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A Signaling Network of Thyroid-Stimulating Hormone

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Abstract

Human thyroid stimulating hormone (TSH) is a glycoprotein secreted by the anterior part of the pituitary gland. TSH plays an important physiological role in the regulation of hypothalamic-pituitary-thyroid axis by modulating the release of the thyroid hormones from the thyroid gland. It induces iodine uptake by the thyroid, promotes thyroid epithelial differentiation and growth, and protects thyroid cells from apoptosis. Impairment of TSH signal transduction pathway leads to thyroid disorders such as goitre, hypothyroidism and hyperthyroidism, which can have complex clinical manifestations. TSH signaling is largely effected through two separate pathways, the adenylate cyclase and the phospholipase C pathways. In spite of its biomedical importance, a concise signaling map of TSH pathway is not available in the public domain. Therefore, we have generated a detailed signaling map of TSH pathway by systematically cataloging the molecular reactions induced by TSH including protein-protein interactions, post-translational modifications, protein translocation events and activation/inhibition reactions. We have cataloged 40 molecular association events, 42 enzyme-substrate reactions and 16 protein translocation events in TSH signaling pathway resource. Additionally, we have documented 208 genes, which are differentially regulated by TSH. We have provided the details of TSH pathway through NetPath (http://www.netpath.org), which is a publicly available resource for human signaling pathways developed by our group. We have also depicted the map of TSH signaling using NetSlim criteria (http://www.netpath.org/netslim/) and provided pathway maps in Wikipathways (http://www.wikipathways.org/). We anticipate that the availability of TSH pathway as a community resource will enhance further biomedical investigations into the function and effects of this important hormone.

Keywords: Homeostasis; Basic Metabolic Rate; HPT Dysregulation; Camp; PKA; Osteoporosis; Cretinism; Myxedema; Thyrotoxicosis; Endocrine Signaling

Introduction

Thyroid-stimulating hormone (TSH) is synthesized and secreted by the anterior lobe of the pituitary gland. TSH acts on thyroid follicular epithelium and triiodothyronine (T3) and thyroxine (T4) hormones regulate the synthesis and release of TSH at the pituitary level and indirectly affect TSH production by their effect on TRH. These hormones act on diverse types of cells in human body and are involved in the maintenance of basic metabolism. TSH secretion is regulated by thyrotropin releasing hormone (TRH) secreted by hypothalamus. TSH belongs to a subset of the cysteine-knot growth factor super family. It is a heterodimer consisting of one alpha and one beta subunit associated by non-covalent bonds [1]. TSH is closely related to luteinizing hormone (LH), chorionic gonadotropin (CG) and follicle stimulating hormone (FSH). These hormones share the same alpha subunit encoded by *CGA* gene [2] but have different beta subunits. The beta subunit of TSH is encoded by the *TSHB* gene. Excess amount of alpha subunits can be found in the free form in normal pituitary and normal placenta [1,3] indicating that the synthesis of the alpha and beta subunits is regulated independently. The alpha and beta subunits consist of 116 and 138 amino acid residues, respectively.

TSH mediates its effect through its cognate receptor, thyrotropin receptor or TSHR, located primarily on the cell surface of the thyroid follicular cells. TSHR belongs to the G protein-coupled receptor superfamily of integral membrane proteins. TSHR contains 2 subunits including a large ectodomain (alpha or A subunit) and a small ectodomain (beta or B subunit), which interacts with G proteins to initiate signaling. Binding of TSH with TSHR on thyroid epithelial cells stimulates production of iodine transporter, thyroglobulin, and thyroid peroxidase proteins, which are essential for the synthesis and secretion of thyroid hormones. A high concentration of TSH results in increased endocytosis of colloid from lumen to follicular cells and also increases release of T3 and T4 into the circulation. Low concentration of TSH lowers thyroid hormone synthesis and secretion [4]. Thyroid disorders are one of the commonest endocrine disorders affecting public health

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in form of cretinism, myxedema, thyrotoxicosis, and goitre of various subtypes [5,6]. Though the pathophysiology of thyroid disorders is known to certain extent, the underlying molecular interactions are ill-understood. In this context, a detailed documentation of molecular reactions induced by TSH, as reported in scientific literature till date, will be highly useful. Currently, a comprehensive resource of human TSH pathway is not available as a public resource. Therefore, we took an initiative to study molecular reactions occurring downstream to TSH-TSHR interactions and also documented the genes that are differentially regulated by TSH. We also generated a detailed TSH pathway model, which comprises of 104 proteins. We have made the TSH signaling pathway data available as a part of NetPath at http://www.netpath.org/pathway/NetPath_23, a resource for human signaling pathways previously developed by our group [7]. A concise map of TSH signaling pathway developed based on a set of confidence criteria is also available at http://www.netpath.org/netslim/TSH_pathway.html. We also uploaded TSH pathway in WikiPathways at http://www.wikipathways.org/index.php/Pathway:WP2032.

Manual curation strategies

We carried out an extensive search of literature relevant to TSH signaling pathway. As the first step towards this, we have created a pathway resource for molecular reactions, which occur upon stimulation by TSH. We have used PathBuilder [8] to annotate features of TSH pathway. Inclusion criteria for molecular reactions in TSH signaling pathway are (i) molecular reactions must be induced by TSH-TSHR interaction, (ii) the reactions must have been proven *in vivo,* with the exception of ligand-receptor interactions, (where even *in vitro* experimental evidence is considered), (iii) experiments must have been conducted in a mammalian system, with a preference to reactions from the human system. The proteins involved in the various reactions reported in mammalian system other than human should have orthologs in human.

Molecular associations

We have captured the protein-protein interactions (PPIs) under two categories as direct and complex. Direct interaction refers to a binary interaction, which represents either a homomeric or a heteromeric PPI. On the other hand, when proteins have been analyzed as components of a complex using co-precipitation assays, and where topology of binary association of each protein components of such a complex is unknown, such reactions were termed as complex. Additional information on protein domains and motifs, subcellular localization, post-translational modifications (PTMs), experimental methodologies and biological systems used in the investigation were provided. We have also documented the species of interacting proteins as well as the species of the cell line in which the experiment was carried out.

Enzyme-substrate reactions

PTMs brought about by enzyme-substrate reactions can change physiochemical properties, structural conformation, subcellular localization and the activity of the proteins. Therefore, PTMs assume critical role in signaling events [9-12]. We documented PTMs including phosphorylation, dephosphorylation, glycosylation and proteolytic cleavage reactions in the context of TSH pathway. Whenever an enzyme was proved to modify a substrate, then those reactions were curated as direct catalytic events. However, as immediate upstream enzymes were not reported for many of the PTMs, we have referred to them as induced. For every enzyme-substrate reactions, we have also documented additional details on protein species, type of modification and the host cell line in which reactions were studied. Site and residue information of PTMs if available, were mapped to specific sequence as provided in Ref Seq database [13]. We have also narrated information on enzymes, substrates and host cell line for every enzyme-substrate reaction in the 'Comments' section.

Protein translocation events

We have documented translocation of molecules across various sub-cellular compartments upon stimulation of TSH. We also documented the dependence of such translocation events on PPIs or PTMs, wherever such information is available. We have used standard Gene Ontology (GO) terms for denoting various subcellular compartments [14].

Activation/Inhibition

Apart from PPIs, PTMs and protein translocations, actiavtion/inhibition reactions of proteins were also reported in TSH signaling [15-18]. Such reactions could not be listed under molecular association, enzyme-substrate or translocation reactions. Ideally, these reactions can be specifically targeted to investigate their role in TSH signaling

Gene regulation

Genes that are differentially expressed upon TSH stimulation were documented. This list of genes was obtained from various experimental platforms including DNA microarrays, Northern blotting, serial analysis of gene expression and quantitative RT-PCR.

Results and Discussion

In all, we cataloged 40 molecular association events, 42 enzyme-substrate reactions and 16 protein translocation events shown in Figure 1(included as supplementary data). PTM site and residue information were available for 8 proteins. We have also catalogued more than 208 genes, which were reported to be differentially regulated at the mRNA level by TSH stimulation (Table 1). Curated data have been reviewed at various levels by curators and reviewers. We have also involved a Pathway Authority (PPM, who is a co-author) in the review process.

Based on the TSH pathway map assembled here, TSHR is known to interact with multiple G-alpha subunits such as *GNA12*, *GNA13*, *GNAQ*, *GNAO1*, *GNAI2*, *GNAI1*, *GNAI3*, *GNAS*, *GNA11* [19]. TSHR activation leads to the dissociation of the GTP bound Gα subunit from the Gβγ subunit heterodimer. These subunits further regulate the activities of adenylate cyclases, phospholipase C [20] and ion channels. Activation of the adenylate cyclases leads to the generation of cAMP [21,22] and subsequent activation of PKA dependent and independent pathways [23] mainly PKA/CREB and cAMP-*RAP1A*/*RAP1B* system, respectively. TSH also activates the RAS and PI3K dependent mitogenesis pathways [24,25]. Various studies have also proved the association of TSHR with *JAK1*, *JAK2*, *STAT3* [26], *HSPA5*, *CALR*, CANX [27] and *ATP1A1* [28].

Features annotated for pathway	Number of reactions	Number of links to published research articles
Molecular associations	40	34
Enzyme-substrate reactions	42	51
Transport events	16	16
Gene regulation	208	26

Table 1: Statistics for data annotated for TSH pathway.

Visualization of TSH signaling pathway

We applied NetSlim criteria [29] to TSH signaling pathway data and selected 60 molecules involved in 44 reactions, which were made available in NetSlim resource (http://www.netpath.org/netslim/TSH_pathway.html). These selected reactions were depicted as TSH pathway map using PathVisio [30] as shown in Figure 2 (included as supplementary data). A NetSlim map with citation is also provided with each node (molecules) linked to corresponding NetPath molecule page and the edges hyperlinked to their respective PubMed identifiers. The arrangement of molecules in the map was derived from i) inhibition/activation assays, ii) canonical pathways; and iii) review articles. The NetSlim version of the TSH pathway map can be downloaded from NetSlim database in different formats such as .gpml, .GenMAPP, .png and .pdf. The data collected for TSH pathway can be visualized using visualization software called Cytoscape [31,32]. We have uploaded NetSlim version of TSH pathway to WikiPathways in order to reach wider sections of users. Wikipathways is an open, public platform dedicated to the curation of biological pathways by and for the scientific community [33]. The pathway data is freely available for download in a variety of community standard formats.

Availability and data formats

The TSH pathway is made freely available through NetPath and NetSlim. We have provided a textual description for each of the reactions for TSH pathway in NetPath. The scientific community can involve in the corrections and enrichment of data by providing critical comments. Graphical representation of TSH pathway as seen in NetPath and NetSlim databases are provided in Figure 3 (included as supplementary data). The comprehensive set of curated data in TSH pathway can be downloaded from NetPath at http://www.netpath.org/pathway/NetPath_23. NetSlim version of the TSH pathway information can be downloaded from NetSlim at http://www.netpath.org/netslim/TSH_pathway.html. In addition, we have also provided the list of genes, which are differentially regulated by this signaling pathway in tab-delimited and Microsoft Excel formats. The pathway data is compatible with various standard data exchange formats including Proteomics Standard Initiative-Molecular Interaction (PSI-MI version 2.5) [34], Biological Pathways Exchange (BioPAX level 3) [35] and Systems Biology Markup Language (SBML 4.1.0) [36]. PSI-MI is used to represent interactions and experiments. SBML is used for simulation models of molecular pathways and mainly discovered for representation and exchange of pathway data.

Conclusions

The TSH pathway reactions cataloged in this study are annotated from published research articles using a set of defined criteria. We documented various reactions reported to be induced by TSH to execute physiological function on their target cells. Also, TSH pathway data obtained in this study would provide an appropriate platform to design high-throughput experiments to further investigate this signaling pathway. It will also provide a basic resource to integrate the ever increasing information on TSH signaling components in the future. We hope that our effort would also facilitate biomedical investigations pertaining to complex regulation of HPT axis and thereby provide insight into molecular networks in the regulation of HPT axis. The queries, suggestions and critical comments from the scientific community can enrich TSH pathway information.

Conflict of Interests

The author(s) declare that they have no competing interests.

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