A network map of netrin receptor UNC5B-mediated signaling

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Abstract

UNC-5 Homolog B (UNC5B) is a member of the dependence receptor family. This family of receptors can induce two opposite intracellular signaling cascades depending on the presence or absence of the ligand and is thus capable of driving two opposing processes. UNC5B signaling has been implicated in several cancers, where it induces cell death in the absence of its ligand Netrin-1 and promotes cell survival in its presence. In addition, inhibition of Netrin-1 ligand has been reported to decrease invasiveness and angiogenesis in tumors. UNC5B signaling pathway has also been reported to be involved in several processes such as neural development, developmental angiogenesis and inflammatory processes. However, literature pertaining to UNC5B signaling is scarce and scattered. Considering the importance of UNC5B signaling, we developed a resource of signaling events mediated by UNC5B. Using data mined from published literature, we compiled an integrated pathway map consisting of 88 UNC5B-mediated signaling events and 55 proteins. These signaling events include 27 protein-protein interaction events, 33 catalytic events involving various post-translational modifications, 9 events of UNC5B-mediated protein activation/inhibition, 27 gene regulation events and 2 events of translocation. This pathway resource has been made available to the research community through NetPath (<http://www.netpath.org/>), a manually curated resource of signaling pathways (Database URL: http://www.netpath.org/pathways?path_id=NetPath_172). The current resource provides a foundation for the understanding of UNC5B-mediated cellular responses. The development of resource will serve researchers to explore the mechanisms of UNC-5B signaling in cancers.

Keywords Carcinoma · Tumorigenesis · Netrin signaling pathway · Gene set enrichment · Molecular association · Dependence receptors

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Abbreviations

UNC5B	UNC5 netrin receptor B
PP2A	Protein Phosphate 2A
NTN1	Netrin-1
TS	Thrombospondin domain
DCC	Deleted in colorectal cancer
DAPK	Death-associated protein kinase
IG	Immunoglobulin Domain
DD	Death Domain
PPI	Protein-protein interaction
PTM	Post-translational modification

Introduction

UNC-5 Homolog B (UNC5B) is a well-known member of the dependence receptor family. This class of receptors is known for its peculiar ability to induce two opposite intracellular signals: one in the presence of the ligand and another in its absence (Goldschneider and Mehlen 2010). The dependence receptor family includes more than a dozen members including p75 neurotrophin receptor (p75NTR), deleted in colorectal cancer (DCC), patched (Ptc) and neogenin (*NEOI*) among others.

UNC5B signaling has been associated with a plethora of diverse functions. UNC5B is known to promote cell death in the absence of its ligand- Netrin-1 (NTN1) whereas ligand availability results in cell survival (Llambi et al. 2001). It plays an essential role in arbitrating the blood vessel formation and repulsive effect of axonal migration through association with its ligand NTN1 (Potente et al. 2011); (Lu et al. 2004). UNC5B expression has been reported to be decreased in bladder cancer cells as compared to normal counterparts and lower UNC5B expression in tumors was associated with high recurrence rates and poor survival (Liu et al. 2013). On the contrary, increased expression of UNC5B in bladder cancer has been reported to decrease tumor cell proliferation (Kong et al. 2016). UNC5B is also known to modulate bone morphogenetic protein signaling in human adipose-derived stem cells thereby suggesting its role in bone regeneration (Hu et al. 2018). Netrin-1-UNC5B pathway has also been implicated to promote defective accumulation of adipose tissue macrophages and insulin resistance in murine models fed with high-fat diet (Ramkhalawon et al. 2014).

UNC5B is a member of the UNC5 receptor family. Mammals possess four UNC5 receptors in the cell membrane, UNC5A (UNC5H1), UNC5B (UNC5H2), UNC5C (UNC5H3) and UNC5D (UNC5H4) (Paradisi et al. 2013). In humans the gene encoding for UNC5B is located on chromosome 10q22.1 *UNC5B* encodes a 945 amino acid protein with 2 isoforms formed by alternative splicing. UNC5B is a single pass transmembrane receptor protein composed of an extracellular region, a transmembrane region, and a

cytoplasmic region. Extracellular region embraces two immunoglobulin domains (IG) and two thrombospondin domains (TS). This region is involved in ligand binding. The crystal structure of the cytoplasmic portion of UNC5B has been resolved (Wang et al. 2009). It encompasses three domains namely ZU5, UPA and DD (death domain). The cytoplasmic region contains a caspase-3 cleavage site (Llambi et al. 2001) which is engaged in downstream signaling leading to apoptosis.

Netrin family members serve as ligands for UNC5 receptors, among which Netrin-1 (Tanikawa et al. 2003; An et al. 2016), Netrin-3 (Wang et al. 1999) and Netrin-4 (Hayano et al. 2014; Lejmi et al. 2014) have been reported to bind UNC5B. However, Netrin-1 is the most studied ligand of UNC5B. Netrin-1 has been previously reported to be involved in embryonic development of the nervous system (Graef et al. 2003; Serafini et al. 1994), developmental angiogenesis (Larrivee et al. 2007; Wilson et al. 2006) and modulation of inflammatory processes (Tadagavadi et al. 2010). In addition, it has been reported to promote tumor cell invasiveness and angiogenesis in glioblastomas (Shimizu et al. 2013). Inhibition of Netrin-1 in glioblastomas has been reported to result in decreased invasiveness and tumor angiogenesis and therefore has been proposed as a potential therapeutic target (Sanvoranart et al. 2016).

Netrin-1 establishes a survival cue during the development of the nervous system. Netrin-1 triggers interaction of UNC5B and brain-specific GTPase PIKE-L. This interaction prompts PI3K cascade which suppresses apoptosis. Netrin-1 and its receptors take part in the homeostatic maintenance of intestinal epithelium. Netrin-1 also plays a role in developmental angiogenesis (Guenebeaud et al. 2010; Mehlen and Guenebeaud 2010; Tang et al. 2008). Human UNC5A-C expression is downregulated in various cancers like colorectal, breast, ovary, uterus, stomach, lung and kidney tumors (Thiebault et al. 2003). Netrin-1 is overexpressed and synthesized by autocrine signaling in metastatic breast cancer, lung cancer, neuroblastoma and pancreatic cancer. The underlying reason is likely to be a change in promoter activity (Guenebeaud et al. 2010; Mehlen and Guenebeaud 2010).

UNC5B is involved in a number of biological processes including nervous development, angiogenesis, and homeostasis of intestinal epithelium. In order to understand these biological processes, UNC5B signaling needs to be studied in detail. Furthermore, UNC5B and its receptor Netrin-1 play an important role in cancer development. Based on these previous findings, we chose this receptor for our study, and curated various signaling events mediated by UNC5B. As of May 15, 2018, only 157 articles pertaining to UNC5B or UNC5H2 were reported in PubMed, suggesting limited literature on the subject. To our knowledge, there are no resources providing a comprehensive view of the UNC5B-mediated signaling pathway for data analysis. In the current study, we have documented UNC5B signaling events in absence or

presence of its ligands in various cell lines and model organisms. The detailed signaling pathway map generated in the current study will aid in better understanding of the UNC5B pathway in health and disease.

Materials and methods

Annotation of UNC5B signaling events

Literature survey using PubMed was performed to recover research article related to UNC5B signaling using search words, “UNC5B” and “UNC5H2”. Signaling events occurring under the stimulation of Netrin binding to UNC5B were identified from the literature. Further these events were categorized into enzyme-catalyzed events, protein-protein interactions (PPIs), activation/inhibition and site-specific post-translational modification (PTMs) events with respect to their activity and gene regulation events. Manual curation of these events was carried out as per previously described NetPath annotation criteria (Kandasamy et al. 2010) for a series of signaling pathways such as RANKL/RANK (Raju et al. 2011), AGE/RAGE (Soman et al. 2013), and gastrin (Subbannayya et al. 2014). PathBuilder, a manual curation software for NetPath was used for manual documentation of signaling events (Kandasamy et al. 2009).

Information related to protein-protein interactions (PPIs), site-specific post-translational modification (PTMs) and activation/inhibition reactions of proteins were obtained from research articles studying human and other cells/cell lines. The genes which were reported to be regulated through the UNC5B receptor in different human cell types were also listed. Each reaction annotated in this pathway is linked to the respective article from which the data was curated or extracted. A textual description for each reaction specifying the cell lines and the ligand used for stimulation have been provided. The curated signaling events underwent two rounds of internal reviews and one round of external review.

Visualization of the signaling pathway

PathVisio (version 3.2.4) was used for drawing the pictorial representation of the pathway reactions (van Iersel et al. 2008). A topological arrangement of reactions was carried out to depict signaling events induced by netrin binding to the UNC5B receptor. The topology was determined using literature evidence. In addition, genes which were transcriptionally regulated by UNC5B were depicted in the pathway along with their functional role obtained by inhibition, activation, silencing and/or mutation studies.

Results and discussion

Curation of UNC5B mediated signaling events

In the current study, a total of 157 research articles were screened from PubMed using the search terms ‘UNC5B OR UNC5H2’ until May 15, 2018. UNC5B-mediated signaling events in absence or presence of its ligands in various cell lines and model organisms were documented. A total of 29 research articles were found to contain potential UNC5B signaling events. Of these, 55 unique proteins were identified in the study which showed participation in total 88 signaling events. Curation resulted in the cataloging of 33 catalytic events involving various post-translational modifications, 9 activation/inhibition events, 27 molecular association events, 17 gene regulation events and 2 transport events. To our knowledge, this is the first pathway resource cataloging UNC5B-mediated signaling events.

Development of UNC5B-mediated signaling map

The UNC5B pathway page can be accessed from Net Path (http://www.netpath.org/pathways?path_id=NetPath_172). The UNC5B pathway page provides a short description and statistics of the UNC5B-mediated signaling events. Each annotated molecule is linked to its relevant NetPath molecule page. The NetPath molecule page provides various information including gene symbol, protein summary, alternate names and links to various online resources including Entrez Gene, RefSeq, Human Protein Reference Database (HPRD), Online Mendelian Inheritance in Man (OMIM) and Swiss-Prot. In addition, it also provides its interactors and the association of the molecules with various pathways available in NetPath. Figure 1 displays a pictorial representation of the complete set of curated reactions in the UNC5B pathway. The signaling map depicts various signaling modules including ERK1/ERK2, p38 MAPK and PI3K-AKT pathways. The UNC5B pathway page in NetPath furnishes information on the pathway, along with molecules participating in the pathway and the statistics for the number of molecules and the annotated reaction for the pathway. Each of these molecules have been linked to the molecule page in NetPath which gives a brief description about the selected molecule. The molecule page links each protein captured in NetPath to other proteins-centric resources including Entrez gene (Maglott et al. 2011); (Brown et al. 2015), Human Protein Reference Database (HPRD) (Keshava Prasad et al. 2009), (Prasad et al. 2009), OMIM (Hamosh et al. 2005) and Swiss-Prot (Boeckmann et al. 2003). The UNC5B signaling pathway data can be downloaded from NetPath in various standard data exchange formats such as BioPAX level 3.0 (Demir et al. 2010), SBML level 2.1 (Hucka et al. 2003) and PSI-MI version 2.5 (Hermjakob et al. 2004).

Fig. 1 An illustrated view of Netrin-UNC5B signaling. The pathway map of UNC5B signaling represents the interaction between Netrins and UNC5B receptor and the downstream signaling events triggered by these interactions. These induced signaling events include protein-protein interactions, post-translational modifications, transport, activation/

inhibition and gene regulation events. These events have been represented by various color codes as shown in the legend. The details of post-translational events including site and residue have also been provided in the map

Summary of UNC5B-mediated signaling pathway

UNC5B interacts with death associated protein kinase (DAPK) which significantly promotes its apoptotic activity. DAPK is constitutively associated with UNC5B and auto-phosphorylates and inactivates itself in presence of Netrin-1 (Llambi et al. 2005). In the absence of Netrin-1, Protein Phosphatase 2A (PP2A) binds to the receptor complex and dephosphorylates DAPK, thus initiating the apoptotic pathway. In contrast, binding of Netrin-1 to UNC5B recruits PP2A inhibitor CIP2A to the complex which prevents the dephosphorylation and activation of DAPK and hence the initiation of the cell death program (Guenebeaud et al. 2010). Under normal growth conditions, ZU5 domain of UNC5B interacts with the UPA and DD regions of this receptor, fashioning a closed L-shaped supramodular conformation and thus keeping it in the inactive state. In response to death signals, the supramodule region opens up, exposing the hidden death domain thereby bolstering apoptosis (Wang et al. 2009). p53 also promotes apoptosis through UNC5B by binding to intron-1 of UNC5B and increasing its mRNA

expression. However, when Netrin-1 binds to UNC5B, p53-dependent apoptosis is inhibited (Tanikawa et al. 2003). Netrin-1 activates PI3K/Akt signaling through UNC5B. Akt phosphorylates PIKE-A at Ser-472, resulting in its association with UNC5B. This blocks UNC5B apoptotic fragmentation and inhibits apoptosis. Activated Akt in turn phosphorylates MDM2 on Ser-166 and Ser-186. These phosphorylation events promote its translocation into the nucleus. MDM2 binds p53 in the nucleus and targets it for proteasomal degradation. p53 degradation in presence of netrin-1 causes down-regulation of UNC5B, promoting survival (He et al. 2011).

In the current signaling map, UNC5B was found to influence several members of the MAPK family including MAPK1/3 (Koch et al. 2011), MAPK14 (Lv et al. 2015), MAP2K2 and MAP2K1 (Lv et al. 2015). In addition, several other signaling modules including PI3K-AKT (Tang et al. 2008; He et al. 2011), FAK (An et al. 2016) and RAF1 (An et al. 2016) were found to be activated. UNC5B signaling was found to regulate the gene expression of members of the cytokine family including *IL1A*, *IL10*, *IL6*, *CCL2* (*MCP-1*) and *TNF* (Tadagavadi et al. 2010). In addition, several genes

including *ICAM1*, *VCAM1*, *SELE* (Wang et al. 2008), *ARHGEF12* (*LARG*), *RGMA*, *NEO1* (Mediero et al. 2015), *CTGF* (*CCN2*) (Qi et al. 2015), *ITGB4*, *PTPA* (*PP2A*) (An et al. 2016), *COL1A1* and *ALPL* (Sato et al. 2017) were found to be regulated by UNC5B.

Conclusions

UNC5B signaling is involved in nervous development, angiogenesis, and turnover of the intestinal epithelium. Furthermore, UNC5B and its receptor Netrin-1 play a role in carcinogenesis and cancer progression. However, since data pertaining to UNC5B signaling was scattered, we generated a comprehensive signaling pathway map by cataloging UNC5B-mediated signaling events from literature. We anticipate that the availability of a detailed pathway map of UNC5B signaling will serve as a useful resource for researchers to identify signaling modules playing important roles in normal physiology and disease and expand the current understanding on this unique pathway. In addition, the UNC5B pathway map will serve as a baseline to identify diagnostic markers and therapeutic targets for cancers and aid in designing experiments towards a better understanding on UNC5B signaling.

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Compliance with ethical standards

Declaration of interest The authors report no declarations of interest.

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