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A Comprehensive Curated Reaction Map of Leptin Signaling Pathway

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Abstract

Leptin, a peptide hormone, regulates endocrine function and maintains body weight homeostasis by regulating food intake and energy expenditure via hypothalamus-mediated effects. Impairment in leptin signaling induces obesity, a major health problem worldwide. Leptin signaling regulates other physiological processes such as angiogenesis, hematopoiesis and also has effects on the reproductive, cardiovascular and immune systems. Despite its biomedical importance, a comprehensive catalog of the signaling events activated by leptin is not available as a public resource. Therefore, we initiated cataloging of the molecular reactions in this pathway to develop a detailed reaction map of leptin signaling. For the benefit of scientific community, we provided the leptin signaling pathway through NetPath (http://www.netpath.org), a freely accessible pathway resource previously developed by our group. We anticipate that leptin/leptin receptor signaling map and the data comprising these signaling events will enable future biomedical investigations.

Keywords: Cytokine; Leptin resistance; Adipocyte; Energy homeostasis; PathVisio; NetSlim

Introduction

Leptin is a peptide hormone primarily synthesized and secreted by adipocytes of white fat [1]. It is also expressed in secondary sites including stomach, placenta and skeletal muscle [1,2]. Leptin activates various pathways through leptin receptor encoded by the *LEPR* gene [3]. Leptin receptor (LEPR), also called OB receptor (OBR), is a member of gp130 family of cytokine receptors [4]. LEPR is expressed in many tissues including the hypothalamus of the brain, adipose tissue, heart, placenta, lung and liver [1,4-6]. Six isoforms of LEPR-LEPRa, LEPRb, LEPRc, LEPRd, LEPRe and LEPRf have been reported [7,8]. LEPRb is the longest leptin receptor and is described in most of the signal transduction studies [9,10]. LEPRe is the secretory isoform and is known to control circulating leptin levels [11]. Role of LEPRa, LEPRc, LEPRd and LEPRf in leptin signaling is not clear [8]. Leptin associates with its receptor in 1:1 stoichiometry and forms a tetrameric receptor/ligand complex [3,12]. LEPRb forms homodimer and can also form heterodimer with LEPRa and LEPRc [13,14]. Homodimer of LEPRb is found to be essential to transduce leptin dependent signaling [14,15]. Besides, it is also reported that heterodimers of LEPRa and LEPRb lack signaling capacity [14]. LEPRb does not have intrinsic kinase activity. Therefore, it mediates multiple signaling pathways through cytoplasmic kinases including Janus Kinase 2 (JAK2) [16].

Leptin was detected in various regions of the brain including hypothalamus, cerebral cortex and cerebellum [17]. It was found to be transported to these areas of the brain across the blood brain barrier [18]. Impairment in this transport of leptin across blood brain barrier is considered to be one of the factors that contribute to leptin resistance and thus obesity [19]. Leptin plays a major role in the regulation of energy homeostasis and neuroendocrine function [20,21]. High serum leptin levels are mainly associated with obesity. Leptin concentration is higher in obese individuals. Obesity is rather related to leptin tolerance or resistance but not due to leptin deficiency [22,23].

Leptin is also known to regulate reproduction, bone homeostasis and immune signaling [24,25]. In humans, females have twice the amount of circulating leptin than that of males [26]. A certain level of circulating leptin has implication in the maintenance of menstrual cycles and normal reproductive function [27]. Serum leptin levels determine the onset of puberty and also vary during pregnancy [28,29]. Excess levels of leptin, defective leptin signaling and leptin resistance are associated with abnormal reproductive function [30]. Previously, studies have shown that mice without a functional LEPR were obese and infertile [31,32]. Impaired leptin signaling in these obese mice had poor outcome in embryo implantation [33]. Leptin also stimulates bone growth and acts as a suppressor of bone resorption [34,35]. Being a proinflammatory cytokine, leptin also plays an important role both in innate and adaptive immunity [36]. Elevated levels of leptin in serum are associated with several autoimmune diseases including encephalomyelitis and rheumatoid arthritis [37,38] and in several chronic inflammatory conditions such as nonalcoholic hepatitis and Behcet's disease [39,40]. Further, involvement of leptin in angiogenesis and proliferation of hematopoietic cells has been reported [41-43]. Ishikawa and coworkers have shown that leptin and its receptor are overexpressed in gastric carcinoma [44]. Leptin stimulation has been shown to induce progression of estrogen dependent breast cancer cells and to promote cell proliferation of human acute myelogenous leukemia cells [45].

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Leptin has been implicated in various physiological functions. From a biomedical point of view, perhaps it is one of the most important proteins. However, the signaling pathway reactions triggered by LEP-LEPR interaction have not been cataloged in public signaling pathway resources. Therefore, we sought to create a comprehensive map of the leptin signaling pathway, which would serve as a reference for leptin signaling. Our approach to develop leptin pathway map include manual annotation of individual biochemical reactions induced by leptin. We have classified these molecular reactions into protein-protein interactions (PPIs), enzyme-substrate reactions, activation/inhibition and protein translocation events. In addition, we have also cataloged genes, which are differentially regulated by leptin signaling. Besides, transcriptional regulators of these genes were also documented.

Methodology

PubMed and Information Hyperlinked over Proteins (iHOP) were searched for articles, which described leptin signaling. We screened these articles for i) protein-protein interactions; ii) post-translational modifications; iii) altered localization of proteins; iv) activation/inhibition of proteins and v) transcriptional regulation of genes and their regulators; induced by stimulation of LEPR with leptin. We have documented individual information curated from full text of every article into PathBuilder, an in-house curation tool, previously developed by our group [46]. The criteria for such curation included: i) each reaction must have been observed under the stimulation of LEPR by leptin and should not be under the influence of multiple ligand-receptor stimulations; ii) the proteins in a reaction must be mammalian proteins; iii) reactions must have been carried out in mammalian cell lines and iv) gene expression data was captured only from normal human cells.

Protein-protein interactions (PPIs)

PPIs were curated as either binary or complex interactions. Binary reactions represent homomeric or heteromeric interaction between two molecules. Complex reactions constitute protein complexes of three or more proteins where topology of association between components is not reported. For every PPI, we have documented i) the species of the interacting proteins; ii) cellular location; iii) species of cell line in which the experiment was performed; iv) PubMed identifier (hyperlinked to its corresponding abstract) from which the reaction was captured; and v) a brief comment on PTM dependence, domains or motifs involved and the names of cell lines in which the reactions were carried out.

Enzyme-substrate reactions

Post-translational modifications (PTMs) can bring about changes in the physicochemical properties of a given protein and thereby can recruit the substrate protein to different subcellular localization [47]. Besides, PTMs can also activate or inhibit the activity of proteins. For example, phosphorylation and dephosphorylation reactions have been shown to result in activation and/or inactivation of particular proteins [48,16,49]. Therefore, we included a comprehensive search for PTMs including phosphorylation, dephosphorylation, acetylation, deacetylation and proteolytic cleavage, among others, pertaining to the leptin signaling pathway. However, we could capture phosphorylation and dephosphorylation events and not any other types of PTMs. We categorized these PTM events as direct or induced. Direct PTMs were those reactions for which the enzyme responsible for the reaction was known. The upstream enzyme was also documented as a protein interactor of the substrate. However, where such enzyme was not yet been identified, the reactions were designated as 'induced PTMs'. For most PTMs, we have also annotated the site and residue information, which were further mapped to protein sequences provided in RefSeq database. In summary, for each enzyme-substrate reaction, we have captured the following information i) the type of PTM; ii) the site and residue of modification; iii) the cellular location; iv) the species of the enzyme and the substrate involved in the reaction; v) the species of cell line; vi) activation or inhibition status of the substrate in response to PTMs as reported by specific assays and vii) PubMed identifier of the article describing the reaction. In addition, a description on the enzyme, substrate, site and residue of modification, host cell line in which the reaction was proved was provided for each enzyme-substrate reaction in 'Comments'.

Activation/Inhibition reactions

Leptin signaling was reported to activate or inhibit certain proteins as evaluated by specific assays. We documented activation and inhibition of such molecules only if these molecules were not reported to be components of any PPI or PTMs in leptin pathway.. In other words, proteins curated in "Activation/Inhibition" section will be a unique set of molecules influenced by this signaling pathway. For every activation/inhibition event, we have captured i) the species of the protein activated or inhibited; ii) the subcellular localization in which the reaction was reported; iii) species of cell line in which the experiment was carried out; iv) PubMed identifier; v) cell line in which the reaction was reported and vi) a brief description of the protein being activated/inhibited.

Protein translocation

We have documented protein translocation events that were triggered by leptin signaling. These events can be dependent on PTMs or physical interactions. In addition to protein's altered localization, we have also documented i) the species of the protein being studied; ii) species of cell line in which the experiment was carried out; iii) the PTM dependence on translocation and iv) research article hyperlinked to its abstract in PubMed. A brief description is provided in the 'Comments' section comprising the details of primary and altered localization of the protein, the PTM dependence and the cell lines in which the reaction was studied. We used controlled vocabularies as defined by Gene Ontology (GO) for denoting subcellular localization [50].

Gene regulation

We have annotated genes that are transcriptionally regulated by leptin signaling in normal human cells. This includes the genes whose differential regulation was associated with leptin pathway as investigated by DNA microarrays, Northern blotting, serial analysis of gene expression and quantitative PCR. The cell type in which these genes were identified to be regulated was also documented. In addition, we also cataloged transcriptional regulators of up- or down-regulated genes which were detected through various promoter assays pertaining to leptin signaling.

Results and Discussion

Our manual curation effort to capture leptin pathway reactions resulted in cataloging 108 molecules from 130 screened articles. The 108 molecules contributed to 54 direct and 8 complex PPIs, 20 direct and 77 induced catalytic events, 10 activation and 11 translocation events. We have also cataloged 31 and 79 genes, which were transcriptionally up- or down-regulated, respectively. In addition, we have also curated transcriptional regulators for ten of these differentially regulated genes. Each reaction curated in leptin pathway underwent a series of internal review and also a review by a Pathway Authority (G.A. who is a co-author). We would like to adopt this model for curation of pathways in

the future where we involve experts on individual pathways as Pathway Authorities and include them as co-authors on descriptions of the corresponding pathways. This will also help us in keeping the pathways updated.

Signaling modules in leptin pathway

The signaling modules are well-known sub-pathways without specific or precise boundaries in interaction networks but are commonly identified by a name such as mitogen-activated protein kinase (MAPK) or JUN N-terminal kinase (JNK) pathway. They provide the framework for the identification and representation of a group of molecules which are accepted to have specific lineage and function within interaction networks. Our curation effort of leptin pathway has captured various signaling modules reported to be induced by LEPR activation in various research articles pertaining to leptin signaling such as JAK/STAT, RAS/RAF/MAPK, IRS1/PI-3K, AMPK/ACC and PLC-gamma modules.

JAK/STAT signaling module

The stimulation of LEPR with leptin induces autophosphorylation and activation of JAK2 [51]. Activated JAK2 mediates phosphorylation of LEPR at Tyr-986, Tyr-1078 and Tyr-1141 residues (accession # NP_002294.2) and thus provides binding for downstream signaling molecules such as signal transducer and activator of transcription (STAT) proteins [52,53]. Binding of STATs to the phosphorylated residues of LEPR leads to the tyrosine phosphorylation of STATs by JAK2 [54]. Activated STATs dimerize, disassociate from the receptor and translocate into the nucleus [55]. In the nucleus, they bind to their specific target nucleotide sequences resulting in the transcription of genes such as suppressor of cytokine signaling 3 (*SOCS3*) and TIMP metallopeptidase inhibitor 1 (*TIMP1)* [56,57]. SOCS3 induced by STATs associates with Tyr-986 of LEPR and represses leptin-mediated signaling. Thus, SOCS3 mediates feedback inhibition of LEP/LEPR pathway [47]. Suppressor of cytokine signaling 7 (SOCS7), another member of the SOCS family, attenuates leptin signaling by interacting with phosphorylated STATs to prevent their nuclear translocation [58]. Another negative regulator of leptin signaling is protein tyrosine phosphatase, non-receptor type 1 (PTPN1). Cytosolic PTPN1 dephosphorylates JAK2 and STAT3 thereby negatively regulates leptin signaling [59].

RAS/RAF/MAPK signaling module

Mitogen-activated protein kinase 1/3 (MAPK1/3) pathway is another signaling module regulated by leptin signaling. Protein tyrosine phosphatase, non-receptor type 11 (PTPN11) was initially suggested as a negative regulator of leptin signaling [48] but later it was revealed that PTPN11 can also enhance leptin signaling by activating MAPK1/3 through growth factor receptor-bound protein 2 (GRB2). Activation of LEPR induces PTPN11 phosphorylation [48,53]. Phosphorylated PTPN11 provides a binding site for GRB2 leading to the activation of MAPK1/3 pathway through RAS-RAF-MEK signaling module [60].

IRS/PI-3K signaling module

Leptin regulates IRS/PI-3K by promoting the interaction and formation of SH2B/JAK2/IRS complex. In response to leptin stimulation, SH2B adaptor protein 1 (SH2B1) recruits insulin receptor substrates 1 and 2 (IRS1 and IRS2) to JAK2 and thus, inducing their phosphorylation [61]. Phosphorylated IRS1/2 further interact with p85 subunit of phosphatidylinositol 3-kinase (PI-3K) [62]. The stimulation of PI-3K leads to the activation of protein kinases such as protein kinase B (AKT1) and downstream signaling cascades such as mammalian target of rapamycin (MTOR) [63], nitric oxide synthase 3 (NOS3) [64], phosphodiesterase 3A, cGMP-inhibited (PDE3A) [65] and results in the regulation of glycogen synthase kinase 3 alpha/beta (GSK3A/B) proteins [61]. In addition, activated AKT also activates IkappaB kinases (IKKs), which induce the activation and nuclear translocation of NF-kappaB [63].

AMPK/ACC signaling module

Leptin signaling activates 5'-AMP-activated protein kinase (AMPK) module. AMPK is activated in response to a rise in AMP/ATP ratio [66]. Activated AMPKs switch off the ATP-consuming process, i.e., fatty acid biosynthesis by inactivating a key enzyme in the fatty acid biosynthesis-acetyl-CoA carboxylase (ACC). Activated AMPKs are also responsible for switching on the catabolic processes including stimulation of fatty acid oxidation in the skeletal muscle, which produces ATP [67,68]. Thus, AMPKs regulates various processes such as glucose homeostasis, hepatic gluconeogenesis and lipid metabolism [69].

PLC-gamma signaling module

Stimulation of LEPR with leptin induces the activation of Phospholipase C gamma (PLC-gamma)[70,71]. Activated PLC-gamma hydrolyses phospholipid phosphatidylinositol-4,5-bisphosphate (PIP2) to generate inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). These products regulate intracellular calcium levels and protein kinase C activation [72,70].

Graphical representation and Visualization of LEP/LEPR signaling pathway

We have curated 108 molecules involved in 62 PPIs, 97 catalytic events, 10 activation and 11 translocation events. We recently developed NetSlim [73], as an extension of NetPath, to retrieve a rather confident set of reactions from the curated data. NetSlim criteria included i) each reaction should be supported by two or more research articles and ii) the reaction proved in high-throughput experiments should have support from at least one low-throughput experiment. If a particular reaction was not supported by two different research articles the following criteria were applied: i) the reaction should be proved in two different cell types; ii) in case of protein- protein interactions, the interaction were considered if the two proteins existed as components of a complex; and iii) if the reactions were reported in expert review articles.

We applied NetSlim criteria to leptin signaling pathway data and selected 73 molecules involved in 89 reactions, which were made available in NetSlim resource (http://www.netpath.org/netslim/Leptin_pathway.html). These selected reactions were depicted as leptin pathway map as shown in Figure 1. We generated this pathway diagram using PathVisio [74], which is an improved visualization tool incorporating features of GenMAPP [75].

In the NetSlim version of leptin pathway map, each molecule represented as node in the map is linked to its corresponding NetPath molecule page and specific protein pages of other databases such as HPRD [76,77]. The edge of each reaction is hyperlinked to its respective PubMed identifiers (Map with citation). The reactions that occur downstream of LEPR stimulation are represented by solid or dashed edges. Direct reactions are represented by solid edges and dashed edges symbolize induced or indirect. Protein-protein interactions, enzyme-catalysis reactions, activation/inhibition reactions and translocation events are represented in different colors as indicated in the legend. The arrangement of molecules in the map was derived from i) inhibitor

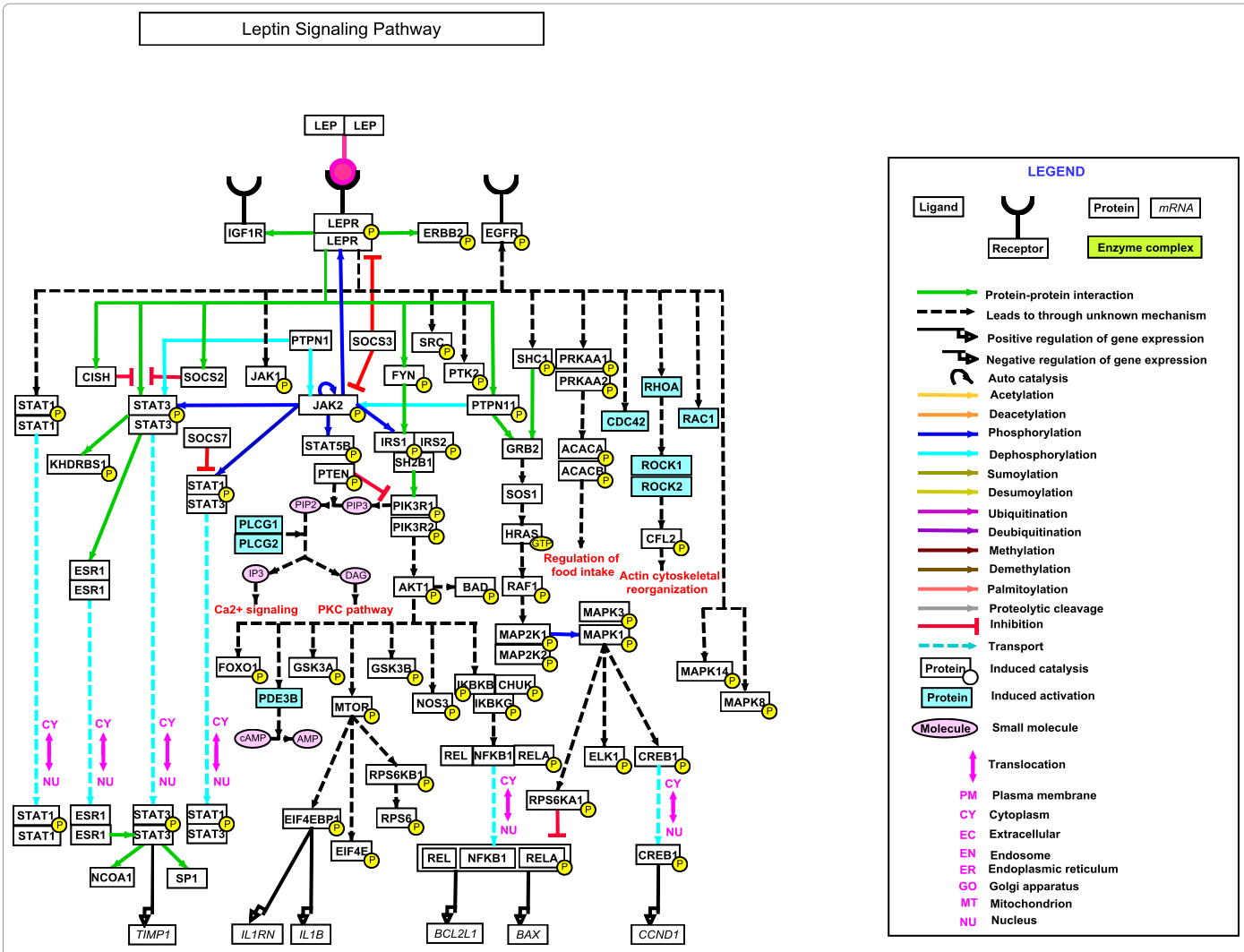

Figure 1: Representation of leptin signaling network model using PathVisio. Leptin signaling reactions selected using NetSlim criteria were used for obtaining the pathway diagram. The molecules and reactions are represented by nodes and edges, respectively. The rectangular nodes represent the gene symbols of proteins and the oval nodes represent small molecules. A legend is provided online along with pathway map at http://www.netpath.org/netslim/Leptin_pathway.html. Apart from the molecules involved in the PPIs, PTMs and protein transport reactions, we have also included molecules such as PDE3B, RHOA, RAC1, CDC42 and ROCK1/2, whose protein activity is reported to be induced by leptin receptor stimulation. The nodes are topologically arranged to represent various signaling modules activated by the leptin-leptin receptor signaling system such as JAK/STAT, RAS/RAF/MAPK, IRS/PI-3K, AMPK/ACC and PLC-gamma modules.

based assays; ii) mutation based assays; iii) knock out studies; iv) canonical pathways; and v) review articles. The NetSlim version of the leptin pathway map can be downloaded from NetSlim database in various formats such as .gpml, .GenMAPP, .png and .pdf.

Data formats and availability

NetPath is a resource of signaling pathways previously developed by our group [78]. We submitted leptin pathway data curated in this study into NetPath as an additional pathway. NetPath and NetSlim resources are made freely accessible by scientific community. Overview of leptin pathway as seen in NetPath and NetSlim databases are provided in Figure 2 and Figure 3 (included as supplementary data), respectively. Complete set of curated reactions in leptin pathway can be downloaded from NetPath at www.netpath.org/pathway/NetPath_22. The reactions in NetSlim version of the leptin pathway can be downloaded from NetSlim at http://www.netpath.org/netslim/Leptin_pathway.html. The pathway data from NetPath and NetSlim is available in various standard community data exchange formats such as Proteomics Standards Initiative for Molecular Interaction (PSI-MI version 2.5), Biological PAthway eXchange (BioPAX level 3) and Systems Biology Markup Language (SBML version 2.1) [79-81]. The availability of data in these formats allows interoperability with various pathway analysis software tools such as Cytoscape [82]. Gene regulation data is made available in tab-delimited and Microsoft Excel formats.

Conclusion

Leptin signaling pathway map was developed based on data retrieved from relevant published research articles on leptin signaling. The data presented in this study summarizes the role of leptin in the activation of multiple signaling modules implicated in various

physiological processes. In view of its biomedical importance, we anticipate that the availability of a comprehensive pathway map will further accelerate the research on leptin and its associated functions. We also seek active participation of biomedical community for the qualitative and quantitative enrichment of leptin pathway information. Biomedical experts can send their suggestions and critical comments through http://www.netpath.org/comments. Leptin pathway map is freely downloadable at www.netpath.org/pathway/NetPath_22 and http://www.netpath.org/netslim/Leptin_pathway.html in various data formats.

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