these effects are quite different and may have different kinetics or timing. For instance, enhancement of the antiviral activity of interferon-γ by TH is achieved nongenomically and genomically by T4-dependent activation of the mitogen activated protein kinase /extracellular signal-regulated kinase 1/2 (MAPK/ ERK1/2) which can phosphorylate serine (Ser142) located on DNA binding domain of TRβ1 - a nuclear receptor of T3. This phosphorylation leads to dissociation of TRβ1 from corepressor proteins, the NCoR (nuclear receptor co-repressor) and SMRT (silencing mediator of retinoid and TH receptors) allowing for transactivation of T3-specific target genes. TETRAC and TRIAC (see ligands), which can inhibit nongenomic actions of TH, can also block the T4 potentiation of the antiviral and immunomodulatory actions of the interferon-γ, even though these analogues have no direct effect on the interferon-γ action. There is increasing evidence that nongenomic and genomic actions of TH may overlap with genomic and nongenomic effects of estrogens and testosterone in tumor cells. The thyroid hormone, estrogene and dihydrotestosterone have similar ERK1/2-dependent proliferative actions on the estrogen receptor α (ERα)-positive human breast cancer cells. TH and steroids also have interacting nongenomic and genomic actions in heart and brain cells. The binding affinity of T4 to its plasma-membrane receptor modulates intracellular protein trafficking of the ERα and TRβ1 receptors from the cytoplasm to nucleus. T4 -transduced activation of the ERK1/2 promotes its nuclear uptake and ERK1/2-dependent phosphorylation of the TRβ1, ERα and signal transducers and activators of transcription 3 and 1α (STAT1α, STAT3). In the nucleus of T4-treated cells, the TRβ1, ERα, STAT1α and STAT3 transcription factors were found to be co-immunoprecipitated with the activated ERK1/2. The complexing of TRβ1 and ERK1/2 was relatively rapid and detected with 1.4- and 7.8-fold increases in TRβ1 in 30 and 40 min of T4 treatment, respectively. This effect of T4 was observed as early as in 10 min and persisted for up to 90 min. The increase in nuclear TRβ1 was assumed to be originated from the pool of cytosolic fraction of the TRβ1. Interestingly, the nongenomic action of T4 has been shown to block the p53-mediated proapoptotic activity of resveratrol, a polyphenolic compound found in grapes and wine (SIRT1 activator), by disrupting an ERK1/2-nucleoprotein complex. Although, inhibition of T4 binding at the cell surface receptor can restore the apoptotic action of the resveratrol (Lin et al., 2002).

The action of T4 on cellular signal transduction is initiated at a cell-surface by activation of an integrin receptor - αvβ3 (identified in 2005). The TH plasma-membrane receptor was previously reported as a putative G protein-coupled receptor (GPCR) that preferentially binds T4 and 3,5,3',5'-tetraiodothyroacetic acid (TETRAC), an antagonist of the nongenomic actions of TH (Lin et al., 1998; Lin et al.,1999). Extracellular binding of T4 to its transmembrane receptor was demonstrated to activate the signal transduction cascade which included G-proteins, PLC, PKC, Ras, Raf-1, MAPK kinase (MEK), the MAPK (ERK1/2) and downstream pathways (Davis et al., 2000).

The current model of nongenomic actions of TH is based on transduction of the hormone signal through the membrane by the integrin αvβ3, which can preferentially bind T3 to S1 and T4 to S2 sites of the integrin (Davis et al., 2009). The signal transduction may result in pro-proliferative and pro-angiogenic effects achieved in a ligand -dependent manner via the αvβ3/ERK1/2 cascade or by the hormone activated phosphatidylinositol 3-kinase (PI3K), respectively. The αvβ3 integrin is concentrated largely in plasma membranes of endothelial cells, vascular smooth muscle cells, various cancer cells, osteoclasts and platelets. Hormone-binding domain of the integrin receptors includes two recognition sites that are capable of binding T3 (S1) or T4 and T3 (S2). S2 site can bind both T4 and T3, though the affinity for T3 binding to the S2 site is lower than that for T4. Binding T4 to integrin αvβ3 (without cell entry) may mediate nongenomic actions of TH by activation of extracellular-regulated kinases 1/2 (ERK1/2), which transduces the hormone signal into complex cellular and nuclear events including angiogenesis and tumor cell proliferation. This T4-induced pathway can stimulate shuttling of TRβ1 receptor from the cytoplasm to the nucleus and increase the TRβ-mediated transactivation of specific genes. Moreover, phosphorylation of serine 142 located on DNA binding domain (DBD) of the cytoplasmic/nuclear TRβ1 is thought to facilitate transcription derepression by dissociation of corepressors (NCoR and SMRT) and recruiting coactivators and mediators (SRC-1, CBP, p300, pCAF, TRAP-DRIP). The nongenomic action via

integrin receptors may be specifically inhibited by TETRAC, a deaminated T4 analogue that can displace T4 and T3 from both sites (S1, S2) but does not mimic the agonist functions of the hormones through the integrin receptors. This T4-analogue blocks thyroid hormone effects on angiogenesis and cancer cell proliferation and might have some benefits in cancer treatment. In the chick chorioallantoic membrane (CAM) model, the cells treated with TETRAC - an agonist for TRs (in genomic action), significantly enhance the expression of thrombospondin, an antiangiogenic gene. This effect has been shown to complement the anti-VEGF and anti-bFGF actions of TETRAC. In contrast, protein ligands present in extracellular matrix (ECM) and containing an Arg-Gly-Asp (RGD) motif fully inhibits T3 actions initiated at S1 site (PI3K pathway) and does not affect T3 actions initiated at S2 site and ERK1/2-dependent cell proliferation. The S1 site can bind T3 exclusively and rapidly transduces the hormone signal via PI3K leading to cytoplasm-nucleus shuttling of TRα1 and expression of HIF1A gene encoding the hypoxia-inducible factor-1α, alpha subunit (HIF-1α) (Davis et al., 2009).

The similar activation of the HIF-1α transcription may be also initiated intracellularly by interaction of T3 with cytoplasmic fraction of TRβ1 (see diagram 5).The liganded TRβ1 mediates action of T3 on expression of specific genes, including pro-angiogenic genes through binding to the regulatory subunit p85α of PI3K (see diagram 5) followed by activation of downstream cascade of protein kinase Akt/PKB, mammalian target of rapamycin (mTOR) and its substrate - p70S6K that is involved in cellular protein synthesis (Kenessey and Ojamaa, 2006; Moeller et al., 2006). Activation of mTOR is rapid, with detectable phosphorylation within the minutes after T3 treatment. This pathway may lead to up-regulation of specific genes including: HIF1A and HIF-1α target genes; SLC2A1 (GLUT1) - solute carrier family 2 (facilitated glucose transporter), member 1; PFKP - phosphofructokinase, platelet; SLC16A4 (MCT4) - solute carrier familymonocarboxylate transporter 4; RCAN2 regulator of calcineurin 2 (ZAKI-4). Rapamycin - an inhibitor of mTOR, abrogates the thyroid hormone-dependent induction of the ZAKI-4α, suggesting the role of sequential activation of the kinases in the PI3K pathway. Treatment of endothelial cells with T3 leads to activation of the Akt/PKB and endothelial nitric oxide synthase (eNOS) that is abolished by PI3-kinase inhibitors, but not by the inhibitors of transcription. The PI3K-mediated effects are important to angiogenesis and other recently appreciated cell functions but not to tumor cell division. The T3 liganded TRβ1 in cytoplasm may also nongenomically initiate a process that result in transcription of the ATP1A1 (ATPase, Na+/K+-ATPase) and insertion of the gene product into the plasma membrane (Davis et al., 2009).

Regardless of TRβ1, also TRα1 can interact with the p85α subunit of PI3K in a T3 -dependent manner, leading to phosphorylation of Akt and activation of downstream signaling pathways. The other nongenomic actions of the TH have been also shown to modulate cellular ion fluxes, sodium current (I(Na)), inward rectifying potassium current (IKir), sodium pump (Na, K-ATPase) and ERK1/2-regulated Na/H exchanger (NHE) encoded by SLC9C1 - solute carrier family 9 subfamily C (Na+-transporting carboxylic acid decarboxylase), member 1. Both, the genomic and nongenomic actions of TH have been shown to proceed the transcription and activity of the sarcoplasmic reticulum Ca(2+)-ATPase (calcium pump). T4 and rT3 but not T3 may act through a truncated form of TRα1 (TRΔα1) located in cytoplasm, wherein the liganded TRΔα1 may take part in conversion of soluble actin to fibrous (F) actin that is important to cell motility. Certain of these actions appear to interfere with genomic function of the TRβ receptors. The nongenomic effects of TRβ ligands occur rapidly and are unaffected by inhibitors of transcription or translation processes.

THs exert important physiological actions by both genomic and nongenomic effects in mitochondria. T3 and T2 - a thyroid hormone metabolite, regulate mitochondrial genome transcription and nongenomically initiated mitochondrial processes such as cellular respiration and thermogenesis. T2 metabolite binds and activates the mitochondrial cytochrome-c-oxidase Va, whereas T3 binds to two truncated TRα1 isoform (p28 and p43). Whereas the role of p28 remains unknown, p43 protein is a T3-dependent transcription factor of the mitochondrial genome, acting via dimeric complexes involving two other truncated forms of nuclear receptors: mtRXR and mtPPAR. All these mitochondrial actions as well as expression of other nuclear TH receptors (e.g. TRα1, TRα2) may have an impact on thyroid hormone availability and the TRβ function in the cell.

Concluding this section, the nongenomic actions of TH that are mediated by TRβ is fast (10-40min), frequently reported to result in pro-proliferative, pro-angiogenic, anti-apoptotic effects and may be simplified into two main signaling cascades: 1) extracellular-T4/ αvβ3-integrin/ PLC/ PKCα/ ERK1/2/ TRβ1-Ser_{142} phosphorylation that among others can result in specific gene transactivation or transrepression (see diagram 5N4); 2) cytoplasmic-T3/ TRβ1/ CSH_2-p85α-p110(PI3K)/ Akt/ mTOR phosphorylation leading to transcription of PI3K-specific genes such as HIF1A and GLUT1 (diagram 5N1, 5N1b, 5N1c). Specific inhibitors of these pathways are shown on diagram 5.

Interaction

TRβ has been shown to interact with: BRD8, CCND1, NCOA1, NCOA6, NCOR2, NR2F6, PPARGC1A and RXRA.

Crosstalk signaling with proteins of nuclear hormone receptor superfamily.

Apart from TRβ proteins, the other T3 regulated nuclear receptors may affect the thyroid hormone levels and the TRβ function in cells. T3 and T4 exert a pleiotropic effect on cellular homeostasis and are mediated by protein products of both the THRB and THRA genes. The THRA encodes TRα1, TRα2, TRα3 and some truncated variants TRΔα1, TRΔα2, p28 and p43. The TRα1 can display both a nuclear and cytoplasmic location, and is the only thyroid hormone receptor that is imported into the mitochondrial matrix as p28 and p43 truncated variants. The thyroid hormone receptor beta gene may produce TRβ1, TRβ2, TRβ4 (in humans) and two additional isoforms expressed in rats: TRβ3 and TRΔβ3. The TRα1, TRβ1, TRβ2 as well as TRβ3 can bind T3 and mediate T3-dependent actions, thus, the proteins are bona fide receptors, whereas the TRα2, TRα3 or TRΔα1 TRΔα2, TRΔβ3 and TRβ4 do not bind the hormone and their function remains to be elucidated. The TRα2 and TRα3 have longer carboxy-terminal domains (AF_2) that does not bind T3 and weakly binds DNA, thus, the variants act as dominant negative antagonists of T3 signalling. The truncated variant TRΔβ3 lacks the DNA-binding domain but retains T3 binding activity and acts as a dominant-negative antagonist. The human variant TRβ4 that is a C-terminal spliced variant of TRB1 lacks T3 - binding ability and acts as an endogenous dominant-negative isoform. The TRβ4 weakly but significantly inhibits transcription mediated by functional T3 receptors.

The function of the TRβ proteins may be influenced by the other members of the receptor superfamily that may lead to either synergistic or antagonistic effects. The nuclear receptor superfamily includes the estrogen receptor-like subfamily liganded by estrogene (ER) or 3-ketosteroids: glucocorticoid (GR), progesterone (PR), androgen (AR), mineralocortycoid (MR) and the thyroid hormone receptor-like subfamily that consists of the nuclear receptors for the thyroid hormone (TRα, TRβ), liver X receptor-like proteins (LXR, FXR), vitamin D receptor-like proteins (VDR, PXR, CAR), retinoic acid receptors (RARs) and peroxisome proliferator-activated receptors (PPARs). This subfamily also include the heme receptors: Rev-ErbAα encoded by NR1D1 gene that regulates various cellular function including circadian cycle and the ratio of TRα1/TRα2 isoforms as well as Rev-erbβ (NR1D2) identified in the THRB locus (3p24.2, see diagram 2). These heme binding receptors were identified previously as "orphan" (unknown ligand and/or DNA target) receptors. The members of the thyroid hormone receptor-like subfamily are classified as type II nuclear receptors, which are retained in the nucleus and can bind to DNA regardless of the ligand binding status and usually form heterodimers with Retinoid X Receptor-like subfamily transcription factors, which includes the retinoid X receptor (RXR), hepatocyte nuclear factor-4 (HNF4), testicular receptor (TR2, TR4) and photoreceptor cell-specific nuclear receptor (PNR). The class II receptors include the members of the estrogen receptor-like subfamily as well.

Interactions in the nuclear superfamily could be illustrated on the basis of crosstalk signaling between TRs and PPARs. This interaction appears to be important in TRs -mediated adipogenesis and carcinogenesis. Majority of the effects of PPARs and TRs were found to be opposing during the crosstalk, however the cooperative effects were reported as well (Lu and Cheng, 2009). PPARs liganded by prostaglandins, prostacyclins or a conjugated linoleic acids (CLAs) and T3 liganded TRs can reciprocally affect the target gene expression. In humans, the DBDs of TRs and PPARs are highly homologous and can bind to the same half-site sequence that is present in both, TRE and PPRE elements. The canonical DR1 PPRE consists of two direct repeats of AGGTCA with a 1 bp spacer, whereas the DR4 TRE is built by the same direct repeats, separated with a 4 bp spacer. TRs can competitively bind to PPREs of PPAR target genes. Moreover, either TRs or PPARs compete with the other receptor for binding to RXR that may result in decreased availability of RXR and reduced transcriptional activity of the PPAR target genes. Consequently, the PPARγ agonist rosiglitazone is able to reverse the effects of dexamethasone and to increase serum T3 and T4 levels. Interestingly, in rats with a high-fat diet and in a hyperthyroid state, administration of the PPARα agonist Wy14,643 restores glucose tolerance by enhancing glucose-stimulated insulin secretion and relieves the effect of hyperthyroidism. These data suggest that PPARα activity may restore the pancreatic islet function affected by abnormal T3/TR signaling. Furthermore, the genomic crosstalk of these two receptors may occur also via nongenomic actions of the receptors (Lu and Cheng 2009).

Ligands and metabolites

L-thyroxine (T4), a major secretory product of thyroid gland, and 3, 5, 3'-triiodo-L-thyronine (T3), the most active form of thyroid hormone are naturally occurring ligands for TRβ receptors. T3 is a tyrosine-based derivative of T4 that is produced by the thyroid gland in response to thyroid-stimulating hormone (TSH) from the anterior pituitary. The TSH is released by thyrotropin-releasing hormone (TRH) from the paraventricular

nucleus (PVN) of the hypothalamus and T4 synthesis is controlled by negative feedback loop: hypothalamic-pituitary thyroid (HPT) axis. Free thyroid hormones in the circulation act negatively on the pituitary and hypothalamus, thus reducing the release of TRH, TSH and finally T4 and T3 concentration in plasma. In the nucleus, the thyroid hormones bind TRβ with high affinity and specificity with K_d values in the nM range. Most T4 and T3 in the circulation are bound to proteins including Thyroxine-binding globulin (TBG), transthyretin and albumin. This proteins are responsible for carrying the thyroid hormones in the bloodstream.

Under normal conditions only a small fraction of T3 is generated by the thyroid gland, the remainder of T3, which is available for binding sites in the plasma and body cells, is synthesized by mono-deiodination of T4, that is inactive and needs to be converted to T3 what occurs in peripheral tissues. The reaction is catalyzed by type 1 (DIO1, EC1.97.1.10) or type 2 (DIO2, EC 1.97.1.11) iodothyronine deiodinases (selenoproteins), the first is abundant in kidney, liver and thyroid whereas the last one is mainly present in brown adipose tissue, pituitary and central nervous system. DIO1 is sensitive to inhibition by the anti-thyroid drug propylthiouracil (PTU). The enzyme activity of the kidney and liver is responsive to the nutritional status of an organism and is found to be more active during states of accelerated glucose metabolism. Most T3 molecules are produced by enzymatic outer ring deiodination (ORD) of T4. Alternative, inner ring deiodination (IRD) of T4 yields the metabolite rT3. ORD is regarded as an activating pathway and IRD as an inactivating pathway. DIO1 shows the ORD and IRD activity, DIO2 only ORD activity and the third iodothyronine deiodinase - DIO3 (expressed above all in brain tissue) mediates only the degradation of thyroid hormone since it has only IRD activity. T3 and rT3 undergo further deiodination to the common metabolite 3,3'-diiodothyronine (3,3'T2), which is generated by IRD of T3 and by ORD of rT3. Recent evidence for binding of T2 by a subunit of mitochondrial cytochrome c oxidase and its stimulation appears to be of a receptor/effector nature, showing as well that the T2 metabolite may have an important biological role that influences cellular respiration.

Apart from T4, T3, rT3 and 3,3'T2, the other thyroid hormone derivatives have been shown to take part in iodothyronine-like endogenous signaling, which may includes T2 (3,5-diiodo-L-thyronine), TAMs (thyronamines), and sulfate or glucuronic acid derivatives of thyroid hormones.

The most commonly studied TRs agonists are: TRIAC (3,5,3'-triiodothyroacetic acid showing thyromimetic activity, TRβ selective), TETRAC (tetraiodothyroacetic acid, an inhibitor of T4-transduced, αvβ3-integrin-mediated pathway), DITPA (3,5-diiodothyropropionic acid), DIMIT (3,5-dimethyl-3'-isopropylthyronine), GC-1 (3,5-dimethyl-4-(4'-hydroxy-3'-isopropylbenzyl)-phenoxy acetic acid (sobetirome), a TRβ-selective), KB-141 (3,5-dichloro-4-[(4-hydroxy-3-isopropylphenoxy) phenyl] acetic acid), SKF 94901(3,5-dibromo-3'-pyridazinone-L-thyronine).

TETRAC expresses nucleus-mediated thyromimetic activity, though current studies demonstrated its role in suppression of T4-induced αvβ3-integrin/ERK1/2 pathway (Davis et al., 2009). Covalently bonded to a nanoparticle, TETRAC acts exclusively at the cell surface TH αvβ3 integrin receptor without the thyromimetic activity and TR-mediated effects in nucleus.

TRs agonist relative potencies: GC-1~T3~TRIAC~T4>>rT3 have been determined for both coactivator recruitment and corepressor dissociation.

The TRβ and liver selective thyromimetics (STRMs) such as GC-1 (sobetirome), KB2115 (eproterome), KB141 and MB07811 have been developed to selectively lower serum total and LDL cholesterol without affecting HDL cholesterol levels and induce weight loss without deleterious effects on heart and combat other aspects of metabolic disease. GC-1 is the first TRβ agonist. KB141 is a selective TRβ agonist that can bind with an affinity 14-fold higher than that for TRα and is a useful candidate for attenuating the features of metabolic syndrome. This agonist lowers cholesterol, causes significant weight reduction in primates and has a 10-fold window in which therapeutic increases in metabolic rate are seen without cardiac hypertrophy or tachycardia therefore is a promising candidate for treating obesity, hyperlipidemia and diabetes. KB2115 has been described as a TRβ-selective agonist that is preferentially taken up by the liver and is also effective in patients on statin therapy. MB07811 is a liver-selective pro-drug that after hepatic activation through enzymatic cleavage is converted to the active form MB07344 - a strong TRβ ligand binding the other TRs with significantly lower affinity. Selective targeting with TRβ agonists may present an innovative strategy for searching for TRβ-specific effects.

The most commonly studied antagonists are: DIBRT (3,5-dibromo-4-(3'5'-diisopropyl-4'-hydroxyphenoxy)benzoic acid) and NH-3 (4-(4-hydroxy-3-isopropyl-5-(4-nitrophenylethynyl) benzyl)-3,5-dimethylphenoxy) acetic acid (NH-3, a low affinity TR antagonist). The TRβ-selective (but not TRα-selective) agonists and antagonists are expected to alter gene expression in a TRβ biased manner that can differ from T3, which binds the two TRs with similar affinity. Amiodarone (Amio) and dronedarone (Dron) are drugs used to

discriminate between TRα1 or TRβ1 regulated genes in central and peripheral TH metabolism. A major metabolite of Amio, desethylamiodarone, acts as a TRα1 and TRβ1 antagonist, whereas the major metabolite of Dron -debutyldronedarone acts as a selective TRα1 antagonist that allows the TRβ1 effects to become apparent.

Homology

Human THRB gene is reported to be conserved in Euteleostomi and has orthologs identified in: Pan troglodytes (Chr3, NCBI protein reference sequence: XP_001163850.1), Macaca mulatta (Chr2, XP_001090554.1), Canis lupus (Chr23, XP_862690.2), Bos taurus (Chr27, XP_002698801.1), Mus musculus (Chr14 7.08 cM, NP_001106888.1), Rattus norvegicus (Chr15 p16, NP_036804.2), Danio rerio (Chr19, NP_571415.1) and Gallus gallus (Chr2, erythroblastic leukemia viral (v-erb-a) oncogene homolog 2, avian, NP_001239150.1); according to the NCBI HomoloGene. Since the THRB shows multiple alignment, pairwise similarity scores and evolutionary distances, additional putative orthologs are likely in a variety of different species and can be viewed using alignment tools (BLAST, NCBI Resources).

This gene is highly homologous to ERBB2 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)). A connection between TRβ1 and cancer became evident when the chicken TRα1 ortholog was characterized as the c-erbA proto-oncogene, the cellular counterpart of the retroviral v-erbA oncogene. However, a growing number of evidences suggest that TRβ could serve as a tumor suppressor.

THRB is a member of a large superfamily of nuclear hormone receptor genes (see interactions) and displays extensive structural and functional similarity with the paralogous gene - THRA, which evolved from a common ancestor gene, duplicated 500 Mya. The gene is duplicated at least in vertebrates, birds and amphibians. Divergent evolution of the genes increased the variety of cellular responses to TH in vertebrates. The paralogous THRB and THRA show 77% of mRNA coding sequence (CDS) identity and 84% of protein identity. The similarities were determined based on the NCBI BLAST protein seq.: TRβ1 (NP_000452.2) ver. TRα1 (NP_955366.1); results: identities: 310/367(84%); positives: 342/367(93%); gaps: 0/367(0%); method: Compositional matrix adjust. NCBI BLAST mRNA CDS seq.: TRβ1 (NM_000461.4) ver. TRα1 (NM_199334.3); results: identities: 854/1113(77%); score 814 bits(902); gaps: 16/1113(1%). Comparative DNA analysis of the THRB and THRA revealed 10 conserved fragments of at least 261bp in length (73-90% of DNA identity between the genes), located in THRB introns: downstream of exon 1 (4x); 2a (2x), 2b, 3 (2x) and 4. These Alu-like elements were found in 5'-end of THRA sequence as well as in NR1D1 and NR1D2 genes. Moreover, two full-length of ADAR-binding sequence motif flanking A-to-I RNA editing sites (Ramaswami et al., 2012) are present in introns before exon 2a and 2c of the THRB. This consensus sequence is also present in THRA and NR1D1 genes.

The other reported paralogs for THRB gene are: VDR, NR4A3, NR4A1, NR1I2, RARG, NR1I3, RARA, NR1H2, NR4A2, NR1H4, RARB, NR1H3.

Interestingly, the THRB has close structural and functional relatives in NR1D2 (in the same locus), which encodes Rev-erbβ as well as THRA encoding TRα1 and TRα2 isoforms and NR1D1 gene expressing Rev-erAα. The THRB and NR1D2 genes are also linked to the RARB encoding RARβ. Since it has been shown that the THRA/NR1D1 locus is also linked to the RARA gene, these data suggest that the two receptor gene clusters RARA/THRA/NR1D1 and RARB/THRB/NR1D2 were generated by a single large-scale duplication. Moreover, the THRA gene shares a partial overlap with the NR1D1 gene that influences the TRα1/TRα2 ratio. Similar regulation may occur in case of the TRβ transcripts and the products of the genes sharing the same DNA sequence with the THRB (see diagram 1 and 2). The genes located in the same locus may produce long naturally occurring antisense transcripts (cis-NATs) forming sense-antisense pairs with a single stranded DNA or transcripts of the THRB. These sense-antisense pairs may activate a pseudogene-mediated regulation (see Pseudogene).

Reference conserved domains on TRβ1 protein (Homo sapiens, NP_001121649.1): 1) cd06961: NR_DBD_TR superfamily, DNA-binding domain of thyroid hormone receptors, Heterodimer interface of human Thyroid Hormone; 2) cd06935: NR_LBD_TR superfamily, The ligand binding domain of thyroid hormone receptor, coactivator recognition site (polypeptide binding site), dimer interface (polypeptide binding site).

Reference conserved domains on TRβ2 (Homo sapiens): 1) cd06929: NR_LBD_F1 (aa : 281-454), Ligand-binding domain of nuclear receptor family 1; 2) cd06916 : NR_DBD_like (aa : 122- 194) DNA-binding domain of nuclear receptors is composed of two C4-type zinc fingers.

Animal models

Animal models imply close associations between aberrant expression of TRβ or TRβ mutants and pathogenesis of some diseases, such as dominant or reccessice Generalized Resistance to Thyroid Hormone (GRTH) and Follicular Thyroid Carcinoma (FTC). Creation of a mouse model that harbors a knockin mutation of TRβ has facilitated

the study of the molecular actions of TRβ mutants in vivo. Knock-out studies in mice suggest that the different TH receptors may mediate different functions, though several studies of TRs actions revealed near complete overlaps in their effects. Tissue-specific expression of TRβ isoforms is thought to be a major factor responsible for the observed differences in phenotypes, including those that are affected by TRβ mutations. In mice knock-outs, the TRβ abnormalities may affect the following systems: endocrine/exocrine glands (increased thyrotroph cell number, enlarged thyroid gland, abnormal pituitary gland physiology); hearing/vestibular/ear (abnormal cochlea morphology, increased or absent threshold for auditory brainstem response, sensorineural hearing loss); homeostasis/metabolism (increased circulating thyroid-stimulating hormone level); nervous system (increased thyrotroph cell number, abnormal pituitary gland physiology, decreased cochlear outer hair cell number, abnormal retinal cone cell morphology); skeleton (spiral ligament degeneration), vision/eye (abnormal retinal cone cell morphology).

THRB gene, like mice Thrb ortholog, encodes TH receptor isoforms TRβ1 TRβ2 and TRβ4. Moreover, function of these receptors may be influenced by two additional isoforms: TRβ3 and TRΔβ3, expressed in rat models (Williams et al., 2000). TRβ1, TRβ2, and TRβ3 are bona fide T3 receptors that bind DNA, T3 and regulate expression of T3-responsive target genes. Studies of Trβ and Trβ2 knockout mice indicated that Trβ1 is essential for development of auditory function, whereas Trβ2 is not required, but that Trβ2 alone is essential for development of mid-wavelength (MW) cones photoreceptors. In contrast, both Trβ1 and Trβ2 are required for regulation of hypothalamic-pituitary-thyroid axis. The Trβ2 deletion in mice induces a complete and selective loss of MW-cone opsin without significant changes in total cone numbers. TRβ3 and TRΔβ3 variants are transcribed using third promoter (P3) positioned upstream of human exon 5 and downstream of second promoter (P2) of TRβ2 (see diagram 3). TRΔβ3 mRNA lacks rat exon B (315 nt) encoding a fragment of DNA-binding domain, present in TRβ3, which contains both exon A (342 nt) and B of rat TRβ. Start codons of TRβ3 has been identified in frame in various animal Thrb sequences including mouse, dog, chicken but not in human, chimpanzee and macaque. Nevertheless, none of these ATG codons are positioned within a favorable Kozak translation initiation sequence context and the lack of murine TRβ3 or TRΔβ3 expressed sequence tags (NCBI EST) suggests that in rats, expression from P3 promoter is differentially regulated. TRβ3 is a functional T3 receptor and the most potent isoform, but dependent on the sequence context of TRE elements, whereas TRΔβ3 retains T3-binding activity but lacks a functional DNA-binding domain and does not activate target gene transcription. Therefore, this isoform acts as a modulator (potent antagonist) of TRβ3 when coexpressed at low concentrations. At higher concentrations, TRΔβ3 is a TRE-selective and cell-specific antagonist of TRα1, Trβ1, and TRβ3. Identified in humans TRβ4 is a C-terminal variant that lacks the ligand binding domain (truncated variant of TRβ1), thus may function as a potent endogenous antagonist, however its expression and function in animals is unknown.

Mice with targeted deletions in TR genes have provided understanding of the possible roles of the different TRβ isoforms. Knockout mice that are unable to produce theTRα1 receptor show subnormal body temperature and mild abnormalities in cardiac function, whereas mice which lack expression of both TRα isoforms were severely hypothyroid and die within the first few weeks of life. Mice with disruptions of the entire beta gene (TRβ1 and TRβ2) exhibit elevated TSH levels and deafness suggesting its role in auditory system, while mice with mutations disturbing only TRβ2 had elevated TSH, but normal hearing. These mutants allow determination of which functions of the different receptor isoforms are redundant and which are not. TRs play important roles in the pathogenesis of thyroid cancers and hepatocellular carcinoma (HCC). For instance, v-erbA, a mutant form of TR lacking ligand-binding ability, triggers HCC development in transgenic mice. Similarly, $TR\beta^{PV}$ (Kaneshige et al., 2000) mutation harmoring mice develope thyroid cancers (see exemplary mutants below).

There are various mouse knock-outs for THRB:

- $TR\beta^{PV/PV}$; mutant thyroid hormone receptor kindred PV (Kaneshige et al., 2000); Synonyms: TRβPV; Allelic composition: homozygous $TR\beta^{PV/PV}$ and heterozygous $TR\beta^{PV/+}$; Mutation details: PV has an unusual mutation in exon 10, a C-insertion at codon 448, which produces a frameshift of the carboxyl-terminal 14 amino acids of TRβ1. PV was derived from a patient (called PV) with severe RTH characterized by elevated thyroid hormone levels accompanied by normal TSH, short stature, goiter, and tachycardia. This naturally occurring mutation shows lost T3-binding, transactivation activities, and displays dominant negative activity. Moreover, PV strongly interferes with the transactivation activity of wild-type TRs in vitro and unlike the missense mutations or single amino acid deletion of TRβ found in other patients, this unique frame-shifted mutated sequence is immunogenic, for which high-affinity specific antibodies have been developed. TRβ PV mutant has been obtained by using homologous recombination and the CreyloxP System. Affected

systems: endocrine/exocrine glands, homeostasis/metabolism; Human disease model: 1) Thyroid Hormone Resistance (NCBI OMIM: 274300, 188570); TRβ PV/+ and TRβ PV/PV mice faithfully reproduce human RTH - the TRβPV mutation was initially identified in a patient who has the syndrome of resistance to thyroid hormone (RTH). Mice expressing a single PV allele showed the typical abnormalities of thyroid function found in heterozygous humans (mutation of one copy of human THRB) with RTH that is characterized by a reduced sensitivity of tissues to the action of thyroid hormones. 2) Thyroid Carcinoma (OMIM: 188470), pituitary tumors; $TR\beta^{PV/PV}$ mice but not $TR\beta^{PV/+}$, spontaneously develop follicular thyroid carcinoma and pituitary tumors with tumor progression similar to human cancer. The homozygous PV mice exhibit severe dysfunction of the pituitary-thyroid axis, abnormal bone development and impaired weight gains. This phenotype is distinct from that seen in mice with a null mutation in the TRβ gene ($TR\beta^{-/-}$) demonstrating the interference of the mutant TRβ with the functions of the wild-type TRβ. $TR\beta^{PV/PV}$ mutants may serve also as molecular model for nongenomic actions of T3 condributing to thyroid carcinogenesis. The mutant mice allows the elucidation of oncogenic activity of the TRβ(PV) through cytoplasmic effects of T3 (see diagram 5 N1). This nongenomic action is mediated by interaction of PV with p85α regulatory subunit of PI3K to activate the downstream AKT/mTOR, $p70^{S6K}$ and PI3K-integrin-linked kinase-matrix metalloproteinase-2 signaling pathways. The PV-mediated PI3K activation leads to increased cell proliferation, motility, migration, and metastasis. In this regulation, a nuclear receptors corepressor NCoR competes with PV for binding to the p85α that result in reduction of the AKT-mTOR-$p70^{S6K}$ signaling. The NCoR protein levels are significantly lower in thyroid tumor cells than in wild type thyrocytes, allowing more effective binding of PV to p85α to activate the PI3K pathway, thereby contributing to tumor progression in the $TR\beta^{PV/PV}$ mutant mice (Guigon and Cheng, 2009).

- $Thrb^{tm1Df}$; thyroid hormone receptor beta; targeted mutation 1, Douglas Forrest ; Synonyms: Thrb-, TRbeta- Allelic composition: homozygous, $Thrb^{tm1Df}/Thrb^{tm1Df}$ involves: 129 S1/Sv * C57BL/6J; Mutation details: Insertion of a neomycin cassette into exon 3, disrupts both the beta1 and beta2 isoforms of this gene. This transcript revealed a deletion of exon 3 sequences, and fused beta1 exon 2 to exon 4 resulting in an aberrant open reading frame, which terminates early into exon 4. No functional protein is predicted from this transcript, as the essential DNA binding and T3 binding domains not present; Affected systems: endocrine/exocrine glands, hearing/vestibular/ear, homeostasis/metabolism, nervous system but not behavior/neurological phenotype; Human disease model : Thyroid Hormone Resistance, Generalized, Autosomal Recessive - GRTH (OMIM: 274300).

- $Thrb^{tm3Few}$; thyroid hormone receptor beta; targeted mutation 3, Frederic E Wondisford; Synonyms: GS125 KI, TR-BetaGS; Allelic composition: homozygous, $Thrb^{tm3Few}/Thrb^{tm3Few}$, involves: 129* C57BL/6; Mutation details: Missense mutations were introduced at codons 125 and 126 (exon 3), resulting in Glu to Gly and Gly to Ser substitutions.

The substitutions were within the P-box of the first zinc finger and were shown, in vitro, to abolish DNA-binding while retaining the ability to interact with T3 and cofactors. Western blot analysis showed endogenous levels of protein in homozygous mutant mice; Affected systems: endocrine/exocrine glands, hearing/vestibular/ear, homeostasis/metabolism, nervous system; vision/eye; Human disease model: Thyroid Hormone Resistance, Generalized, Autosomal Recessive, GRTH (OMIM: 274300).

- $Thrb^{tm2Few}$; thyroid hormone receptor beta; targeted mutation 2, Frederic E Wondisford; Synonyms: $TRbeta^{delta337T}$; Allelic composition: homozygous, $Thrb^{tm2Few}/Thrb^{tm2Few}$, involves: 129X1/SvJ * C57BL/6; Mutation details: The deletion of 3 base pairs in exon 6, corresponding to a deletion that results in thyroid horomone resistance in humans, was introduced via site-directed mutagenesis along with a neomycin selection cassette inserted into intron 5. The mutation in exon 6 affects the ligand-binding domain which is common to both isoforms produced from this locus; Affected systems: behavior/neurological, homeostasis/metabolism, nervous system; Human disease model: Thyroid Hormone Resistance, Generalized, Autosomal Dominant, GRTH (OMIM: 188570).

- $Thrb^{tm2Few/+}$; thyroid hormone receptor beta; targeted mutation 2, Frederic E Wondisford; Synonyms: $TRbeta^{delta337T}$; Allelic composition: heterozygous, $Thrb^{tm2Few}/Thrb^{+}$, involves: 129X1/SvJ * C57BL/6; Mutation details: (see $Thrb^{tm2Few}/Thrb^{tm2Few}$); Affected systems: behavior/neurological, homeostasis/metabolism, nervous system; Human disease model: Thyroid Hormone Resistance, Generalized, Autosomal Dominant, GRTH (OMIM: 188570).

- $Thrb^{tm1Df/tm1.1Syc}$; thyroid hormone receptor beta; targeted mutation 1, Douglas Forrest; Synonyms: Thrb-, TRbeta-; Allelic composition: heterozygous, $Thrb^{tm1Df}/Thrb^{tm1.1Syc}$, involves: 129S1/Sv * 129S6/SvEvTac * C57BL/6 * C57BL/6J; Mutation details: see above; Affected systems: endocrine/exocrine glands, mortality/aging, tumorigenesis, respiratory system; Human disease

model: Thyroid Carcinoma, Follicular; FTC (OMIM: 188470).
- $Thrb^{tm1Few}$; thyroid hormone receptor beta; targeted mutation 1, Fredric E Wondisford; Synonyms: TRbeta2 null; Allelic composition: homozygous mutant mice $Thrb^{tm1Few}/Thrb^{tm1Few}$, involves: 129S4/SvJae; Mutation details: A PGK-neo cassette replaced the transcription site, the entire Thrb2-specific exon, and the splice donor acceptor site. RT-PCR analysis of pituitary RNA confirmed the preservation of the Thrb1 isoform, as well as the absence of the Thrb2 isoform. Affected systems: endocrine/exocrine glands, homeostasis/metabolism but not hearing/vestibular/ear.
- $Thrb^{tm4Few}$; thyroid hormone receptor beta; targeted mutation 4, Frederic E Wondisford; Synonyms: TR-Beta-, TrbetaKO ; Allelic composition: homozygous, $Thrb^{tm4Few}/Thrb^{tm4Few}$ involves: involves: 129 * C57BL/6; Mutation details: Exon 3 was replaced with a self-excising PGK-neo cassette. The deletion of exon 3 putatively results in an aberrant open reading frame caused by the fusion of exons 2 and 4. Using a C-terminal mAb, protein was undetected by Western blot analysis of homozygous mutant mice; Affected systems: all mentioned at the begining of this section.
More information on the Mouse Genome Informatics website (Mouse Genome Database (MGD)).

Mutations

Note
9555 human THRB variants (NCBI dbSNP) includind 8007 single nucleotide polymorphisms (SNPs) and 326 human variants (20 studies, NCBI dbVar) have been recorded in the NCBI databases. Moreover, 42 pathogenic variants of clinical significance including 31 germline SNP, 4 copy gain, 3 deletions and 3 insertions of the gene have been catalogued in the NCBI ClinVar database (see Table 1). These allelic variants have been identified to impair hormone binding, DNA binding or ligand-dependent conformational changes. Some of them inhibits homodimer formation or stabilizes homodimer ligand-dependent conformational changes. The majority of the mutated TRβ receptors lost their trans-activation function and exhibited dominant-negative activity.

Germinal

There are 31 annotated germline THRB allelic variants in the NCBI dbSNP and ClinVar but only 15 reported in dbVar of the NCBI. Most of them are SNPs and insertions or deletions are less frequently found. The most relevant SNPs are those located on hormone binding domain (see diagram 6 and table 1). Some of them inhibits homodimer formation or stabilizes homodimer ligand-dependent conformational changes.
The majority of the mutated TRβ receptors lost their function of transactivation (e.g. DIO1, GH1) or transrepression (e.g. TSHB, TRH) in T3-dependent manner.
These variants usually act as dominant-negative mutants. Most of the germline, clinically associated mutations of the TRβ receptor have been identified in patients with autosomal recessive or dominant, generalized thyroid hormone resistance (RTH, OMIM: 188570, 274300 respectively) as well as selective pituitary thyroid hormone resistance (OMIM: 145650).
Patients with RTH have got impairment of the mechanism negatively regulating the feedback of T4/T3 to the hypothalamic TRH and pituitary TSH genes by the mutated TRβ receptors.
There are also studies showing the mutations in TRβ-DNA binding domain, in 5' and 3' mRNA untranslated regions (UTRs) and intronic variants with potential disease association.
These allelic variants have been identified to impair transcription, alternative splicing, translation or TRβ protein function such as hormone binding, DNA binding, ligand-dependent conformational changes or corepressors/coactivators dissociation/association function (see references).

Somatic

There are no reference variants reported as somatic in the NCBI dbSNP and ClinVar databases, however 3 allele variants (ID: nsv429603, nsv429566, nsv429555) are present in the NCBI dbVar.
Morover, several studies tested the hypothesis that the functions of TRβ could be impaired in various cancer tissues by somatic mutations (see references).
For instance, Puzianowska-Kuznicka et al. (2002) tested this hypothesis in selected human thyroid papillary cancer. Based on cancer-derived cDNAs, they found that the mean expression levels of TRβ1 mRNA and TRα1 mRNA were significantly lower, whereas the protein levels of both were higher in cancer tissues compared to healthy thyroid samples. Sequencing of TRβ1 and TRα1 cDNAs, cloned from 16 papillary cancers, revealed that mutations affected receptor amino acid sequences in 93.75% and 62.5% of cases, respectively.
In contrast, no mutations were identified in healthy thyroid controls, and only 11.11% and 22.22% of thyroid adenomas had such TRβ1 or TRα1 mutations, respectively.
The authors summarized that the findings suggest a possible role for mutated thyroid hormone receptors in the tumorigenesis of human papillary thyroid carcinoma (NCBI OMIM: 188550).

	NCBI ClinVar accession no.:	Variation: mRNA (protein)	Phenotype; GRTH=Thyroid hormone resistance, generalized; PRTH=Thyroid hormone resistance, selective pituitary	Position on DNA ref.seq: NC_000003.11
1	RCV000013381.1	c.700G>A (p.Ala234Thr)	GRTH, autosomal dominant	24185030
2	RCV000013395.1	c.727C>T (p.Arg243Trp)	GRTH, autosomal dominant	24185003
3	RCV000013394.1	c.728G>A (p.Arg243Gln)	GRTH, autosomal dominant	24185002
4	RCV000013367.1	c.929T>C (p.Met310Thr)	GRTH	24169205
5	RCV000013382.1	c.947G>A (p.Arg316His)	GRTH	24169187
6	RCV000013369.1	c.949G>A (p.Ala317Thr)	GRTH, autosomal dominant	24169185
7	RCV000013384.1	c.958C>T (p.Arg320Cys)	GRTH, autosomal dominant	24169176
8	RCV000013390.1	c.959G>T (p.Arg320Leu)	PRTH	24169175
9	RCV000013380.1	c.959G>A (p.Arg320His)	GRTH, autosomal dominant	24169175
10	RCV000013368.1	c.964G>C (p.Asp322His)	GRTH	24169170
11	RCV000013370.1	c.994G>A (p.Gly332Arg)	GRTH, autosomal dominant	24169140
12	RCV000013397.1	c.1009A>G (p.Thr337Ala)	PRTH	24169125
13	RCV000013366.1	c.1010_1012delCAC (p.Thr337_Arg338delinsArg)	GRTH, autosomal recessive	24169122-24169124
14	RCV000013386.1	c.1012C>T (p.Arg338Trp)	PRTH	24169122
15	RCV000013385.1	c.1012C>T (p.Arg338Trp)	GRTH, autosomal dominant	24169122
16	RCV000013363.1	c.1020G>C (p.Gln340His)	GRTH, autosomal dominant	24169114
17	RCV000013379.1	c.1033G>A (p.Gly345Ser)	GRTH, autosomal dominant	24169101
18	RCV000013362.1	c.1033G>C (p.Gly345Arg)	GRTH, autosomal dominant	24169101
19	RCV000013372.1	c.1034G>T (p.Gly345Val)	GRTH, autosomal dominant	24169100
20	RCV000013371.1	c.1034G>A (p.Gly345Asp)	GRTH	24169100
21	RCV000013373.1	c.1040G>A (p.Gly347Glu)	GRTH, autosomal dominant	
22	RCV000013396.1	c.1148G>A (p.Arg383His)	GRTH, autosomal dominant	24164613
23	RCV000013393.1	c.1302C>A (p.Cys434Ter)	GRTH, autosomal dominant	24164459
24	RCV000013387.1	c.1313G>A (p.Arg438His)	GRTH, autosomal dominant	24164448
25	RCV000013374.1	c.1324A>G (p.Met442Val)	GRTH, autosomal dominant	24164437
26	RCV000013378.1	c.1327A>G (p.Lys443Glu)	GRTH, autosomal dominant	24164434
27	RCV000013391.1	c.1336T>C (p.Cys446Arg)	GRTH, autosomal dominant	24164425
28	RCV000013375.1	c.1349T>A (p.Leu450His)	GRTH	24164412
29	RCV000013377.1	c.1357C>A (p.Pro453Thr)	GRTH, autosomal dominant	24164404
30	RCV000013364.1	c.1358C>A (p.Pro453His)	GRTH, autosomal dominant	24164403
31	RCV000013392.1	c.1373T>C (p.Val458Ala)	GRTH, autosomal dominant	24164388
32	RCV000013389.1	c.1376T>G (p.Phe459Cys)	GRTH	24164385
33	RCV000051721.1	see: dbVar nsv530242	Hydronephrosis	19956874-25579464
34	RCV000051720.1	see: dbVar nsv530241	Developmental and morphol. dely	11504802-38961034
35	RCV000051718.1	see: dbVar nsv530239	Developmental and morphol. dely	93949-29290273
36	RCV000051097.1	see: dbVar nsv529645	Cleft palate, isolated, Cleft upper lip	93949-37189567
37	RCV000013383.1	L325F	PRTH	see in ClinVar
38	RCV000013399.1	1-BP INS, 1590T	PRTH	see in ClinVar
39	RCV000013398.1	1-BP DEL, CODON 438C	GRTH, autosomal dominant	see in ClinVar
40	RCV000013388.1	1-BP INS, 1644C	GRTH	see in ClinVar
41	RCV000013376.1	1-BP INS, 1627C	GRTH, autosomal dominant	see in ClinVar
42	RCV000013365.1	EX4-10DEL	GRTH, autosomal recessive	see in ClinVar

Table 1. Pathogenic variants of clinical significance, according to NCBI ClinVar.

Diagram 6

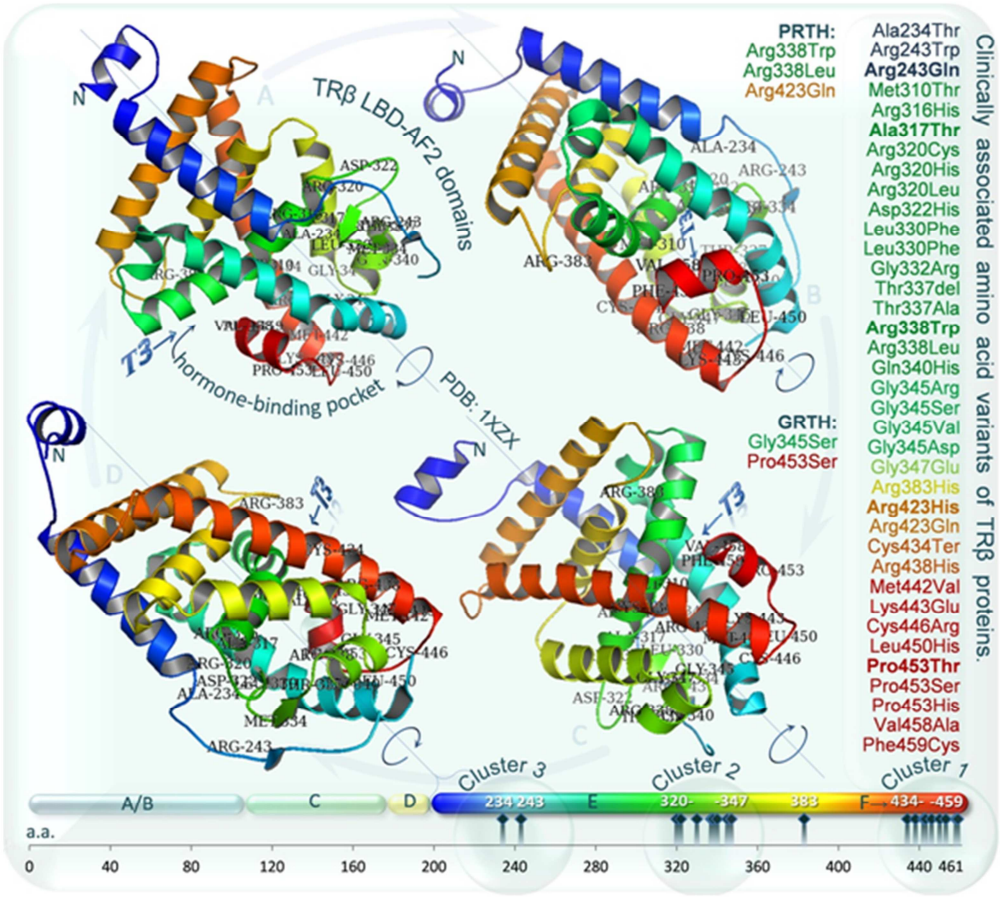

Diagram 6. Clinically asociated amino acid variants of TRβ proteins. Graphic representation of three mutational "hot spots" in the TRβ ligand-binding domain, in which natural mutations have clustered. Crystallographic structure of the TRβ ligand binding domain (LBD, E) complexed with triiodothyronine (T3) and C-terminal domain T3-dependent transactivation (F) are shown on all four sides (A,B,C,D) to visualize listed (on the right) reference variants, which colour corresponds to each α-helix structures of LBD (rainbow colored, N-terminus in blue, C-terminus in red). Mutations in the LBD may be associated with resistance to thyroid hormone (RTH). The most frequent mutations in RTH are bolded. Substitutions associated with pituitary-specific RTH (PRTH, R338L, R338W, R429Q) and generalized RTH (GRTH, P453S, G345S) are given. Mutations of GRTH (P453S, G345S) impair both TRβ2 and TRβ1 function proportionally, whereas variants of PRTH disproportionately disrupt the function of TRβ2 (Wan et al., 2005). An increased inability of the mutants to properly release the nuclear corepressors is postulated to inhibit the T3-mediated transactivation or transrepression of target genes. The TRβ mutants function in a dominant-negative fashion to interfere with the transcription activity of other wild-type thyroid hormone receptors (TRα) leading to resistance in peripheral tissues and dysregulation of the hypothalamic-pituitary thyroid axis (Dumitrescu and Refetoff, 2013). The conserved T3 binding domain was visualized using PyMOL 1.3 Molecular Graphics System, on the basis of crystallographic structure file (PDB: 1XZX) of the RCSB Protein Data Bank and The NCBI Conserved Domains Database (CDD, ref.c.d.: cd06961, NR_LBD_TR). For a more extensive listing of mutations, see references.

The similar conclusion can be found in studies of Kamiya et al. (Kamiya et al., 2002), who have cloned and sequenced 22 cDNAs obtained from the human renal clear cell carcinoma (OMIM: 144700). Somatic mutations were found in 7 TRβ1 and 3 TRα1 samples. These findings are consistent with the results obtained on hepatocellular carcinoma HCC (Lin et al., 1999). However, some data from direct genomic DNA sequencing provided an evidence that somatic THRB gene mutations may not be as common in differentiated thyroid cancers, in which hypermethylation of the gene was shown to be a major mechanism responsible for down-regulation of the gene expression. Indeed, THRB has been proposed to serve as a novel epigenetic marker for early detection and prognosis of high grade serous ovarian cancer (Kashuba et al., 2013) and identified to be frequently methylated in prostate cancer, breast cancer, non-small cell lung cancer and acute lymphoblastic leukemia (Dmitriev et al., 2009; Dmitriev et al., 2012; Ling et al., 2010; Vasiljevic et al., 2011). Searching the cBio Cancer Genomics Portal (Cerami et al., 2012), currently providing access to data from more than 5000 tumor samples from 20 cancer studies revealed that somatic mutations were the most frequently found in Skin Cutaneous Melanoma (up to 5.7% cases altered in the database).

The mutations were also reported in stomach adenocarcinoma (4.1%), kidney renal clear cell carcinoma (3.6%), colorectal cancer (2.8%), pancreatic adenocarcinoma (2.4%), colon and rectum adenocarcinoma (1.9%), head and neck squamous cell carcinoma (1.8%), lung adenocarcinoma (1.6%), ovarian serous cystadenocarcinoma (1.6%), uterine corpus endometrioid carcinoma (1.3%), sarcoma (1.2%), lung squamous cell carcinoma (1.1%), breast invasive carcinoma (0,9%) and acute myeloid leukemia (0,5%).
Most of the cancer mutations were identified as substitutions, however deletions were the most frequently found in renal clear cell. There were also reported amplifications in pancreatic adenocarcinoma, kidney chromophobe renal cell carcinoma and sarcoma (Gao et al., 2013; see cBioPortal in external links). It is noticeable that the reported alteration frequency can vary in different studies depending on the target populations, number of tested samples, analytical methods and histopathological classification of the tumors.
Somatic mutations/sequence variants have been shown to be created post-transcriptionally in various cancer-derived transcripts (Klimek-Tomczak et al., 2006; Chen et al., 2013). Adenosine (A) to inosine (I) RNA editing of AZIN1 was demonstrated to be increased in the hepatocellular carcinoma and suggested as a potential driver in the pathogenesis of human cancers, particularly HCC. ADAR1-mediated A-to-I RNA editing was shown to change the RNA nucleotide sequence relative to that of the encoding DNA that was reported to result in cancer development and progression (Huang et al., 2013). ADAR belongs to the family of RNA specific adenosine deaminase, which acts on double-stranded RNA (dsRNA) substrats including those created by long naturally occurring antisence transcripts (cis- and trans-NATs) such as Rev-erbα/TRα2 or intra-THRB transcripts (see diagram 1). Then, inosine, which in RNA can pairs with cytosine (C) uracil (U) or adenine (A, generating wobble pairs), is recognized as guanosine (G) by ribosomes (translation) and reverse transcriptase during cDNA synthesis. In fact, the comparison of whole genome sequences and deeply sequenced transcriptomes of human lymphoblastoid cell line GM12878 and a Han Chinese individual (YH) allowed for identification of two mismatches corresponding to A/I(G) ediging sites within THRB sequence (DNA ref. sec. position: 24167619, 24321108) as well as several editing sites in NR1D2 gene (see diagram 1, genomic context, Ramaswami et al., 2012). The next-generation sequencing used in the study permitted comparing genomic DNA and RNA sequencing data from the same individuals. These preliminary findings, however obtained from non-cancerous samples, may indicate that the ADAR-mediated RNA editing may change the sequence of THRB pre-mRNA post-transcriptionally (Ramaswami et al., 2012) and that this process may be impaired in numerous cancers (Huang et al., 2013).

Implicated in

Thyroid related disorders and cancers

Note

THRB gene mutations (NCBI OMIN : 190160) are known to be a cause of several disorders including autosomal recessive or dominant, generalized thyroid hormone resistance (GRTH; NCBI OMIM: 188570, 274300 respectively) as well as selective pituitary thyroid hormone resistance (PRTH; OMIM: 145650). Some forms of peripheral resistance to TH observed in familial euthyroid hyperthyroxinemia (OMIM: 145680) also appear to have a defect in the nuclear receptor for TH (Winter and Signorino, 2001). This gene has been also implicated in cancers such as follicular or papillary thyroid carcinoma (FTC, PTC; OMIM: 188470, 188550 respectively). Disturbances of the THRB gene are frequent findings in numerous cancers including renal cell cancer (RCC; OMIM: 144700) as well.
TRβ is a member of thyroid hormone receptors subfamily that mediates genomic and nongenomic actions of thyroid hormone (TH, T4/T3) that can influence cell growth, metabolism, apoptosis, and metastasis. TRβ mutations are involved in the reduced sensitivity to TH, short stature, attention-deficit hyperactivity disorder, autoimmune thyroid disease, erythroleukemia, hepatocellular carcinoma, and thyroid carcinoma (Rosen et al., 2011). The reduced sensitivity to TH may include defects of transport, metabolism and action of TH. TRβ mutations have been identified to affect some of these processes (Dumitrescu and Refetoff, 2013). Clinically, effects of TH are observed as changes in metabolic rate, altered lipid metabolism, and characteristic effects on cardiovascular development. Aberrations in the levels of TH can cause multiple disorders, including cardiovascular disease, diabetes mellitus, chronic liver disease and is implicated in various cancers. Interestingly, TH can modulate response to interferon-γ and has potential therapeutic applications in hepatitis B and C (Chi et al., 2013). Knowledge of the molecular mechanisms involved in TH action allows the recognition of the phenotypes caused by defects of TH action including the syndromes of reduced

sensitivity to thyroid hormone (Dumitrescu and Refetoff, 2013).

Carcinogenesis

A close association of TRβ mutations with human cancers has become apparent, however the role of TRβ mutants in the carcinogenesis is still not clear (Weinert et al., 2012). Besides, a growing number of studies suggest that the THRB can function as a tumor suppressor (Guigon et al., 2013). This putative role of the gene is consistent with findings showing that four markers spanning the 3p24-p21.3 region, THRB, AP20R, D3S1029, and D3S32, are regularly eliminated from three human chromosome 3 (chr3)/mouse microcell hybrids (MCHs) during tumor growth in SCID mice. These studies indicated that tumor suppressor gene may be located in this area, as suggested by frequent loss of heterozygosity (LOH) within the region containing the THRB and observed in several types of solid tumors (Kholodnyuk et al., 1997). The loss of normal expression of the THRB gene due to truncation or deletion has been observed in many malignancies including kidney, lung, melanoma, breast, head and neck, uterine cervical, ovarian, and testicular tumors.

Moreover, the epigenetic silencing of the THRB gene is common in human cancers. TRs play important roles in the pathogenesis of hepatocellular carcinoma (HCC).

It has been shown that cloned TRα and TRβ are truncated or mutated at high frequencies in the human HCCs. TRβ1 isoform is essential for genomic actions of T3 in liver, wherein TH can influence hepatoma cell growth, metabolism, apoptosis, and metastasis.

Therefore modulation of the TRβ-mediated actions of TH may have powerful therapeutic potential in clinical applications (Chi et al., 2013). Both TRα and TRβ have been shown to mediate action of T3 that blocks the response to the oncogenic forms of the three ras isoforms (H-ras, K-ras, and N-ras). However, the TRβ isoform has stronger anti-transforming properties than the TRα isoform and importantly can inhibit neuroblastoma tumorigenesis even in hypothyroid mice.

These results show the existence of a transcriptional cross talk between the TRβ and the ras oncogene that may influence relevant processes such as cell proliferation, transformation, or tumorigenesis (García-Silva and Aranda, 2004).

Furthermore, decreased THRB expression by promoter hyper-methylation has been reported in human breast cancer, lung cancer, and thyroid carcinoma, whereas reactivation of the silenced thyroid hormone receptor β gene expression delays thyroid tumor progression (Kim et al., 2013). Aberrant TRβ1 mRNA and protein levels have been reported to be a factor that may contribute to carcinogenesis in clear cell renal cell cancer (ccRCC). In this cancer, TRβ1 mRNA and protein levels were reduced by 70% and 91% in ccRCC and accompanied by absent DIO1 protein (a TRβ1 target gene) and a 58% reduction in tissue T3 concentration when compared to controls obtained from the opposite pole of malignant kidneys. These data provide an evidence of impaired T3 action in ccRCC that is maintained by reduced expression of TRβ1. The observed discordance in the magnitude of the change in TRβ1 mRNA level compared to protein (70/91 % reduction) together with the aberrant splicing of various TRβ1 5'UTRs leading to differences in the ratios of the variants may confirm that TRβ1 expression is subject to complex post-transcriptional regulation at least in ccRCC (Master et al., 2010). At this level, the gene is also regulated by microRNAs that are small endogenous noncoding RNAs binding to 3'UTR of the TRβ mRNA and affecting its level through RNA interpherence (RNAi) phenomenon. miR-21 and miR-146a have been found to inhibit the expression of the THRB by lowering the levels of both, TRβ mRNAs and proteins, suppressed down to 10-28% in papillary thyroid cancer (PTC) (Jazdzewski et al., 2011).

A knock-in mouse harboring a dominant negative Trβ mutation develops metastatic thyroid cancer that suggests the involvement of TRβ in carcinogenesis. The $Thrb^{PV/PV}$ mice (Kaneshige et al., 2000) harboring a knockin dominant negative PV mutation (see animal models), identified in a patient with resistance to thyroid hormone, develops the follicular thyroid carcinoma (FTC). The more aggressive thyroid tumor progression in the $Thrb^{PV/PV}$ mice results not only from the loss of tumor suppressor functions but also gain-of-function in the oncogenic activities of the PV variant to drive thyroid carcinogenesis. Cell-based studies with simian virus-40 (SV40)-induced carcinogenesis demonstrated that TRβ can inhibit tumorigenesis by blocking the oncogenic actions of SV40-Tag via protein-protein interaction. The TRβ was shown to compete with Rb and 53 for binding to SV40-Tag oncoprotein that were accompanied by reduced cell proliferation and delayed cell entry from the cell cycle G1 to the S phase. In another research, estrogen (E2)-dependent growth of MCF-7 cells that express the estrogen receptor, but not TRs, was inhibited by the expression of TRβ in the presence of T3. In a xenograft mouse model, large tumors rapidly developed after inoculation of MCF-7 cells that lacks the TRs expression. Markedly smaller tumors (98% smaller) were found when MCF-7-TRβ cells were inoculated in athymic mice, indicating that TRβ can inhibit the E2-dependent cancer growth. This study provides additional in vivo evidence to support the hypothesis that TRβ

could act as a tumor suppressor in breast cancer development and progression. Moreover, cell-based studies in T47D, a breast cancer cell line, showed that T3 represses STAT5 signaling in TRβ-expressing cells through decreasing STAT5-mediated transcription activity and target gene expression whereas sustained STAT5 signaling was observed in $TR\beta^{PV}$-expressing cells. The $Thrb^{PV}$ mutant increases the activity of STAT5 to increase cell proliferation and the expression of the STAT5 target gene encoding β-casein in the mammary gland. Another transcription factor - STAT3 is frequently found to be activated in breast cancer cell lines and patients with advanced breast cancers. Importantly, the STAT3 as well as STAT1α are found to be activated as a result of nongenomic actions of T4 via αvβ3 integrins (Davis et al., 2009), which are also responsible for activation of cytoplasmic fraction of TRβ1 via ERK1/2-mediated phosphorylation of its Ser_{142} (see nongenomic actions of TH). This pathway does not need to be mediated by genomic-actions of TRβ receptors and results in activation of the STAT3 and STAT1α that finally may lead to pro-proliferative, pro-angiogenic and anti-apoptotic effects. The T4 action through αvβ3 integrins can be selectively blocked with a T4 analogue - TETRAC, without affecting the TRβ-mediated genomic actions of T3.

In various reports, enhanced growth and proliferation of cancer cells are observed at low or high levels of the thyroid hormone, depending on the origin of cells or tissues examined. However, these confusing data could result from activation of different and cell-specific mechanisms involved in genomic and nongenomic actions of T4/T3. TH has been shown to be a ERK1/2-dependent growth factor for Human Myeloma Cells acting via αvβ3 Integrin (Cohen et al., 2011). Several studies have demonstrated as well that T3 promotes growth and proliferation of cancer cells through TRβ1/Oct-1-mediated cyclin D1 activation that was confirmed in papillary thyroid carcinoma cell lines (Perri et al., 2013). Decreased concentration of T3 has been also demonstrated to reduce proliferation of Caki-2 cells in vitro (Poplawski and Nauman, 2008). There are studies indicating that elevated levels of TH may initiate direct effects on proliferation including those engaged in the regulation of cell cycle progression that may at least partially reflect the nongenomic actions of TH. Moreover, $Thrb^{PV/PV}$ mice (see animal models) treated with propylthiouracil (PTU), wchich blocks TH production, have been shown to reduce thyroid tumor growth by 42% when compared to control $Thrb^{PV/PV}$ mice (Lu et al., 2012). The tumor cell proliferation, invasion and metastasis was also decreased and accompanied by marked attenuation of the TRβPV/PI3K/AKT/β-catenin/cyclin-D2 signaling pathway thus, showing a critical role of TH in promoting the thyroid carcinogenesis of $Thrb^{PV/PV}$ mice (Guigon and Cheng, 2009). Importantly, these findings suggest an anti-cancer potential of anti-thyroid drugs (Lu et al., 2012). The autors proposed a model in which the the $TR\beta^{PV}$ mutant directly interacts with PI3K to activate AKT signaling pathway. Suppression of TH in these cells, downregulates the membrane receptor integrin αvβ3 switching off a nongenomic action of T4. Furthermore, PTEN was found to be activated in these cells that can decrease the formation of PIP3, repress p-AKT and its downstream β-catenin and GSK3β signaling pathways, finally leading to inhibition of cell proliferation (Lu et al., 2012). In addition, $TR\beta^{PV}$ mutant is known to activate the $TR\beta^{PV}$/PI3K/AKT signaling cascade via binding to p85α regulatory subunit of the PI3K competing with NCoR, which can also bind to p85α and repress this pathway (Guigon and Cheng, 2009).

Besides, elevated levels of TRβ1 expression have been reported to reduce cell proliferation, malignant phenotype and to enhance apoptosis, indicating the suppressive role of the receptor, which is T3-dependent at the genomic level. Furthermore, FTC-236 cells, stably expressing TRβ, exhibited lower cell proliferation and migration through inhibition of β-catenin signaling pathways when compared to FTC-236 without TRβ. There are also studies indicating that the phenotype of tumors induced in hypothyroid hosts is more mesenchymal and their invasiveness and metastatic behaviour are enhanced. These findings are in line with reports documenting reduced tissue T3 in human gliomas (Nauman et al., 2004). Moreover, the reduced TRβ1 expression and tissue hypothyroidism have been also reported in clear cell renal cell cancer (ccRCC). The level of T4 did not differ between normal and ccRCC tissues, whereas the concentration of T3 was reduced by 58% in ccRCC and was accompanied by 92% decrease of DIO1 mRNA - a TRβ1 target gene (Master et al., 2010). These results are in agreement with genomic and nongenomic actions of TH that could be executed in ccRCC via T4-activated αvβ3-integrin/ERK1/2 pathway (T4 levels were not altered) or TRβ1/p85/PI3K/Akt/mTOR pathway but not necessarily through TRβ-mediated genomic actions of T3 (low levels of TRβ1, DIO1, T3). These disturbances are likely to be involved in the process of carcinogenesis or in maintaining a proliferative advantage to malignant cells. Indeed, tetraidothyroacetic acid (TETRAC), a thyromimetic agonist of TRβ that can also block the T4 integrin (αVβ3) receptor at the cell surface, has been shown to inhibit growth of human renal cell carcinoma xenografts (Yalcin at al., 2009) and human medullary thyroid carcinoma (MTC) xenografts in the nude mouse (Yalcin at al., 2008). Interestingly, both the MEK/ERK- and PI3K/Akt-dependent

pathways mediate CD74-induced tumorigenesis of ccRCC and it is known that TRβ1 is involved in these signal cascades (see genomic and nongenomic actions of TH). The CD74 overexpression could not significantly induce the expression of TRβ target genes: HIF1A or HIF2A, what is in agreement with the low levels of T3 in ccRCC. T3 is required not only for genomic but also nongenomic actions mediated by TRβ1 in cytoplasm (see diagram 5) contributing to expression of PI3K-dependent genes, which includes the HIF1A (HIF-1α), SLC2A1 (GLUT1) and RCAN2 (ZAKI-4) genes. At the same time, TRβ1 nuclear import (cytoplasmic/nuclear localization) and its transcription factor activity depend on phosphorylation of TRβ1 Ser_{142} by ERK1/2.

Simultaneously, $TR\beta^{PV}$ mutant has been demonstrated to activate cytoplasmic actions of T3 via binding to CSH_2 domain of p85α (see diagram 5 N1c) (Furuya et al., 2009). This nongenomic action is mediated by direct protein-protein interaction of $TR\beta^{PV}$ with p85α regulatory subunit increasing the catalytic activity of p110 of phosphatidylinositol 3-kinase (PI3K) to activate the downstream AKT/mTOR, $p70^{S6K}$ and PI3K-integrin-linked kinase-matrix metalloproteinase-2 signaling pathways. The $TR\beta^{PV}$-mediated PI3K activation leads to increased cell proliferation, motility, migration, and metastasis (Furuya et al., 2009), but these effects are TH-dependent (Lu et al., 2012). In addition, a nuclear receptor corepressor (NCoR) as well as wild-type TRβ1 competes with TRβPV for binding to the C-terminal SH_2 domain (CSH_2) of p85α. Up-regulation of NCoR in thyroid tumor cells reduces AKT-mTOR-$p70^{S6K}$ signaling. In contrast, lowering cellular NCoR by siRNA knockdown in tumor cells results in over-activation of PI3K-AKT signaling. Importantly, NCoR protein levels are significantly lower in thyroid tumor cells than in wild type thyrocytes that allows for more effective binding of PV to p85α to activate the PI3K pathway, thereby contributing to tumor progression (Furuya et al., 2009). Furthermore, the suppressive role of TRβ has been demonstrated using MCF-7 cell line in xenograft models of estrogen-dependent tumorigenesis. The TRβ-mediated inhibition of tumor growth has been elucidated via down-regulation of JAK-STAT-cyclin D pathways (Park et al., 2013). Tumor suppressor function of TRβ has been demonstrated in a mouse model of metastatic follicular thyroid carcinoma as well (Zhu et al., 2010).

According to the findings mentioned above, it could be hypothesized that TH may act as a growth, pro-angiogenic, pro-proliferative and anti-apoptotic factor when initiated at the nongenomic level (Cohen et al. 2011; Davis, 2009), whereas in nucleus, TRβ1 could serve as a suppressor itself or mediating some genomic actions of T3 on specific genes involved in retardation of tumor growth and progression. (Martínez-Iglesias et al., 2009b; Kim at al., 2013). TRβ-dependent transrepression (see genomic actions of TH) is thought to be a mechanism that may have an important function in suppression of transforming effects of at least several oncogenes. The inhibitory action of T3 on ras-mediated transformation (Garcìa-Silva and Aranda, 2004) can be enhanced by over-expression of corepressors and reversed by silencing of the corepressors. This shows an important functional role of endogenous corepressors in suppression of transformation and tumorigenesis by TRβ1. All these findings raise the possibility that TRβ could act as a tumor suppressor in tumorigenesis. However, the presence of several TR isoforms, various TH metabolites, multiple transcription cofactors as well as simultaneous activation of the genomic and nongenomic actions of TH make its final effect more pleiotropic and less clear.

Thyroid carcinoma, papillary (PTC)

OMIM: 188550, MedGen UID: 66773.

Synthesis and release of TH by follicular cells in the thyroid gland is regulated through the hypothalamic-pituitary thyroid (HPT) axis, a negative feedback loop controlled by both, the TRβ1 and TRβ2 isoformes. Nonmedullary thyroid cancer (NMTC) includes thyroid cancers of follicular cell origin and accounts for more than 95% of all thyroid cancer cases (Vriens et al., 2009). The remaining cancers originate from parafollicular cells - medullary thyroid cancer (MTC). NMTC is classified into: follicular, papillary, Hurthle cell, and anaplastic carcinoma. Dominant-negative $TR\beta^{PV}$ mutant (Kaneshige et al., 2000) which lacks the C-terminus of the receptor (see animal models), causes severe disruption of the HPT axis, goiter, TSHomas, and metastatic follicular thyroid carcinoma (FTC). A double mice knockout of both TRα and TRβ results in a higher incidence of follicular thyroid carcinoma and increased aggressiveness in a skin cancer model. These animal models indicate the meaning of TRs in the pathogenesis of FTC.

Disease

Papillary thyroid cancer (PTC) is the most common subtype of FNMTC (familial NMTC), accounting for 72- 85% of cases. PTC occurs more frequently in women and in the 20-55 year age group. PTC appears as an irregular solid or cystic mass in a normal thyroid parenchyma and is characterized by distinctive nuclear alterations including grooves, pseudoinclusions, and chromatin clearing. PTCs that are smaller than 1 cm are referred to as papillary microcarcinomas. These tumors have been identified in up to 35% of individuals at autopsy, suggesting that they may be extremely common although rarely clinically relevant. PTC can also be

multifocal but is typically slow growing with a tendency to spread to lymph nodes and usually has an excellent prognosis. Activation of the mitogen-activated protein kinase (MAPK) pathway as a result of mutations or somatic recombination is found in the majority of PTCs (Bonora et al., 2010).

Prognosis

Depending on source, the overall 5-year survival rate for PTC is 96-97%, whereas a 10-year survival rate is 93%.

For younger patients, the prognosis is better than for patients older than 45 years (Ito et al., 2013; Biersack and Grünwald, 2005).

Cytogenetics

Germline mutations were found in approximately 5% of NMTC, occurring as a primary feature FNMTC or as a minor component of a familial cancer syndrome (familial adenomatous polyposis, Carney complex) that are hereditary.

Moreover, several cases of PTC including differentiated PTC have been reported to be associated with the RTH syndrome (Ramos-Prol et al., 2013). Furthermore, TRβ has been found to be a major target gene for microRNAs in PTC (Jazdzewski et al., 2011). Both, miR-21 and miR-146a have been reported to inhibit the expression of the TRβ mRNA and protein, lowered down to 10-28% in PTC (Jazdzewski et al., 2011). In addition, 70% of PTCs have been shown to harbor point mutations of the BRAF and RAS genes or RET/PTC rearrangements, all of which can activate the mitogen-activated protein kinase pathways (Witt et al., 2013). For more information see Mutations.

Thyroid carcinoma, follicular (FTC)

Note

OMIM: 188470, MedGen UID: 64630.

TRs have been shown to serve as tumor suppressors in a mouse model of metastatic follicular thyroid carcinoma (Zhu et al., 2010). See Animal models.

Disease

Follicular thyroid cancer (FTC) accounts for approximately 15% of NMTC and occurs more commonly in women over 50 years of age. FTC is defined by invasive features that result in infiltration of blood vessels and full penetration of the tumor capsule as well as the absence of the nuclear alterations, which characterize papillary carcinoma. FTC is rarely multifocal and usually does not metastasize to the regional lymph nodes but tends to spread via the bloodstream to the lung and bones. The Oncocytic follicular carcinoma (Hurthle cell, oxyphilic) is an important histologic variant of FTC, composed of eosinophilic cells replete with mitochondria (Bonora et al., 2010).

Prognosis

The overall 5-year survival rate for FTC is 91%, whereas 10-year - 85% (Biersack and Grünwald, 2005).

Cytogenetics

FTCs are known to harbor RAS mutation, PAX8/PPARγ rearrangement and activation of the PTEN/AKT pathway. These mutations are also mutually exclusive and identified in 70% of follicular carcinomas. Molecular classifiers measure the expression of a large number of genes on a microarray chip providing a substantial negative predictive value pending further validation. Aberrant THRB gene expression is thought to be implicated in FTCs (see Mutations and Animal models of FTC).

Thyroid hormone resistance, generalized, autosomal dominant (GRTH)

Note

OMIM: 188570, MedGen UID: 424846.

Resistance to TH (RTH), a syndrome of reduced end-organ responsiveness to TH, was identified in 1967 (Refetoff et al., 1967) but linkage between a TRβ locus on chromosome 3 and the RTH phenotype was demonstrated in 1988 (Usala et al., 1988). Recent discoveries of genetic defects that reduce the effectiveness of TH through altered cell membrane transport and metabolism broadened the definition of reduced TH sensitivity to include all defects that interfere with the biological activity of TH secreted in normal amounts (Dumitrescu and Refetoff, 2013). A number of humans with a syndrome of TH resistance have been identified to have mutations in the THRB gene (Dumitrescu and Refetoff, 2013). Clinically, such individuals show a type of hypothyroidism characterized by goiter, elevated serum concentrations of T3, T4 and near normal serum concentrations of TSH. More than half of affected children show attention-deficit disorder, which shows the role of thyroid hormones in brain development. THRB gene mutations produce two forms of generalized resistance to TH (GRTH) - autosomal recessive and dominant. The first one is less common and described in a family containing deletion of all coding sequences of the THRB gene that is inherited as an autosomal recessive trait (Takeda et al., 1992). The more common form of RTH is inherited in a dominant mode and is characterized by defects in only one allele of THRB, usually a missense mutation. The mutant THRB allele produces mutant TRβ protein that cannot mediate effects of T3 and acts by interfering with the function of the wild-type TRs (wt-TRs), finally contributing to a dominant negative effects (DNEs). The majority of the mutated TRβ receptors abolish ligand binding, lost their trans-activation function, and exhibited

dominant-negative activity. The TRβ mutants may disturb the wt-TRs binding to TREs or preserve the ability to dimerize with a partner (e.g. RXR). The DNE can be exerted also through reduced association with cofactors (CoAs) or increased affinity for corepressors (CoRs), which have been found to play a role in the autosomal dominant RTH. Indeed, mutants that fail to interact with coactivators or are defective in T3-induced release of corepressors have been identified in RTH patients. Importantly, the presence in a TRβ mutant of an additional mutation that abolishes either DNA binding, dimerization or the association with a CoR can result in the abrogation of the DNE (Dumitrescu and Refetoff, 2013). Moreover, RTH has been found to be modulated in vivo by the corepressor - NCoR1 (Fozzatti et al., 2011). Thus, full and potent dominant-negative activity of TRβ mutant requires functional DBD to retain the ability to bind DNA and to form homodimers and RXR/TR heterodimers.

The findings reveal that dominant-negative activity in RTH is mediated by transcriptionally inactive complexes containing TR mutants bound to TREs.

TRH is estimated to occur in approximately 1 per 40000 newborns (Refetoff and Dumitrescu, 2007). The gene defect remains unknown in 15% of subjects with RTH.

Familial occurrence of RTH has been documented in about 75% of cases, whereas incidence of sporadic cases has been reported in 21.0% of cases that is in agreement with estimate of the frequency of de novo mutations of 20.8%. RTH has been found with equal frequency in both male and female gender. The prevalence may vary among different ethnic groups however appears to have wide geographic distribution among Caucasians, Africans, Asians and Amerindians (Dumitrescu and Refetoff, 2013).

Disease

The majority of patients with RTH are identified by their persistent elevation of circulating free TH levels association with non-suppressed serum TSH and higher doses of exogenous TH are required to obtain appropriate secretion of pituitary TSH as well as the metabolic responses in peripheral tissues. The apparent resistance to TH may vary in severity and the magnitude of the hormonal resistance is mainly dependent on the nature of TRβ mutations. RTH shows a variable clinical presentation, however the common features of the RTH syndrome may include: elevated levels of free T4 and to a lesser degree T3, normal or slightly increased level of TSH responding to TRH, goiter and the absence of the metabolic consequences of TH excess. The frequency of the most frequently observed manifestations are as follows: thyroid gland: goiter 66-95%, tachycardia 33-75%, emotional disturbances 60%, hyperkinetic behaviour 33-68%, attention deficit hyperactivity disorder 40-60, learning disability 30%, mental retardation (IQ 2 SD 29-47, recurrent ear and throat infections 55% (Dumitrescu and Refetoff, 2013).

Diagnosis is based on the clinical findings and standard laboratory tests including searching for germline mutations by sequencing of THRB exons. Nevertheless, a new role of mutations/polymorphisms within intronic sequences is recognised to affect alternative splicing and other events of post-transcriptional processing of TRβ RNA (Alberobello et al., 2011). Thus, the further association studies involving whole THRB sequencing (376 609 bp) would be needed to be carried out in patients with TRH symptoms who have no mutations in the THRB exons. Recently, the alternative splicing have been shown to produce human TRβ4 isoform, a carboxyl-terminal splicing variant of TRβ1 that contains a stop codon due to the presence of an intronic 137-bp insertion located between exon 7 and 8. TRβ4 lacks the ligand binding domain and thus, may modulate T3 action as an endogenous dominant-negative protein. This variant is expressed in various human tissues regardless of mutation status in the coding sequence and may interfere with the function of wild-type TR isoforms (Tagami et al., 2011).

Prognosis

RTH affected individuals have elevated serum TH levels and normal or elevated TSH but are usually clinically euthyroid and require no treatment. However, the clinical presentation of RTH is variable and requires differential diagnosis excluding all other possible causes of hyperthyroxinemia. Most patients have normal growth and development, and lead a normal life at the expense of high TH levels and a small goiter. In some cases, abnormalities may be found in: connective tissue, head and neck, metabolism/homeostasis, abdomen, cardiovascular system, ear, eye, endocrine system, integument, musculature, nervous system, respiratory system, skeletal system and increased upper to lower segment ratio. Goiter has recurred in every patient who underwent thyroid surgery. As a consequence, some patients have been submitted to several thyroidectomies or treatments with radioiodide (Dumitrescu and Refetoff, 2013).

Cytogenetics

Mutations in THRB gene have been identified in approximately 85% cases of the RTH, however other genes such as MCT8 and SECISBP2 are believed to be associated with the disease as well (Bottcher et al., 2007). TRβ dominant negative mutants have been shown not only to fail its function in a transcriptional response to T3 but also

to interfere with wild-type TRα and TRβ actions. Haplotyping of intragenic polymorphic markers showed that, in most instances, identical mutations have developed independently in different families (Dumitrescu and Refetoff, 2013). Among 457 families 170 different mutations have been identified and 78 of the mutations were shared by more than one family.

Majority of the families (430) were found to have single nucleotide substitutions (SNP) resulting in a single amino acid substitution (419), stop codons producing truncated proteins (11). In 20 families, deletions, insertions and a duplication were identified.

Most mutations were found in exon 9 and 10, however they were present in exon 6, 7, 8 as well. Unrelated families (33) shared the R338W mutation. Variants: R243Q, A317T, R338W, R423H and P453T were found in more than 15 families.

All TRβ gene mutations were located in the functionally relevant domain of T3-binding and its adjacent hinge region (see diagram 6). Three mutational clusters containing CpG hot spots have been identified (Dumitrescu and Refetoff, 2013). For more disease information, see references.

Thyroid hormone resistance, generalized, autosomal recessive (GRTH)

Note

OMIM: 274300, MedGen UID: 333543.

Recessively inherited resistance to thyroid hormone (RTH) is a rare autosomal disorder usually caused by mutations in the THRB gene. The loss of both THRB alleles may result in severe abnormalities reflecting unresponsiveness to TH.

Disease

A family with deletion of all coding sequences of the THRB gene has been reported to be inherited as an autosomal recessive trait (Takeda et al., 1992). The complete lack of TRβ in this family produces severe deafness, contributing to mutism and monochromatic vision (see Animal Models). Heterozygous individuals that express a single TRβ gene have no clinical or laboratory abnormalities. It has been demonstrated that this is not due to compensatory overexpression of a single normal allele of the THRB nor that of the THRA gene. However, normally expressed TRα1 is capable of partially substituting for the TRβ function (Dumitrescu and Refetoff, 2013).

Cytogenetics

The following homozygous mutations in the THRB have been identified: THRBdel (deletion of both alleles of THRB), T337del, I280S, G347, R316C (Ferrara et al., 2012). A novel rare homozygous mutation in the gene in position 1216 (G to A transition, codon 311) resulting in novel Glu-311-Lys (p.E311K) substitution has been reported as well. The homozygous patient was characterized by severe symptoms of RTH. Both parents were heterozygous, suggesting autosomal recessive mode of the inheritance (Slezak et al., 2012).

Thyroid hormone resistance, selective pituitary (PRTH)

Note

OMIM: 145650, MedGen UID: 333543.

In contrast to GRTH, PRTH is characterized by resistance in the pituitary gland but not in peripheral tissues. Note that the anterior pituitary (secreting TSH) and in particular hypothalamus (releasing TRH) are brain structures wherein TRβ2 is predominantly expressed. The presence of the TRβ2 isoform in cochlea, retina is extremely important during development (see Expression and specific functions). Due to the tissue-specific expression and function of the TR isoforms all tissues other than the pituitary have been grouped together under the term peripheral tissues.

Disease

This form of resistance to thyroid hormone is pituitary-selective and is characterized by hyperthyroidism and TRH-stimulated, inappropriate secretion of TSH (Gershengorn et al., 1975). Subjects with PRTH may have equally high levels of serum TH and non-suppressed TSH. These individuals may appear to be hypermetabolic, restless and may have sinus tachycardia or other thyrotoxic effects. In GRTH, the TH response of both the pituitary and peripheral tissues is disrupted, whereas in PRTH (designated also central), the ability of the pituitary to sense (and down-regulate) elevated TH is selectively impaired. Simultaneously, the peripheral tissues remains relatively TH-responsive that results in peripheral thyrotoxicity (Wan et al., 2005). It has been proposed that PRTH syndrome is associated with T3 receptor mutants that selectively impair β2 isoform function in pituitary and hypothalamic cells (Wan et al., 2005). The wild-type TRβ2 isoform has been reported to display an enhanced T3 response relative to the TRβ1, expressed broadly in almost all tissues. In the normal subjects, and in GRTH, TRβ2 in the pituitary can sense rising T3 levels in advance of TRβ1 in the peripherial tissues, preventing the thyrotoxicity. In contrast, the THRB mutations associated with pituitary RTH (see diagram 6) disproportionately disrupt the pituitary's ability to sense and suppress elevated T3 levels in advance of the peripheral tissues, producing symptoms of the thyrotoxicity (Wan et al., 2005).

Prognosis

Prognosis depends on clinical manifestations and laboratory testing (see GRTH). There are some

difficulties in differentiating thyrotropin secreting pituitary microadenoma from pituitary-selective thyroid hormone resistance accompanied by pituitary incidentaloma (Akiyoshi et al., 1996). Importantly, THRB mutations have been found to be similar in both diseases (Dumitrescu and Refetoff, 2013).

Cytogenetics

Both forms, PRTH and GRTH are linked to mutations in THRB expressing TRβ1 TRβ2 and TRβ4 isoforms in tissue-specific pattern (see Diagram 2 and 3). Despite striking differences among the clinical presentations between these forms of RTH, there is a lack of direct genotype-phenotype correlation and almost identical THRB gene mutations have been observed in PRTH or GRTH patients. Nevertheless, there are several association studies showing such correlations. Germline mutations associated with GRTH (P453S, G345S) have been reported to impair both TRβ2 and TRβ1 function proportionally, whereas mutations associated with PRTH (R338L, R338W, R429Q) have been demonstrated to disproportionately disrupt TRβ2 function (Wan et al., 2005). Moreover, TRβ mutants R383H and R429Q have been shown to have greater impairment of transactivation on negatively than positively regulated promoters. These two mutants are candidates for predominantly PRTH, even though they have been clinically described as generating both, GRTH and PRTH. It has been proposed that the substitution of these charged amino-acids could disrupt the unique property of TRβ2 to bind coactivators through multiple contact surfaces. This may result in a decrease in T3-mediated feedback suppression. Consequently, the mutation affects predominantly TRβ2 mediated action of TH (Dumitrescu and Refetoff, 2013). Another proposed mechanism for PRTH is a "double-hit" combining a SNP and the mutant R338W (Alberobello et al., 2011). Recent studies have demonstrated that an intron enhancer region may play a critical role in the pituitary expression of the TRβ2 isoform. It has been hypothesized that intronic polymorphisms in the intronic region could modulate the pituitary expression of the mutated gene contributing to the clinical presentation of RTH. The combined coding mutation such as missense R338W and two common SNPs (rs2596623T, rs2596622C) located in the intron enhancer region of the THRB gene can generate a tissue-specific dominant-negative conditions for development of the pituitary-selective RTH. Moreover, the results suggest that rs2596623T may lead to pituitary over-expression of the mutant allele (Alberobello et al., 2011).

A novel TRβ variant - G339S has been found in several members of a family with elevated TSH, normal or low serum T4 and autoimmune thyroid disease (AITD) that was confounded with initially diagnosed RTH. This variant would not have an effect on the hypothalamic-pituitary-thyroid axis as determined by thyroid hormone binding in vitro and thyroid function tests in vivo (Larsen et al., 2013).

Somatic mutations in the THRB have been identified in some TSH-secreting pituitary tumors (e.g. TSHomas). These mutations can be identical to those occurring in the germline. However, because their expression is limited to thyrotrophs, the phenotypes that of TSH induced thyrotoxicosis. It is postulated that defective TR interfering with the negative regulation of TSH by TH is responsible for the development of the pituitary tumor (Refetoff and Dumitrescu, 2007). Interestingly, TRβ4, a dominant negative variant of TRβ1, has been proposed to affect the function of wild type TRs in the TSHomas (Tagami et al., 2011).

To be noted

Note

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