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TECHNICAL REPORT

A pathway map of prolactin signaling

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Abbreviations

PRL	Prolactin
PRLR	Prolactin receptor
PL	Placental lactogen
GH	Growth hormone
JAK2	Janus kinase 2
STAT1	Signal transducer and activator of transcription 1 91 kDa

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MAPK	Mitogen activated protein kinase
PI3-Kinase	Phosphoinositide 3-kinase
AKT1	v-akt murine thymoma viral oncogene homolog 1
RAC1	ras-related C3 botulinum toxin substrate 1
IRS1	Insulin receptor substrate 1
IRS2	Insulin receptor substrate 2
MTOR	Mammalian target of rapamycin
GSK3B	glycogen synthase kinase 3 beta
PAK1	p21 protein (Cdc42/Rac)-activated kinase 1
BioPAX	Biological PAthway eXchange
PSI-MI	Proteomics Standards Initiative for Molecular Interaction
SBML	Systems Biology Markup Language

Introduction

Prolactin (PRL) is a pleiotropic polypeptide hormone secreted primarily by the lactotrophic cells of anterior pituitary gland in vertebrates (Freeman et al. 2000). This hormone family includes placental lactogen (PL) and growth hormone (GH) (Corbacho et al. 2002). Prolactin plays a major role in lactation and reproduction and has been shown to have a multitude of effects relating to growth, development, metabolism, immunoregulation and protection (Ben-Jonathan et al. 2006). The prolactin signaling pathway is initiated by the binding of prolactin with the prolactin receptor (PRLR), a member of class I cytokine receptor superfamily (Binart et al. 2000), which is expressed in a variety of tissues. The PRLR comprises of an extracellular ligand binding domain, a transmembrane domain and an intracellular domain. The PRLR lacks intrinsic kinase activity and transduces signal through kinases that interact with its cytoplasmic tail. Three constitutively active variants of the receptor have been reported in humans (Goffin et al. 2010). Though the signaling reactions downstream of the longest isoform of prolactin receptor have been well established, little is known about prolactin signaling initiated by six other isoforms (Bouilly et al. 2011). Studies also indicate that binding affinity of the human prolactin receptor to nonhuman prolactin is lower than human prolactin (Utama et al. 2009). The prolactin receptor also binds to hPL and hGH leading to the activation of downstream pathways. However, we have not considered these reactions in the current study. This study reports only those reactions, which occur upon stimulation of prolactin receptor with prolactin, based on the criteria described previously (Nanjappa et al. 2011).

Availability of signaling pathway information is useful to the biomedical research community, especially for systems biology approaches. Considering this, we have developed 'NetPath' as a resource of ligand-receptor specific signal transduction pathways (Kandasamy et al. 2010). As a part of this, we have carried out manual annotation of available information from the published literature for ligand-receptor signaling pathways (Raju et al. 2011a; Nanjappa et al. 2011; Telikicherla et al. 2011; Goel et al. 2012). Similarly, in this study, we enriched publicly available information pertaining to prolactin-prolactin receptor dependent signaling pathways and also generated a graphic map depicting the prolactin signaling pathway.

Materials and methods

A literature survey was carried out using PubMed for retrieving articles related to prolactin signaling using search terms 'Prolactin', 'Prolactin Receptor' and 'Prolactin signaling'. We have categorized the molecular information into protein-protein interactions (PPIs), enzyme-substrate reactions (post-translational modifications), protein translocation events, activation/inhibition reactions and gene regulation. We have used PathBuilder, a software used for managing manually curated signaling data (Kandasamy et al. 2009). Information relevant to protein-protein interactions, enzyme-catalyzed reactions, subcellular translocation events, activation/inhibition reactions of proteins were captured from human and other mammalian cells/cell lines. Prolactin-induced transcriptional regulation of genes was also documented. In addition, we have also included information on transcriptional regulators downstream of prolactin signaling. For each category of events, along with the specific data, information has been provided for the source of protein, type of experiment and interaction location. The PubMed identifier of the research article, from which a particular reaction was annotated, has been provided as a hyperlink.

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The reactions pertaining to prolactin signaling pathway in NetPath were filtered using NetSlim criteria described previously by our group (Nanjappa et al. 2011; Raju et al. 2011a, b). The layout of the molecules in prolactin pathway is based on the experimental evidence gathered from the curated research articles. The pathway diagram was drawn using PathVisio (van Iersel et al. 2008). The prolactin pathway representation has been provided in NetSlim (http://www.netpath.org/netslim/) (Raju et al. 2011b).

Results and discussion

To our knowledge, this is the first pathway resource for specific molecular reactions that occur upon stimulation of PRLR by prolactin. We have screened over 20,000 research articles and annotated the reactions from 202 articles. We could document 87 PPI events in prolactin signaling. Additionally, we annotated 97 catalytic reactions and 21 protein translocation events under prolactin stimulation. Further, we have also documented 118 genes that were reported to be differentially regulated by prolactin in various human cells. A total of 116 molecules have been included in the prolactin pathway based on our approach. We have included the data for prolactin signaling in NetPath (accessible at http://www.netpath.org/pathways?path_id=NetPath_56).

Molecular reactions that fulfill NetSlim standards were selected and graphically depicted using PathVisio. To generate the prolactin pathway illustration we have used only 84 molecules, which fulfilled NetSlim criteria, out of the 116 molecules annotated. The illustrative map of the pathway reactions is provided in Fig. 1. The prolactin pathway

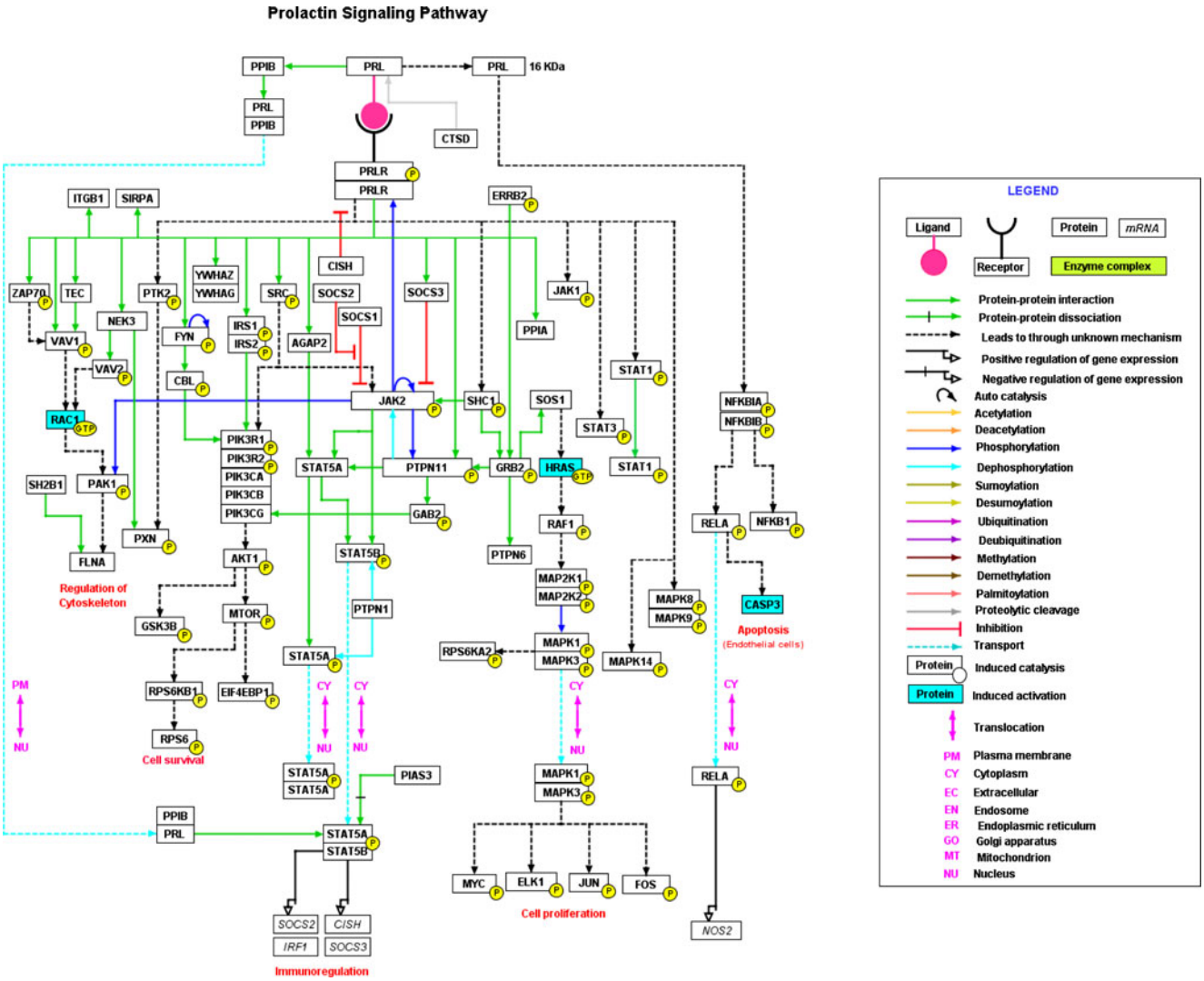

Fig. 1 Schematic representation of the prolactin signaling network in NetSlim. Prolactin signaling pathway represents a set of reactions induced by the binding of prolactin to the prolactin receptor. Multiple colors differentiate protein-protein interactions, enzyme-catalysis events and translocation reactions in the pathway. The major downstream signaling modules that are activated include JAK/STAT, RAS/RAF/MAPK, PI3-Kinase/AKT and RAC signaling

map can be accessed from NetSlim at http://www.netpath.org/netslim/NetSlim_56. The prolactin signaling map (map with citation) in NetSlim has citations for each reaction(s) linked to the reference articles in PubMed. Description of each protein involved in the signaling has been linked to its respective page in NetPath and to the molecule page of other resources such as HPRD (Goel et al. 2011). Genes which are downstream targets of the prolactin pathway are linked to their corresponding description under NCBI.

In humans, a 23 kDa full length prolactin and a 16 kDa N-terminal fragment of prolactin (16 K prolactin) have been reported. Full length prolactin has been reported to stimulate angiogenesis whereas 16 K PRL fragment has potent anti-angiogenic and vasoconstrictive activities (Struman et al. 1999). The 16 kDa prolactin fragment does not appear to bind to the PRLR (Corbacho et al. 2000) but is able to activate programmed cell death in the endothelial cells (Martini et al. 2000) (Fig. 1). The principal signaling pathway activated by prolactin-PRLR interaction is the JAK/STAT pathway. Prolactin binding induces the dimerization of the receptor and activation of JAK2, a kinase constitutively associated with PRLR (Pezet et al. 1997). JAK2 phosphorylates multiple tyrosine residues of PRLR and enables the binding of signal transducer and activator of transcription (STAT) proteins (DaSilva et al. 1996). Tyrosine-phosphorylated STAT5 dissociates from the receptor, dimerizes and translocates into the nucleus where it binds to the promoters of target genes. Even though JAK/STAT pathway is considered as one of the major downstream pathways for cytokine receptor signaling, prolactin also activates the MAP kinase pathway. Das et al., 1996 described the participation of RAS/RAF/MAPK cascade in prolactin mediated cell proliferation in mammary epithelial cells (Das and Vonderhaar 1996). Activation of the PI3-Kinase pathway upon prolactin exposure has been reported in multiple target cells. The pathway activation is dependent upon SRC family kinases in multiple cell types and it promotes survival of lymphoid cells (Dominguez-Caceres et al. 2004; Clevenger et al. 2003; Piazza et al. 2009). In response to prolactin, insulin receptor substrate 1 and 2 (IRS1 and IRS2) are phosphorylated in COS cells transfected with PRLR and have been shown to be associated with the regulatory subunit of PI3-kinase (Yamauchi et al. 1998). PI3-Kinase further activates AKT1 and leads to the activation of MTOR and its downstream targets (Bishop et al. 2006).

Prolactin also regulates cytoskeletal re-organization through the activation of the RAC pathway. The activation of Rho family of small GTP binding proteins such as RAC leads to the activation of p21 protein (Cdc42/Rac)-activated kinase 1 (PAK1) (Rider et al. 2007). These pathways and others, such as those involving tyrosine kinases Fyn, Tec, the guanine nucleotide exchange factor Vav, the phosphatase SHP-2, the signaling suppressors SOCS and CIS (Clevenger et al. 2003), prolyl isomerase cyclophilin A (Clevenger et al. 2009) are presented in the NetPath web resource.

Prolactin signaling pathway information is compatible with multiple data exchange formats such as PSI-MI version 2.5 (Hermjakob et al. 2004),BioPAX level 3 (Demir et al. 2010) and SBML 2.1 (Hucka et al. 2003). The data for the prolactin signaling pathway is freely available for download in the formats mentioned above. The pathway diagram can be downloaded from NetSlim in .gpml, .pdf, and .png formats. We have also provided prolactin pathway information in Wikipathways (http://www.wikipathways.org/index.php/Pathway:WP2037).

Conclusions

Analysis of signaling events that occur upon interaction of specific ligand-receptor combinations are crucial for our understanding of biological processes that they regulate. We anticipate that the prolactin signaling map available through NetPath (http://www.netpath.org/pathways?path_id=NetPath_56) will facilitate further studies on prolactin associated signaling and related disorders. We will continue to update the prolactin pathway on a regular basis as more published literature becomes available.

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