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Rubicon: LC3-associated phagocytosis and beyond

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Abstract

Rubicon (*Rubcn*) was initially identified as a component of the Class III PI3K complex and a negative regulator of canonical autophagy and endosomal trafficking. However, Rubicon has attracted the most notoriety because of its critical role in LC3-associated phagocytosis (LAP), a form of non-canonical autophagy that utilizes some components of the autophagy machinery to process extracellular cargo. Additionally, Rubicon has been identified as a key modulator of the inflammatory response and viral replication. In this review, we discuss the known functions of Rubicon in LAP and other signaling pathways and examine the disease pathologies associated with Rubicon dysfunction in animal models and humans.

Keywords

autophagy; LC3-associated phagocytosis; autoimmunity; immunology; interferon; endosome

Introduction

The *Rubcn* gene was first identified in 1996 from the cDNA library of human myeloid cell line KG-1 and named KIAA0226 [1]. In 2009, two research groups simultaneously identified KIA0226 as a novel Beclin 1-binding protein, and hence dubbed as Rubicon (RUN domain and cysteine-rich domain containing, Beclin 1 - interacting protein) [2, 3]. Rubicon is ubiquitously expressed in most tissue and organs [4, 5], but the mRNA expression of *Rubcn* is most abundant in the spleen, testis, cerebral cortex, and lymph node compared with other tissues (Figure 1A–B). The human *Rubcn* gene is located at chromosome 3q29 that can encodes three protein isoforms. The mouse *Rubcn* gene is located at chromosome 16 containing 23 exons that produce two protein isoforms by alternative splicing [6]. Protein alignment reveals an 84% sequence similarity between human and mouse Rubicon and that Rubicon is conserved among vertebrate species [7].

The Rubicon protein is comprised of multiple functional domains that modulate a variety of intracellular signaling cascades via interaction with its binding partners. It contains a RUN domain, which interacts with GTPases, two serine-rich regions (S-R), a coiled-coiled

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domain (CCD), multiple helix-coil-rich repeats, and a cysteine-enriched FYVE-like [8, 9]. These different domains mediate specific protein-protein interactions that dictate its downstream function (Figure 1C). Furthermore, Rubicon can undergo phosphorylation events that can affect the protein interaction and downstream signaling [9].

Since its characterization almost a decade ago, Rubicon has been found to be involved in many signaling pathways and cellular responses, with its role in LC3-associated phagocytosis (LAP) attracting the most attention. Just as Julius Caesar crossing the river Rubicon committed his troops to war with Rome, activating the protein Rubicon can commit a cell to LAP, while inhibiting canonical autophagy. The ability to distinguish canonical autophagic processes from non-canonical ones is an area of great interest, as canonical autophagy serves a critical role in cellular quality control and the ability to specifically modulate non-canonical autophagy via Rubicon could prove to be beneficial therapeutically. In this review, we will examine the multi-faceted role of Rubicon in both canonical and non-canonical autophagy, immunity, and inflammatory diseases.

Rubicon in macroautophagy

Macroautophagy (hereafter autophagy) is catabolic cell survival pathway by which eukaryotic cells sequester components of their cytoplasm in de novo autophagosomes for degradation and recycling during times of energetic stress, such as starvation [10]. This process is classically considered non-selective in nature is largely orchestrated by the ATG family of proteins [11]. Sensing of energy deficits, predominately by AMP-activated kinase (AMPK), results in the inhibition of mTOR complex 1 (mTORC1) activity, which keeps autophagy in check during times of nutrient abundance [12]. In response to AMPK activity and mTORC1 inactivation, the autophagy pre-initiation complex composed of ATG13, FIP200, and ULK1/2, is formed [13]. ULK1 then phosphorylates Ambra1, a Beclin 1-binding partner, linking the activity of the pre-initiation complex, to the Class III PI3K complex, which is responsible for the generation of phosphatidylinositol-3-phosphate (PI(3)P), which plays a critical role in multiple cellular trafficking pathways and is a vital recruitment signal for the downstream ubiquitin-like conjugation systems of autophagy, the ATG5–12 and LC3-PE conjugation systems [14, 15].

Recent studies have described 3 functionally, molecularly, and location distinct Class III PI3K complexes (herein called PI3KC3) that operate during autophagy. PI3KC3s commonly contain VPS34, the catalytic subunit, Beclin 1, and VPS15 (also called p150), and the specificity of PI3KC3 are determined by different complex components which bind Beclin 1 [16]. The PI3KC3 containing ATG14 (also called Barkor or ATG14L) is required for starvation-induced autophagy and is targeted to forming autophagosomes. In addition, ATG14 has been shown to augment PI(3)P production by VPS34, indicating that during canonical autophagy, ATG14 serves as both a localization agent and activity regulator of the PI3KC3 [17].

A second PI3KC3 lacks ATG14 but contains UVRAG (UV radiation resistance-associated gene), a Beclin 1-binding protein that promotes Beclin 1-VPS34 interactions as well as Vps34 activity [18]. The role of the UVRAG-containing PI3KC3 as been controversial, as

some studies have supported its role in autophagosome formation [18, 19] while other studies have challenged this role and rather highlighted this PI3KC3's major role in endocytosis, endosomal trafficking, autophagosome maturation via its interaction with class C-VPS/HOPS [17, 20, 21].

The third PI3KC3 contains both UVRAG and Rubicon, and unlike the preceding two PI3KC3, this complex is a negative regulator of autophagy, interacting at multiple steps in the autophagic pathway. This inhibitory complex is partly induced by the master autophagy negative regulator, mTORC1. Under nutrient-rich conditions, mTORC1 binds and phosphorylates UVRAG, amplifying the association of UVRAG with Rubicon and the inhibition of autophagy [22]. Originally identified as a Beclin 1-binding partner localizing at the early and late endosomes, Rubicon was also described as a VPS34-bidining partner via its RUN domain, and this interaction inhibited VPS34 lipid kinase activity and autophagosome formation [16, 23]. Thus, Rubicon-deficient cells demonstrate increased autophagic activity, with increased ATG16L puncta, decreased levels of p62, $LC3^{+}$ puncta, and LC3-II conversion [5, 7, 23]. However, Rubicon also plays a role in inhibiting the autophagosomal maturation stage, as Rubicon-deficient cells showed a higher ratio of autophagolysosomes to autophagosomes, compared to control cells [20].

While macroautophagy is considered the main canonical autophagic pathway and nonspecifically active, the autophagy machinery can also be selectively targeted to variety of internal substrates, such as damaged organelles (mitophagy for mitochondria) [24], macromolecules (lipophagy for lipids) [25], aggregated proteins (aggrephagy) [26], intracytoplasmic microbes (xenophagy), or phagocytosed particles such as dying cells or extracellular pathogens (LC3-associated phagocytosis or LAP) (Figure 2) [27–29]. While the role of Rubicon in most forms of non-canonical autophagy have yet to be explored, recent studies have identified Rubicon as a molecule required for LAP.

Rubicon in LC3-associated phagocytosis

LC3-associated phagocytosis (or LAP) is a form of non-canonical autophagy triggered by the uptake of a particle that engages an extracellular receptor, such as Toll-like receptors (TLR), Fc receptors (FcR), or a phosphatidylserine receptor (PtdSer-R). Signaling through these receptor families during phagocytosis results in the recruitment of some, but not all, of the autophagy machinery to the cargo-containing, single-membraned vesicle, termed the LAPosome [5, 27, 29]. This autophagic machinery facilitates the lipidation and embedding of LC3-II in the LAPosome membrane, which mediates its subsequent fusion to the lysosomes wherein the cargo is efficiently processed for degradation and the proper immune response is initiated [5]. As LAP is induced by a variety of stimuli, including pathogens [5, 29], immune complexes [30], and dying cells [27, 28, 31], LAP is considered a conserved mechanism for inducing tolerance to exogenous threats, as LAP-deficient cells and animal models respond to these threats with exaggerated inflammation and pathology [5, 27, 28].

While LAP shares much of its machinery with canonical autophagy, LAP is both molecularly and functionally distinct. LAP does not require the activity of the pre-initiation complex, described above, nor is it affected my mTOR modulation [5, 27, 29, 30]. Similarly,

ATG14 is dispensable for LAP, which exclusively utilizes the UVRAG-containing PI3KC3, and its LAPosome-localized production of PI(3)P mediates the downstream recruitment of the ATG5–12 and LC3-PE ubiquitin-like conjugation systems [5]. Similar to canonical autophagy, E3-ligase complex ATG7 and ATG10 mediates the conjugation of ATG5 to ATG12 in association with ATG16L1 to form a stabilizing, multimeric complex. Conversion of cytosolic LC3-I to lipidated LC3-II is mediated by ATG4, which cleaves the LC3 precursor allowing it to be subsequently conjugated to the lipid, phosphatidylethanolamine (PE), via the activity of ATG7 and ATG3 [5]. The aforementioned ATG5/12/16L1 complex is also required for the conversion of LC3-I to LC3-II. This lipidated LC3-II is now bound to the LAPosome membrane and is required for fusion to lysosomes [5, 32] (Figure 2).

However, whereas Rubicon association with the UVRAG-containing PI3KC3 had an inhibitory role during canonical autophagy, Rubicon is required for efficient LAP [5, 28, 33]. Rubicon-deficient cells undergo normal levels of phagocytosis, yet fail to recruit LC3-II to the cargo-containing phagosome [5, 28, 33]. The Rubicon-UVRAG-containing PI3KC3 translocates to the LAPosome, independently of pre-initiation complex activity. This association or stability of the entire PI3KC3 at the LAPosome seems to rely heavily on the presence of VPS34, as the loss of VPS34 results in the loss of Beclin 1, UVRAG, and Rubicon from the LAPosome. Whereas Rubicon inhibits VPS34 lipid kinase activity during canonical autophagy, Rubicon-deficient cells failed to produce significant amounts of PI(3)P in response to LAP stimuli [5].

Rubicon's promotion of PI(3)P by VPS34 serves two critical roles during LAP – the recruitment of the ATG5–12 and LC3-PE conjugation systems and the stabilization and activation of the NOX2 complex, the major NADPH oxidase in phagocytes [5, 34]. Two components of this multimeric complex, gp91^{phox} and p22^{phox}, are constitutively associated in the membranes of intracellular vesicles. TLR of FcR stimulation during phagocytosis triggers the translocation of cytosolic factors Rac1, p47^{phox}, p67^{phox}, and p40^{phox} to the phagosome to form the active NOX2 complex, which produces reactive oxygen species (ROS) in the phagosomal lumen [5, 33, 34]. The NOX2 subunit p40^{phox} binds PI(3)P, and in the absence of PI(3)P generated via Rubicon's activity on VPS34, p40^{phox} fails to associates with the LAPosome and ROS production is impaired [5, 34].

NOX2-mediated ROS production is required for LAP, and Rubicon plays an additional role in promoting that pathway [33, 35]. Studies demonstrates that Rubicon directly interacts with the p22^{phox} subunit of NOX2 to stabilize the complex for optimal ROS production [5, 33]. In the absence of ROS (for example, in Rubicon or NOX2-deficient cells or in the presence of a ROS scavenger, such as Tiron or Catalase), recruitment of downstream LAP components, like ATG16L1, ATG7, and LC3-II, is impaired [5]. However, LAPosomes within NOX2$^{-/-}$ cells contain wild type levels of PI(3)P, and exogenous induction of superoxides (by H_20_2) can increase LC3-II localization [5]. The reliance of LAP on these two signaling factors, PI(3)P and ROS, and the ability of Rubicon to interact with both of the mediating complexes (Beclin 1 via CCD domain [33]; VPS34 via RUN domain [16]; p22^{phox} via S-R domain [33]) positions Rubicon to be a vital part of the LAP pathway.

Rubicon in endosomal trafficking

Endosomal trafficking involves the sorting of cellular cargo through a series of sequentially maturing vesicles, classically from early endosomes to late endosomes to ultimately lysosomes, where the cargo is degraded and/or processed [36]. As many pathogens encode proteins that subvert either sequestration by or function of the endosomal trafficking pathway, understanding its molecular mechanisms is of clinical significance. The transition from early-to-late endosome initiated with the recruitment of the small GTPase Rab7 to the $Rab5^+$ early endosomes, followed by Rab5 displacement and activation of Rab7, which is required for endosome maturation. Rab7 is activated by the guanine nucleotide exchange factor (GEF) class C-VPS/HOPS (homotypic fusion and vacuole protein sorting) complex, which promotes GTP binding to Rab7 [37].

As described above, UVRAG interacts with and positively regulates the class C-VPS/HOPS complex [21]. Rubicon is highly enriched on $Rab5^+$ early endosomes, which in turn would prevent UVRAG interactions with late endosome localized class C-VPS/HOPS complex. Once active, Rab7 competes for Rubicon binding, relinquishing UVRAG and promoting the UVRAG-class C-VPS/HOPS complex. The net result of this activity is amplification of Rab7 activity and early-to-late endosome maturation [8]. Biologically, Rubicon acts as a negative regulator of endocytic trafficking, as cells that overexpress Rubicon contain abnormal lysosomal morphology and decreased transport and degradation of internalized receptors (such as EGFR) to the lysosome [7]. Conversely, Rubicon-deficient cells demonstrate a defect in recycling of transferrin receptor back to the plasma membrane [7, 8].

Rubicon in the inflammatory response

Both canonical and non-canonical autophagy have been implicated in regulation of the inflammation in response to a variety of pathogens [38]. In the presence of cytosolic DNA, Rubicon is released from PI3KC3, which activates canonical autophagy and aids in the removal of the pathogen [39]. In response to *Aspergillus fumigatus* infection, animals deficient for LAP (including $Rubcn^{-/-}$ animals) demonstrated a defect in the clearance of this fungal pathogen and a significant increase in the production of pro-inflammatory cytokines [5]. Similarly, $Rubcn^{-/-}$ macrophages produce increased amounts of pro-inflammatory cytokines during efferocytosis, the process of engulfing and clearing dying cells [28].

Recent studies have indicated that Rubicon can act as a sentinel in inflammatory response, possibly independent of autophagy or LAP. Rubicon is responsible for the feedback inhibition of the CBM complex (assembly of CARD-9, BCL-10 and MALTI). employed during Dectin-1 and RIG-I stimulation [9, 40]. CARD 9 is a key molecule utilized by various PRR signaling pathways [9, 40, 41]. To avoid excessive release of inflammatory cytokines, Rubicon targets CARD9 to disrupt the CBM complex and disengage the signaling activities [9].

Several PRRs (like TLRs and RIG-1, STING & DAI) are involved in recognition and response to viruses [42]. However, many viruses (such as HIV, herpes virus, Kaposi’s

sarcoma-associated herpesvirus [KSHV], and influenza) have adapted mechanisms to evade detection by manipulating the autophagy pathway [43, 44]. KSHV inhibits autophagosome maturation via its interaction with Rubicon [45]. Hepatitis C virus (HCV) expresses NS3–NS5B, which can induce the expression of Rubicon protein and delay the autophagosome maturation [46, 47]. HCV also delays induction of UVRAG, which aids in the accumulation of viruses in the autophagosome during the early stages of HCV infections [46, 47].

Recently, it has been shown that high expression of Rubicon results in inhibition of IFN signaling and prevents establishment of anti-viral state [48, 49]. In H1N1 influenza virus and vesicular stomatitis viruses (VSV), Rubicon interacts with IRF3 and is responsible for proteasomal degradation or dephosphorylation of IRF3 [48]. Type I interferon and Type III interferons are inhibited by Rubicon upon interaction with NEMO suppresses anti-viral state in hepatitis B virus (HBV) patients [49]. Rubicon has also been shown to have an inhibitory effect on VSV, influenza A virus (IAV), Enterovirus 71 (EV71) and Sendai virus (SeV) [49].

Rubicon in disease pathologies

Rubicon is involved in a plethora of signaling pathways at the cellular level. Rubicon has also been implicated in several disease states in both human and mouse model systems (Figure 3). The first documentation of Rubicon's effects on human health was reported in 2013, wherein a homozygous mutation in *Rubcn* was identified in a consanguineous family with early onset recessive ataxia [50]. Recessive ataxia is a group of rare neurological disorders characterized by incoordination of gait and limbs, dysarthria, and impaired eye movements [51]. Based on the major sites of degeneration, it can be further classified into cerebellar, spinocerebellar and sensory ataxias. While mutations in the genes that encode mitochondrial, DNA repair, membrane cytoskeleton, and cytosolic chaperone proteins have been identified in ataxia patients, a homozygous frameshift mutation in *Rubcn* (c.2624delC, p.Ala875ValfsX164) was found to co-segregate with a novel form of early onset recessive ataxia [4]. *In vitro* studies further demonstrate that the truncated Rubicon lost its ability to co-localize with Rab7 at late endosomes, linking defective endosomal trafficking to the disease development [50]. Rubicon has also been implicated in other human pathologies, such as non-alcoholic fatty liver disease (NAFLD), cholestasis [52], and LPS-induced stroke [53], though the molecular mechanisms are largely unknown.

Defective immune responses against self-antigens are at the center of the development autoimmune and autoinflammatory disorders. Studies have shown that mutations that impair autophagic pathways play a role in the development of autoimmune syndromes, as GWAS have revealed associations between human patients of autoimmune diseases with mutations or single nucleotide polymorphisms (SNP), in autophagic genes controlling the autophagic pathway [54, 55].

Systemic lupus erythematosus (SLE) is a systemic, multifactorial autoimmune disease, with pathogenesis and severity linked to defective efferocytosis [56]. Animal models with impaired clearance of dying cells develop symptoms of a SLE-like syndrome with aging, including increased inflammation, cross-presentation, and lymphocyte hyperactivity. In

addition, polymorphisms in *Atg5* were identified among the risk loci for SLE, supporting a role for autophagic processes in this disease [57].

Strikingly, *Rubcn*$^{-/-}$ mice (or mice with other defects in the LAP, but not canonical autophagy pathway) develop an SLE-like syndrome with aging, and pathology was shown to be associated with an impairment in the clearance of dying cells that occur under homeostatic conditions [28]. Apoptotic cells are considered “immunologically silent”, and as such wild type phagocytes that efferocytose them typically produce anti-inflammatory cytokines, such as TGFβ and interleukin-10 (IL-10), while actively suppressing pro-inflammatory cytokines, such as tumor-necrosis factor (TNF), IL-1, and IL-12 [58]. However, *Rubcn*$^{-/-}$ phagocytes produce increased levels of IL-1β and IL-6 and significantly less anti-inflammatory cytokines, such as IL-10, upon such engulfment [28]. With age, *Rubcn*$^{-/-}$ mice display significantly increased serum levels of pro-inflammatory cytokines, serum and kidney autoantibodies, interferon signature gene expression, and kidney pathology, all characteristics of human SLE [28]. Whether or not mutations in *Rubcn* are linked to any autoimmune or autoinflammatory diseases is currently being investigated.

We now recognize that intestinal microbiota can regulate the development and function of the immune system, playing an important role in inflammatory bowel disease (IBD), including Crohn’s disease (CD) and ulcerative colitis (UC). Human commensal *Bacteroides fragilis*, which is packaged into outer membrane vesicles (OMVs) for delivery to intestinal dendritic cells, has adapted beneficial immunomodulatory properties. OMVs containing *B. fragilis* activates the LAP to maintain an immunotolerant gut environment, thus protecting the host from IBD/ colitis. Likewise, *Rubcn*$^{-/-}$ mice fail to elicit T_{reg} differentiation in response to *B. fragilis* OMVs, demonstrating that Rubicon (and LAP) are critical for immunotolerance [59].

Rubicon and the LAP pathway at large has been demonstrated to play a critical role in the clearance of and immunological response to a variety of pathogens. Rubicon is required for the control of pathogens such as *Aspergillus fumigatus* [60], *Listeria monocytogenes*, and *Burkholderia pseudomallei* [61–64]. Conversely, during *Candida albicans* infection, Rubicon promotes the survival of the fungus [9, 65].

Rubicon has also been recently identified as a modulator of hepatitis B and C virus infection [46, 49]. HBV is a globally prevalent liver disease caused by the hepatitis B virus infection that can lead to cirrhosis and hepatocellular carcinoma. A recently study found that patients with HBV infection have increased Rubicon expression in peripheral blood and liver, further enhancing viral replication and antagonizing the type I interferon response [49]. Similarly, HCV induces Rubicon expression, which is beneficial for viral replication [46].

Conclusions

The identification of Rubicon as a key player in the immune response and autoimmunity allows researchers to examine the role of autophagy in a new light. As Rubicon participates in both canonical and non-canonical autophagy (albeit in opposing directions), as well as functions possibly unrelated to autophagy, it is poised to be a candidate for

immunomodulatory therapies. As Caesar's crossing of the river Rubicon represented a point of no return in his quest for Rome, perhaps engaging the protein Rubicon represents a pivotal point in immunological fate.

Abbreviations:

AMPK	AMP-activated protein kinase
ATG	autophagy
BCL-10	B-cell lymphoma/leukemia 10
C-VPS/HOPS	homotypic fusion and vacuole protein sorting complex
CARD9	caspase-associated recruitment domain 9
CBM	CARD9, BCL-10, and MALT1
CCD	coiled-coiled domain
CD	Crohn's disease
DAI-1	DNA-dependent activator of IFN-regulatory factor 1
EGFR	epidermal growth factor receptor
EV71	Enterovirus 71
FcR	Fc (Fragment, crystallizable) receptor
FIP200	FAK-interacting protein of 200 kDA
FYVE	Fab1, YOTB, Vac1 (vesicle transport protein), and EEA1
GEF	guanine nucleotide exchange factor
GTPase	guanosine triphosphate-ase
GWAS	genome wide association studies
HBV, HCV	hepatitis B virus, hepatitis C virus
HIV	human immunodeficiency virus
IAV	influenza A virus
IBD	inflammatory bowel disease
IFN	interferon
IL	interleukin
IRF	interferon regulatory factor
KSHV	Kaposi's sarcoma-associated herpesvirus

LAP	LC3-associated phagocytosis
LC3	light chain 3
MALT1	mucosa-associated lymphoid tissue lymphoma translocation protein 1
mTOR	mammalian target of rapamycin
NAFLD	non-alcoholic fatty liver disease
NEMO	NF-kappa-B essential modulator
NOX2	NADPH (nicotinamide adenine dinucleotide phosphate) oxidase
OMV	outer membrane vesicle
PE	phosphatidylethanolamine
PI(3)P	phosphatidylinositol 3-phosphate
PI3KC3	class III PI3K complex
PRR	pathogen recognition receptor
PtdSer-R	phosphatidylserine receptor
Rab5, Rab7	Ras-related protein in brain
RIG-I	retinoic acid-inducible gene I
ROS	reactive oxygen species
Rubicon	RUN domain and cysteine-rich domain containing, Beclin 1 - interacting protein
RUN	RPIP8, UNC-14, and NESCA
S-R	serine-rich
SeV	Sendai virus
SLE	systemic lupus erythematosus
SNP	single nucleotide polymorphism
STING	stimulator of interferon genes
TGF	transforming growth factor
TLR	Toll-like receptor
TNF	tumor necrosis factor
Treg	regulatory T cell

UC	ulcerative colitis
ULK1/2	Unc-51 like autophagy activating kinase
UVRAG	UV radiation resistance-associated gene
VPS34	VPS15: vacuolar protein sorting 34, vacuolar protein sorting 15
VSV	vesicular stomatitis viruses

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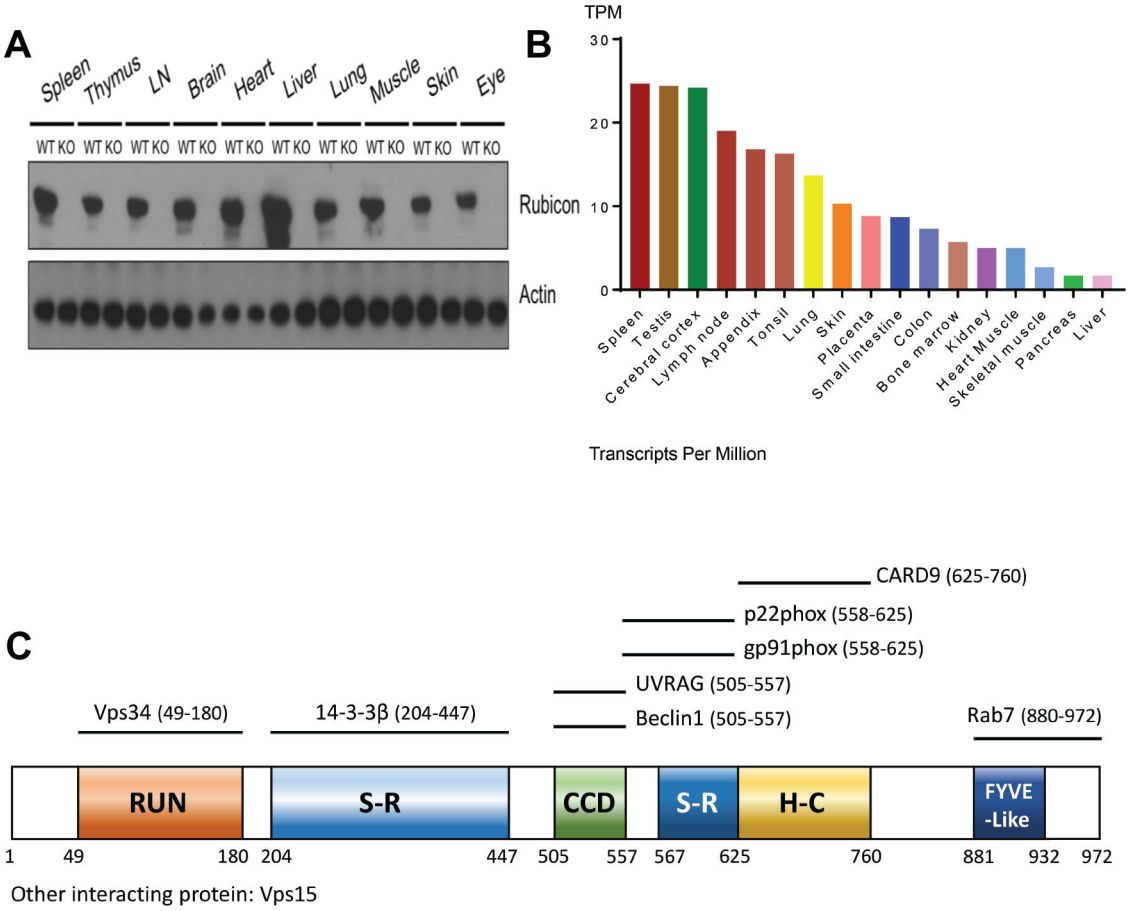

Figure 1. Protein structure and expression pattern of Rubicon various tissues

A. The expression of mouse Rubicon in multiple organs/tissues accessed by immunoblotting, originally published in [5].

B. *Rubcn* is detected in many human tissues by mRNA sequencing (data were retrieved and tailored from proteinatlas, a publically available protein expression database)

C. The schematic protein structure of human Rubicon and known sites of interaction with its binding partners. Rubicon contains multiple functional domains that mediate the protein function by interacting with other proteins. RUN, RUN domain; S-R, serine-rich region; CCD, coiled-col domain; H-C, helix-coil-rich region; FYVE-like, FYVE-like domain.

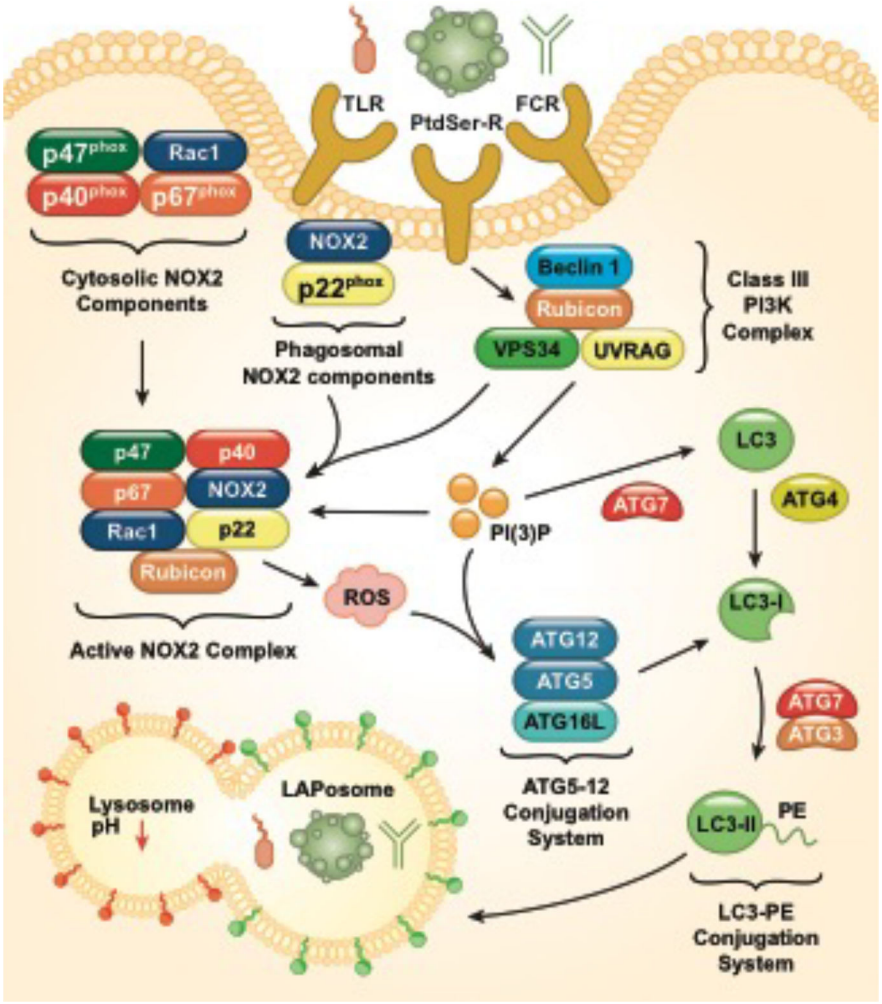

Figure 2: LC3-associated phagocytosis (LAP)

Upon engulfment of stimuli that engage Toll-like receptors (TLR), phosphatidylserine receptors (PtdSer-R), or Fc receptors (FCR), components of the LAP pathway are recruited to the cargo-containing LAPosome. The Class III PI3K complex, composed of Beclin-1, VPS34, UVRAG, and Rubicon, assembles and associates with the vesicle and is critical to the sustained and localized production of PI(3)P at the LAPosome. PI(3)P serves two roles —the recruitment of the downstream conjugation systems (ATG5–12 Conjugation System and LC3-PE Conjugation System) and the stabilization of the NOX2 complex for the production of ROS. The active NOX2 complex is assembled upon receptor engagement when cytosolic NOX2 components (p47phox, p40phox, p67phox, and Rac1) join phagosomal NOX2 components (NOX2 and p22phox) at the LAPosome. Of note, Rubicon interaction is also required for the stabilization of the NOX2 complex. Both ROS and PI(3)P are required for the subsequent lipidation and translocation of LC3-II to the single membrane of the LAPosome, and LC3-II is required for fusion to the lysosome and maturation of LAPosome.

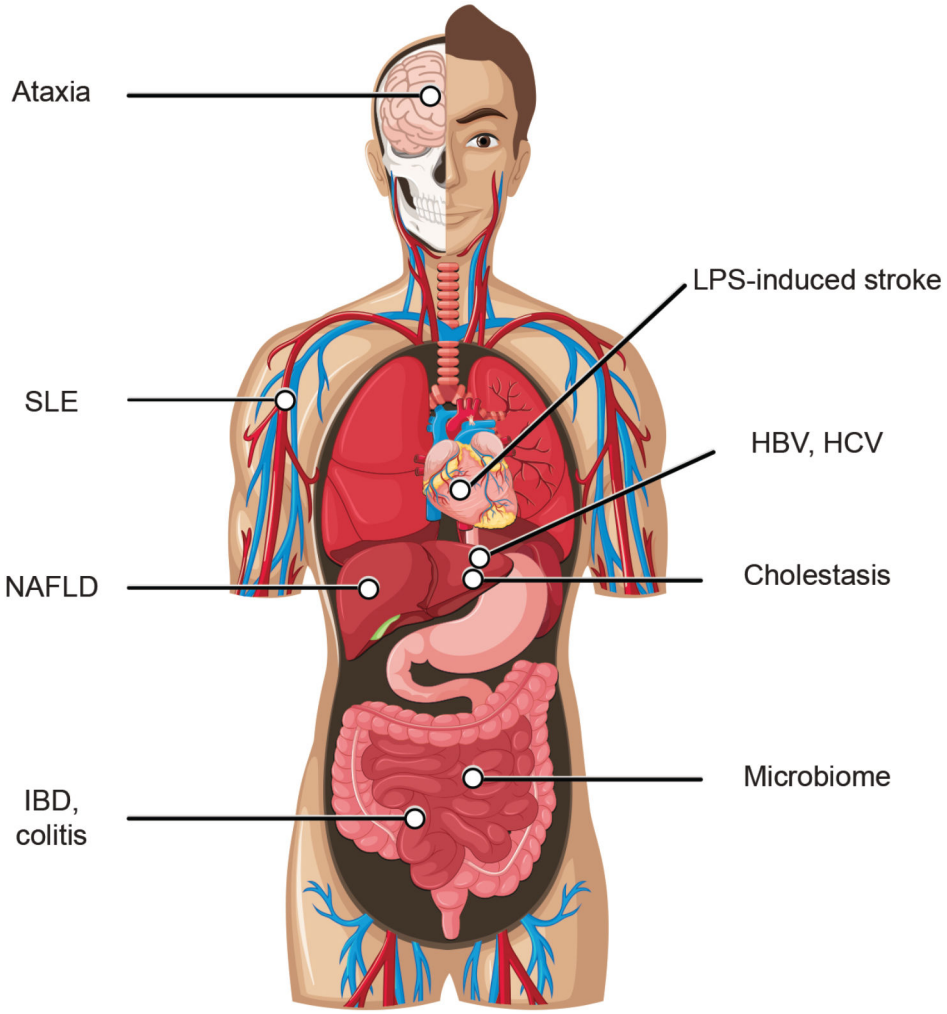

Figure 3: Rubicon-associated diseases in animals and humans

Illustration of pathologies associated with aberrant Rubicon expression or function, to date.