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Cancer-related genes and ALS

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1. ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that is characterized by the progressive degeneration of both upper motor neurons in the motor cortex and lower motor neurons in the brainstem and spinal cord. Recent advances in human genetics have identified more than 30 ALS-causing genes or genetic loci that include the *fused in sarcoma* (*FUS*) gene. In addition, a set of studies suggested a mutual relationship between cancer and ALS. The *hpo* gene, *Drosophila MST* was newly identified as a novel genetic modifier of the *cabeza* (*caz*), *Drosophila FUS*. The Hippo pathway negatively regulates the control of organ growth and tumor suppression. Moreover, the *p53* tumor suppressor was found to genetically interact with *caz*. Frontotemporal lobar degeneration (FTLD) is characterized by the degeneration of neurons in the frontal and temporal lobes, and consists of a spectrum with ALS. Fusion protein nucleophosmin–human myeloid leukemia factor 1 (NPM-hMLF1), which is associated with the pathologies of myelodysplastic syndrome and acute myeloid leukemia, was recently shown to suppress defects in the *Drosophila* FTLD model expressing the human *FUS* gene. Further studies in the field are expected to elucidate epidemiological, genetic, and histopathological links between cancer and ALS/FTLD, and will lead to the development of therapeutic strategies. We herein summarize previous and current findings that support mutual links between cancer and ALS/FTLD.

2. INTRODUCTION

ALS is a neurodegenerative disorder that is characterized by the progressive degeneration of both upper motor neurons in the motor cortex and lower motor neurons in the brainstem and spinal cord (1). ALS patients develop progressive muscle weakness and ultimately die within 3-5 years without the assistance of an artificial respirator (1). Approximately 10% of ALS patients have a familial history of disease (FALS), whereas the remainder are classified as sporadic (SALS). Recent studies on human molecular genetics identified more than 30 genes or genetic loci that are mutated in patients with FALS and some with SALS (2). Among the genes involved in ALS, mutations in the following four have been implicated in the majority of cases (2). Mutations in these genes include missense mutations in the *superoxide dismutase 1* (*SOD1*) gene, the *TARDBP* gene encoding TAR-DNA-binding protein-43 (TDP-43) and *fused in sarcoma/ translocation in liposarcoma* (*FUS/ TLS* or *FUS*) gene, and GGGGCC hexanucleotide expansions in the *C9orf72* gene (2). The *FUS* gene was originally identified in myxoid liposarcoma patients with the chromosomal translocation t(12; 16) (q13; p11) as a fusion gene with the CAAT enhancer-binding homologous protein, a growth arrest and DNA-damage inducible member of the C/EBP family of transcription factors (3, 4). FUS belongs to the FET family exhibiting DNA/RNA-binding activities and contains several domains including a serine-tyrosine-glycine-glutamine domain, three glycine-arginine-rich regions, one RNA-recognition motif, and one zinc finger domain (5, 6). Previous studies revealed that FUS is involved in multiple RNA metabolic pathways, including transcription, the splicing and transport of mRNA, post-translational modifications, and miRNA biogenesis (7).

Recent studies have emphasized a mutual relationship between cancer and neurodegenerative disorders, including ALS. In this review, we discuss epidemiological and molecular implications between cancer and ALS. We then summarize novel findings on genetic links between cancer-related genes and the ALS causing-gene *FUS* obtained from studies using relevant *Drosophila* models. These findings will contribute to our understanding of the underlying molecular mechanisms and also to the development of therapeutic strategies for both cancer and ALS.

3. CANCER AND ALS - DIFFERENT AGE-RELATED DISEASES –

3.1. Epidemiological relationship between cancer and ALS

Cancer is a complex disease that is characterized by uncontrolled cellular proliferation, survival, and defects in proper differentiation and death. A number of studies have attempted to elucidate the epidemiological relationship between cancer and ALS. The risk of ALS was shown to be elevated in the first year after a cancer diagnosis (8). In contrast, a lower risk of cancer has been reported in ALS patients after the diagnosis of ALS than in healthy individuals (8). Other studies investigated the relationship between the risks of different cancer types and ALS (8-10). A significantly elevated risk of ALS (positive correlation) was reported in survivors of melanoma and tongue cancer. (8-10) On the other hand, a significantly reduced risk of ALS (inverse correlation) was observed in survivors of brain, prostate, and lung cancers (8, 9). Furthermore, a recent population-based analysis of a unique database revealed an overall decreased hazard (hazard ratio 0.80, p=0.014, 95% confidence interval 0.66–0.96) for any type of cancer in 1,081 ALS patients (11). These findings suggest that although opposite findings are mixed, an epidemiological relationship exists between ALS and cancer. Therefore, an investigation of common pathological signaling cascades or factors between these two diseases is warranted.

3.2. Evidence of common signaling cascades

To date, a set of genes or signaling cascades involved in cancer has been reported in ALS studies. A microarray analysis on samples obtained from ALS patients revealed that many candidate genes for biomarkers of ALS are those related to cancer development (12). For example, p38 mitogen-activated protein kinase (p38 MAPK) signaling pathways are well-known cascades involved in various types of cancers, including prostate cancer, breast cancer, bladder cancer, liver cancer, lung cancer, transformed follicular cancer, and leukemia (13). Similar to these cancers, the aberrant expression and activation of p38 MAPK signaling pathways has been reported with the progression of ALS (14). Pharmacologically, the p38 MAPK inhibitor, SB203580, suppressed defects in transgenic mice carrying the human mutant-formed *SOD1* gene (*SOD1*^{G93A}) (15).

In addition to p38 MAPK signaling pathways, the activation of the Src/c-abl signaling pathway was reported in a study on FALS induced pluripotent stem cell (iPSC)-derived motor neurons carrying mutations in the *SOD1* gene (16). Src is overexpressed and strongly activated in a wide range of cancers, including breast, colon, pancreatic, and other miscellaneous cancers (17). Bosutinib, an FDA approved dual Src/ c-abl inhibitor for the treatment of chronic myeloid leukemia (CML), increased the survival of SALS iPSC-derived motor neurons and FALS iPSC-derived motor neurons carrying mutations in the *SOD1*, *TDP-43* (*TARDBP*), and *C9orf72* genes *in vitro* (16). Moreover, bosutinib prolonged the survival of transgenic

mice carrying human mutant *SOD1*^{G93A} (16). Thus, these signaling cascades are expected to become therapeutic targets in not only cancer, but also ALS. Some biological pathways in which microRNAs or long non-coding RNAs are involved also appear to be common between cancer and ALS (18).

4. THE HIPPO PATHWAY – ITS DISCOVERED ROLE IN ALS FROM A *DROSOPHILA* STUDY

4.1. Overview of the Hippo pathway

The canonical Hippo pathway controls cell numbers, organ sizes, tissue regeneration, and stem cell maintenance in multicellular organisms by regulating cell proliferation and promoting apoptosis (19). The essential role of the Hippo pathway in growth control was originally identified in *Drosophila* (19). This pathway in mammals contains the serine/threonine kinases, STE20-like protein kinase 1 (MST1) and MST2, large tumor suppressor homolog 1 (LATS1) and LATS2, the scaffolding protein Salvador homolog 1 (SAV1), the scaffolding proteins MOB kinase activator 1A (MOB1A) and MOB1B, the transcriptional co-activators Yes-associated protein (YAP) and transcriptional co-activator with the PDZ-binding motif (TAZ), and TEA domain-containing sequence-specific transcription factors (TEAD1) to TEAD4 (19). To regulate gene expression, YAP and TAZ form complexes with TEADs as their main partners and also with other partners, such as SMADs, T-box transcription factor 5 (TBX5), RUNT-related transcription factor 1 (RUNX1), and RUNX2 (19). The Hippo pathway is regulated by multiple upstream factors, including the Crumbs homolog (CRB) complex, thousand and one amino acid protein (TAO) kinases, the cell polarity kinase MAP/microtubule affinity-regulating kinase 1 (MARK1), G protein-coupled receptors (GPCRs), and E-cadherin (19). When the Hippo pathway is activated, MST1/MST2, which form complexes with SAV1, start to phosphorylate and activate LATS1/LAT2 and MOB1A/MOB1B (19). Following their activation, LATS1/LATS2 phosphorylate their downstream targets YAP and TAZ (19). Phosphorylated YAP and TAZ are then exported from the nucleus and are accumulated in the cytoplasm by the 14-3-3 protein or degraded by proteasomes in a β-transducin repeat-containing E3 ubiquitin protein ligase (β-TRCP)-dependent manner (19). Under these conditions, the nuclear activities of YAP and TAZ are inhibited (19). Consequently, TEADs form complexes with another transcription co-factor vestigial-like protein 4 (VGL4) to suppress the expression of target genes involved in cell proliferation, migration, survival, and stem cell functions (19). In contrast, when the Hippo pathway turns off, YAP and TAZ accumulate in the nucleus to activate the expression of these target genes by forming complexes with TEAD (19). Therefore, the Hippo pathway negatively regulates the nuclear functions of YAP and TAZ. A schema of the Hippo pathway is summarized in Figure 1.

4.2. The Hippo pathway in human cancer and tissue repair/regeneration

The essential role of the Hippo pathway in the control of organ growth and tumor suppression has been investigated in many studies. Its function was originally derived from the discovery of overgrowth phenotypes in *Drosophila* carrying a loss-of-function allele of the *Hippo* (*hpo*) gene, a *Drosophila* homologue of the *MST1/MST2* gene, and the *Warts* (*wts*) gene, a *Drosophila* homologue of the *LATS1*/*LATS2* gene, or overexpression of the *Yorkie* (*yki*) gene, a *Drosophila* homologue of the *YAP* and *TAZ* genes (20-27). Similar overgrowth phenotypes observed in the liver and heart with increasing cell numbers were reported in MST1/MST2$^{-/-}$, LAST1/LATS2$^{-/-}$, and *YAP*-overexpressing mice (28-35). These findings demonstrate that the Hippo pathway negatively regulates tumor growth. There are several lines of evidence to suggest that aberrations of the Hippo pathway are associated with several human cancers (36). Elevated expression levels and the strong nuclear localization of YAP have been reported in several solid tumors, including liver, lung, breast, skin, colon, and ovarian cancers (36-44). In addition, the *TAZ* gene is overexpressed in approximately 85% of high-grade human breast cancers, while the amplification of the *TAZ* gene has been found in 15–20% of these breast cancer patients (41-45). Although the mechanisms involved in the transformation of normal cells to tumor cells associated with the overexpression of the *YAP* or *TAZ* gene currently remain unclear, previous findings suggest that additional acquired cancer cell phenotypes, such as cancer stem cell characteristics, epithelial-to-mesenchymal transition, drug resistance, and the inhibition of senescence, are related to these mechanisms (36). Moreover, defects in normal methylation on the *YAP* gene promoter, the epigenetic regulation of MST1/MST2 and LATS1/LATS2, or an abnormality in the proteins that control *YAP* gene transcription or protein stability may be involved in the hyper-amplification of the *YAP* and *TAZ* genes (46-53).

Stem cell activation and progenitor cell expansion are generally involved in tissue repair and regeneration (19). Recent studies demonstrated that YAP and TAZ regulate the balance among stem cells, progenitor cells, and differentiated cells (19). Enhanced YAP and/or TAZ activity is associated with the expansion of stem and progenitor cells in the liver, intestine, pancreas, heart, skin, and central nervous system (33, 34, 37, 54-58). For example, the increased expression of the *YAP* gene has been

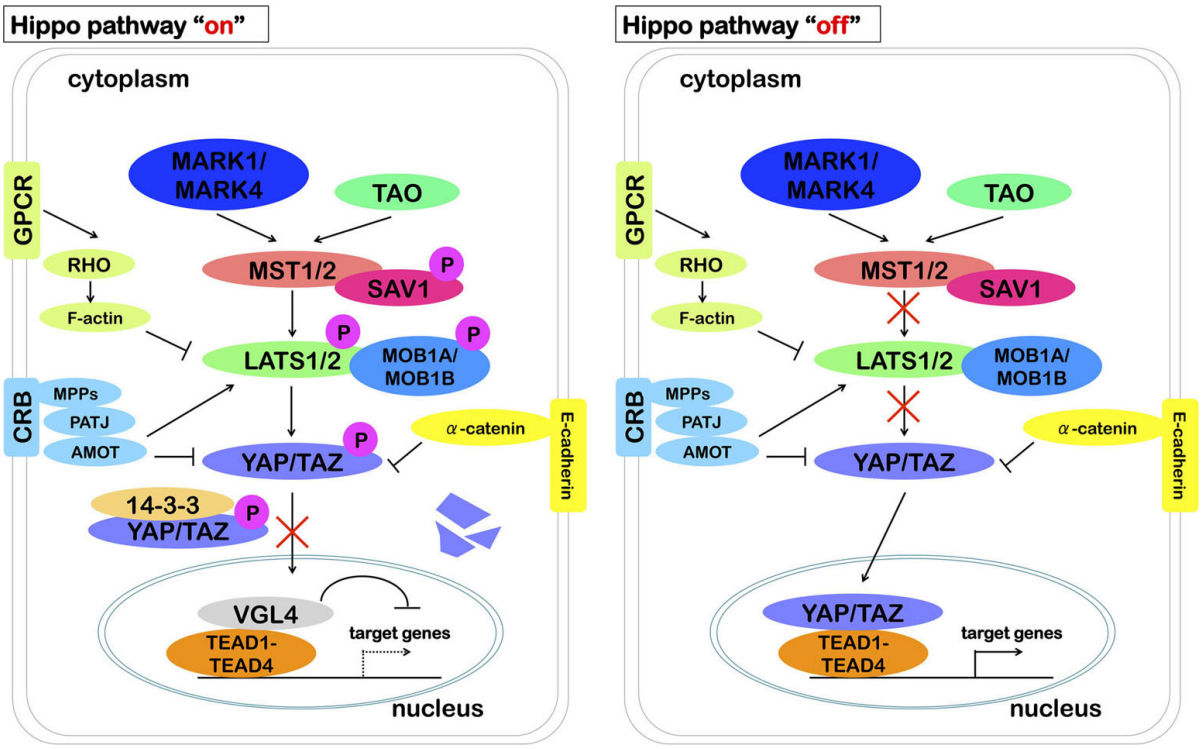

Figure 1. The Hippo signaling pathway and its mode of action in mammals. The Hippo pathway does not have specific extracellular signaling peptides or receptors; this pathway is regulated by multiple upstream components, including the Crumbs homolog (CRB) complex, thousand and one amino acid protein (TAO) kinases, the cell polarity kinases MAP/microtubule affinity-regulating kinase 1 (MARK1) and MARK4, G protein-coupled receptors (GPCRs), and E-cadherin. When the Hippo pathway is activated, mammalian STE20-like protein kinase 1 (MST1) and MST2, which form complexes with Salvador homolog 1 (SAV1), start to phosphorylate and activate large tumor suppressor homolog 1 (LATS1) and LATS2 as well as MOB kinase activator 1A (MOB1A) and MOB1B. Following their activation, LATS1/LATS2 phosphorylate their downstream targets, the Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ). Phosphorylated YAP and TAZ are exported from the nucleus and are accumulated in the cytoplasm by the 14-3-3 protein or degraded. Under these conditions, the nuclear activities of YAP and TAZ are inhibited. Consequently, TEADs form complexes with another transcription co-factor vestigial-like protein 4 (VGL4), but not with YAP or TAZ, to suppress the expression of target genes involved in cell proliferation, migration, survival, and stem cell functions. In contrast, when the Hippo pathway is deactivated, YAP and TAZ accumulate in the nucleus to drive the expression of these target genes by forming complexes with TEAD. Therefore, the Hippo pathway negatively regulates the nuclear functions of YAP and TAZ.

observed in dextran sodium sulphate (DSS)-treated mice, which is a model used to examine intestine injury, inflammation, and subsequent regenerative responses (59). The deletion of the *YAP* gene in the colonic epithelium of mice results in severe defects in DSS-induced intestinal regeneration responses (60). Other findings suggest that the liver-specific deletion of both the *MST1* and *MST2* genes in mice resulted in the expansion of oval cells, which is a progenitor cell population associated with liver repair following hepatocyte injury (30). In the case of the heart, the overexpression of the non-phosphorylatable *YAP* gene (YAP^{S127A}) in the adult mouse heart promoted heart regeneration after myocardial infarction (54). Furthermore, novel evidence was reported for the relationship between the down-regulation of the Hippo pathway and repair responses in spinal motor neurons following injury in mice (60). The deletion of the *MST1* gene in mice prolongs post-traumatic spinal motor neuron survival mediated by the enhancement of autophagy (60). Therefore, the elevated expression of the *YAP* gene in the nucleus or the inactivation of the Hippo pathway may be of therapeutic benefit for the regeneration of the injured gut, heart, liver, and neurons.

4.3. Hippo, *Drosophila* MST, as a novel modifier of motor neuron degeneration induced by the knockdown of *cabeza*, *Drosophila FUS*

Drosophila has a single FUS homolog, cabeza (caz) (61, 62). We established *Drosophila* ALS models through the knockdown of the *caz* gene (63). The neuron-specific knockdown of the *caz* gene induces a locomotive defect in adult flies that is accompanied by morphological defects in the presynaptic terminals of third instar larval motor neurons (63). We previously identified the *rhomboid-1* and *rhomboid*-3 genes as genetic interactants with the *caz* gene that are positive regulators of the MAPK signaling pathway (64). This evidence is consistent with that of a mammalian study, suggesting that more extensive genetic screening with the *caz*-knockdown *Drosophila* ALS model will contribute to the identification of genes and signaling

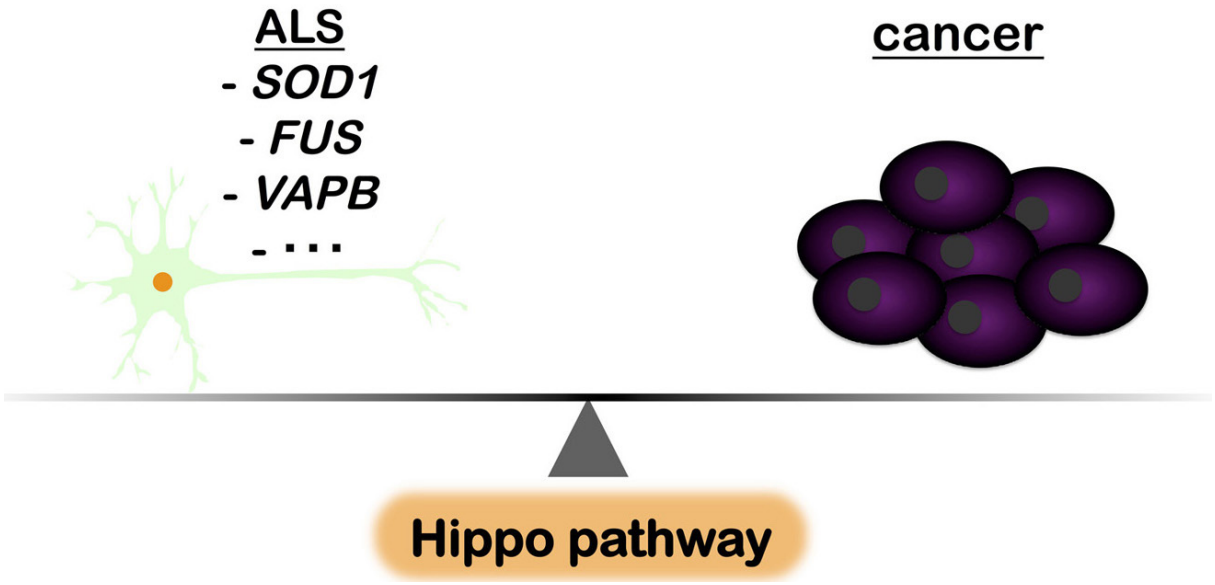

Figure 2. Hypothetical drawing of the balance between ALS and cancer mediated by the Hippo pathway. Epidemiological, genetic, and histopathological evidence suggests mutual links between ALS and cancer. The Hippo pathway is a well-known tumor suppressor pathway and may also activate some pathways in *SOD1*-, *FUS*-, and *VAPB*-related ALS pathologies. Based on the epidemiological inverse correlation between cancer and ALS, the Hippo pathway may be a key factor for understanding the mechanisms responsible for both cancer and ALS.

pathways that are related to the pathology of human ALS (18).

As a novel genetic modifier of the *caz*-knockdown *Drosophila* ALS model, we recently discovered the *hpo* gene, *Drosophila MST* (65). The strong suppression of the rough eye phenotype induced by the knockdown of the *caz* gene was observed in *caz* knockdown flies crossed with flies carrying a loss-of-function allele of the *hpo* gene (65). On the other hand, a loss-of-function allele of the *wts* or *yki* gene exerted no effect on the rough eye phenotype induced by the knockdown of the *caz* gene, indicating that the *caz* gene negatively regulates the *hpo* gene, but not via the canonical Hippo pathway. We also found that defects in climbing ability and an aberrant morphology in presynaptic terminals, which mimic human ALS symptoms, were suppressed by the crossing of *caz* knockdown flies with flies carrying a loss-of-function allele of the *hpo* gene (65). A recent study reported that Strip, a component of the striatin-interacting phosphatase and kinase complex, is required for the proper development of the synapse structure at the neuromuscular junction (NMJ) in *Drosophila* (66). Strip has been reported to negatively regulate the activity of the Hippo pathway (66). Strip genetically interacts with Enabled, an actin assembly/elongation factor and the presumptive downstream target of Hippo signaling, to regulate the local organization of actin at synaptic termini (66). This regulation occurs independently of the *yki* gene (66). Therefore, the Strip-Hippo pathway plays a critical role in synaptic development, linking cell signaling to actin organization (66). The negative regulation of the *hpo* gene via the *caz* gene may be mediated by the newly identified non-canonical Strip-Hippo pathway (65). Further analyses are necessary to clarify this point.

We found that *Hippo* mRNA levels were increased in *caz* knockdown flies, implying that the function of Hippo itself was activated (65). We observed the accumulation of the nuclear caz protein in *caz* knockdown flies crossed with flies carrying a loss-of-function allele of the *hpo* gene (65). Based on the close relationship between the Hippo pathway and autophagy responses, we investigated whether the mechanisms responsible for the observed rescue were mediated by autophagy responses (65). Consistent with our evidence, other studies suggested a critical role for the Hippo pathway in *in vivo* models of other FALS (transgenic mice carrying human mutant *SOD1*^{G93A} and a *Drosophila* model carrying the mutant form of the human *VAMP-associated protein B* gene) (67, 68). Moreover, in ventral motor neurons of postmortem spinal cord sections from a SALS patient, stronger immunopositive signals against phospho-MST1 were detected than in samples from healthy controls (66). Therefore, MST1/MST2, the core component of the Hippo pathway, is a novel therapeutic target not only for several FALS, but also for SALS. Based on the epidemiological inverse correlation between cancer and ALS, this well-known tumor suppressor may be a key factor for elucidating the mechanisms responsible for cancer and ALS (Figure 2).

5. ROLE OF THE TUMOR SUPPRESSOR p53 IN ALS

5.1. Evidence for pathological roles of p53 in *SOD1*-, *TDP-43*-, and *C9orf72*-related ALS

The transcription factor p53, "the guardian of the genome", plays crucial roles in the prevention of tumors by multiple cellular responses, including the apoptosis of damaged cells, maintenance of genomic stability, inhibition of angiogenesis, and modulation of cell metabolism and the tumor microenvironment (69, 70). p53 is inactivated in nearly all tumors, which are caused by mutations in the *p53* (*TP53*) locus or by the degradation of the p53 protein in an overexpressed Mdm2-dependent manner (69, 70). Mdm2 is a RING-Finger ubiquitin E3 ligase that recognizes the N-terminal transactivation domain of p53 (69). Previous studies demonstrated that the nuclear expression levels of p53 were elevated in the spinal cord and motor cortex of ALS patients (71, 72). Moreover, p53 co-localized with activated caspase-3, TUNEL staining, phosphorylated pRb and E2F-1, G1 to S phase regulators in the nucleus, suggesting that p53 and these cell cycle regulators promote neuronal cell death in the ALS spinal cord (72). Consistent with these findings, *in vivo* rodent and *in vitro* cell models carrying the wild-type or mutant form of the *SOD1*, *TDP-43*, or *C9orf72* gene emphasized the role of p53 in ALS as follows (73). A higher level of DNA damage, elevated p53 activity, and the expansion of apoptotic cells were observed in cultured cells transfected with the mutant form of the *SOD1* gene (73, 74). On the other hand, the deletion of the mouse *p53* gene in transgenic mice carrying the mutant form of the *SOD1* gene did not alter disease onset or progression (75, 76). In the case of TDP-43-related ALS, the low-grade overexpression of wild-type *TDP-43* in HeLa cells caused p53-dependent G2/M phase arrest (77). Moreover, the ectopic expression of the wild-type *TDP-43* gene or mutant form of the *TDP-43* (*TDP-43*^{A315T}) gene led to p53-mediated apoptosis in neural stem/progenitor cells and immature neurons of the developing mouse telencephalon (78). p53-mediated apoptosis has also been observed in iPSC-derived motor neurons carrying the mutant form of the *TDP-43* (*TDP-43*^{G298S}) gene (78). Increased DNA damage and the nuclear activation of p53 have been reported in iPSC-derived motor neurons carrying the mutant form of the *C9orf72* gene (79). These findings collectively imply a pathological role for p53 in several ALS.

5.2. *p53* as a genetic interactant with *caz*, *Drosophila FUS*

We examined possible genetic interactions between *caz*, *Drosophila FUS*, and *p53* using our established *caz* knockdown fly model (63). Flies with the eye-specific *caz* knockdown showed the morphologically aberrant rough eye, as we reported previously (64, 65). Two different knockdown alleles of *p53* that target different regions of *p53* mRNA (UAS-p53-IR and UAS-p53-IR$_{68-165}$) suppressed the morphologically aberrant rough eye induced by the *caz* knockdown (*GMR-GAL4/Y; UAS-caz-IR/UAS-p53-IR, GMR-GAL4/Y; UAS-caz-IR/UAS-p53-IR*$_{68-165}$) (Figure 3E and 3F) more significantly than the responder control of UAS-p53-IR flies (*GMR-GAL4/Y; UAS-Caz-IR/UAS-GFP-IR*) (Figure 3D). The knockdown of *p53* alone exhibited an apparently normal eye morphology (*GMR-GAL4/Y; +/UAS-p53-IR* and *GMR-GAL4/Y; +/UAS-p53-IR*$_{68-165}$) (Figure 3B and 3C). The suppressive effects observed were not potential background mutations or off-target effects because two independent knockdown lines of *p53* showed similar suppressive effects. These findings indicated that *caz* genetically interacts with *p53*.

We previously reported that apoptosis at least partially contributes to the morphologically aberrant rough eye induced by the *caz* knockdown (64). Apoptotic motor neuronal death occurs in human ALS (80), and p53-dependent and independent cell death pathways are both known to exist (72, 77, 78). The genetic interaction between *caz* and *p53* may also be related to p53-dependent apoptosis. Other studies reported p53-mediated apoptosis in iPSC-derived motor neurons carrying the mutant form of the *FUS* gene. Mutations in the *FUS* gene may affect gene expression indirectly by altering miRNA levels. Of several microRNAs, miR-375 is deregulated in FUS mutant motor neurons (81). The overexpression of miR-375 protects motor neurons from DNA damage-induced apoptosis by targeting the *TP53* gene (82, 83). More recently, a gene expression analysis showed that miR-34a and miR-504 were also both down-regulated in human ALS motor neurons (84). MiR-34a and miR-504 share many molecular pathways related to apoptosis (85, 86). MiR-34a is a direct transcriptional target of the *TP53* gene, while miR-504 is a direct upstream negative regulator of p53 protein expression (85-87). A pathological role for p53 has been reported in *SOD-1*, *TDP-43*, and *C9orf72* -related ALS as described above (73, 74, 77-79). Moreover, the spinal motor neurons of ALS patients have been shown to have higher levels of the p53 protein, which may contribute to apoptosis-mediated neuronal death (71, 72). Therefore, p53-mediated apoptosis may play a crucial role in motor neuron cell death during ALS.

6. The NPM-hMLF1 FUSION PROTEIN AS A NOVEL SUPPRESSOR OF FTLD REVEALED WITH A *DROSOPHILA* MODEL

6.1. Clinical and genetic spectra between ALS and FTLD

Frontotemporal lobar degeneration (FTLD) is one of the most common forms of young-onset dementia, accounting for approximately 10-20% of

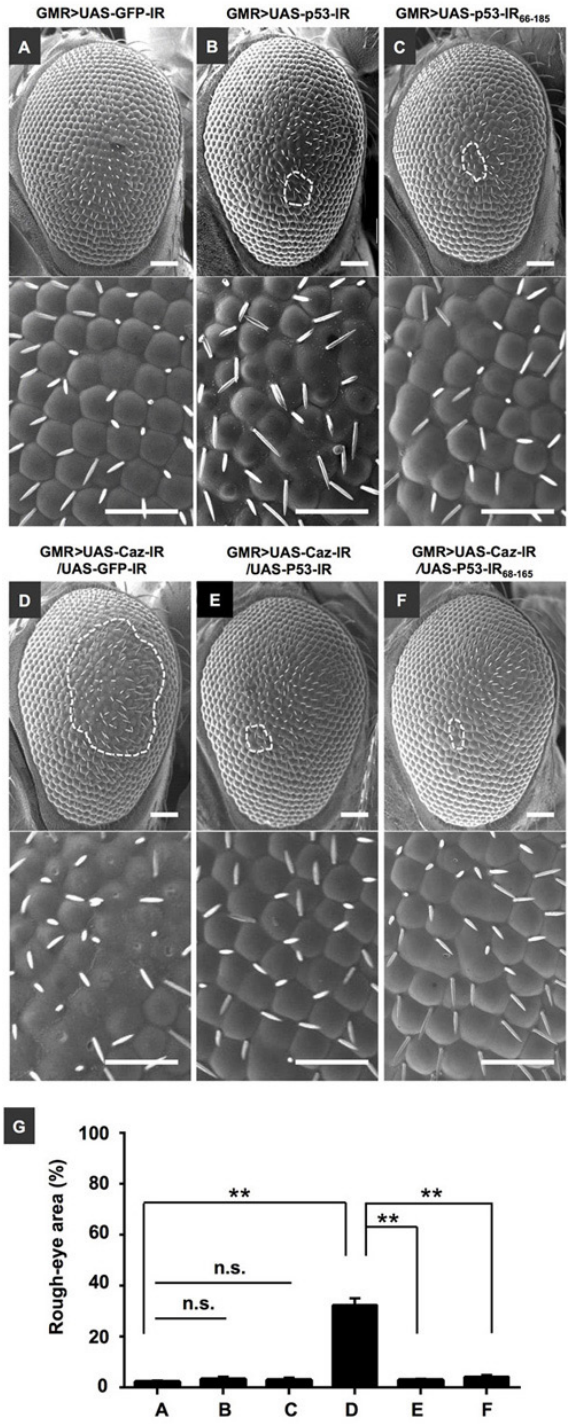

Figure 3. The rough eye morphology induced by the *caz* knockdown is suppressed by the knockdown of *p53*. Fly stocks were maintained at 25 °C on standard food containing 0.65% agar, 10% glucose, 4% dry yeast, 5% cone flour, and 3% rice powder. Flies carrying *w; UAS-p53-IR*; + (BDSC41638) (UAS-p53-IR), and w; P(y $^{+t7.7}$ w^{+mC}=UAS-rCD2.RFP.UAS-GFPi)^{attP40} (BDSC 56181) (UAS-GFP-IR) were obtained from the Bloomington *Drosophila* Stock Center (BDSC) in Indiana. Flies carrying *w; UAS-p53-IR*$_{68-165}$, + (VDRC103001) (UAS-p53-IR$_{68-165}$) were obtained from the Vienna *Drosophila* RNAi center (VDRC). The establishment of lines carrying *GMR-GAL4* was as described previously (117). Fly lines carrying *UAS-caz-IR* have been described previously (63-65). Adult flies were anesthetized with diethyl ether, mounted on a stage, and inspected under the scanning electron microscope V-7800 (Keyence Inc.) in the low vacuum mode. (A) GMR>UAS-GFP-IR (*GMR-GAL4/Y*; *UAS-GFP-IR/+*), (B) GMR>UAS-p53-IR (*GMR-GAL4/Y*; *UAS-p53-IR/+*), (C) GMR>UAS-p53-IR$_{68-165}$ (*GMR-GAL4/Y*; *UAS-p53-IR*$_{68-165}$*/+*), (D) GMR>UAS-Caz-IR/UAS-GFP-IR (*GMR-GAL4/Y*; *UAS-Caz-IR/UAS-GFP-IR*), (E) GMR>UAS-*Caz*-IR/ UAS-p53-IR (*GMR-GAL4/Y*; *UAS-Caz-IR/UAS-p53-IR*), (F) GMR>UAS-*Caz*-IR/ UAS-p53-IR$_{68-165}$ (*GMR-GAL4/Y*; *UAS-Caz-IR/ UAS-p53-IR*$_{68-165}$). Higher magnification images are shown in the lower panels. Posterior is to the right, and dorsal to the top. Flies were developed at 28 °C. The scale bars indicate 50 μm. (G) Quantified data of rough eye areas. The rough areas of the compound eyes are marked with dotted lines. The quantification of each marked area (n = 3) was performed using image processing and analysis in Java. Dunnett's test was used to statistically compare differences between four (A-D) or three (D-F) groups. n.s. indicates $p > 0.05$. ** indicates $p < 0.01$. Error bars indicate standard errors of the means.

all dementias worldwide (88). FTLD is characterized by the degeneration of neurons in the frontal and temporal lobes, such that FTLD patients show defects in or abnormal behavior, defects in personality, and language disorders (88). Clinically, approximately 50% of ALS patients exhibit slight defects in cognitive functions and behavior, and more than 15% may ultimately develop frontotemporal dementia (89, 90). Moreover, FTLD is pathologically characterized by typical protein inclusions in degenerating neurons, similar to ALS (91).

Approximately 60% of patients with FTLD show protein inclusions immunoreactive with ubiquitin and TDP-43, and this has been categorized as FTLD-TDP (91). In addition to FTLDTDP, four more subtypes may be classified based on the major protein inclusion constituents: FTLDtau containing the tau protein, FTLD-FUS containing the FUS protein, FTLDUPS, which has the inclusions of proteins associated with the ubiquitin-proteasome system, and FTLDni, which has no inclusions (91). Approximately 30-40% of patients with FTLD have a familial history, whereas the remainder of cases are classified as sporadic (88). Mutations in three main genes are identified in FTLD, including the *microtubule-associated protein tau* (*MAPT*), *granulin* (*GRN*), and *C9orf72* genes (88). Of note, more than 50 mutations in the *FUS* gene have been identified to date in ALS, while mutations in the *FUS* gene have rarely been reported in FTLD, indicating that the majority of FTLD-FUS cases have inclusions with wild-type FUS (91). Therefore, although ALS and FTLD show significant heterogeneity in their clinical symptoms, the overlap in genetics and pathology prompted us to consider ALS and FTLD as a spectrum (91).

6.2. The leukemic fusion protein NPM-hMLF1 generated by the t(3;5)(q25.1;q34) chromosomal translocation in MDS/AML

Myelodysplastic syndrome (MDS) is a clonal disease that arises from the expansion of hematopoietic stem cells with genetic mutations (92). MDS is characterized by morphological dysplasia, ineffective hematopoiesis leading to cytopenias, and a risk of transformation to acute myeloid leukemia (AML) (92). AML is a clonal myeloid neoplasm that involves the maturation arrest of myeloid progenitors and the dysregulated proliferation of blast cells in the bone marrow (93). Secondary AML is categorized as AML with myelodysplasia-related changes (AML-MRC), which accounts for 24-48% of all AML cases (93). In AML-MRC, chromosomal abnormalities, such as -7/del(7q), del(5q), +8, and del(20q), are similar to those found in pure MDS (94). On the other hand, chromosome rearrangements, including balanced translocations, are less common in AML-MRC (94). The most frequent chromosome translocations observed in AML-MRC occur in the 5q31-q35 genomic region, at which the *nucleophosmin* (*NPM*) gene is located (94, 95). The human *myeloid/myelodysplastic leukemia factor 1* (*hMLF1*) gene is identified at the locus 3q25.1 as the partner of the *NPM* gene in the t(3;5)(q25.1;q34) translocation, resulting in the generation of the fusion gene *NPM-hMLF1* (94, 95). The incidence of this chromosomal translocation is approximately 0.5% of patients with AML, and is observed in all age groups, but is more common in younger patients (96, 97). Clinically, patients with t(3;5)(q25;q35) show a 10-year survival rate of 34%, suggesting that the appearance of t(3;5)(q25;q35) is associated with an intermediate prognosis (96).

The chimeric NPM-hMLF1 protein consists of 426 amino acids by the fusion of the initial 175 amino acids from NPM with 251 amino acids from hMLF1; therefore, it lacks the initial 16 amino acids from hMLF1 (98). Although the precise mechanism of leukemogenesis associated with the NPM-hMLF1 fusion protein remains unclear, hMLF1 is not expressed in normal hematopoietic tissues, suggesting that the NPM-hMLF1 fusion protein leads to the overexpression of the *hMLF1* gene in hematopoietic cells (99). Previous studies suggested that hMLF1 is overexpressed in more than 25% of AML-MRC cases (100). NPM is a non-ribosomal RNA-binding phosphoprotein that is typically localized in the nucleolus and functions as a shuttle protein by transporting ribosomal ribonucleoproteins between the nucleus and cytoplasm in the assembly of ribosomes (101, 102). Although the hMLF1 protein mainly localizes in the cytoplasm, the NPM-hMLF1 fusion protein mainly localizes in the nucleus (103). These findings indicate that haploinsufficiency of wild-type NPM and its unusual cytoplasmic expression may also contribute to leukemogenesis (104).

6.3. NPM-hMLF1, suppressor of defects in a *Drosophila* FTLD/ALS model expressing the human *FUS* gene

We recently demonstrated that the fusion protein NPM-hMLF1 suppressed defects in a *Drosophila* FTLD/ALS model carrying the human *FUS* gene (105). The co-expression of the *NPM-hMLF1* gene suppressed the abnormal compound eye morphology induced by the human *FUS* gene (105). Furthermore, the co-expression of the *NPM-hMLF1* gene partially rescued the pharate adult lethal phenotype induced by the motor neuron-specific expression of the human *FUS* gene, suggesting that NPM-hMLF1 modifies the FUS-related FTLD pathology (105). Normally, expression level of target protein drived by the GAL4-UAS system is higher at 28 °C than at 25°C. However, we showed that the driving of human *FUS* gene expression at 28 °C rather down-regulated the levels of the human FUS protein itself and the endogenous caz protein compared to that at 25°C, resulting in the

severe aberrant eye morphology phenotype (105). We also reported that this down-regulation was mediated by proteasome-dependent degradation (105). The co-expression of the *NPM-hMLF1* gene recovered human FUS protein levels, but not those of the caz protein (105). Although the precise mechanisms responsible for the observed rescue remain unclear, we showed the co-localization of human FUS with NPM-hMLF1 mainly in the nucleus, indicating that NPM-hMLF1 binds to hFUS to protect it from degradation (105). On the other hand, we observed that the co-expression of the *NPM-hMLF1* gene slightly increased the solubility of the human FUS protein, suggesting that the refolding of human FUS protein aggregates by NPM-hMLF1 is applicable as a novel therapy for the pathology of human FTLD/ALS (105).

Of note, NPM contains both nuclear localization signal (NLS) and nuclear export signal (NES) sequences. Under oncogenic stress conditions, NPM stabilizes p53 by binding directly to ARF and recruiting it to nucleoli, which results in the inactivation of Mdm2 and accumulation of p53 (106-108). Similarly, hMLF1 also contains the NLS and NES sequences and stabilizes p53 by suppressing COP1 activity through CSN3, the subunit 3 of the COP9 signalosome (CSN) in the nucleus (109). CSN plays a role in the regulation of E3-cullin RING ubiquitin ligases (109). These findings suggest that the shuttling functions of both NPM and hMLF1 are critical for the stabilization of p53 in the nucleus. Importantly, NPM-hMLF1 impairs p53 activation induced by genomic stress and oncogenic cellular stress (110). The precise mechanisms underlying the impairment of p53 activation have not yet been elucidated in detail; however, it has been reported that the shuttling imbalance in NPM and hMLF1 due to the generation of NPM-hMLF1 may disturb the regulation of p53 stability, thereby inducing leukemogenesis (110). Although further studies are needed to confirm this hypothesis, the inactivation of p53 appears to be critical in the observed rescue because caz has potential as a negative regulator of p53 (Figure 3).

Recent studies reported that the unconventional repeat-associated non-ATG (RAN) translation of GGGGCC repeats in the sense strand and GGCCCC repeats in the antisense strand were closely related to the pathogenesis of *C9orf72*-ALS/FTLD (111, 112). RAN translation generates five dipeptide-repeat proteins (DRPs), including glycine–alanine (GA), glycine–arginine (GR), proline–alanine (PA), proline–arginine (PR), and glycine–proline (GP). DRP inclusions have been detected in *C9orf72*-ALS/FTLD patients (111, 112). Importantly, expressed poly-GR and poly-PR, but not poly-GA, poly-GP, or poly-PA, in neuronal cells localize to the nucleolus and induce the translocation of the nucleolar component NPM, leading to nucleolar stress and cell death (113, 114). A previous study demonstrated that the overexpression of the *NPM* gene inhibited apoptosis in cells expressing poly-GR and poly-PR (113). In addition, the knockdown of the *Nlp* gene, the *Drosophila* ortholog of the *NPM* gene, enhanced the pharate adult phenotypes induced by poly-GR_{50} (115). Although it currently remains unclear whether the ectopic expression of the *NPM* gene may rescue the FUS-related ALS and FTLD pathologies, a suggested mechanism is the co-expression of the *NPM-hMLF1* gene, which produces the initial 175 amino acids of NPM, contributing to the suppression of the phenotypes of the *Drosophila* FTLD/ALS model expressing the human *FUS* gene.

The pathological role of FUS in AML-MRC with the t(3;5)(q25;q35) chromosome translocation currently remains unknown, but may demonstrate novel links in this leukemic cancer-related gene and FTLD/ALS-causing genes. A schematic model of this rescue is summarized in Figure 4.

7. PERSPECTIVES

Two age-related diseases, cancer and ALS, show apparently opposing endpoints: uncontrolled cell survival and proliferation in cancer and progressive neuronal death in ALS (116). We reviewed several epidemiological, genetic, and histopathological findings indicating mutual links between these two diseases. We also summarized recently discovered key pathways and factors associated with the two diseases *in vivo*. Current studies on p53 and Hippo have indicated its potential as a therapeutic target for not only cancer, but also ALS. Of note, the leukemic fusion protein NPM-hMLF1 suppresses defects in a *Drosophila* FTLD/ALS model carrying the human *FUS* gene. It will be interesting to clarify whether this fusion protein exerts similar suppressive effects in other *Drosophila* models targeted to various ALS-causing genes. Approximately 75% of causative factors for human diseases are conserved in *Drosophila* (18). Based on the accumulation of extensive knowledge in genetics and developmental biology, *Drosophila* models have been playing important roles in the study of human diseases, including ALS and cancer (18). In the future, these unique studies with *Drosophila* models will contribute to the development of novel therapeutic strategies for both cancer and ALS.

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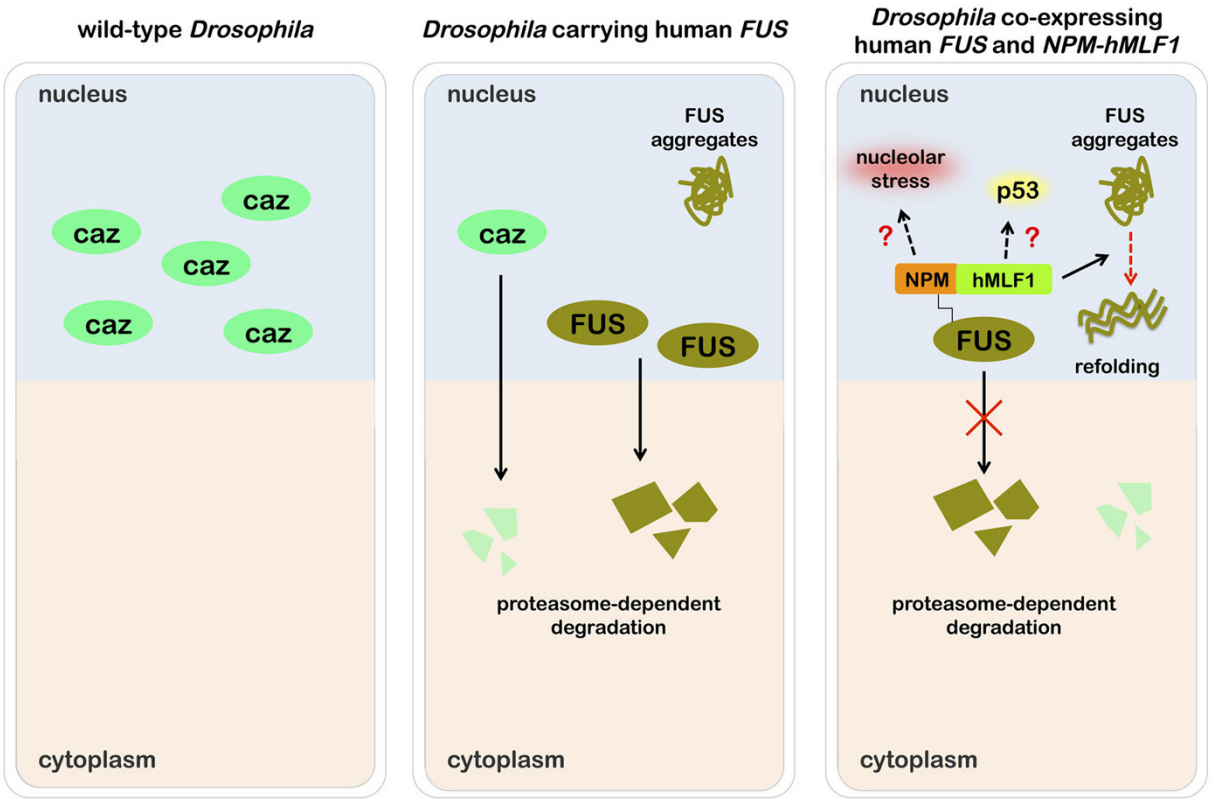

Figure 4. The co-expression of *NPM-hMLF1* suppressed defects in the *Drosophila* FTLD model expressing the human *FUS* gene. In wild-type flies, caz is mainly localized in the nucleus (left). In flies carrying the human *FUS* gene, endogenous caz and ectopically expressed human FUS are down-regulated in a proteasome-dependent manner, leading to the loss of function of FUS and formation of FUS aggregates in the nucleus (middle). In flies co-expressing human *FUS* with the *NPM-hMLF1* gene, NPM-hMLF1 in the nucleus may bind to human FUS, but not to endogenous caz, in order to protect against protein degradation, and slightly refolds FUS aggregates. In addition, parts of NPM and hMLF1 may affect nucleolar stress and p53 activation.

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