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A network map of thrombopoietin signaling

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Abstract

Thrombopoietin (THPO), also known as megakaryocyte growth and development factor (MGDF), is a cytokine involved in the production of platelets. THPO is a glycoprotein produced by liver and kidney. It regulates the production of platelets by stimulating the differentiation and maturation of megakaryocyte progenitors. It acts as a ligand for MPL receptor, a member of the hematopoietic cytokine receptor superfamily and is essential for megakaryocyte maturation. THPO binding induces homodimerization of the receptor which results in activation of JAKSTAT and MAPK signaling cascades that subsequently control cellular proliferation, differentiation and other signaling events. Despite the importance of THPO signaling in various diseases and biological processes, a detailed signaling network of THPO is not available in any publicly available database. Therefore, in this study, we present a resource of signaling events induced by THPO that was manually curated from published literature on THPO. Our manual curation of thrombopoietin pathway resulted in identification of 48 molecular associations, 66 catalytic reactions, 100 gene regulation events, 19 protein translocation events and 43 activation/inhibition reactions that occur upon activation of thrombopoietin receptor by THPO. THPO signaling pathway is made available on NetPath, a freely available human signaling pathway resource developed previously by our group. We believe this resource will provide a platform for scientific community to accelerate further research in this area on potential therapeutic interventions.

Keywords BioPAX · Cancer · Hematopoiesis · Thrombocytopenia · Thrombocytosis

Abbreviations

THPO	Thrombopoietin
MPL	MPL proto-oncogene, thrombopoietin receptor
JAK2	Janus kinase 2
STAT	Signal transducer and activator of transcription protein
MAPK	Mitogen-activated protein kinase
PIK3	Phosphatidylinositol-4,5-bisphosphate 3-kinase
MPN	Myeloproliferative neoplasms
RT-PCR	Real-time polymerase chain reaction
BioPAX	Biological Pathway Exchange
SBML	Systems Biology Markup Language
PSI-MI	Proteomics Standards Initiative for Molecular Interaction`
PTM	Post-translational modification

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Introduction

Thrombopoietin (THPO) is a 332 amino acid-long protein, which is highly glycosylated and has a molecular mass of approximately 70 kDa (Wolber and Jelkmann 2002). Production of THPO predominantly occurs in the liver while other organs including kidney, lung, spleen, bone marrow and brain also secrete the hormone in small amounts (Nomura et al. 1997). The gene encoding THPO protein is located on chromosome 3q26.33-q27 in humans (Suzukawa et al. 1995). THPO is a cytokine that has been reported to play an important role in proliferation and differentiation of megakaryocyte progenitors (Bacon et al. 1995; Kaushansky et al. 1995). It acts in early and late stages of megakaryocyte lineage to promote the proliferation of megakaryocyte progenitors and increases the ploidy of these cells (de Sauvage et al. 1996). Pro-platelet processes are formed from polyploid megakaryocytes, which later fragment into platelets (Kaushansky 2005). It has been shown that THPO and MPL knockout mice have reduced number of platelets and megakaryocytes indicating the importance of THPO signaling in maintaining the high number of platelets in blood (Murone et al. 1998). It also stimulates the growth of other blood cells including granulocytes, erythrocytes and monocytes (Wolber et al. 1999; Kaushansky et al. 1996).

Receptor for THPO is MPL (THPOR), which belongs to the type I hematopoietic cytokine receptor family (Vigon et al. 1992). The MPL gene is located on chromosome 1p34 (Le Coniat et al. 1989). MPL is predominantly expressed on the surface of megakaryocyte progenitors, platelets and hematopoietic stem cells (HSCs), where it plays a major role in maturation of megakaryocytes, regulation of platelet production, maintenance and self -renewal of HSCs (de Sauvage et al. 1996, Debili et al. 1995, Qian et al. 2007).

Thrombopoietin levels in blood are inversely related to number and size of platelets in healthy individuals. Platelet homeostasis is maintained by internalization of THPO from blood by high affinity THPO receptors (MPL) on platelets followed by degradation (Dahlen et al. 2003). Altered plasma THPO levels have been reported in several clinical conditions, including several hematological diseases associated with thrombocytopenia and thrombocytosis (Emmons et al. 1996; Kosugi et al. 1996; Cerutti et al. 1997). Thrombocytopenia, a disorder characterized by low levels of platelets in blood, has been reported in patients suffering from Immune Thrombocytopenia (ITP), HIV, Hepatitis C virus infection and cancer (Emmons et al. 1996; Ballem et al. 1992; Wang et al. 2004; Elting et al. 2001). Cancer patients who undergo chemotherapy have been reported to suffer from thrombocytopenia (Winer et al. 2015). THPO receptor agonists such as romiplostim and eltrombopag are used in thrombocytopenic patients to induce proliferation and differentiation of megakaryocytes (Erickson-Miller et al. 2009; Winer et al. 2015). In physiological conditions such as reactive thrombocytosis or thrombocythemia, platelet numbers as well as THPO levels are abnormally high in blood (Wolber and Jelkmann 2002; Cerutti et al. 1997). Reactive thrombocytosis is associated with inflammatory conditions including diseases such as acute myocardial infarction and unstable angina pectoris (Senaran et al. 2001). Plasma THPO levels have also been found to be high in cigarette smokers as compared to non-smokers and it increases platelet activation including platelet-leukocyte adhesion and P-Selectin expression on platelets in smokers (Lupia et al. 2010). Platelet activation increases the risk of cardiovascular diseases including atherosclerosis and thrombosis in smokers (Lupia et al. 2010).

Upon binding to its receptor, THPO induces various biochemical events to promote proliferation, differentiation and cell survival. THPO binding to the receptor MPL leads to the homodimerization of the receptor, which is followed by JAK2 activation. JAK2 phosphorylates tyrosine residues of receptor and creates docking sites for various signaling molecules including SHC, GRB2, SOS, VAV and CBL to initiate intracellular signaling (Murray 2007; Miyakawa et al. 1996). The major signaling pathways activated by THPO includes JAK/STAT, MAPK/ERK and PIK3/AKT (Bacon et al. 1995, Geddis et al. 2001, de Sauvage et al. 1996, Rojnuckarin et al. 1999). JAK/STAT and MAPK pathways are involved in proliferation and maturation of megakaryocyte progenitors while PI3K/AKT pathway is required for cell cycle progression (Geddis et al. 2001; Nakao et al. 2008). THPO, in combination with other cytokines, also supports survival of hematopoietic stem cells (Qian et al. 2007).

Impact of THPO signaling in cancer development is also reported in a few studies. In acute myeloid leukemia (AML), THPO has been reported to promote survival and proliferation of leukemic cells (Pulikkan et al. 2012). Mutations in MPL receptor have been identified in myeloproliferative neoplasms due to which it remains constitutively active and results in increased proliferation of hematopoietic cells (Defour et al. 2016). One of the characteristic features of myeloproliferative neoplasms (MPN) is formation of spontaneous megakaryocytic colonies. Suppression of MPL in megakaryocytes from patients of MPN in vitro decreases their colony forming ability (Li et al. 1996). In colorectal cancer, THPO facilitates the self-renewal of tumor initiating cells by activation of Wnt signaling pathway and potentiates metastasis of these cells to liver (Wu et al. 2015). In majority of cancers including head and neck cancer, patients are afflicted by thrombocytosis, which has been found to be responsible for the poor prognosis (Rachidi et al. 2014). Higher platelet count in oral squamous cell carcinoma has been shown to have direct correlation with tumor size, metastasis and overall survival rate (Lu et al. 2007). Platelets secrete various cytokines and growth factors, which play an important role in migration, invasion and

proliferation of cancer cells (Pilatova et al. 2013; Bambace and Holmes 2011).

THPO plays a vital role in cellular processes such as proliferation, differentiation and cell survival. Therefore, it is necessary to develop a THPO signaling network. In this study, we have curated literature information pertaining to THPO signaling from published literature and developed a pathway map to facilitate better understanding of THPO-induced signaling.

Materials and methods

PubMed search was performed for thrombopoietin pathway using the keywords such as thrombopoietin, thrombopoietin signaling pathway, THPOR, c-MPL and THPO. The articles were manually screened to retrieve the molecular associations, enzyme-substrate reactions, activation/inhibition reactions, gene regulation events and protein transport reactions involved in thrombopoietin pathway. The information was curated using PathBuilder, a web-based in-house tool developed by our group (Kandasamy et al. 2009). A detailed map of thrombopoietin was developed and submitted to NetPath (Kandasamy et al. 2010). Several other signaling pathways were curated which include; oxytocin receptor signaling network (Chatterjee et al. 2016), brain derived neurotropic factor (BDNF) pathway (Sandhya et al. 2013), aryl hydrocarbon receptor (Yelamanchi et al. 2016), macrophage migration inhibitory factor (Subbannayya et al. 2016); and endothelial TEK tyrosine kinase (Khan et al. 2014) signaling pathways, which are available in NetPath (Kandasamy et al. 2010). A map of pathway reactions involved in thrombopoietin pathway was generated using PathVisio (van Iersel et al. 2008). In addition, a confident signaling network of THPO is drawn by following the NetSlim criteria as described previously (http://www.netpath.org/netslim/) (Raju et al. 2011). All curated molecular events were reviewed by a pathway authority (subject expert in the field) to confirm the authenticity of the reactions pertaining to thrombopoietin pathway.

Results and discussion

Data integration and development of thrombopoietin signaling pathway

Literature search of all the key terms related to thrombopoietin signaling in PubMed database fetched more than 4700 research articles. These articles were screened for information related to signaling events induced by THPO. We have catalogued 48 molecular associations, 66 catalytic reactions involving post-translational modifications (PTMs), 19 protein translocation events and 43 activation/inhibition reactions along with information related to the host organism. Under

catalytic reactions, PTMs captured through annotation included phosphorylation and ubiquitination. PTMs were assessed on two modes: direct and indirect. In direct reactions, upstream enzyme was curated for specific substrate in the published literature. Indirect reactions include those where the type of modification has been experimentally proven but no information exists about its immediate upstream enzyme. The information about modification, site, residue, position, species involved, type of experiment and PubMed IDs were also captured. Genes regulated by THPO which have been validated using microarray and non-microarray techniques including northern blotting, serial analysis of gene expression and quantitative RT-PCR were also documented. One hundred genes were found to be regulated by THPO-MPL signaling, out of which 77 genes were upregulated and 23 downregulated.

Data curation and availability of data formats

The THPO pathway data is freely available in NetPath (http://www.netpath.org/pathways?path_id=NetPath_140) (Kandasamy et al. 2010). The NetPath page of THPO pathway has description of the pathway along with the number of molecules and reactions involved. A high confidence map of THPO signaling containing molecular events which follow NetSlim criteria has been included (Raju et al. 2011). A brief description of the THPO pathway as well as the pathway map has been provided in the NetSlim page of THPO pathway and can be downloaded from http://www.netpath.org/netslim/THPO_pathway.html. The data in NetPath and NetSlim maps is compatible with various standard data exchange formats including Proteomics Standards Initiative Molecular Interaction XML format (PSI-MI) (Kerrien et al. 2007), a standard format for data representation in proteomics to facilitate data comparison, exchange and verification; BioPAX (http://www.biopax.org/) (Demir et al. 2010). a standard language that enables exchange, visualization and analysis of biological pathway data; and the Systems Biology Markup Language (SBML) (http://sbml.org/), a computer-readable format for representing models of biological processes (Hucka et al. 2003). These pathway maps can be downloaded in GenMAPP, GPML and PDF formats.

Summary of signaling events regulated by the THPO signaling pathway

The various signaling events induced by THPO binding to its receptor MPL is illustrated in Fig. 1). Thrombopoietin binds to the extracellular domain of receptor MPL, which leads to the homodimerization of the receptor to initiate intracellular signaling. Receptor dimerization allows JAK kinases, which are bound to cytoplasmic domains of MPL receptor, to cross-phosphorylate and hence activate each other (Tortolani et al. 1995). Activated JAK2 kinase
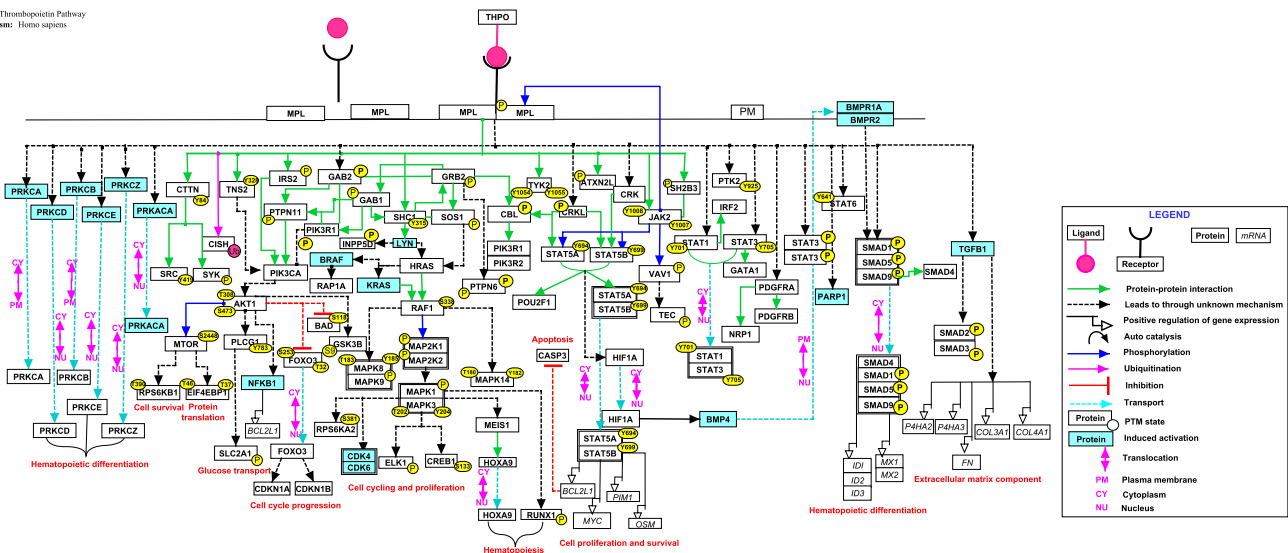
Fig. 1 A schematic representation of signaling reactions induced by thrombopoietin. The pathway map was generated using PathVisio tool and is available in NetPath. Different types of reactions in THPO pathway map are distinguished with different colors and arrows as provided in the legend. Solid and indirect arrows represent direct and indirect reactions respectively. The major pathways activated by THPO are JAK/STAT, MAPK/ERK and PIK3/AKT

phosphorylates tyrosine residues on distal portion of MPL receptor which leads to the recruitment of various proteins to the receptor via their Shc homology (SH2) or phosphotyrosine-binding (PTB) motifs (Bacon et al. 1995; Ezumi et al. 1995). JAK2 also phosphorylates and activates STAT family of latent transcription factors (Murray 2007; Bacon et al. 1995). Phosphorylation causes dimerization of STAT proteins allowing their translocation to the nucleus where they stimulate transcription of genes like *BCL2L1, cyclin D1* and *c-MYC* which have been reported to play an important role in cell survival and proliferation (Li 2008). STAT3 and STAT5 are predominantly phosphorylated in response to THPO (Miyakawa et al. 1996; Bacon et al. 1995).

THPO also activates the phosphatidylinositol-3-kinase (PI3K) pathway and the mitogen activated protein kinase (MAPK) pathways. In PI3K pathway, THPO activates PI3K, which leads to phosphorylation and activation of its downstream effector AKT (Geddis et al. 2001). AKT promotes cell cycle progression by phosphorylating transcription factor FOXO3, preventing its translocation to nucleus, which inhibits expression of cell cycle inhibitor CDKN1B and cell surface death receptor FAS protein (Nakao et al. 2008; Tanaka et al. 2001). Phosphorylation of BAD protein by AKT prevents its inhibitory effects on anti-apoptotic protein BCL2L1 thereby promoting cell survival (del Peso et al. 1997). In MAPK pathway, THPO recruits SOS1 (guanine nucleotide exchange factor) which activates RAS (Rojnuckarin et al. 1999). RAS activates RAF kinase proteins which then phosphorylate and activate cascade of MAP kinases including MAPK1, MAPK3, MAPK8, MAPK9 and MAPK14. These MAP kinases activate cell cycle regulatory genes including CDK4/6, ELK1 and CREB1 that have been

known to be involved in proliferation and differentiation of megakaryocytes (Rojnuckarin et al. 1999; Ritchie et al. 1999).

THPO signaling also plays an important role in the development of hematopoietic stem cells. In mouse embryonic stem cells, THPO has been shown to activate BMP4 which will lead to phosphorylation of SMAD proteins (Pramono et al. 2016). Phosphorylated SMAD proteins translocate to nucleus where they associate with coactivators and regulate expression of genes including RUNX1, GATA1, GATA2, MSX and TAL1 which are involved in HSC development (Jeanpierre et al. 2008; Pramono et al. 2016). In human UT7 cells, thrombopoietin increases the expression of MEIS1 and induces its interaction with HOXA9 resulting in the nuclear accumulation of this complex which plays key role in HSC development (Kirito et al. 2004).

THPO has been reported to be involved in development and maintenance of some cancers. PI3K/AKT/mTOR pathway activated by THPO helps in survival and proliferation of MPL expressing cells in acute myelogenous leukemia (Pulikkan et al. 2012). THPO/MPL signaling promotes proliferation and survival of leukemia cells by upregulation of BCL2L1 through activation of JAK/STAT and MEK/ERK pathways (Chou et al. 2012; Nishikawa et al. 2014). In hepatoblastoma cells, THPO induces proliferation, migration, chemo invasion and cell survival by activating PI3K/Akt and NF-KB pathways (Romanelli et al. 2006).

Conclusions

The manually curated and freely available detailed map of THPO pathway developed in this study will facilitate better

understanding of thrombopoietin-induced signaling and the impact of thrombopoietin-mediated physiological disorders such as thrombocytopenia and cancer. More molecules and reactions are now under thrombopoietin pathway in public data, which will be exchanged automatically across databases, and will become a part of several gene-set enrichment tools. This will pave the way for discovery of more diseases where this pathway has potential role. All valuable suggestions and critical comments from scientific community are welcome through NetPath http://www.netpath.org/comments.

Compliance with ethical standards

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

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