Review Article
What is the functional role of the thalidomide binding protein cereblon?

Xiu-bao Chang, A. Keith Stewart

Mayo Clinic College of Medicine, Mayo Clinic Arizona, Scottsdale, AZ 85259, USA.

Abstract: It has been found that nonsense mutation R419X of cereblon (CRBN) is associated with autosomal recessive non-syndromic mental retardation. Further experiments showed that CRBN binds to the cytosolic C-terminus of large-conductance Ca^{++} activated potassium channel (BK_{Ca}) α-subunit and the cytosolic C-terminus of a voltage-gated chloride channel-2 (ClC-2), suggesting that CRBN may play a role in memory and learning via regulating the assembly and surface expression of BK_{Ca} and ClC-2 channels. In addition, it has also been found that CRBN directly interacts with the α1 subunit of AMP-activated protein kinase (AMPK) and prevents formation of a functional holoenzyme with regulatory subunits β and γ. Since AMPK is a master sensor of energy balance that inhibits ATP-consuming anabolic pathways and increases ATP-producing catabolic pathways, binding of CRBN with α1 subunit of AMPK may play a role in these pathways by regulating the function of AMPK. Furthermore, CRBN interacts with damaged DNA binding protein 1 and forms an E3 ubiquitin ligase complex with Cullin 4 where it functions as a substrate receptor in which the proteins recognized by CRBN might be ubiquitinated and degraded by proteasomes. Proteasome-mediated degradation of unneeded or damaged proteins plays a very important role in maintaining regular function of a cell, such as cell survival, dividing, proliferation and growth. Intriguingly, a new role for CRBN has been identified, i.e, the binding of immunomodulatory drugs (IMiDs), e.g. thalidomide, to CRBN has now been associated with teratogenicity and also the cytotoxicity of IMiDs, including lenalidomide, which are widely used to treat multiple myeloma patients. CRBN likely plays an important role in binding, ubiquitination and degradation of factors involved in maintaining function of myeloma cells. These new findings regarding the role of CRBN in IMiD action will stimulate intense investigation of CRBN's downstream factors involved in maintaining regular function of a cell.

Keywords: CRBN, ClC-2, AMPK, BK_{Ca}, DDB1, Cul4A, E3 ubiquitin ligase

Introduction

Genetic linkage analysis revealed that a gene located at 3p25-pter is associated with autosomal recessive nonsyndromic mental retardation (ARNSMR) [1]. Single-strand conformational polymorphism analysis and direct DNA sequencing identified a nonsense mutation in the coding region of a gene named as cereblon (CRBN) [2]. The nomenclature of CRBN was based on its putative role in cerebral development and the presence of a large (237-amino acid) highly conserved ATP-dependent Lon protease domain [2]. However, whether this protein possesses ATP-dependent Lon protease activity or not has not been convincingly proved. In addition, why nonsense mutation that leads to truncation of the C

-terminal 24 amino acid of this gene resulted in mental retardation is not well illustrated yet. CRBN has recently been recognized as the target of immunomodulatory drugs (IMiDs) and the binding of IMiDs to CRBN is teratogenic [3]. Furthermore, IMiD binding to CRBN is cytotoxic to multiple myeloma cells and absence of CRBN confers IMiDs resistance. Although IMiDs are widely used in treating multiple myeloma patients, the molecular mechanism of the treatment with IMiD is not well understood. Furthermore, the overall normal physiologic function of this protein is not well illustrated. In this review, we will summarize the potential functional role of this protein and attempt to facilitate further studies to unveil the functional role of this protein and the molecular mechanism of IMiDs-

mediated cancer cell death.

Brief view of CRBN gene and protein

Human CRBN gene is located on chromosome 3 at 3p26.2 [1, 4]. The size of CRBN genomic DNA is 30,111 bases that contain 11 predicted exons [2, 5] coding for 442 amino acids [2]. Mouse CRBN promoter region contains a NF-E2-related factor 2 binding site and the expression of mouse CRBN is significantly increased upon exposure of the cells to hypoxia/reoxygenation [6]. The apparent molecular weight of CRBN protein is ~ 51 kDa [7]. Human CRBN contains the N-terminal part (237-amino acids from 81 to 317) of ATP-dependent Lon protease domain without the conserved Walker A and Walker B motifs, 11 casein kinase II phosphorylation sites, 4 protein kinase C phosphorylation sites, 1 N-linked glycosylation site and 2 myristoylation sites [2]. Rat CRBN protein contains a regulator of G protein-signaling-like domain and a leucine zipper motif [7]. CRBN is widely expressed in testis, spleen, prostate, liver, pancreas, placenta, kidney, lung, skeletal muscle, ovary, small intestine, peripheral blood leukocyte, colon, brain and retina [5, 7-10] where CRBN is located in cytoplasm, nucleus and peripheral membrane [3, 5, 7-9, 11].

CRBN protein may play an important role in central nervous system development, especially in memory and learning

Deletion of genomic DNA on chromosome 3 that includes CRBN is associated with mental retardation [4, 12, 13], suggesting that wild-type CRBN may play a role in memory and learning. The findings that CRBN is highly expressed in human brain [2, 10] support this conclusion. Further analysis revealed that an R419X nonsense mutation of CRBN leads to the truncation of the C-terminal 24 amino acids and is directly associated with ARNSMR [2], indicating that wild-type CRBN protein plays an important role in memory and learning. This result also indicates that the C-terminal 24 amino acids are crucial for CRBN function. However, the molecular mechanism of CRBN function involved in memory and learning remains unsolved and the pathogenic consequences caused by the truncation in CRBN gene are not clear.

Shortly after identification of CRBN as a potential candidate involved in memory and learning, rat CRBN was identified as a large-conductance Ca^{++} activated potassium channel (BK_{Ca}) α-subunit (Slo) binding protein in rat brain [7]. Mammalian BK_{Ca} channels are widely expressed in neuronal and non-neuronal tissues including epithelia, smooth muscle and sensory cells, composed of four identical α-subunits and four β-subunits [14, 15] and located on the plasma membrane [15]. CRBN binding to Slo subunits may play an important role in regulating assembly by disruption of tetramer formation and in reducing the functional BK_{Ca} channels on plasma membrane surface by holding them in endoplasmic reticulum. Of note, a functional defect of the BK_{Ca} channel is associated with autism and mental retardation [16]. In addition, BK_{Ca} channel over-expression causes impairment of learning and memory [17]. Furthermore, mice lacking BK_{Ca} channels show cerebellar dysfunction in the form of abnormal conditioned eye-blink reflex, abnormal locomotion and pronounced deficiency in motor coordination, perhaps due to the consequences of cerebellar learning deficiency [18]. Thus, it is most likely that CRBN participates in memory and learning by regulating the cell surface expression of functional BK_{Ca} channels [7, 8].

However, normal function of the central nervous system requires not only anion channels and cation channels, but also neurotransmitter transporters and receptors. Thus, BK_{Ca} should not be the sole channel that plays an important role in the central nervous system. Interestingly, it has been found that the Lon region of CRBN interacts with the C-terminal tail of ClCs, such as ClC-1 and ClC-2 [9]. ClCs are ubiquitously expressed as homodimers [19, 20] at the plasma membrane where they function as inwardly rectifying chloride channels [9]. These chloride channels play important roles in the regulation of cellular excitability, in trans-epithelial transport and in cell volume regulation [21]. It has been revealed that ClC-2 is expressed in brain [22] and detected in neuronal cell bodies and synapses [23, 24], suggesting that ClC-2 may play a role in control of neuronal excitability. Thus, CRBN binding to ClC-2 may play a role in control of neuronal excitability by preventing the formation of a functional ClC-2 homodimer on the plasma membrane surface. However, since no convincing data have been presented, the functional role of CRBN binding to ClCs needs to be further elucidated. It is tempting to postulate, however, that the well-recognized sedative properties of thalidomide may in some way be related to the drug's inhibition of CRBN.

CRBN may play a role in energy-balance by interacting with AMP-activated protein kinase (AMPK)

In searching for more CRBN partners in the brain, rat CRBN was used as a bait in a yeast two hybrid system to screen rat brain cDNA [11]. Interestingly, it was found that rat CRBN directly interacts with the α1 subunit of AMPK. The following results clearly indicate that this binding is specific: 1) AMPK α1 subunit was co-immunoprecipitated with CRBN; 2) CRBN as well as AMPK β and AMPK γ1 were co-immunoprecipitated with AMPK α1; 3) Pull-down experiments showed that either wild-type AMPK α1 or T172D- or D157A-mutated AMPK α1 can bind to the purified (from E. coli) glu-tathione-S-transferase-CRBN fusion protein; 4) Double immunohistostaining of hippocampal neurons indicated that some of the AMPK α1 subunits are co-localized with CRBN [11]. Further experiments suggested that a small region in the C-terminus, from 393 to 422, of AMPK α1 subunit would be the critical region for high affinity binding to CRBN.

AMPK holoenzyme is a serine/threonine protein kinase comprising a heterotrimer of a catalytic α subunit and two regulatory subunits β and γ. Interestingly, CRBN binding to AMPK α1 subunit competes with AMPK γ1 for AMPK complex formation [11]. This competition resulted in decreased activation of AMPK. Thus, expression of rat, mouse or human CRBN in HEK293FT cells or other cells significantly suppressed the activation of endogenous AMPK [11]. Due to the suppression of AMPK activation by over-expression of exogenous CRBN, the phosphorylation of the AMPK substrate acetyl coenzyme A carboxylase was also significantly decreased [11]. In contrast, knockdown of endogenous CRBN dramatically up-regulated the endogenous AMPK activity [11]. Thus, CRBN functions as a negative regulator of AMPK.

The heterotrimeric protein of AMPK is highly expressed in skeletal muscle, liver, kidney, brain, mammary glands, heart, lung and adipose tissue [25] where it functions as a master sensor of energy balance that inhibits ATP-consuming anabolic pathways and increases ATP-producing catabolic pathways. For example, during acute exercise, as muscles contract, ATP is efficiently hydrolyzed to ADP and inorganic phosphate. Two ADP molecules are catalyzed by adenylate kinase to generate one ATP molecule

and one AMP molecule. As more AMP is produced during muscle contraction, the AMP:ATP ratio is dramatically increased. Once the ratio of AMP:ATP has risen, two AMP molecules will replace the bound ATP molecules at the cystathionine beta synthase domains of the AMPK γ subunit [26] which in turn induces conformational changes of the protein and exposes the T172 of AMPK α subunit to an upstream AMPK kinase [27]. Upon phosphorylation of T172 on AMPK α subunit by AMPK kinase, the holoenzyme becomes activated. The activated AMPK acts as a metabolic switch that regulates many different pathways involving protein metabolism, cell growth, apoptosis, lipid metabolism and carbohydrate metabolism. For example, the activated AMPK promotes relocation of glucose transporter 4 (GLUT4) from the cytosolic pool to the plasma membrane (by an unknown mechanism) [28-36]. Once GLUT4 is relocated to plasma membrane, it will facilitate the cellular uptake of glucose which will be used to generate ATP by glycolysis. In the meantime, activation of AMPK will: 1) stimulate the hepatic fatty acid oxidation and ketogenesis; 2) inhibit cholesterol synthesis, lipogenesis and triglyceride synthesis; 3) stimulate skeletal muscle fatty acid oxidation; and 4) modulate (down) insulin secretion by pancreatic beta-cells.

CRBN forms an E3 ubiquitin ligase complex with damaged DNA binding protein 1 (DDB1) and Cullin-4 (CUL4)

The DDB1, CUL4 and regulator of cullin 1 (ROC1) complex is an identified cullin-RING ubiquitin ligase that regulates DNA repair [37-43], DNA replication [44-47] and transcription [48]. Although the DDB1 in this complex might directly recruit substrates to the E3 ubiquitin ligase, ubiquitination of several known substrates suggested that this ubiquitination process might require additional cellular factors [40, 49]. In searching for additional cellular factors that might participate in ubiquitination in E3 ubiquitin ligase complex, CRBN, along with other 30 proteins, was identified as a potential factor or substrate receptor contributing to ubiquitination of cellular proteins and named as DDB1-CUL4-associated factor (DCAF) [50].

Although DCAF proteins were suggested to be factors serving as the substrate-recruiting modules or substrate receptors for the E3 ubiquitin ligase machinery [50, 51], the functional role of CRBN in this complex is still unknown. Given the

fact that CRBN also binds to BK_{Ca} [7, 8], ClC-2 [9] and AMPK [11], it is possible that CRBN might function as a substrate-recruiter to bind to these proteins for ubquitination by the E3 ubiquitin ligase machinery. However, even if it is the case that binding of BK_{Ca}, ClC-2 and AMPK to CRBN leads to ubiquitination and proteasome-mediated degradation, it is still not clear whether CRBN will bind to other proteins or not.

Thalidomide, an IMiD drug, was prescribed in 1950s to pregnant women as a treatment for their morning sickness. However, treatment with this sedative drug caused birth defects [52-54]. In searching for thalidomide target proteins, thalidomide was immobilized on ferriteglycidyl methacrylate beads and used to pull out the thalidomide binding proteins from human Hela cell extracts [3]. CRBN and DDB1 were specifically pulled out from these cell extracts [3]. Interestingly, CRBN can be auto-ubiquitinated *in vitro* and *in vivo* [3]. Thus, it is possible that auto-ubiquitination of CRBN may play a self-regulatory role, i.e., 1) in the presence of CRBN substrate, CRBN binds to its substrate and leads to ubiquitination of the CRBN-bound substrate and then proteasome-mediated degradation of the ubiquitinated substrate; 2) in the absence of CRBN substrate, CRBN is auto-ubiquitinated and undergoes proteasome-mediated degradation. In addition, auto-ubiquitination of CRBN was inhibited by thalidomide in a concentration dependent manner [3], suggesting that binding of thalidomide to CRBN may inhibit the function of the CRBN-DDB1-CUL4A-ROC1 E3 ubiquitin ligase complex, but not the other DCAFs-bound DDB1-CUL4A-ROC1 E3 ubiquitin ligase complex.

Although treatment with thalidomide caused birth defects [52-54], the molecular mechanism of the birth defects was not well elucidated. Ito et al [3] have found that the development of pectoral fins and otic vesicles in thalidomide-treated zebrafish embryos was disturbed. The embryos injected with an anti-sense oligonucleotide for zebrafish Crbn yielded specific defects in fin and otic vesicle development, which is similar to those of thalidomide-treated embryos. These defects were rescued by co-injection of zebrafish Crbn mRNA [3]. In addition, thalidomide treatment of zebrafish embryos over-expressing Y374A/W376A-mutated zebrafish Crbn, which prevents thalidomide binding, did not significantly affect otic vesicle size [3]. Furthermore, the expression of fibro-

blast growth factor 8a (Fgf8a) in thalidomide treated embryos was severely reduced and Fgf8a expression was restored upon injection of Y374A/W376A-mutated zebrafish Crbn mRNA, suggesting that thalidomide exerts teratogenic effects by inhibiting CRBN function. What is the molecular mechanism of thalidomide-induced teratogenicity? The review article published recently [55] gave a comprehensive review of the molecular mechanisms of thalidomide-induced teratogenicity. Briefly, many hypotheses, including oxidative stress hypothesis and anti-angiogenesis hypothesis, have been proposed to explain how thalidomide causes birth defects. However, none of these hypotheses has clearly indicated the functional role of CRBN. Therefore, the CRBN substrates involved in development remain to be determined.

In spite of the teratogenic effect of thalidomide, it has been found that thalidomide is useful in treating multiple myeloma patients [56, 57]. Based on this finding, related compounds including thalidomide and its analogues lenalidomide and pomalidomide are widely used nowadays to treat multiple myeloma patients and other hematogenic malignancies. Despite the fact that the availability of the IMiDs has dramatically improved survival for patients with myeloma [58], the molecular mechanism of the action of these IMiDs remains unclear. A recent paper published in Leukemia [59] gave a comprehensive review about the molecular mechanism of the action of IMiDs in multiple myeloma, indicating that these IMiDs possess pleiotropic anti-myeloma properties including immune-modulation, anti-angiogenic, anti-inflammatory and anti-proliferative effects. However, the direct target of these IMiDs is not known. It has been shown that CRBN is the direct target of thalidomide in teratogenic effects [3, 55], implying that CRBN might also be the direct target of the IMiDs used in the treatment of multiple myeloma patients. Interestingly, our recent results indicate that CRBN expression is required for the anti-myeloma activity of IMiDs including lenalidomide and pomalidomide [60], implying that CRBN might be the direct target of these IMiDs.

Although the availability of the IMiDs has dramatically improved survival for patients with multiple myeloma, the majority of the patients treated with IMiDs develop resistance over time by mechanisms that remain unknown [58]. In one study of myeloma cells made resistant to

lenalidomide [61], analysis revealed that dysregulation of Wnt/β-catenin pathway may be one of the possible reasons of lenalidomide resistance. Interestingly, acute lenalidomide treatment increased β-catenin transcription by 3 fold and both acute and chronic exposure of multiple myeloma cells to lenalidomide resulted in enhanced accumulation of β-catenin protein by up to 20 fold [61]. This fact could be interpreted as that the phosphorylated β-catenin protein could be a substrate of CRBN. In the absence of Wnt, *β*-catenin is phosphorylated by casein kinase 1α at Ser45, which primes it for phosphorylation by glycogen synthase kinase-3 α/β at Thr41, Ser37 and then Ser33 (on *β*-catenin) [62]. At least, theoretically, once phosphorylated β-catenin is recognized by CRBN, it should trigger *β*-catenin ubiquitination (by CRBN-DDB1-CUL4-ROC1 E3 ubiquitin ligase complex) and proteasome-mediated degradation. However, in the presence of thalidomide or lenalidomide, CRBN cannot form a functional CRBN-DDB1-CUL4-ROC1 E3 ubiquitin ligase, thus, the phosphorylated β-catenin protein cannot be efficiently ubiquitinated. The consequence of this inhibition effect would result in enhanced accumulation of β-catenin protein and enhanced downstream targets cyclin D1 and c-Myc [61]. Although this interpretation sounds reasonable, up regulation of cyclin D1 and c-Myc does not fit with the ability of lenalidomide to efficiently kill the parental multiple myeloma cells that have not undergone lenalidomide long-term selection. Thus, enhanced accumulation of β-catenin during short term or long term treatment with lenalidomide might be a consequence, but not the major mechanism of lenalidomide resistance. In fact, recently, we have found that the expression of CRBN in the lenalidomide selected multiple myeloma cells is un-detectable and the likely source of resistance. CRBN knockdown by small interfering RNA confers resistance to lenalidomide or pomalidomide [60]. Thus, it will be quite interesting in these studies to examine levels of β-catenin and CRBN in those relapsed patients who become resistant to IMiD treatment.

Conclusion and future prospects

A non-sense mutation of CRBN gene was identified in a kindred with mild mental retardation [2]. However, how such a small truncation in the C-terminus of CRBN causes mental retardation remains unknown. CRBN interacts with BK_{Ca} [7, 63] and ClCs [9] and modulates their functions.

Therefore, it is possible that CRBN participates in memory and learning via modulating large-conductance calcium-activated potassium channel activity and ClC chloride channel activity. In addition, CRBN interacts with the α1 subunit of AMPK [11], prevents the formation of functional AMPK and modulates the multifunctional metabolic sensor AMPK. Recently, CRBN has also been found to be a substrate receptor in the DDB1-CUL4A-ROC1 E3 ubiquitin ligase complex [3, 50].

Although the substrates of CRBN have not been well elucidated yet, CRBN has been found to be the target protein of IMiDs [3, 55]. Treatment of pregnant women and zebrafish or chicks embryos with thalidomide is teratogenic [3, 55], implying that CRBN might play an important role in development. In addition, IMiDs, which possess pleiotropic anti-myeloma properties including immuno-modulation, anti-angiogenic, anti-inflammatory and anti-proliferative effects [59], are widely used to treat multiple myeloma and preferentially target clonogenic cells (or stem like cells) in the side population of multiple myeloma [64].

Thus, although few papers have been published since the identification of CRBN as a factor associated with memory and learning in 2004, published results clearly indicate that CRBN plays important roles in binding proteins for degradation, in cell survival, in memory and learning and in energy balance and fetal development. In addition, inhibition of CRBN function by IMiDs may play an important role in treating multiple myeloma patients. Therefore, future efforts will be made to determine: 1) how CRBN gene expression is regulated; 2) the structural and functional relationship of CRBN; 3) the substrates of CRBN in CRBN-DDB1-CUL4-ROC1 E3 ubiquitin ligase complex; 4) the molecular mechanism of CRBN-mediated memory and learning; 5) the molecular mechanism of IMiDs induced apoptosis; 6) the molecular mechanism of IMiDs-resistance in multiple myeloma; and 7) whether we can combat IMiDs-resistance or not. It is expected that with improved understanding of the molecular mechanisms of CRBN functions, novel therapeutic approaches could be developed in treating multiple myeloma and other cancers.

Abbreviations

AMPK, AMP-activated protein kinase; ARNSMR, autosomal recessive nonsyndromic mental retardation;

BK$_{Ca}$, large-conductance Ca^{++} activated potassium channel; ClC-2, voltage-gated chloride channel-2; CRBN, cereblon; CUL4, Cullin 4; DCAF, DDB1-CUL4A-associated factor; DDB1, damaged DNA binding protein 1; Fgf8a, fibroblast growth factor 8a; GLUT4, glucose transporter 4; IMiDs, immunomodulatory drugs; ROC1, regulator of cullin 1; Slo, BK$_{Ca}$ α-subunit.

Acknowledgements

Please address correspondence to: Dr. Xiu-bao Chang, Or: Dr. A. Keith Stewart, Mayo Clinic College of Medicine, Mayo Clinic Arizona, Scottsdale, AZ 85259, USA. Tel: 480-301-6151; Fax: 480-301-8387, E-mail: xbchang@mayo.edu, Or: stewart.keith@mayo.edu

References

[1] Higgins JJ, Rosen DR, Loveless JM, Clyman JC and Grau MJ. A gene for nonsyndromic mental retardation maps to chromosome 3p25-pter. Neurology 2000; 55: 335-340.

[2] Higgins JJ, Pucilowska J, Lombardi RQ and Rooney JP. A mutation in a novel ATP-dependent Lon protease gene in a kindred with mild mental retardation. Neurology 2004; 63: 1927-1931.

[3] Ito T, Ando H, Suzuki T, Ogura T, Hotta K, Imamura Y, Yamaguchi Y and Handa H. Identification of a primary target of thalidomide teratogenicity. Science 327: 1345-1350.

[4] Dijkhuizen T, van Essen T, van der Vlies P, Verheij JB, Sikkema-Raddatz B, van der Veen AY, Gerssen-Schoorl KB, Buys CH and Kok K. FISH and array-CGH analysis of a complex chromosome 3 aberration suggests that loss of CNTN4 and CRBN contributes to mental retardation in 3pter deletions. Am J Med Genet A 2006; 140: 2482-2487.

[5] Xin W, Xiaohua N, Peilin C, Xin C, Yaqiong S and Qihan W. Primary function analysis of human mental retardation related gene CRBN. Mol Biol Rep 2008; 35: 251-256.

[6] Lee KJ, Lee KM, Jo S, Kang KW and Park CS. Induction of cereblon by NF-E2-related factor 2 in neuroblastoma cells exposed to hypoxia-reoxygenation. Biochem Biophys Res Commun 399: 711-715.

[7] Jo S, Lee KH, Song S, Jung YK and Park CS. Identification and functional characterization of cereblon as a binding protein for large-conductance calcium-activated potassium channel in rat brain. J Neurochem 2005; 94: 1212-1224.

[8] Higgins JJ, Tal AL, Sun X, Hauck SC, Hao J, Kosofsky BE and Rajadhyaksha AM. Temporal and spatial mouse brain expression of cereblon, an ionic channel regulator involved in human intelligence. J Neurogenet 24: 18-26.

[9] Hohberger B and Enz R. Cereblon is expressed in the retina and binds to voltage-gated chloride channels. FEBS Lett 2009; 583: 633-637.

[10] Aizawa M, Abe Y, Ito T, Handa H and Nawa H. mRNA distribution of the thalidomide binding protein cereblon in adult mouse brain. Neurosci Res 69: 343-347.

[11] Lee KM, Jo S, Kim H, Lee J and Park CS. Functional modulation of AMP-activated protein kinase by cereblon. Biochim Biophys Acta 1813: 448-455.

[12] Narahara K, Kikkawa K, Murakami M, Hiramoto K, Namba H, Tsuji K, Yokoyama Y and Kimoto H. Loss of the 3p25.3 band is critical in the manifestation of del(3p) syndrome: karyotype-phenotype correlation in cases with deficiency of the distal portion of the short arm of chromosome 3. Am J Med Genet A 1990; 35: 269-273.

[13] Cargile CB, Goh DL, Goodman BK, Chen XN, Korenberg JR, Semenza GL and Thomas GH. Molecular cytogenetic characterization of a subtle interstitial del(3)(p25.3p26.2) in a patient with deletion 3p syndrome. Am J Med Genet A 2002; 109: 133-138.

[14] Knaus HG, Garcia-Calvo M, Kaczorowski GJ and Garcia ML. Subunit composition of the high conductance calcium-activated potassium channel from smooth muscle, a representative of the mSlo and slowpoke family of potassium channels. The Journal of biological chemistry 1994; 269: 3921-3924.

[15] Berkefeld H, Fakler B and Schulte U. Ca2+-activated K+ channels: from protein complexes to function. Physiological reviews 2010; 90: 1437-1459.

[16] Laumonnier F, Roger S, Guerin P, Molinari F, M'Rad R, Cahard D, Belhadj A, Halayem M, Persico AM, Elia M, Romano V, Holbert S, Andres C, Chaabouni H, Colleaux L, Constant J, Le Guennec JY and Briault S. Association of a functional deficit of the BKCa channel, a synaptic regulator of neuronal excitability, with autism and mental retardation. The American journal of psychiatry 2006; 163: 1622-1629.

[17] Hammond RS, Bond CT, Strassmaier T, Ngo-Anh TJ, Adelman JP, Maylie J and Stackman RW. Small-conductance Ca2+-activated K+ channel type 2 (SK2) modulates hippocampal learning, memory, and synaptic plasticity. The Journal of neuroscience : the official journal of the Society for Neuroscience 2006; 26: 1844-1853.

[18] Sausbier M, Hu H, Arntz C, Feil S, Kamm S, Adelsberger H, Sausbier U, Sailer CA, Feil R, Hofmann F, Korth M, Shipston MJ, Knaus HG, Wolfer DP, Pedroarena CM, Storm JF and Ruth P. Cerebellar ataxia and Purkinje cell dysfunction caused by Ca2+-activated K+ channel deficiency. Proceedings of the National Academy of Sciences of the United States of America 2004;

101: 9474-9478.

[19] Accardi A and Picollo A. CLC channels and transporters: proteins with borderline personalities. Biochimica et biophysica acta 2010; 1798: 1457-1464.

[20] Jentsch TJ. CLC chloride channels and transporters: from genes to protein structure, pathology and physiology. Critical reviews in biochemistry and molecular biology 2008; 43: 3-36.

[21] Jentsch TJ. Chloride channels: a molecular perspective. Current opinion in neurobiology 1996; 6: 303-310.

[22] Smith RL, Clayton GH, Wilcox CL, Escudero KW and Staley KJ. Differential expression of an inwardly rectifying chloride conductance in rat brain neurons: a potential mechanism for cell-specific modulation of postsynaptic inhibition. The Journal of neuroscience : the official journal of the Society for Neuroscience 1995; 15: 4057-4067.

[23] Bosl MR, Stein V, Hubner C, Zdebik AA, Jordt SE, Mukhopadhyay AK, Davidoff MS, Holstein AF and Jentsch TJ. Male germ cells and photoreceptors, both dependent on close cell-cell interactions, degenerate upon ClC-2 Cl(-) channel disruption. The EMBO journal 2001; 20: 1289-1299.

[24] Enz R, Ross BJ and Cutting GR. Expression of the voltage-gated chloride channel ClC-2 in rod bipolar cells of the rat retina. The Journal of neuroscience : the official journal of the Society for Neuroscience 1999; 19: 9841-9847.

[25] Verhoeven AJ, Woods A, Brennan CH, Hawley SA, Hardie DG, Scott J, Beri RK and Carling D. The AMP-activated protein kinase gene is highly expressed in rat skeletal muscle. Alternative splicing and tissue distribution of the mRNA. European journal of biochemistry / FEBS 1995; 228: 236-243.

[26] Adams J, Chen ZP, Van Denderen BJ, Morton CJ, Parker MW, Witters LA, Stapleton D and Kemp BE. Intrasteric control of AMPK via the gamma1 subunit AMP allosteric regulatory site. Protein science : a publication of the Protein Society 2004; 13: 155-165.

[27] Hawley SA, Davison M, Woods A, Davies SP, Beri RK, Carling D and Hardie DG. Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. The Journal of biological chemistry 1996; 271: 27879-27887.

[28] Klip A, Schertzer JD, Bilan PJ, Thong F and Antonescu C. Regulation of glucose transporter 4 traffic by energy deprivation from mitochondrial compromise. Acta physiologica 2009; 196: 27-35.

[29] Cartee GD and Wojtaszewski JF. Role of Akt substrate of 160 kDa in insulin-stimulated and contraction-stimulated glucose transport. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme 2007; 32: 557-566.

[30] Santos JM, Ribeiro SB, Gaya AR, Appell HJ and Duarte JA. Skeletal muscle pathways of contraction-enhanced glucose uptake. International journal of sports medicine 2008; 29: 785-794.

[31] Winder WW, Taylor EB and Thomson DM. Role of AMP-activated protein kinase in the molecular adaptation to endurance exercise. Medicine and science in sports and exercise 2006; 38: 1945-1949.

[32] Luiken JJ, Coort SL, Koonen DP, Bonen A and Glatz JF. Signalling components involved in contraction-inducible substrate uptake into cardiac myocytes. The Proceedings of the Nutrition Society 2004; 63: 251-258.

[33] Krook A, Wallberg-Henriksson H and Zierath JR. Sending the signal: molecular mechanisms regulating glucose uptake. Medicine and science in sports and exercise 2004; 36: 1212-1217.

[34] Luiken JJ, Coort SL, Koonen DP, van der Horst DJ, Bonen A, Zorzano A and Glatz JF. Regulation of cardiac long-chain fatty acid and glucose uptake by translocation of substrate transporters. Pflugers Archiv : European journal of physiology 2004; 448: 1-15.

[35] Richter EA, Derave W and Wojtaszewski JF. Glucose, exercise and insulin: emerging concepts. The Journal of physiology 2001; 535: 313-322.

[36] Winder WW. AMP-activated protein kinase: possible target for treatment of type 2 diabetes. Diabetes technology & therapeutics 2000; 2: 441-448.

[37] Nag A, Bondar T, Shiv S and Raychaudhuri P. The xeroderma pigmentosum group E gene product DDB2 is a specific target of cullin 4A in mammalian cells. Molecular and cellular biology 2001; 21: 6738-6747.

[38] Chen X, Zhang Y, Douglas L and Zhou P. UV-damaged DNA-binding proteins are targets of CUL-4A-mediated ubiquitination and degradation. The Journal of biological chemistry 2001; 276: 48175-48182.

[39] Sugasawa K, Okuda Y, Saijo M, Nishi R, Matsuda N, Chu G, Mori T, Iwai S, Tanaka K and Hanaoka F. UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. Cell 2005; 121: 387-400.

[40] Groisman R, Kuraoka I, Chevallier O, Gaye N, Magnaldo T, Tanaka K, Kisselev AF, Harel-Bellan A and Nakatani Y. CSA-dependent degradation of CSB by the ubiquitin-proteasome pathway establishes a link between complementation factors of the Cockayne syndrome. Genes & development 2006; 20: 1429-1434.

[41] Groisman R, Polanowska J, Kuraoka I, Sawada J, Saijo M, Drapkin R, Kisselev AF, Tanaka K and Nakatani Y. The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially

regulated by the COP9 signalosome in response to DNA damage. Cell 2003; 113: 357-367.

[42] Kapetanaki MG, Guerrero-Santoro J, Bisi DC, Hsieh CL, Rapic-Otrin V and Levine AS. The DDB1-CUL4ADDB2 ubiquitin ligase is deficient in xeroderma pigmentosum group E and targets histone H2A at UV-damaged DNA sites. Proceedings of the National Academy of Sciences of the United States of America 2006; 103: 2588-2593.

[43] Wang H, Zhai L, Xu J, Joo HY, Jackson S, Erdjument-Bromage H, Tempst P, Xiong Y and Zhang Y. Histone H3 and H4 ubiquitylation by the CUL4-DDB-ROC1 ubiquitin ligase facilitates cellular response to DNA damage. Molecular Cell 2006; 22: 383-394.

[44] Liu C, Powell KA, Mundt K, Wu L, Carr AM and Caspari T. Cop9/signalosome subunits and Pcu4 regulate ribonucleotide reductase by both checkpoint-dependent and -independent mechanisms. Genes & development 2003; 17: 1130-1140.

[45] Higa LA, Mihaylov IS, Banks DP, Zheng J and Zhang H. Radiation-mediated proteolysis of CDT1 by CUL4-ROC1 and CSN complexes constitutes a new checkpoint. Nature cell biology 2003; 5: 1008-1015.

[46] Hu J, McCall CM, Ohta T and Xiong Y. Targeted ubiquitination of CDT1 by the DDB1-CUL4A-ROC1 ligase in response to DNA damage. Nature cell biology 2004; 6: 1003-1009.

[47] Bondar T, Ponomarev A and Raychaudhuri P. Ddb1 is required for the proteolysis of the Schizosaccharomyces pombe replication inhibitor Spd1 during S phase and after DNA damage. The Journal of biological chemistry 2004; 279: 9937-9943.

[48] Wertz IE, O'Rourke KM, Zhang Z, Dornan D, Arnott D, Deshaies RJ and Dixit VM. Human De-etiolated-1 regulates c-Jun by assembling a CUL4A ubiquitin ligase. Science 2004; 303: 1371-1374.

[49] Liu C, Poitelea M, Watson A, Yoshida SH, Shimoda C, Holmberg C, Nielsen O and Carr AM. Transactivation of Schizosaccharomyces pombe cdt2+ stimulates a Pcu4-Ddb1-CSN ubiquitin ligase. The EMBO journal 2005; 24: 3940-3951.

[50] Angers S, Li T, Yi X, MacCoss MJ, Moon RT and Zheng N. Molecular architecture and assembly of the DDB1-CUL4A ubiquitin ligase machinery. Nature 2006; 443: 590-593.

[51] Zimmerman ES, Schulman BA and Zheng N. Structural assembly of cullin-RING ubiquitin ligase complexes. Current opinion in structural biology 2010; 20: 714-721.

[52] Miller MT and Stromland K. Teratogen update: thalidomide: a review, with a focus on ocular findings and new potential uses. Teratology 1999; 60: 306-321.

[53] Melchert M and List A. The thalidomide saga.

The international journal of biochemistry & cell biology 2007; 39: 1489-1499.

[54] Knobloch J and Ruther U. Shedding light on an old mystery: thalidomide suppresses survival pathways to induce limb defects. Cell Cycle 2008; 7: 1121-1127.

[55] Ito T, Ando H and Handa H. Teratogenic effects of thalidomide: molecular mechanisms. Cell Mol Life Sci 68: 1569-1579.

[56] van Rhee F, Dhodapkar M, Shaughnessy JD, Jr., Anaissie E, Siegel D, Hoering A, Zeldis J, Jenkins B, Singhal S, Mehta J, Crowley J, Jagannath S and Barlogie B. First thalidomide clinical trial in multiple myeloma: a decade. Blood 2008; 112: 1035-1038.

[57] Singhal S, Mehta J, Desikan R, Ayers D, Roberson P, Eddlemon P, Munshi N, Anaissie E, Wilson C, Dhodapkar M, Zeddis J and Barlogie B. Antitumor activity of thalidomide in refractory multiple myeloma. The New England journal of medicine 1999; 341: 1565-1571.

[58] Stewart AK. Novel therapies for relapsed myeloma. Hematology Am Soc Hematol Educ Program 2009; 578-586.

[59] Quach H, Ritchie D, Stewart AK, Neeson P, Harrison S, Smyth MJ and Prince HM. Mechanism of action of immunomodulatory drugs (IMiDS) in multiple myeloma. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K 2010; 24: 22-32.

[60] Zhu YX, Braggio E, Shi CX, Bruins LA, Schmidt JE, Van Wier S, Chang XB, Bjorklund CC, Fonseca R, Bergsagel PL, Orlowski RZ and Stewart AK. Cereblon expression is required for the anti-myeloma activity of lenalidomide and pomalidomide. Blood 2011;

[61] Bjorklund CC, Ma W, Wang ZQ, Davis RE, Kuhn DJ, Kornblau SM, Wang M, Shah JJ and Orlowski RZ. Evidence of a role for activation of Wnt/beta-catenin signaling in the resistance of plasma cells to lenalidomide. J Biol Chem 286: 11009-11020.

[62] Liu C, Li Y, Semenov M, Han C, Baeg GH, Tan Y, Zhang Z, Lin X and He X. Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell 2002; 108: 837-847.

[63] Higgins JJ, Hao J, Kosofsky BE and Rajadhyaksha AM. Dysregulation of large-conductance Ca2+-activated K+ channel expression in non-syndromal mental retardation due to a cereblon p.R419X mutation. Neurogenetics 2008; 9: 219-223.

[64] Jakubikova J, Adamia S, Kost-Alimova M, Klippel S, Cervi D, Daley JF, Cholujova D, Kong SY, Leiba M, Blotta S, Ooi M, Delmore J, Laubach J, Richardson PG, Sedlak J, Anderson KC and Mitsiades CS. Lenalidomide targets clonogenic side population in multiple myeloma: pathophysiologic and clinical implications. Blood 2011; 117: 4409-4419.