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The cullin7 E3 ubiquitin ligase: A novel player in growth control

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Abstract

Cullin7 (CUL7) is a molecular scaffold that organizes an E3 ubiquitin ligase containing the F-box protein Fbw8, Skp1 and the ROC1 RING finger protein. Dysregulation of the CUL7 E3 Ligase has been directly linked to hereditary human diseases as *cul7* germline mutations were found in patients with autosomal-recessive 3-M and Yakuts short stature syndromes, which are characterized by profound pre- and postnatal growth retardation. In addition, genetic ablation of CUL7 in mice resulted in intra-uterine growth retardation and perinatal lethality, underscoring its importance for growth regulation. The recent identification of insulin receptor substrate 1, a critical mediator of insulin and insulin-like growth factor-1 signaling, as the proteolytic target of the CUL7 E3 ligase, provided a molecular link between CUL7 and a well-established growth regulatory pathway. This result, coupled with other studies demonstrating interactions between CUL7 and the p53 tumor suppressor protein, as well as the simian virus 40 large T antigen oncoprotein, further implicated CUL7 as a novel player in growth control and suggested pathomechanistic insights into CUL7-linked growth retardation syndromes.

Keywords

cullin7; Fbw8; IRS-1; IGF-1; insulin; ubiquitin; proteasome; growth retardation; 3-M syndrome; yakuts short stature syndrome

The CUL7 E3 Ligase Targets Cyclin D1 and Insulin Receptor Substrate 1 for Ubiquitin-Dependent Degradation

The turnover of intracellular proteins by the Ubiquitin (Ub)-Proteasome System (UPS) is a precisely controlled process that regulates a broad spectrum of fundamental cellular functions, ranging from cell cycle progression to signal transduction[1]. Central to the UPS is the recognition of a substrate by an E3 Ub ligase, a step pivotal for initiating the ubiquitination reaction that joins the target protein covalently with lysine 48-linked polyubiquitin chains, thereby leading to its degradation by the 26S proteasome.

The cullin-RING complexes constitute the largest group of E3 ligases, which are characterized by two signature components: a cullin (CUL) protein and the RING (for Really Interesting New Gene) finger protein ROC1 (also termed Rbx1 or Hrt1)[2]. Cullins are molecular scaffolds, capable of integrating both a molecule with substrate-targeting function, and the ROC1 RING

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domain for tethering an E2 Ub conjugating enzyme. In the prototypic SCF (Skp1·CUL1·F-box protein-ROC1) complex, the CUL1 N-terminus binds to the Skp1·F-box protein substrate-module, whereas the C-terminally located cullin domain anchors ROC1, which recruits Cdc34 and/or Ubc4/5 E2 conjugating enzyme to catalyze the transfer of Ub to the substrate protein.

Cullin7 (CUL7, also known as KIAA0076, p185, p193) is the seventh cullin family member identified to date. It was initially isolated as a cellular protein bound to simian virus 40 (SV40) large T antigen.[3,4] As revealed by the subsequent work by Dias et al.[5] and Arai et al.,[6] CUL7 assembles an SCF-like E3 ligase complex composed of the adapter protein Skp1 (S-phase kinase associated protein 1), ROC1 and the WD40 repeat-containing F-box protein Fbw8 (also named Fbx29, Fbw6 or Fbxw8; see Fig. 1A and Table 1). To date, Fbw8 is the only F-box protein known to bind to CUL7 via Skp1,[5,7] underscoring the remarkable selectivity of this cullin protein.

Recently, the CUL7 E3 Ub ligase has been implicated in the proteasomal degradation of two cellular proteins: cyclin D1,[8] and insulin receptor substrate 1 (IRS-1).[9] Cyclin D1 is an important to S-phase cell cycle progression and is subjected regulator of the G_1 to considerable posttranslational regulation (reviewed in ref. [10]). The study by Okabe et al.[8] demonstrated that Fbw8, the substrate recognition subunit of the CUL7 E3 ligase, mediates the ubiquitination of cyclin D1 in a manner that is dependent upon the phosphorylation of cyclin D1 residue T286 by the ERK2 MAP kinase. Conversion of T286 to alanine or knockdown of Fbw8, CUL1 or CUL7 by RNAi resulted in the stabilization of cyclin D1 and prevented cell cycle progression in a number of different cell types tested. However, it should be noted that Lin et al.[11] identified the $SCF^{FBX4\text{-}\alpha B\text{-}crystallin}$ complex as an E3 ligase for the proteolytic turnover of cyclin D1, and demonstrated the requirement for T286 phosphorylation by glycogen synthase kinase 3β for degradation.

By employing a proteomic approach in search for Fbw8 interacting proteins, Xu et al.[9] identified IRS-1 as a proteolytic target of the CUL7 E3 ligase. IRS-1 is a critical component of the signaling pathways downstream of the insulin and insulin-like growth factor 1 (IGF-1) receptor (reviewed in ref. [12]). Upon receptor activation, IRS-1 is phosphorylated at multiple tyrosine residues, and then recruits SH2 (Src homology 2)-containing adaptor proteins for the activation of downstream Akt (via PI3K) and RAS/MEK/ERK (via Grb2/SOS) pathways (see Fig. 2). Haruta et al.[13] observed that IRS-1 was degraded during prolonged exposure to insulin, in a manner that was sensitive to Wortmannin, a PI3K inhibitor, and to rapamycin, a mammalian target of rapamycin (mTOR) inhibitor. It is now believed that the proteolytic turnover of IRS-1 constitutes a negative feedback loop that restrains the magnitude and/or duration of PI3K activation,[14] via a mechanism requiring seryl phosphorylation of IRS-1 by mTOR and its effector kinase S6K (whose activities are stimulated by the PI3K/Akt cascade; see Fig. 2) (reviewed in refs. [14–16]).

The study by Xu et al.[9] demonstrated that Fbw8 binds to IRS-1 and promotes its ubiquitination and proteasomal degradation, and that inactivation/deletion of Fbw8 and CUL7, respectively, accumulated IRS-1. Moreover, Fbw8-induced degradation of IRS-1 was dependent upon mTOR activity and may be mediated by multiple mTOR/S6K target serine residues on IRS-1. Thus, the CUL7 E3 appears to be responsible for mediating mTOR-dependent degradation of IRS-1, thereby functioning as a critical component of the mTOR/IRS-1 negative feedback loop (reviewed in ref. [17]), which fine-tunes the PI3K activity in accordance with the magnitude and duration of mTOR/S6K activities.

In support of this, embryonic fibroblasts of $CUL7^{-/-}$ mice were found to accumulate IRS-1 and exhibit increased activation of IRS-1 downstream pathways Akt and MEK/ERK.[9] However, despite the over-activation of these pro-mitogenic signaling pathways, $CUL7^{-/-}$

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mouse embryonic fibroblasts (MEFs) grew poorly with an increased population of cells arrested in G_1-phase. Furthermore, the CUL7$^{-/-}$ MEFs exhibited upregulation of distinct tumor suppressors that included p16 and hypo-phosphorylated retinoblastoma protein (pRb), a large flat morphology and high levels of β-galactosidase activity, all of which are characteristic features of cells undergoing senescence.

It should be noted that previous studies have implicated a role for the SOCS-containing E3 ligases in IRS-1 degradation,[18] raising the intriguing possibility that multiple E3 Ub ligases may participate in the proteolytic regulation of IRS-1 in response to cellular and environmental cues.

CUL7 may be Associated with Multiple Non-Proteolytic Functions

In comparison with canonical cullin family members (CUL1-5), CUL7 exhibits several atypical features. Composed of 1698 amino acids in humans, CUL7 is of substantially large size. In addition to the highly conserved cullin domain, it contains two distinct motifs: a DOC domain (similar to the DOC1 of the APC/C),[19] and a CPH domain (conserved domain in CUL7, PARC and HERC2 proteins)[20] (Fig. 1B). Finally, CUL7 appears to be present only in higher eukaryotes (vertebrates), suggesting a late phylogenetic origin.[5] Intriguingly, CUL7 was found to mediate interactions with several proteins in a manner that is independent of Fbw8 and that does not affect the stability of the associated proteins (see Table 1), suggesting that the CUL7 E3 ligase may exert both proteolytic (Fbw8-related) and non-proteolytic effects.

NMR studies revealed a direct binding of CUL7 to p53 and showed that the evolutionarily conserved CUL7 CPH domain is the predominant p53 binding site.[20] On p53, the interaction surface was mapped to the tetramerization domain. Given that the oligomerization state of p53 affects both its transcriptional activity and subcellular localization, it was proposed that CUL7 might control p53 function by binding preferentially to the active, tetrameric forms of p53.[20] At present there is no experimental evidence that the CUL7 E3 ligase participates in the proteolytic degradation of p53: under conditions in which MDM2 promoted the formation of high molecular weight species of p53-Ub in vitro, CUL7 supported only the mono- and di-ubiquitination of p53.[21,22] In addition, no accumulation of p53 protein was detected in cells depleted of CUL7 by RNAi,[22] or in the CUL7$^{-/-}$ MEFs.[23] However, one study reported the upregulation of p53 protein level in SHEP N-Myc cells depleted of CUL7.[24] Interestingly, experimental evidence suggests that the CUL7-p53 interaction may contribute to transcriptional regulation: while CUL7 appears to repress p53 in a luciferase reporter assay,[22] p53 seems to be required for upregulation of CUL7 at both mRNA and protein levels after DNA damage induced by etoposide.[21]

It remains an open question whether CUL7 contributes to p53-dependent apoptosis. Initially, Tsai et al.[4] identified a putative BH3 domain in the C-terminus of CUL7 (p193), suggesting that CUL7 might belong to the BH3-only family of pro-apoptotic proteins. It was shown that forced expression of CUL7 in NIH-3T3 cells promoted apoptosis in a manner that was dependent on the integrity of the BH3 domain. Moreover, expression of SV40 large T antigen or Bcl-x_L, an antagonist of BH3-only proteins, prevented apoptosis.[4] However, given the proximity of this putative BH3 motif with the C-terminally located cullin domain, future work is required to determine whether CUL7's cullin domain plays a role in apoptosis. Notably, expression of a CUL7 C-terminal truncation mutant with presumptive dominant interfering activity (designated CUL7 1152 stop) was found to confer resistance to MG132- and etoposide-induced apoptosis in U2OS cells, independent of CUL7's interaction with p53 or PARC.[25] However, a recent report by Kim et al.,[24] identified CUL7 in a functional screen for inhibitors of Myc-induced apoptosis, showing that expression of CUL7 prevented both c-Myc and N-Myc mediated apoptosis and promoted the transformation of neuroblastoma SHEP cells in a

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p53-dependent manner. Further studies are thus needed to dissect the relative roles of CUL7 as a regulator of apoptosis in various cell types and tissues.

It has been more than a decade since the CUL7 protein was reported to bind to SV40 T antigen.[3,4] SV40 is a member of the polyomaviridae family of DNA viruses capable of inducing tumors in rodents. Owing to the ability of T antigen to transform and immortalize mammalian cells in culture, studies using this oncoprotein yield important insights into the mechanisms of transformation. For instance, it is well documented that T antigen interacts and inhibits both p53 and pRb family proteins, thereby disabling critical cellular tumor suppressive mechanisms (reviewed in ref. [26]). DeCaprio and colleagues demonstrated that the association between T antigen and CUL7 is a requirement for SV40 transformation.[27] This observation is in agreement with an earlier study that demonstrated that co-expression of both dominant interfering CUL7 1152 stop and dominant interfering p53 were required for E1A-mediated transformation of embryonic stem cell-derived cardiac myocytes[28] (unlike T antigen, the E1A viral oncoprotein lacks CUL7 and p53 binding activity). Interestingly, transgenic mice expressing CUL7 1152 stop in the myocardium exhibited enhanced cardiomyocyte proliferation following myocardial infarction,[29] which was sufficient to block adverse post-infarction ventricular remodeling. These results are consistent with a CUL7-mediated role in cell cycle progression, although the precise mechanistic underpinnings remain unclear.

T antigen deletion analyses mapped the CUL7 interaction region to a N-terminal motif spanning residues 69 to 83. Moreover, the transformation potential of the T antigen$^{\Delta 69-83}$ mutant was significantly reduced.[27] However, this mutant could transform cells depleted of CUL7, suggesting that T antigen might neutralize a function of CUL7 that protects against cellular transformation.[27] A recent study by Zhao et al.[30] suggested that the CUL7-T antigen interaction may be required for the degradation of the Mre11-Rad50-Nbs1 complex, which plays a critical role in DNA damage response pathways. It is possible that T antigen may recruit the CUL7 E3 ligase to mediate degradation of cellular proteins for viral propagation. It remains to be investigated whether other non-proteolytic partners of CUL7 (Table 1) influence the CUL7 proteolytic function by regulating the interactions between the E3 and targets, and/or the catalytic efficiency of ubiquitination.

CUL7 is a Novel Regulator of Growth

Two recent studies have independently linked mutations of the *cul7* gene to hereditary growth retardation syndromes in humans. Cormier-Daire and colleagues identified 25 *cul7* germline mutations in patients with 3-M syndrome, an autosomal-recessive disorder characterized by pre- and postnatal growth retardation, facial dysmorphism and skeletal anomalies (Table 2).[31] Of these mutations, 19 predict premature termination of translation, with a majority implicated for loss of the functional cullin domain (see Fig. 1B). More recently, Maksimova et al.[32] identified 43 patients from 37 Yakuts families, a geographically isolated ethnic group in Russia, with a short stature syndrome similar to 3-M syndrome (Table 2). A novel mutation in the *cul7* gene, 4582insT, was found in all these families, and is predicted to produce a truncated protein terminating at amino acid 1553 (see Fig. 1B).

In line with the human hereditary syndromes, targeted disruption of the *cul7* gene in mice resulted in severe intrauterine growth retardation (IUGR) with significantly smaller fetuses at later gestational stages and placenta anomalies (see Table 3). Interestingly, disruption of other cullin family members resulted in early embryonic lethality (<E7.5),[33–36] while CUL7 knockout mice develop anomalies in later gestational stages (>E12.5). Deletion of the *fbw8* gene in mice yielded a similar phenotype as CUL7$^{-/-}$ (see Table 3).[7,37] However, while CUL7$^{-/-}$ mice succumb neonataly due to respiratory distress, disruption of the *fbw8* gene resulted in a less severe phenotype with abnormalities mainly restricted to the placenta and

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growth. Approximately 30% of the homozygous Fbw8$^{-/-}$ offspring reached adulthood, albeit displaying body sizes smaller than their wild-type littermates throughout postnatal development. Clearly, these findings establish overlapping function of CUL7 and Fbw8 in growth control, suggesting that CUL7 requires Fbw8 for executing its major growth-regulatory activity. However, the more severe phenotype of the CUL7$^{-/-}$ mice implicates that CUL7 may possess functions that are independent of Fbw8. Of note, impaired proliferation kinetics were observed with the CUL7$^{-/-}$ and Fbw8$^{-/-}$ MEFs, suggesting that both histopathological and cell autonomous effects contribute to the pathogenesis of the CUL7-associated growth retardation syndromes.

cul7 is located on human chromosome 6p21.1. In humans, CUL7 mRNA is expressed in various fetal and adult tissues, with highest transcript levels found in fetal kidney and placenta, as well as adult skeletal muscle, heart and pancreas.[31] High levels of CUL7 mRNA were found in mouse testes.[4] It was revealed that transcript levels of Fbw8 were most abundant in mouse placenta and skeletal muscle, especially of the abdominal walls, diaphragm and intercostal space.[7] It remains to be investigated whether and how the expression profile of CUL7 and Fbw8 is correlated with the activity of this E3 ligase.

Possible Pathomechanisms for CUL7-Linked Growth Retardation Syndromes

How might the loss of CUL7 function contribute to growth retardation? Given that genetic disruption of either CUL7 or Fbw8 in mice profoundly impaired placental and embryonic development, as well as proliferation kinetics in embryonic fibroblasts, and given that short stature is the predominant clinical feature of patients with 3-M and Yakuts syndromes, it is tempting to speculate that diminished proteolytic function of the CUL7 E3 ligase is the principal pathogenic mechanism. In support of this notion, biochemical characterization of a subset of 3-M derived CUL7 mutations in vitro provided direct evidence for a reduced ubiquitination activity.[31] The finding that IRS-1 is a proteolytic target of the CUL7 E3 is particularly intriguing, as altered activity of the IGF-1 pathway in patients with loss-of-function mutations in the *Igf-1*[38] or *Igf-1 receptor*[39–41] gene has also been linked to severe growth retardation defects (Table 2, reviewed in ref. [42]). Mouse knockout studies are in agreement with the prominent role for IGF-1 signaling in growth (see Table 3). IGF-1 and IGF-1 receptor (IGF-1R) knockout mice exhibited birth weights only 60% and 45% of the wild type animals, respectively.[43–45] Similar to the CUL7$^{-/-}$ mice, IGF-1R knockout mice died soon after birth of respiratory failure.[45] Moreover, ablation of the IRS-1 gene resulted in small, insulin-resistant mice.[46,47] In addition, Cho et al.,[48] showed that mice deficient in Akt1, an isoform of Akt downstream of IGF-1/PI3K (Fig. 2), exhibited both pre- and postnatal growth impairment, with significantly reduced body size.

One possible pathogenic mechanism for the CUL7-linked growth retardation comes from studies with the CUL7$^{-/-}$ MEFs. It was observed that while the CUL7$^{-/-}$ MEFs exhibited high levels of IRS-1 and concomitantly, enhanced PI3K/Akt and RAS-MAPK pathways, these cells grew poorly and displayed typical features of oncogene-induced senescence[9] (OIS). It was, therefore, proposed that accumulation of IRS-1 due to a dysfunctional CUL7 E3 ligase, triggers OIS, which in turn might contribute to the growth retardation phenotype observed in patients with 3-M/Yakuts syndromes.

OIS is a tumor suppressive program that is initiated upon sustained oncogenic signaling to prevent malignant transformation (reviewed in refs. [49–51]). Melanocytic nevi (moles) are a well-characterized example of OIS in cancer biology. Nevi are common benign skin tumors, 80% of which harbor the identical B-Raf (V600E) mutation, which is present in the majority of malignant melanomas. It was revealed that nevi displayed OIS phenotypes, thereby

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suggesting a role for OIS in preventing melanocytic nevi to progress into a malignant state.[52–55] Of particular interest, several components in IRS-1 downstream signaling pathways proved capable of inducing OIS: gain-of-function mutation of Ras (V12),[56] B-Raf (V600E)[55] and MEK,[57] as well as constitutive activation of the PI3K/Akt pathway through depletion of the negative regulator Phosphatase and Tensin homolog (PTEN),[58] or transgenic overexpression of Akt,[59] respectively. Interestingly, several hereditary short stature syndromes such as Noonan-, Costello- or LEOPARD syndrome are linked to gain-of-function mutations of the Ras-Erk MAPK pathway (Table 2; reviewed in ref. [60]). Of note, previous studies have linked premature senescence to Werner syndrome, which is associated with short stature as a main clinical feature (reviewed in ref. [61]). Future investigations are required to determine whether high levels of IRS-1 are sufficient to initiate OIS, and whether senescence is a contributing factor to the pathogenesis of growth retardation observed in 3-M/Yakats dwarfism syndromes.

A second hypothetical pathomechanism is based on the increased expression of IGF-1 binding proteins (IGFBP) found in the $CUL7^{-/-}$ and $Fbw8^{-/-}$ MEFs.[7] It is well established that the vast majority of IGF-1 molecules in the extracellular compartment are complexed with IGFBP, and that IGFBP-bound IGF-1 exhibits altered activity (reviewed in ref. [62]). Several lines of evidence link IGFBPs to the pathogenesis of IUGR: transgenic overexpression of IGFBP-1 and -2 were sufficient to cause fetal growth restriction in transgenic mice (see Table 3).[63–65] Moreover, newborns with IUGR were found to have high IGFBP-1 levels that negatively correlated with IGF-1 availability and fetal growth.[66] Pathways downstream of IRS-1 such as PI3K/Akt and Erk were reported to increase the expression and secretion of IGFBPs, thereby possibly constituting a negative feedback loop on IGF-1 signaling.[67–69] Conceivably, aberrant accumulation of IRS-1 in the $CUL7^{-/-}$ MEFs might trigger the upregulation of IGFBPs, leading to the specific inhibition of IGF-1 receptor signaling. However, IUGR is a complex clinical condition that can result from multiple maternal, fetal and placental dysfunctions (reviewed in ref. [70]). It remains to be determined whether the CUL7 E3 ligase (either directly or indirectly) targets additional factors in the IGF-1 signaling network, and/or in other growth-regulatory pathways, thereby further contributing to the pathogenesis of IUGR.

Is CUL7 a Tumor Suppressor or an Oncogene?

Paradoxically, recent studies have associated CUL7 with these two apparently opposing activities. By using a SV40 T antigen model, DeCaprio and co-workers have identified a potential tumor suppressive role for CUL7 in viral transformation.[27] In addition, the mTOR/IRS-1 negative feedback loop was linked to the inhibition of malignancy in cells where mTOR is hyper-activated such as hamartoma syndromes. Intriguingly these syndromes are typically benign, rather than malignant (reviewed in ref. [71]; see Table 2), and it was proposed that the mTOR/IRS-1 negative feedback loop plays a critical role in restraining PI3K activity, thereby halting the progression to malignancy.[72–74] Such a tumor suppression activity might be mediated, at least in part, by the CUL7-mediated targeted degradation of IRS-1. On the contrary, by employing a Myc-induced apoptosis system, Penn and colleagues revealed a growth-promoting function of CUL7, and presented in silico evidence for upregulated CUL7 mRNA level in non-small cell lung carcinoma.[24] While further investigations are required to resolve the “tumor suppressor or oncogene” conundrum, it would not be surprising if CUL7 proved to play both growth promoting and suppressive roles in a context-dependent manner.

Concluding Remarks

As discussed above, the CUL7 E3 ligase is implicated in multiple biological functions, including pre- and postnatal growth, cellular senescence, cell cycle regulation, apoptosis and

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transformation by SV40 T antigen (see Fig. 3). Presumably, execution of these biological functions requires both proteolytic and non-proteolytic activities of the CUL7 E3 ligase.

How does CUL7 function as a unique molecular scaffold by interacting with Skp1-Fbw8 selectively, and by mediating interactions with multiple proteins of both cellular and viral origin (Table 1)? Is it possible that CUL7 "evolves" from CUL1 to assemble an E3 ligase, specifically contributing to complex growth regulatory pathways, such as IGF-1 signaling, demanded by higher organisms? Insights into these questions require structural resolution of the CUL7 E3 ligase and comparison with the previously resolved structures of canonical cullin-based E3s. Structure-based studies will also be critical to reveal the molecular details that govern the interactions between the CUL7 E3 and targets, or its modulator(s), thereby producing information crucial for development of therapeutic and pharmaceutical agents, amenable for both function studies and treatment of human diseases.

Finally, it remains to be explored whether CUL7 has a role in insulin signaling, which potentially impacts insulin resistance and therefore, diabetes. Clearly, given its critical role in growth and association with multiple cellular growth-regulatory pathways, the CUL7 E3 ligase has emerged as an exciting new branch of Ub research.

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Abbreviations

CUL7
: cullin7

Ub
: ubiquitin

SCF
: Skp1·CUL1· F-box protein

IRS-1
: insulin receptor substrate 1, growth factor 1

IGF-1R
: IGF-1 receptor

UPS
: Ub-proteasome system

IUGR
: intrauterine growth retardation

OIS
: oncogene induced senescence

SV40
: simian virus 40

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mTOR
: mammalian target of rapamycin

MEF
: mouse embryonic fibroblast

IGFBP
: IGF-1 binding protein

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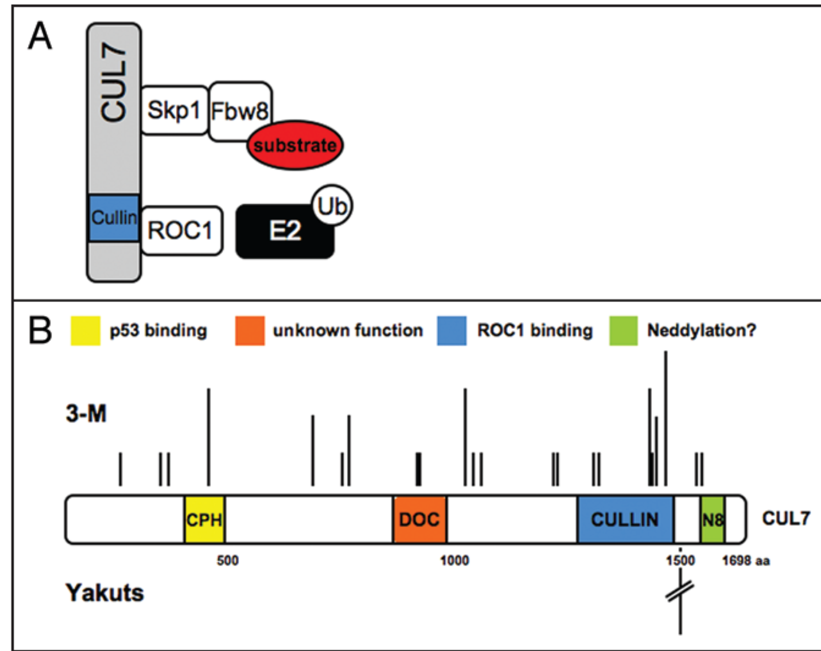

Figure 1.

(A) Composition of the CUL7 E3 Ub ligase complex. The CUL7 protein assembles an SCF-like complex composed of Skp1, Fbw8 and ROC1. While Fbw8 is responsible for substrate protein recognition, ROC1 recruits an Ub-charged E2 Ub-conjugating enzyme for substrate ubiquitination. It remains to be determined how CUL7 binds to the Skp1-Fbw8 heterodimer. (B) Domain organization of the CUL7 protein, as well as localization and relative frequency of CUL7 mutations identified in patients with 3-M (upper half) and Yakuts Short Stature syndrome (lower half). A single mutation (4582insT) of the CUL7 gene, predicted to yield a protein truncated at amino acid position 1553, was found in 43 patients of 37 families with Yakuts short stature syndrome. N8, abbreviation for Nedd8, denotes the CUL7 C-terminal site containing sequence conserved for cullin neddylation, as described previously.[75]

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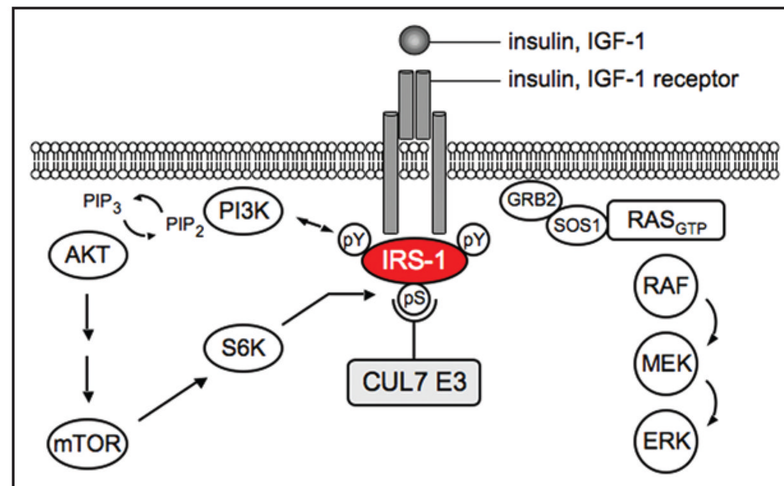

Figure 2.

Role of IRS-1 in insulin and IGF-1 signaling. Upon ligand binding to the receptor, IRS-1 is recruited to the receptor and phosphorylated on tyrosine residues, which serve as docking sites for adaptor proteins of the PI3K/Akt pathway or Ras-Erk MAPK pathway. Akt signaling is restrained by a negative feedback loop via mTOR and its effector serine/threonine kinase S6K. Phosphorylation of multiple serine residues on IRS-1 by mTOR/S6K may create a phosphodegron required for CUL7 E3 ligase-mediated ubiquitination and proteasomal degradation.

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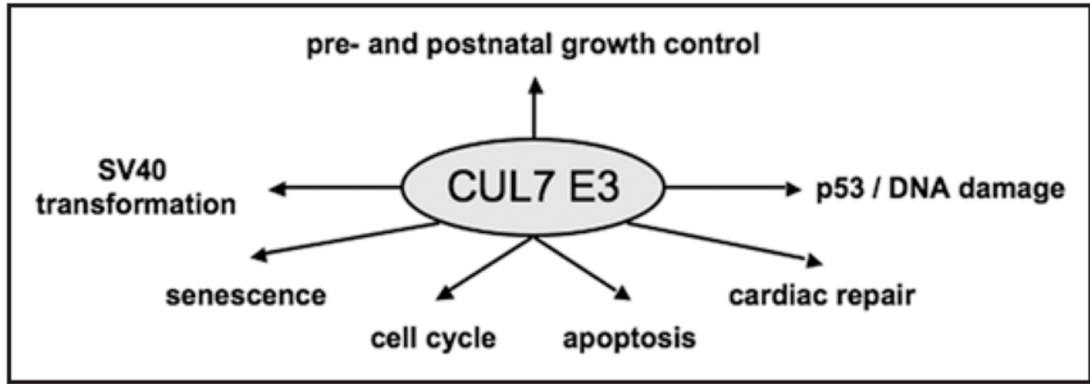

Figure 3.

Biological functions of the CUL7 E3 Ub ligase. As described in the text, the CUL7 E3 is associated with multiple biological functions. Studies on 3-M/Yakuts short stature syndromes[31–32] as well as with mouse knockouts of CUL7/Fbw8,[6,7,37] revealed a prominent role for this E3 in growth control. Moreover, CUL7$^{-/-}$ mouse fibroblasts exhibit senescence phenotype.[9] Using the SV40 T antigen model system, it was observed that the CUL7 interaction with T antigen is required for vial transformation.[27] Given its ability to target cyclin D1 for degradation,[8] the CUL7 E3 may have a role in cell cycle control. In addition, CUL7 was shown to bind to p53.[20–25] The CUL7 E3 appears to be able to regulate apoptosis in both p53-dependent[24] or independent[4] manner. Recent studies have also implicated a role for DNA damage in regulating the p53-CUL7 interactions.[21] Finally, CUL7-mediated cell cycle effects have been implicated in cardiac repair.[28,29]

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Table 1

CUL7 interacting proteins

CUL7-interacting proteins	Comments	Ref.
·The CUL7 E3 components		5, 6
·Skp1	• adaptor capable of tethering the F-box domain and CUL1 • how CUL7 binds to Skp1-Fbw8 remains to be determined	
·Fbw8	• WD-40 repeat-containing F-box protein • targets cyclin D1 (8) and IRS-1 (9) for degradation	8, 9
·ROC1	• RING finger protein capable of recruiting an E2 conjugating enzyme for Ub catalysis • binds to the CUL7 cullin domain	
·Other interacting proteins		
·CUL1	• CUL1 forms a heterodimeric complex with CUL7 in a Fbw8-dependent manner	37
·PARC	• contains cullin, DOC and CPH domains, 60% homologous to CUL7 • CUL7-interacting domains unknown • the significance of the interaction with CUL7 remains to be determined	23, 76
·Glomulin (Fap68)	• binds to the C-terminus of CUL7 • the significance of the interaction with CUL7 remains to be determined	6
·p53	• CUL7 CPH domain binds to the p53 tetramerization domain (20) • enhanced p53-CUL7 interaction by etoposide-induced DNA damage (21) • CUL7 promotes mono- and di-ubiquitination of p53, but not polyubiquitination • CUL7 represses p53-dependent transactivation activity (22)	20–22
·SV40 T antigen	• CUL7-T antigen interaction is required for SV40 transformation (27) • CUL7-T antigen interaction is mediated by the T antigen motif spanning amino acids 69 83 (27) • CUL7 was originally termed p185 (3) or p193 (4), when discovered for interactions with T antigen	3, 4, 27

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Table 2

Human diseases linked to dysregulation of CUL7, or IGF-1 and downstream signaling pathways

Diseases linked to:	Disease names	Gene(s) mutated	Clinical features	Molecular basis	Refs.
CUL7 E3	3-M Yakuts short stature	CUL7	• pre- and postnatal growth retardation • facial dysmorphism • skeletal abnormalities	• majority of 3-M mutations are implicated for loss of the functional cullin domain • common CUL7 mutation in Yakuts patientsyields a C-terminally truncated protein	31, 32
IGF-1/IGF-1R		IGF-1	• pre- and postnatal growth retardation • deafness • mental retardation • osteoporosis	Homozygous mutation converting valine 44 to methionine in IGF-1, reducing IGF-1R binding by 90-fold	38
		IGF-1R	Pre- and postnatal growth retardation	Four families with heterozygote IGF-1R mutations, which decrease the receptor function	39–41
RAS/RAF/MEK	Leopard	SHP2	Common symptoms:	Common molecular culprits:	60
	Noonan	KRAS	•short stature	aberrant activation of the RAS-RAF-ERK pathway	
	Cardo-facio-cutanous (CFC)	BRAF	•facial abnormalities		
	neurofibromatosis (NF1)	MEK1/2	•heart defects		
	Costello	HRAS	•skin abnormalities		
		neurofibromin	•mental retardation		
TSC-mTOR	Hamartoma:	PTEN	Common symptoms:	Common molecular culprits:	71
	Cowden	LKB	•benign tumor	caused by mutations in tumor-suppressor	
	Peutz-Jeghers	TSC	•tumor tissue with	genes that negatively regulate mTOR	
	Tuberous sclerosis		disorganized architecture		

Abbreviations: IGF-1R = IGF-1 receptor.

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Table 3

Mouse models of CUL7 and Fbw8, as well as genes of function in the IGF-1/IRS1 pathways

Gene	Model	Phenotype/observations		Ref.
CUL7	KO	• fetal growth retardation in later gestational stages (>E12.5) • pulmonary anomalies (atelectic lungs, reduced alveolar space)	• 100% of homozygous offspring died at birth due to respiratory failure and cyanosis • dermal and hypodermal hemorrhages in the lower hip	6
		Placenta: • reduced size in later gestational stages (>E12.5) • abnormal spongiotrophoblast development: smaller decidua and spongiotrophoblast layer, fewer secondary trophoblast giant cells	•smaller materal vessel area in the labyrinth layer	
		Mouse embryonic fibroblasts: reduced proliferation rate in culture		
FBW8	KO	• fetal growth retardation in later gestational stages (>E12.5)• 70% of Fbw8$^{-/-}$ offspring died at birth of unknown cause, 30% survived but remain smaller throughout adulthood	•no hemorrhages	7, 37
		Placenta: • reduced size in later gestational stages (>E12.5) • abnormal spongiotrophoblast layer, decidua and trophoblast giant cells not affected	•smaller maternal vessel area in the labyrinth layer	
		Mouse embryonic fibroblasts: reduced proliferation rate in cell culture		
IGF-1R	KO	• 100% of homozygous offspring died at birth due to respiratory failure and cyanosis; size and weight at birth <45% of wild type • generalized organ hypoplasia (including the muscles and skin), anomalies of the nervous system; delayed ossification • placental development not affected*		45
IGF-1	KO	• >95% of homozygous offspring die at birth due to respiratory failure and cyanosis; size and weight at birth <60% of wild type • <5% of IGF-1$^{-/-}$ mice survived birth, but remained smaller throughout life with abnormal development of muscle, reproductive organs (infertility), ossification and skin. • placental development not affected*		43–45
IRS-1	KO	•growth retardation of the fetus in later gestational stages (>E15.5), remain 50 60% smaller throughout adulthood.		46–47

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Gene	Model	Phenotype/observations		Ref.
IGFBP-1	TG	•no organ abnormalities, fertile • reduced body size and weight • generalized organ hypoplasia with exception of brain and spleen	•insulin resistant •fasting hyperglycemia	63
IGFBP-2	TG	• reduced body size and weight	•fasting hypoglycemia	64

Abbreviations: KO = knockout; TG = transgenic mouse model; IGF-1R = IGF-1 receptor.

*despite mice deleted of IGF-1 or IGF-1R revealed no significant changes in placental development, emerging evidence points to a critical role of the IGF system in this process throughout gestation (reviewed in ref. [77]). Placenta weight was positively correlated with cord blood level of IGF-1 and -2,[78,79] and human placenta explant studies demonstrated a key role of IGF-1 and -2 in promoting cytotrophoblast proliferation and differentiation to synzytiotrophblast cells.[80]