MINI-REVIEW

Multifaceted role of SMCR8 as autophagy regulator

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ABSTRACT

Through autophagy intracellular material is engulfed by double membrane vesicles and delivered to lysosomes for degradation. This process requires Rab GTPases, Rab GAPs and Rab GEFs for proper membrane trafficking, since they control vesicle budding, targeting and fusion. Deregulation of autophagy contributes to several human diseases including cancer, bacterial or viral infections and neurodegeneration. This review focuses on the complex roles of the newly identified protein SMCR8 and its interaction partners during formation and maturation of autophagosomes as well as regulation of lysosomal function and further discusses their implication in neurodegenerative diseases such as ALS and FTD.

Introduction

Cellular integrity depends on the equilibrium between protein synthesis and degradation. Macroautophagy, hereafter autophagy, is an intracellular recycling and degradation pathway that is initiated at established organelles and forms closed double membrane vesicles termed autophagosomes. Enclosed in these vesicles is captured heterogeneous cytosolic content such as protein aggregates, organelles and pathogens, which is intraluminally degraded upon fusion of autophagosomes with lysosomes. Since autophagy is highly dependent on membrane trafficking, including membrane fusion, fission and targeting, Rab GTPases as well as their regulators, the Rab GEF activating proteins (GEFs), are important key components for the autophagic process. Among others, several Rab GTPases, GAPs and GEFs like RAB7, Tre-2/Bub2/Cdc16 (TBC) 1 domain family member 25 (TBC1D25), RAB33B, TBC1D2A, RAB3 GTPase activating protein catalytic subunit 1 (RAB3GAP1) and 2, RAB24, RAB1, RAB11, TBC1D14 and the transport protein particle (TRAPP) complex have been studied intensively.

Recent work by several laboratories independently describes the Rab GEF protein Smith-Magenis syndrome chromosome region, candidate 8 (SMCR8) as new autophagy modulator. Until recently, the SMCR8 gene was only known to be deleted in several but not all patients having Smith-Magenis syndrome. Based on predicted structural similarities with folliculin (FLCN), SMCR8 was assigned as DENN (differentially expressed in normal and neoplastic cells) domain-containing Rab GEF protein in a bioinformatics analysis. The DENN domain conducts GDP-GTP exchange for Rab GTPases and comprises 3 subdomains with a central DENN and 2 flanking upstream DENN (uDENN) and downstream DENN (dDENN) modules separated by long linker regions.

Using mass spectrometric (MS) approaches several different groups simultaneously revealed that SMCR8 is affiliated in a complex together with chromosome 9 open reading frame 72 (C9ORF72), also a predicted DENN domain-containing Rab GEF and WD repeat domain 41 (WDR41), and downstream DENN (dDENN) modules separated by long linker regions. As for SMCR8, the cellular function of WDR41 and C9ORF72 remains enigmatic. Insertion of hundreds of GGGGCC hexanucleotide repeats within the first intron of the C9ORF72 gene is a common cause of 2 neurodegenerative diseases termed amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).

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Tight association of the potential Rab GEFs SMCR8 and C9ORF72 with WDR41
was confirmed both at exogenous and endogenous levels in cells as well as with purified components in vitro.\textsuperscript{12,16,20} SMCR8 and C9ORF72 were tightly associated as co-immunoprecipitation of C9ORF72 with SMCR8 was found to be resistant to high amounts of salt and detergent.\textsuperscript{16} In addition, overexpression or depletion of C9ORF72 increased or reduced SMCR8 protein levels, respectively and vice versa.\textsuperscript{15,16,19} Finally, size exclusion chromatography determined co-migration of SMCR8 with C9ORF72 and WDR41 in a complex of about 600 kDa.\textsuperscript{18,20} In summary, these results demonstrate stable formation of an interdependent SMCR8-C9ORF72-WDR41 complex.

The SMCR8-C9ORF72-WDR41 complex possesses GEF activity

Two groups separately showed that the SMCR8-C9ORF72-WDR41 complex provides GEF activity toward RAB8A and RAB39B but also associates with other Rab GTPases including RAB6A, RAB12, RAB25, RAB33A and RAB38.\textsuperscript{12,18} While the catalytic active subunit of the SMCR8-C9ORF72-WDR41 GEF complex is not identified yet, experimental evidence does not point to C9ORF72. First, recombinant C9ORF72 alone did not enhance nucleotide exchange for RAB8A or RAB39B.\textsuperscript{10} Second, C9ORF72 interacted with the small GTPases Arf1 and Arf6 without enhancing GDP exchange.\textsuperscript{23} In contrast, lack of C9ORF72 surprisingly stimulated GTP-bound levels of Arf6.\textsuperscript{23} Third, C9ORF72 preferentially associated with GTP-bound RAB1A and therefore likely is an effector protein and not a GEF of RAB1A.\textsuperscript{24} Interestingly, C9ORF72 or SMCR8 alone interacted or localized with additional, distinct Rab GTPases namely RAB7, RAB11 and RAB31 or with RAB24, RAB32 and RAB7L1/RAB29, respectively.\textsuperscript{12,18,24,25} The association of SMCR8, C9ORF72 or both with several different Rab GTPases give rise to a couple of potential Rab GTPase cascades, which could ensure directional maturation of vesicles, as described for RAB5 and RAB7.\textsuperscript{26} However, it remains to be determined whether SMCR8 by itself possesses GEF activity for any of the associated Rab GTPases or if the whole GEF complex is necessary. Likewise, how SMCR8 or the GEF complex achieves target specificity needs further investigation.\textsuperscript{12,18}

Autophagy modulation by the SMCR8-C9ORF72-WDR41 GEF complex

Intriguingly, numerous Rab GTPases, which associated with the SMCR8-C9ORF72-WDR41 complex, are known autophagy modulators.\textsuperscript{27} Moreover, SMCR8 as well as C9ORF72 were identified as potential autophagy regulators in an image-based RNAi screen.\textsuperscript{20} Further functional characterization of SMCR8 and C9ORF72 confirmed their role in regulating the autophagic process. However, their function remains poorly defined, especially since opposing data on the influence of C9ORF72 depletion on autophagy were reported. While 2 groups observed increased LC3B lipidation upon knockdown of C9ORF72,\textsuperscript{19,25} others demonstrated decreased LC3B lipidation or LC3B-positive puncta.\textsuperscript{12,18,24} The latter was especially apparent when autophagy was induced with the mTOR inhibitor Torin1 or inhibited with BafilomycinA1.\textsuperscript{12,18,24} Accordingly, C9ORF72 overexpression enhanced the number of autophagosomes.\textsuperscript{24} Since impaired initiation or maturation of autophagy lead to a similar LC3B lipidation phenotype, several groups performed autophagy flux assays. This method is based on fusion of red and green fluorescent proteins to LC3B to generate a doubly tagged chimera reporter construct. In immunofluorescence experiments autophagosomes appear red and green and can be distinguished from only red autophagolysosomes due to quenching of the GFP fluorescence as consequence of the acidic pH in lysosomes. While this assay most accurately assesses modulation of autophagy, lack of C9ORF72 was found to simultaneously induce a reduction and an increase in the ratio between autophagosomes and autophagolysosomes.\textsuperscript{18,19} Intriguingly, loss of C9ORF72 caused accumulation of p62-positive protein aggregates,\textsuperscript{12,24} and a decrease in the p62 protein level.\textsuperscript{15} The protein p62 (also known as sequestosome 1 (SQSTM1)) is an autophagic cargo receptor that targets several substrates to autophagosomes by which p62 is subsequently degraded.\textsuperscript{28} Typically, p62 aggregation is caused by maturation defects of autophagosomes while reduced p62 levels are indicative of enhanced autophagic flux.\textsuperscript{29} Due to these contrary effects of C9ORF72 on autophagy, further investigation is required to unequivocally establish how C9ORF72 regulates autophagy.

In SMCR8-lacking cells increased levels of lipidated LC3B and LC3B-positive puncta were observed, which again might be caused by enhanced autophagosome formation or blocked maturation.\textsuperscript{18,20} On one hand several experiments suggested a role of SMCR8 in autophagy initiation. First, depletion of SMCR8 increased the number of WIPI2- (WD repeat domain phosphoinositide-interacting protein), ULK1- (unc-51 like autophagy activating kinase) and FIP200/RB1CC1- (focal adhesion kinase interacting protein of 200 kg Dalton (kDa)/RB1 inducible coiled-coil1) positive puncta, which are all markers of autophagosome initiation sites.\textsuperscript{20} Second, in the flux assay RFP-GFP-LC3B-positive puncta were increased upon SMCR8 knockdown and further accumulated after autophagy block with BafilomycinA1.\textsuperscript{20}
On the other hand, various experiments likewise supported an influence of SMCR8 in autophagosome maturation. In another flux assays series, SMCR8-induced enhancement of the number of autophagosomes was unchanged upon treatment with leupeptin and pepstatin, which block lysosomal degradation. Second, SMCR8 increased the protein level of p62 and the number of p62-positive puncta. In summary, SMCR8 and C9ORF72 seem to both have distinct roles in different phases of autophagy including autophagosome formation and maturation.

**Regulation of the SMCR8-C9ORF72-WDR41 complex by post-translational modifications**

As SMCR8-C9ORF72-WDR41 is a functional GEF complex, the requirement of the GDP exchange capability in the formation of p62-positive protein aggregates was examined by expression of mutant variants of target GTPases. A constitutive active version of RAB39B but not RAB8A was able to reduce accumulation of p62-positive puncta caused by depletion of either SMCR8 or C9ORF72. Interestingly, SMCR8 was phosphorylated by TBK1 at serine 402 and threonine 796. These TBK1-dependent phosphorylations on SMCR8 might enhance the GDP exchange rate toward RAB39B, since expression of a TBK1-dependent phospho-mimicking SMCR8 variant inhibited protein aggregation in SMCR8, C9ORF72 or TBK1 depleted cells like constitutive active RAB39B. Furthermore, RAB39B and the SMCR8-C9ORF72-WDR41 complex interacted with the substrate adaptors p62 and optineurin (OPTN), both of which are also phosphorylated by TBK1. GEF activity regulation via phosphorylation events has previously been observed. For example, upon starvation the ULK1/2 complex phosphorylates DENND3. This enhances the GEF activity of DENND3 toward RAB12 and promotes autophagosome trafficking. In addition to TBK1, AMPK-, mTORC1- and ULK1-dependent phosphorylation sites were detected on SMCR8, whereas C9ORF72 was not found to be posttranslationally modified in these studies. Reconstitution with an ULK1-dependent phospho-mimicking SMCR8 variant left the SMCR8-induced p62-positive protein aggregates unaltered. Thus, regulation and function of these phosphorylations on SMCR8 remain enigmatic.

**Interaction partners of the SMCR8-C9ORF72-WDR41 complex function in autophagy**

SMCR8 was not only phosphorylated by ULK1 but the whole SMCR8-C9ORF72-WDR41 complex interacted with the ULK1 complex. The ULK1 complex is formed by the serine/threonine kinase ULK1, FIP200, autophagy-related protein 13 (ATG13) as well as ATG101 and upon activation phosphorylates multiple substrates, which then drive autophagosome initiation. C9ORF72 and SMCR8 associated with all subunits of the ULK1 complex as revealed by co-immunoprecipitation, pulldown and size exclusion chromatography experiments. In contrast to the interaction between the SMCR8-C9ORF72-WDR41 complex subunits, association of the GEF and ULK1 complexes was enhanced upon starvation, which suggests an important role of the holo-complex in autophagy. Indeed, lack of C9ORF72 inhibited translocation of the ULK1 complex to the nascent phagophore in a RAB1A-dependent manner upon autophagy induction. However, a potential influence of SMCR8 in this process remains to be investigated.

Concurrent with a function of SMCR8 and C9ORF72 in autophagosome formation, their effect on ULK1 kinase activity was investigated. SMCR8 depletion enhanced phosphorylation of the ULK1 substrates ATG13 and ATG14, while C9ORF72 knockdown surprisingly had the opposite effect on ATG13 and none on ATG14 even upon autophagy induction. The kinase activity of the ULK1 complex can be inhibited by disrupting the binding of ATG13 and/or FIP200 to ULK1. Yet, neither overexpression nor lack of SMCR8 impaired association of ULK1 and ATG13 at endogenous levels. Moreover, ULK1 kinase activity is modulated through phosphorylation by upstream kinases such as mTORC1 and AMPK. Upon SMCR8 depletion AMPK kinase activity was unaltered in respect to ULK1 phosphorylation, while a decrease in phosphorylation of the mTORC1-dependent substrates ULK1 and S6K1 (ribosomal protein S6 kinase B1) were reported. In addition, knockdown of C9ORF72 repressed mTORC1 activity as demonstrated via decreased S6K1 phosphorylation and enhanced TFEB (transcription factor EB) translocation into the nucleus. TFEB is a transcription factor that controls gene expression of numerous autophagic and lysosomal proteins. Mechanistically, amino acid availability causes mTORC1-dependent phosphorylation of TFEB on the lysosomal surface, which retains TFEB in the cytoplasm via association with 14–3–3 proteins. Upon amino acid starvation mTORC1 is inactivated and additionally TFEB is dephosphorylated by the calcium-activated phosphatase calcineurin. In consequence, unphosphorylated TFEB is released from 14–3–3 proteins and hence can translocate to the nucleus where TFEB regulates transcription of its target genes. Taken together, SMCR8 and C9ORF72 depletion both
inhibit mTORC1 activity whereas substrate phosphorylation of the downstream kinase ULK1 is modulated antagonistically. This indicates mTORC1-dependent and -independent regulation mechanisms of SMCR8 and C9ORF72 on the ULK1 complex, which requires further investigation.

Transcriptional regulation of various autophagic and lysosomal genes by SMCR8

While studying the modulation of mTORC1 and ULK1 by SMCR8, elevated ULK1 and S6K1 mRNA and protein levels were observed upon SMCR8 depletion. A
subsequent global mRNA expression analysis in cells lacking SMCR8 identified numerous regulated autophagosomal and lysosomal proteins, among them ULK1 and WIP2. While the majority of SMCR8 was clearly cytoplasmic, a small fraction of SMCR8 was also detected in the nucleus and on chromatin at the ULK1 and WIP2 gene loci. The SMCR8-induced gene expression regulation seems to be independent of the SMCR8-C9ORF72-WDR41 GEF complex, given that depletion of C9ORF72 or WDR41 left ULK1 protein levels unaltered. Since bioinformatics analysis software fail to predict a clear nuclear localization sequence or a DNA binding domain, SMCR8 could be classified as a STRaND (shuttling transcriptional regulators and non-DNA binding) protein. STRaNDs translocate from the cytoplasm to the nucleus and control gene expression via association with transcription factors. Many transcription factors are known gene expression modulators of ATG genes, any of which could potentially cooperate with SMCR8. For example, FOXO3 (forkhead box O3), p53 (tumor protein p53), ATF4 (activating transcription factor 4) or ZKSCAN3 (KRAB and SCAN domains 3) all regulate ULK1 gene expression. The latter further modulates WIPI mRNA just as TFEB. Yet, several questions remain unanswered about the gene expression regulation by SMCR8. First, how does SMCR8 translocate into the nucleus and which upstream signals are responsible for its nuclear localization? Potentially, phosphorylation of SMCR8 is involved in its translocation analogous to TFEB. Second, is SMCR8-induced transcriptional regulation coupled with transcription factors and if yes which ones? Also, does SMCR8 trigger histone or DNA modifications to modulate gene expression as shown for several autophagy genes?

Involvement of C9ORF72 and SMCR8 in lysosomal function and disease development

Both, C9ORF72 and SMCR8 are localized in the nucleus, throughout the cytosol and on lysosomes. Concurrent with the latter, swollen lysosomes clustered in the perinuclear region in C9ORF72 knockout cells. Moreover, C9ORF72 knockout mice showed lysosomal defects such as increased protein levels of lysosomal associated membrane protein 1 (LAMP1), prosaposin and progranulin as well as proteolytically processed cathepsin D and L. Consistent with these observations, several lysosomal proteins including LAMP2 were found upregulated in their mRNA and protein abundance in SMCR8 knockdown cells. Interestingly, lysosomal dysfunction is connected to ALS and FTD. These neurodegenerative diseases are characterized by formation of intracellular protein aggregates in neurons. A large cohort of ALS/FTD patients carry hexanucleotide repeat expansions in the C9ORF72 intron region that confer cellular toxicity via several non-exclusive mechanisms. First, sense and antisense hexanucleotide transcripts accumulate as nuclear RNA aggregates, which typically sequester RNA binding proteins. Second, the hexanucleotide transcripts are translated and resulting polypeptides form cytoplasmic aggregates. Third, the GGCGCC repeat expansion interferes with C9ORF72 mRNA expression leading to reduced C9ORF72 protein levels. However, the contribution of decreased C9ORF72 protein abundance to ALS/FTD is controversially discussed. In zebrafish C9ORF72 knockdown induced a motor deficiency, whereas C9ORF72 knockout mice displayed immunological phenotypes and a shortened life-span but no overt neurodegeneration occurred. However, lack of C9ORF72 or SMCR8 impaired clearance of p62-positive aggregates and resulted in reduced survival of cultured neurons when aggregate prone proteins like Ataxin-2 (ATXN2) were overexpressed. ATXN2 associates with RNA to control mRNA stability as well as metabolism, is prone to aggregate and is frequently mutated in ALS/FTD patients. Other genetic modifications promoting ALS/FTD include several autophagy cargo receptors, namely p62 and OPTN as well as regulatory autophagic proteins such as TBK1 and charged multivesicular body protein 2b (CHMP2B). Finally, neuronal cells of ALS patients carrying mutated C9ORF72 display impaired autophagy induction after Bafilomycin A1 treatment.

Concluding remarks

Multiple groups recently focused their attention on SMCR8 and identified overlapping and distinct tasks for this DENN domain-containing protein in the autophagosomal and lysosomal pathway. In summary, SMCR8 controls gene expression and as subunit of the SMCR8-C9ORF72-WDR41 complex provides proper lysosomal function and modulates autophagy via mTORC1, ULK1 and its target Rab GTPase RAB39B (Fig. 1). All of these regulation mechanisms might contribute to the pathogenesis underlying ALS/FTD.

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No potential conflicts of interest were disclosed.

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References

[1] Itoh T, Kanno E, Uemura T, Waguri S, Fukuda M. OATL1, a novel autophagosome-resident Rab33B-GAP, regulates autophagosomal maturation. J Cell Biol 2011; 192:839-53; PMID:21383079; https://doi.org/10.1083/jcb.201008107

[2] Carroll B, Mohd-Naim N, Maximiano F, Frasa MA, McCormack J, Finelli M, Thoresen SB, Perdios L, Dai-gaku R, Francis RE, et al. The TBC/RabGAP Armus coordinates Rac1 and Rab7 functions during autophagy. Dev Cell 2013; 25:15-28; PMID:23562278; https://doi.org/10.1016/j.devcel.2013.03.005

[3] Spang N, Feldmann A, Huesmann H, Bekbulat F, Schmitt N, Hielb C, Koziolek-Drechsler I, Clement AM, Moosmann B, Jung J, et al. RAB3GAP1 and RAB3-GAP2 modulate basal and rapamycin-induced autophagy. Autophagy 2014; 10:2297-309; PMID:25495476; https://doi.org/10.4161/15548627.2014.994359

[4] Meresse S, Gorvel JP, Chavrier P. The rab7 GTPase resides on a vesicular compartment connected to lysosomes. J Cell Sci 1995; 108( Pt 11):3349-58; PMID:8586647

[5] Spang N, Feldmann A, Huesmann H, Bekbulat F, Schmitt N, Hielb C, Koziolek-Drechsler I, Clement AM, Moosmann B, Jung J, et al. RAB3GAP1 and RAB3-GAP2 modulate basal and rapamycin-induced autophagy. Autophagy 2014; 10:2297-309; PMID:25495476; https://doi.org/10.4161/15548627.2014.994359

[6] Meresse S, Gorvel JP, Chavrier P. The rab7 GTPase resides on a vesicular compartment connected to lysosomes. J Cell Sci 1995; 108( Pt 11):3349-58; PMID:8586647

[7] Munafo DB, Colombo MI. Induction of autophagy causes dramatic changes in the subcellular distribution of GFP-Rab24. Traffic 2002; 3:472-82; PMID:12047555; https://doi.org/10.1034/j.1600-0854.2002.30704.x

[8] Lamb CA, Nuhlen S, Judith D, Frith D, Snijders AP, Behrends C, Tooze SA. TRAPP complex and ATG9 trafficking. Mol Biol Cell 2012; 197:659-75; PMID:22613832; https://doi.org/10.1083/jcb.201111079

[9] Smith AC, McGavran L, Robinson J, Waldstein G, Magee RE, Smith AC, Richter SF, Kondo I, Dobyns WB, Patel PI, Lupo J. Molecular analysis of the Smith-Magenis syndrome: a possible contiguous-gene syndrome associated with del(17)(p11.2). Am J Hum Genet 1991; 49:1207-18; PMID:1746552

[10] Zhang D, Iyer LM, He F, Aravind L. Discovery of Novel DENN Proteins: Implications for the Evolution of Eukaryotic Intracellular Membrane Structures and Human Disease. Front Genet 2012; 3:283; PMID:23248642; https://doi.org/10.3389/fgene.2012.00283

[11] Marat AL, Dokainish H, McPherson PS. DENN domain proteins: regulators of Rab GTPases. J Biol Chem 2011; 286:13791-800; PMID:21330364; https://doi.org/10.1074/jbc.R110.217067

[12] Sellier C, Campanari ML, Julie Corbier C, Gaucheret A, Kolb-Chenyi N, Oulad-Abdelghani M, Ruffenach F, Page A, Ciura S, Kabashi E, et al. Loss of C9orf72 impairs autophagy and synergizes with polyQ Ataxin-2 to induce motor neuron dysfunction and cell death. Embo J 2016; 35(12):1276-97; PMID:27103069; https://doi.org/10.15252/embj.201593350

[13] Blokhuis AM, Koppers M, Groen EJ, van den Heuvel DM, Dini Modigliani S, Anink JJ, Fumoto K, van Diggelen F, Snelting A, Sodar P, et al. Comparative interactomics analysis of different ALS-associated proteins identifies converging molecular pathways. Acta Neuropathol 2016; 132:175-96; PMID:27164932; https://doi.org/10.1007/s00401-016-1575-8

[14] Xiao S, MacNair L, McLean J, McGoldrick P, McKeever P, Soleimani S, Keith J, Zinman L, Rogaeva E, Robertson J. C9orf72 isoforms in amyotrophic lateral sclerosis and frontotemporal lobar degeneration. Brain Res 2016; 1647:43-9; PMID:27134035; https://doi.org/10.1016/j.brainres.2016.04.062

[15] Sullivan PM, Zhou X, Robins AM, Paushter DH, Kim D, Smolka MB, Hu F. The ALS/FTLD associated protein C9orf72 associates with SMCR8 and WDR41 to regulate the autophagy-lysosome pathway. Acta Neuropathol Commun 2016; 4:51; PMID:27193190; https://doi.org/10.1186/s40478-016-0324-5

[16] Amick J, Rozniak-Ferguson A, Ferguson SM. C9orf72 binds SMCR8, localizes to lysosomes and regulates mTORC1 signaling. Mol Biol Cell 2016; 27(20):3040-51; PMID:27559131; https://doi.org/10.1091/mbc.E16-01-0003

[17] Levine TP, Daniels RD, Gatta AT, Wong LH, Hayes MJ. The product of C9orf72, a gene strongly implicated in neurodegeneration, is structurally related to DENN Rab-GEFs. Bioinformatics 2013; 29:499-503; PMID:23329412; https://doi.org/10.1093/bioinformatics/bts725

[18] Yang M, Liang C, Swaminathan K, Herrlinger S, Lai F, Shiekhattar R, Chen JF. A C9ORF72/SMCR8-containing complex regulates ULK1 and plays a dual role in autophagy. Sci Advances 2016; 2:e1601167; PMID:27617292; https://doi.org/10.1126/sciadv.1601167

[19] Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollin P, J. C9orf72 isoforms in amyotrophic lateral sclerosis and Frontotemporal Lobar Degeneration. Brain 2016; 132:175-96; PMID:27164932; https://doi.org/10.1007/s00401-016-1575-8

[20] Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollin P, J. C9orf72 isoforms in amyotrophic lateral sclerosis and Frontotemporal Lobar Degeneration. Brain 2016; 132:175-96; PMID:27164932; https://doi.org/10.1007/s00401-016-1575-8
FTD. Neuron 2011; 72:257-68; PMID:21944779; https://doi.org/10.1016/j.neuron.2011.09.010

[22] DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H,Adamson J, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 2011; 72:245-56; PMID:21944778; https://doi.org/10.1016/j.neuron.2011.09.011

[23] Sivadasan R, Hornburg D, Drepper C, Frank N, Jablonka S, Hansel A, Lojewski X, Sterneckert J, Hermann A, Shaw PJ, et al. C9ORF72 interaction with coflin modulates actin dynamics in motor neurons. Nat Neurosci 2016; 19(12):1610-8; PMID:27723745; https://doi.org/10.1038/nn.4407

[24] Webster CP, Smith EF, Bauer CS, Moller A, Hautbergue DS, Peterson TR, Choi Y, Gray NS, Yaffe MB, et al. The mTORC1-mediated inhibition of growth factor signaling forms the molecular basis for selective autophagy. Mol Biol Cell 2015; 26:1218-31; PMID:25925668; https://doi.org/10.1016/j.molcel.2013.12.014

[25] Settembre C, Zoncu R, Medina DL, Vetrini F, Erdin S, Jung CH, Seo M, Otto NM, Kim DH. ULK1-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Mol Biol Cell 2009; 20:1992-2003; PMID:19225151; https://doi.org/10.1091/mbc.E08-12-1249

[26] Egan DF, Chun MG, Vamos M, Zou H, Rong J, Miller CJ, Lou HJ, Raveendran-Panicker D, Yang CC, Sheffer DI, et al. Small Molecule Inhibition of the Autophagy Kinase ULK1 and Identification of ULK1 Substrates. Mol Cell 2015; 59:285-97; PMID:26118643; https://doi.org/10.1016/j.molcel.2015.05.031

[27] Egan DF, Chun MG, Vamos M, Zou H, Rong J, Miller CJ, Lou HJ, Raveendran-Panicker D, Yang CC, Sheffer DI, et al. Small Molecule Inhibition of the Autophagy Kinase ULK1 and Identification of ULK1 Substrates. Mol Cell 2015; 59:285-97; PMID:26118643; https://doi.org/10.1016/j.molcel.2015.05.031

[28] Hara T, Takamura A, Kishi C, Iemura S, Natsume T, Guan JL, Mizushima N. FIP200, a ULK-interacting protein, is required for autophagosome formation in mammalian cells. J Cell Biol 2008; 181:497-510; PMID:18443221; https://doi.org/10.1083/jcb.200712064

[29] Ganley IG, Lam du H, Wang J, Ding X, Chen S, Jiang X, Ueki K, Takahashi M, Watabe H, et al. The C9orf72 protein interacts with ULK1 and is essential for autophagy. EMBO J 2016; 35(15):1656-76; PMID:27334615; https://doi.org/10.15252/embj.201694401

[30] Pinkiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, Øravt A, Bjørkøy G, Johansen T. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem 2007; 282:24131-45; PMID:17580304; https://doi.org/10.1074/jbc.M702824200

[31] Futai M, Mizushima N. Atg101, a novel mammalian autophagy receptor. Mol Biol Cell 2005; 16:709-18; PMID:25925668; https://doi.org/10.1038/nature09204

[32] Hoffmann NJ, Parker BL, Chaudhuri R, Fisher-Wellman KH, Kleinert M, Humphrey SJ, Yang P, Holliday M, Trefely S, Fazakerley DJ, et al. Global Phosphoproteomic Analysis of Human Skeletal Muscle Reveals a Network of Exercise-Regulated Kinases and AMPK Substrates. Cell Metab 2015; 22:922-35; PMID:26437602; https://doi.org/10.1016/j.cmet.2015.09.001

[33] Behrends C, Sowa ME, Gygi SP, Harper JW. Network organization of the human autophagy system. Nature 2010; 466:68-76; PMID:20562859; https://doi.org/10.1038/nature09204

[34] Jung CH, Lee J, Lee SW, Kim YM, Otto NM, Cao J, Kundu M, Kim DH. ULK1-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Mol Biol Cell 2009; 20:1992-2003; PMID:19225151; https://doi.org/10.1091/mbc.E08-12-1249

[35] Rink J, Ghigo E, Kalaidzidis Y, Zerial M. Rab conversion of mTORC1-mediated inhibition of growth factor signal-
Biol 2015; 17:288-99; PMID:25720963; https://doi.org/10.1038/ncb3114

[46] Settembre C, Di Malta C, Polito VA, Garcia Arencibia M, Vetrini F, Erdin S, Erdin SU, Huytn H, Medina D, Colella P, et al. TFEB links autophagy to lysosomal biogenesis. Science 2011; 332:1429-33; PMID:21617040; https://doi.org/10.1126/science.1204592

[47] Settembre C, De Gegl R, Mansetto G, Saha PK, Vetrini F, Visvikis O, Huytn H, Carissimo A, Palmer D, Klisch TJ, et al. TFEB controls cellular lipid metabolism through a starvation-induced autoregulatory loop. Nat Cell Biol 2013; 15:647-58; PMID:23604321; https://doi.org/10.1038/ncb2718

[48] Lu M, Muers MR, Lu X. Introducing STRaNDs: shuttling transcriptional regulators that are non-DNA binding. Nat Rev Mol Cell Biol 2016; 17(8):523-32; PMID:27220640; https://doi.org/10.1038/nrm.2016.41

[49] Fullgrabe J, Klionsky DJ, Joseph B. The return of the nucleus: transcriptional and epigenetic control of autophagy. Nat Rev Mol Cell Biol 2014; 15:65-74; PMID:24326622; https://doi.org/10.1038/nrm3716

[50] Pietrocola F, Izzo V, Niso-Santano M, Vacchelli E, Galuzzi L, Maiuri MC, Kroemer G. Regulation of autophagy by stress-responsive transcription factors. Semin Cancer Biol 2013; 23:310-22; PMID:23726895; https://doi.org/10.1016/j.semcancer.2013.05.008

[51] Chauhan S, Goodwin JG, Manyam G, Wang J, Kumat AM, Boyd DD. ZKSCAN3 is a master transcriptional repressor of autophagy. Mol Cell 2013; 50:16-28; PMID:23434374; https://doi.org/10.1016/j.molcel.2013.01.024

[52] Chua JP, Reddy SL, Merry DE, Adachi H, Katsuno M, Sobue G, Robins DM, Lieberman AP. Transcriptional activation of TFEB/ZKSCAN3 target genes underlies enhanced autophagy in spinobulbar muscular atrophy. Hum Mol Genet 2014; 23:1376-86; PMID:24150846; https://doi.org/10.1093/hmg/ddt527

[53] Schroder B, Wrocklage C, Pan C, Jager R, Kosters B, Schafer H, Elsässer HP, Mann M, Hasilik A. Integral and associated lysosomal membrane proteins. Traffic 2007; 8:1676-86; PMID:17897319; https://doi.org/10.10111/j.1600-0854.2007.00643.x

[54] O’Rourke JG, Bogdanic L, Yanez A, Lall D, Wolf AJ, Muhammad AK, Ho R, Carmona S, Vit JP, Zarrow J, et al. C9orf72 is required for proper macrophage and microglial function in mice. Science 2011; 332:1429-33; PMID:21617040; https://doi.org/10.1126/science.1204592

[55] Smith KR, Damiano J, Franceschetti S, Carpenter S, Canafoglia L, Morbin M, Rossi G, Pareyson D, Mole SE, Staropoli JF, et al. Strikingly different clinicopathological phenotypes determined by progranulin-mutation dosage. Am J Hum Genet 2012; 90:1102-7; PMID:22608501; https://doi.org/10.1016/j.ajhg.2012.04.021

[56] Brady OA, Zheng Y, Murphy K, Huang M, Hu F. The frontotemporal lobar degeneration risk factor, TMEM106B, regulates lysosomal morphology and function. Hum Mol Genet 2013; 22:685-95; PMID:23136129; https://doi.org/10.1038/hmg/dd475

[57] Lang CM, Fellerer K, Schwenk BM, Kuhn PH, Kremmer E, Edbauer D, Capell A, Haass C. Membrane orientation and subcellular localization of transmembrane protein 106B (TMEM106B), a major risk factor for frontotemporal lobar degeneration. J Biol Chem 2012; 287:19355-65; PMID:22511793; https://doi.org/10.1074/jbc.M111.365098

[58] Cruts M, Engelborghs S, van der Zee J, Van Broeckhoven C. C9orf72-Related Amyotrophic Lateral Sclerosis and Frontotemporal Dementia. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Bartolome HM, et al. GeneReviews(R). Seattle (WA), 1993

[59] Blokhuis AM, Groen EJ, Koppers M, van den Berg LH, Pastenkamp RJ. Protein aggregation in amyotrophic lateral sclerosis. Acta Neuropathol 2013; 125:777-94; PMID:23673820; https://doi.org/10.1007/s00401-013-1125-6

[60] Neumann M, SamPATHU DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, et al. Ubiquitinated TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun 2006; 351:602-11; PMID:17084815; https://doi.org/10.1016/j.bbrc.2006.10.093

[61] Neumann M, SamPATHU DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006; 314:130-3; PMID:17023659; https://doi.org/10.1126/science.1134108

[62] Buratti E, Baralle FE. The multiple roles of TDP-43 in pre-mRNA processing and gene expression regulation. RNA Biol 2010; 7:420-9; PMID:20639693; https://doi.org/10.4161/rna.7.4.12205

[63] Weder ND, Aziz R, Wilkins K, Tampi RR. Frontotemporal dementias: a review. Ann Gen Psychiatry 2007; 6:15; PMID:17565679; https://doi.org/10.1186/1744-859X-6-15

[64] Almeida S, Gascon E, Tran H, Chou HJ, Gendron TF, Degroot S, Tapper AR, Sellier C, Charlet-Berguerand N, Karydas A, et al. Modeling key pathological features of frontotemporal dementia with C9orf72 repeat expansion in iPSC-derived human neurons. Acta Neuropathol 2013; 126:385-99; PMID:23836290; https://doi.org/10.1007/s00401-013-1149-y

[65] Donnelly CJ, Zhang PW, Pham JT, Haeusler AR, Mistry NA, Vidensky S, Daley EL, Poth EM, Hoover B, Fines DM, et al. RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. Neuron 2013; 80:415-28; PMID:24139042; https://doi.org/10.1016/j.neuron.2013.10.015

[66] Lagier-Tourenne C, Baughn M, Rigo F, Sun S, Liu P, Li HR, Jiang J, Watt AT, Chun S, Katz M, et al. Targeted degradation of sense and antisense C9orf72 RNA foci. Acta Neuropathol 2013; 125:777-94; PMID:23673820; https://doi.org/10.1007/s00401-013-1125-6

[67] Pietrocola F, Izzo V, Niso-Santano M, Vacchelli E, Galuzzi L, Maiuri MC, Kroemer G. Regulation of autophagy by stress-responsive transcription factors. Semin Cancer Biol 2013; 23:310-22; PMID:23726895; https://doi.org/10.1016/j.semcancer.2013.05.008
[69] Mori K, Arzberger T, Grasser FA, Gijselinck I, May S, Rentzsch K, Weng SM, Schludi MH, van der Zee J, Cruts M, et al. Bidirectional transcripts of the expanded C9orf72 hexanucleotide repeat are translated into aggregating dipeptide repeat proteins. Acta Neuropathol 2013; 126:881-93; PMID:24132570; https://doi.org/10.1007/s00401-013-1189-3

[70] Mori K, Lammich S, Mackenzie IR, Forne I, Zilow S, Kretzschmar H, Edbauer D, Jannsen S, Kleinberger G, Cruts M, et al. hnRNP A3 binds to GGGGCC repeats and is a constituent of p62-positive/TDP43-negative inclusions in the hippocampus of patients with C9orf72 mutations. Acta Neuropathol 2013; 125:413-23; PMID:23381195; https://doi.org/10.1007/s00401-013-1088-7

[71] Mori K, Weng SM, Arzberger T, May S, Rentzsch K, Kremmer E, Schmid B, Kretzschmar HA, Cruts M, Van Broeckhoven C, Lewis J, Miller TM, Harms MB, Falchook AE, Subramony S, et al. The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. Science 2013; 339:1335-8; PMID:23393093; https://doi.org/10.1126/science.1232927

[72] Zu T, Liu Y, Banez-Coronel M, Reid T, Pletnikova O, Mori K, Arzberger T, Gijselinck I, May S, Rentzsch K, Weng SM, Schludi MH, Mackenzie IR, Kretzschmar H, Edbauer D, Subramony SH, et al. RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia. Proc Natl Acad Sci U S A 2013; 110:E4968-77; PMID:24248382; https://doi.org/10.1073/pnas.1315438110

[73] Gijselinck I, Van Langenhove T, van der Zee J, Sleepers K, Plijmers S, Kleinberger G, Jannsen S, Betens K, Van Cauwenbergh C, Pereson S, et al. A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study. Lancet Neurol 2012; 11:54-65; PMID:22154785; https://doi.org/10.1016/S1474-4422(11)70261-7

[74] Waite AJ, Baumer D, East S, Neal J, Morris HR, Ansorge O, Blake DJ. Reduced C9orf72 protein levels in frontal cortex of amyotrophic lateral sclerosis and frontotemporal degeneration brain with the C9ORF72 hexanucleotide repeat expansion. Neurobiol Aging 2014; 35:1779 e5-e13; PMID:24559645; https://doi.org/10.1016/j.neurobiaging.2014.01.016

[75] Koppers M, Blokhuis AM, Westeneng HJ, Terpstra ML, Zundel CA, Vieira de Sa R, Schellevis RD, Waite AJ, Blake DJ, Veldink JH, et al. C9orf72 ablation in mice does not cause motor neuron degeneration or motor deficits. Ann Neurol 2015; 78:426-38; PMID:26044557; https://doi.org/10.1002/ana.24453

[76] Sudria-Lopez E, Koppers M, de Wit M, van der Meer C, Westeneng HJ, Zundel CA, Youssef SA, Harkema L, de Bruin A, Veldink JH, et al. Full ablation of C9orf72 in mice causes immune system-related pathology and neoplastic events but no motor neuron defects. Acta Neuropathol 2016; 132:145-7; PMID:27206760; https://doi.org/10.1007/s00401-016-1581-x

[77] Ciura S, Lattante S, Le Ber I, Latouche M, Tostivint H, Brice A, Kabashi E. Loss of function of C9orf72 causes motor deficits in a zebrafish model of amyotrophic lateral sclerosis. Ann Neurol 2013; 74:180-7; PMID:23720273

[78] Yokoshi M, Li Q, Yamamoto M, Okada H, Suzuki Y, Kawahara Y. Direct binding of Ataxin-2 to distinct elements in 3′ UTRs promotes mRNA stability and protein expression. Mol Cell 2014; 55:186-98; PMID:24954906; https://doi.org/10.1016/j.molcel.2014.05.022

[79] Elden AC, Kim HJ, Hart MP, Chen-Plotkin AS, Johnson BS, Fang X, Armakola M, Geser F, Greene R, Lu MM, et al. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 2010; 466:1069-75; PMID:20740007; https://doi.org/10.1038/nature09320

[80] Chio A, Calvo A, Moglia C, Canosa A, Brunetti M, Barberis M, Restagno G, Conte A, Bisogni G, Marangi G, et al. ATXN2 polyQ intermediate repeats are a modifier of ALS survival. Neurology 2015; 85:251-8; PMID:25527265; https://doi.org/10.1212/WNL.000000000001159

[81] Lattante S, de Calbiac H, Le Ber I, Brice A, Ciura S, Kabashi E. Loss of function of C9orf72 causes ataxia. J Neurosci 2012; 32:14175-84; PMID:23014617; https://doi.org/10.1523/JNEUROSCI.3138-12.2012

[82] Maruyama H, Morino H, Ito H, Izumi Y, Kato H, Watanabe Y, Kinoshita Y, Kamada M, Nodera H, Suzuki H, et al. Mutations of optineurin in amyotrophic lateral sclerosis. Nature 2010; 465:223-6; PMID:20428114; https://doi.org/10.1038/nature08971

[83] Cirulli ET, Lasseigne BN, Petrovski S, Sapp PC, Dion PA, Leblond CS, Couthouis J, Lu YF, Wang Q, Krueger BJ, et al. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. Science 2015; 347:1436-41; PMID:25700176; https://doi.org/10.1126/science.aaa3650

[84] Freischmidt A, Wieland T, Richter B, Ruf W, Schaeffer V, Muller K, Marroquin N, Nordin F, Hübbers A, Weydt P, et al. Haploinsufficiency of TBK1 causes familial ALS and frontotemporal dementia. Nat Neurosci 2015; 18:631-6; PMID:25410659; https://doi.org/10.1038/nn.4000

[85] Al-Sarraj S, King A, Troakes C, Smith B, Maekawa S, Bodi I, Rogelj A, Al-Chalabi A, Hortobágyi T, Shaw CE. p62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS. Acta Neuropathol 2011; 122:691-702; PMID:22100132; https://doi.org/10.1007/s00401-011-0911-2

[86] Skibinski G, Parkinson NJ, Brown JM, Chakraborti L, Lloyd SL, Hummerich H, Nielsen JE, Hodges JR, Spillantini MG, Thysgaard T, et al. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. Nat Genet 2005; 37:806-8; PMID:16041373; https://doi.org/10.1038/ng1609