TRIB2 and the ubiquitin proteasome system in cancer

Mara Salomè, Joana Campos, Karen Keeshan*

Paul O'Gorman Leukaemia Research Centre, Institute of Cancer Sciences, University of Glasgow, Scotland

*Corresponding author: Karen Keeshan. Paul O'Gorman Leukaemia Research Centre, Institute of Cancer Sciences, University of Glasgow, Scotland

Tel: 0044 141 301 7895

Email: Karen.keeshan@glasgow.ac.uk

Key words: TRIB2, ubiquitin proteasome, cancer, leukaemia

Abstract:

Tribbles family of pseudokinase proteins are known to mediate the degradation of target proteins in drosophila and mammalian systems. The main protein proteolysis pathway in eukaryotic cells is the ubiquitin proteasome system (UPS). The TRIB2 mammalian family member has been well characterised for its role in murine and human leukaemia, lung and liver cancer. One of the most characterised substrates for TRIB2-mediated degradation is the myeloid transcription factor CCAAT enhancer binding protein α (C/EBPα). However, across a number of cancers, the molecular interactions that take place between TRIB2 and factors involved in the UPS are varied and have differential downstream effects. This review summarises our current knowledge of these interactions and how this information is important for our understanding of TRIB2 in cancer.

***TRIB2* gene dysregulation in Cancer**

Tribbles homolog 2 (TRIB2) is a pseudokinase that functions as a molecular adaptor mediating degradation and changes to signalling cascades[1]. *TRIB2* is also shown to be a potent oncogene in a variety of malignancies, including myeloid[2] and lymphoid[3] leukaemia, melanoma[4], lung cancer[5], and liver cancer[6]. The oncogenic activity of *TRIB2* is linked to its dysregulated expression, rather than its dysregulation via mutation in the majority of cancers. We analysed *TRIB2* gene in the Catalogue Of Somatic Mutations In Cancer (COSMIC) database (http://cancer.sanger.ac.uk, [7], the most comprehensive source of curated analysed somatic mutations in human cancer to date. This analysis has allowed us determine whether *TRIB2* is altered at the genomic level across a panel of tumour samples identified by the tissue of origin. From a total number of 23983 unique tumour samples, 86 were found with missense (51%), synonymous (46%), nonsense (2%) and inframe deletion (1%) mutations in *TRIB2* (Fig. 1A). Interestingly, although the overall point mutation rate is low, tumours with documented roles for TRIB2 oncogene such as malignant melanoma and lung cancer do have detectable TRIB2 point mutations. In tissues matching Acute Myeloid Leukaemia (AML), which has a very strong association with TRIB2 oncogenic activity, no mutations were retrieved from COSMIC curated data provided by scientific literature and resequencing results from the Cancer Genome Project at the Wellcome Trust Sanger Institute (1/1942 haematopoietic and lymphoid tissue samples identified with a TRIB2 mutation corresponded to a Multiple Myeloma patient sample). Indeed no mutations have been found in ~ 75 AML samples analysed by exome sequencing with good coverage across all exons (Ross Levine, personal communication). Interrogation of other genomic alterations affecting TRIB2 in the COSMIC database identified subsets of tumour tissues with *TRIB2* overexpressed (Fig. 1B). Overall the frequency rate for these alterations is higher and includes lung, skin, liver and hematopoietic and lymphoid tissues samples. Of note, 4/9 samples overexpressing *TRIB2* in the Haematopoietic and lymphoid tissues matched AML samples. Given that there is strong evidence for *TRIB2* oncogenic function in these tumours, these analyses suggest that elevated *TRIB2* expression has potential implications in other tumour tissue contexts yet to be explored e.g. endometrium (endometrioid carcinoma), central nervous system (astrocytoma grade V), prostate and large Intestine samples (both matching adenocarcinoma). Other *TRIB2* gene alterations include copy number variation (CNV), which albeit rare, are found across different tumours tissues and mainly associated with increased copy number (all except for breast, thyroid and kidney which showed CNV loss) (Fig.1C). Hypermethylation, associated with chromatin silencing, was found exclusively in prostate and large intestine tumour tissues (Fig. 1D). Together these analyses suggest that *TRIB2* oncogenic activity is related to its elevated gene expression rather than associated with different genomic alterations or mutations.

It is important to understand how *TRIB2* gene expression is regulated given its elevated expression in cancer. *TRIB2* gene expression has been shown to be regulated by a number of genes in normal and malignant haematopoietic cells, including Notch1[8], MEIS1[9], E2F1 and C/EBPα[10], GATA2 and FOG1[11], PITX1[12] and TAL1/E protein complex[3]. A number of microRNAs also regulate the expression levels of *TRIB2*, including but not limited to miR-511, miR-1297[13], miR-99[14,15], miR-155[16], and let-7[17]. An example of how *TRIB2* expression is regulated in AML has been shown where E2F1 cooperates with the C/EBPα p30 oncogenic isoform to activate the *TRIB2* promoter in AML cells. Indeed, wild type C/EBPα p42 is bound to the TRIB2 promoter in normal granulocyte macrophage progenitor (GMP) cells. This study detailed the feedback loop between TRIB2, C/EBPα p42 and p30, and E2F1 that contributes to AML cell growth and survival[10]. The central mechanism of TRIB2-induced AML identified in this study is based on specific TRIB2-C/EBPα interactions, with C/EBPα being a key substrate of TRIB2 induced proteasomal degradation. The proteasomal mediated degradation of TRIB substrates and interaction with proteins of the ubiquitin proteasome system (UPS) are central to TRIB function in a number of cancers and will be discussed further.

TRIB2: the ruler of E3 ubiquitin ligase activities

The degradation of damaged or no longer necessary proteins is mainly regulated by the UPS. The targets of this pathway are marked by the addition of a ubiquitin tag. Ubiquitin is a short peptide covalently bound to a lysine (Lys) residue on the target. This complex protein modification requires the activity of at least three enzymes: an E1 ubiquitin activating enzyme, an E2 ubiquitin conjugating

enzyme and an E3 ubiquitin ligase. The E3 ligase family comprise over 1000 enzymes with different substrate specificities. Moreover homologous ubiquitin like proteins (UBL) and deubiquitinases (DUBs), which hydrolyse ubiquitin moieties, represent additional layers of regulation for the proteolysis of specific targets[18]. In addition to the 26S proteasome complex, an immunoproteasome complex is present in cells of haematopoietic origin and potentially relates to the higher sensitivity of proteasome inhibition in leukaemic cells[19].

The first connections between TRIB and protein degradation functions were identified in drosophila, where drosophila tribbles was shown to promote degradation of its targets; String (homologous of the mammalian CDC25 phosphatase, positive regulator of cell cycle progression) and slow border cells (Slbo) (promoter of cell migration and homologous of the human C/EBPα)[20]. The members of the C/EBP family of transcription factors are involved in regulation of differentiation in many tissues and control proliferation through interaction with cell cycle proteins. In the haematopoietic system C/EBPα acts as a tumour suppressor that promotes cell cycle arrest and induces granulocytic differentiation through inhibition of E2F activity[21,22]. Both mammalian TRIB1 and TRIB2 have been shown to retain the degradative functions toward the C/EBPα protein and promote C/EBPα p42 degradation and this function underlies their oncogenic potential in leukaemia[2,23,24]. Moreover, TRIB2-dependent C/EBPα p42 degradation is mediated by the proteasome and constitutive photomorphogenesis 1(COP1). COP1 is an E3 ubiquitin ligase recruited by TRIB2 for the interaction with C/EBPα, suggesting TRIB2 functions as a scaffold protein to colocalise the enzyme with its target[25]. The COP1 binding site in the C-terminus is conserved among the TRIB family of proteins and indeed COP1 is the E3 ligase responsible for TRIB1-mediated degradation of C/EBPα[23] and TRIB3-mediated degradation of acetyl-coenzyme A carboxylase (ACC) in fasting condition[26]. TRIB2 also regulates C/EBPα protein levels in liver cells, and mediates C/EBPα degradation in lung cancer models[5,6]. In the latter case, the E3 ligase TRIM21 was identified to function in TRIB2-mediated C/EBPα degradation. With regard to specific TRIB2 substrates, another member of the C/EBP family, C/EBPβ, has been shown to be degraded by TRIB2 in adipogenesis. In this study, TRIB2 functions as a negative regulator of adipogenesis associated with Akt inhibition[27]. Another example of TRIB-dependent degradation of target proteins in cancer is represented by Activating transcription factor 4 (ATF4) which occurs during hypoxia in human tumour cells overexpressing TRIB3[28]. Interestingly, the murine ortholog of TRIB3 Neuronal cell death-inducible putative kinase (NIPK) has also been associated with downregulation of ATF4 transcriptional activity, but in absence of proteolytic degradation[29]. The protein interaction of ATF4 with TRIB2 or TRIB1 as not been reported.

Cell context, especially with regard to protein-protein interactions is highly relevant in deciphering the function of many signalling molecules, including TRIB proteins. TRIB2 has been implicated as a positive or negative mediator of proteasomal degradation. For example, TRIB2 interacts with the substrate binding subunit of the ubiquitin complex SKP1-CUL1-F-box βTrCP in liver cancer cells[6]. This complex is responsible for Yes-associated protein (YAP) degradation, but TRIB2 binding on βTrCP subunit leads to YAP stabilization and transactivation of YAP target genes. The C-terminal domain of TRIB2 was shown to be responsible for the interaction with βTrCP in these liver cancer cells. Noteworthy in the same study, TRIB2 also targets C/EBPα for degradation, but this was not via βTrCP E3 ligase. As previously discussed, COP1 or TRIM21 are known to mediate TRIB2 dependent C/EBPα degradation in AML and lung cancer, whereas the E3 ligase has not been clarified in liver cells. TRIB2 and the E3 ligase Smad ubiquitination regulatory factor 1 (SMURF1) also interact in liver cancer cells. SMURF1 is the enzyme responsible for TRIB2 ubiquitination and proteasomal dependent degradation and SMURF1 inhibition triggers TRIB2-dependent carcinogenesis in the liver[30]. Nonetheless, TRIB2 is also a partner of SMURF1 in the inhibition of the Wnt signalling effector β-catenin and TCF4. Indeed, deletion of the SMURF1 binding sites on the TRIB2 protein abolished its ability to downregulate β-catenin and transcription factor 4 (TCF4)[31]. COP1 or βTrCP were also described as additional modulators in this same pathway[32]. Together these data reveal the complexity of TRIB2 and UPS interactions in liver cancer cells, and highlight the importance of cell context in TRIB family protein function.

UPS aberration in leukaemia

Dysregulation of E3 ligase enzymatic activity has been reported in different malignancies. Transforming mutant variants of the E3 ubiquitin ligase Casitas B-Lineage Lymphoma proto-oncogene (cClb) have been found in human myeloid malignancies and all these mutations were associated with loss of E3 ubiquitin ligase activity in addition to a malignant gain of function[33]. Mutations in the E3 ligase FBW7 have been associated with oncogenic signalling mechanisms in different cancers including NOTCH1 activation in T-ALL[34]. Moreover, the recent profiling of the somatic mutation landscape of epigenetic regulators in paediatric cancer identified the deubiquitinase USP7 as one of the most frequent mutations in T-ALL[35]. This DUB is associated with chromatin remodelling and it is referred as an "eraser" among epigenetic modifiers. It's physiological function is to stabilize the histone H2B (as well as other targets), removing ubiquitin subunits from the substrates. In 8% of T-ALL cases sequence variation translates to loss of function of the protein and the consequent epigenetic alteration is associated with the leukaemic disease[35]. This case supports the important role of ubiquitination signalling beyond terminal protein degradation in leukaemic transformation. The proteolytic pathways of UPS and autophagy in AML cell lines are directly involved in the response to chemotherapeutic drugs cytarabine and doxorubicin. Studies show that the response to chemotherapy is highly dependent on the basal level of activation of the UPS in leukaemic cells [36]. It is possible to measure the proteasome expression and activities in the plasma of AML, ALL and MDS patient samples and higher levels of UPS protein and activities are detectable in patients plasma samples[37]. Importantly, the levels of proteasome activities can be used to risk stratify for the prognosis of different blood tumours. Since it is likely that the activities detected in the AML patients' plasma is reflecting the activities in the leukaemic cells, screening of the proteasome activities in the plasma samples from patients could be advantageous not only as a biomarker, but also to identify the most suitable therapeutic combination taking advantage of the new drugs available in clinical trials targeting the UPS system.

Targeting UPS in blood tumours

Proteasome inhibitors

Leukaemic cells express high levels of proteasomes[38] and this is likely one of the reasons why therapeutic proteasome inhibition has shown better results in blood cancers than in solid tumours[39]. Multiple toxicity mechanisms of proteasome inhibition have been validated in preclinical studies. These comprise NFkB inhibition through stabilization of its regulator IkBα; cell cycle arrest due to deregulation of cyclins and other cell cycle regulator proteins; induction of a proapoptotic state through stabilization of p53 and Bax and downregulation of Bcl-2; ROS production; transmembrane mitochondrial potential gradient dissipation; aggresome formation; endoplasmic reticulum (ER) and unfolded protein response (UPR)[40].

Eight classes of proteasome inhibitors (PI) have been recognised across natural compounds and synthetic molecules (peptide aldehydes, peptide vinyl sulfones, syrbactins, peptide boronates, peptide α'β'-epoxyketones, peptide ketoaldehydes, β-lactones,and oxatiazol-2-ones). Only representative members of some classes have reached the stage of clinical trials investigations in cancer. Many have failed due to lack of specificity and or toxicity in preclinical studies. The main PIs of interest in the treatment of haematological malignancies include three peptide boronates, two peptide epoxyketones and one marine natural product, b-lactone. The boronate compound Bortezomib (BTZ) is a reversible inhibitor of the proteasome and the immunoproteasome and the first PI ever entered in clinical trials[41]. BTZ is currently approved for used as single agent or in combination therapies in multiple myeloma or mantle cell lymphoma patients. Moreover a number of clinical trials have been assessing its efficacy in aggressive disease like acute leukaemias (CALGB (Alliance) Study 10502, COG AAML 1031)[42]. To overcome toxicity and resistance challenges with BTZ, a great effort has been spent in the design and characterization of next generation inhibitors. Among them Carfilzomib has been approved by the FDA for the treatment of relapsed/refractory multiple myeloma[43], and Oprozomib, Ixazomib, Marizomib and Delanzomib have already reached clinical trials investigation. The activity of immunoproteasome specific inhibitors is also currently being assessed in preclinical investigation in models of immunological disorders[41].

Other UPS targets

The recent interest in targeting the UPS system has brought attention to the identification of druggable targets upstream of the proteasome, which by their nature are predicted to lead to more specific inhibition and fewer side effects. In this category there are E1 activating enzyme inhibitors, E2 conjugating enzyme inhibitors, E3 ubiquitin ligases inhibitors and DUB enzyme inhibitors[44]. Indeed the inhibition of the UBL modifier Nedd8 is currently in clinical trials for AML and MDS[45]. More than one E3 ligases have been chosen as potential targets in the drug discovery process for treatment of haematological malignancies and many small molecules have been studied. Examples among the most targeted E3 ligases are MDM2, involved in p53 regulation[46], and SCF (Skp1-Cullin-Fbox) multi-subunit E3 ligases[47].

Concluding remarks and Future perspective

Given that C/EBPα stability is tightly regulated at molecular level and that TRIB2 mediated dysregulation of this pathway is linked to AML, liver and lung carcinogenesis, targeting the related network of E3 ligases could open new exciting therapeutic windows, resulting in a more specific and possibly more effective treatment of these malignancies. There is an urgent need for alternative therapeutic strategies in the treatment of blood diseases. Increasing interest has been raised around the modulation of proteolytic pathways such as autophagy and UPS, which often play critical roles in cancer cells. TRIB2, as a signalling pathway modulator and binding partner of different E3 ubiquitin ligase enzymes has gained attention over the past decade as a major regulator in solid tumours and different leukaemia subtypes. TRIB2 has been proposed as a valid biomarker for diagnosis and progression of melanoma, correlating higher TRIB2 expression levels with advanced stages of the disease[48]. Given the strong preclinical data regarding TRIB2 in AML, the screening of TRIB2 protein expression in leukaemia patients would be extremely useful to complement the current information available on TRIB2 carcinogenesis and mutational profile.

Figure 1. Genomic alterations in *TRIB2* gene. (A) Histogram presentation of point mutations in *TRIB2* gene in tumour samples using the COSMIC database (top). Pie chart referring to the frequency of missense (51%), synonymous (46%), nonsense (2%) and deletion inframe (1%) point mutations in the identified tumour samples (lower). (B) Histogram presentation of gain of *TRIB2* gene expression in the tumour samples indicated. Among them 4/9 Haematopoietic and lymphoid tissues samples match AML as part of the Acute Myeloid Leukaemia study (COSU377) from The Cancer Genome Atlas [TCGA] in which over expression is defined after a z-score>2. (C) Histogram displaying tumour tissues where copy number variations (CNV) of the *TRIB2* gene was identified, with the respective frequency in tumour samples. (D) Histogram presenting tumour tissues where hypermethylation of the *TRIB2* gene locus was identified and respective frequency. Data was retrieved by v72 of COSMIC database (http://www.sanger.ac.uk) and only tissues displaying *TRIB2* alterations are shown. NS*, not specified with histology matching malignant melanoma; NS, not specified

References

[1] Lohan F, Keeshan K. (2013) The functionally diverse roles of tribbles. Biochem. Soc. Trans. **41**, 1096–1100.

[2] Keeshan K, He Y, Wouters BJ, Shestova O, Xu L, Sai H, et al. (2006) Tribbles homolog 2 inactivates C/EBPalpha and causes acute myelogenous leukemia. Cancer Cell. **10**, 401–411.

[3] Sanda T, Lawton LN, Barrasa MI, Fan ZP, Kohlhammer H, Gutierrez A, et al. (2012) Core Transcriptional Regulatory Circuit Controlled by the TAL1 Complex in Human T Cell Acute Lymphoblastic Leukemia. Cancer Cell. **22**, 209–221.

[4] Zanella F, Renner O, Garcia B, Callejas S, Dopazo A, Peregrina S, et al. (2010) Human TRIB2 is a repressor of FOXO that contributes to the malignant phenotype of melanoma cells. Oncogene. 1–10.

[5] Grandinetti KB, Stevens TA, Ha S, Salamone RJ, Walker JR, Zhang J, et al. (2011) Overexpression of TRIB2 in human lung cancers contributes to tumorigenesis through downregulation of C/EBPα. Oncogene. 1–8.

[6] Wang J, Park J-S, Wei Y, Rajurkar M, Cotton JL, Fan Q, et al. (2013) TRIB2 Acts Downstream of Wnt/TCF in Liver Cancer Cells to Regulate YAP and C/EBPα Function. Molecular Cell. **51**, 1-15

[7] Forbes SA, Beare D, Gunasekaran P, Leung K, Bindal N, Boutselakis H, et al. (2015) COSMIC: exploring the world's knowledge of somatic mutations in human cancer. Nucleic Acids Res. **43**, D805–11.

[8] Wouters BJ, Jordà MA, Keeshan K, Louwers I, Erpelinck-Verschueren CAJ, Tielemans D, et al. (2007) Distinct gene expression profiles of acute myeloid/T-lymphoid leukemia with silenced CEBPA and mutations in NOTCH1. Blood. **110**, 3706–3714.

[9] Argiropoulos B, Palmqvist L, Yung E, Kuchenbauer F, Heuser M, Sly LM, et al. (2008) Linkage of Meis1 leukemogenic activity to multiple downstream effectors including Trib2 and Ccl3. Experimental Hematology. **36**, 845–859.

[10] Rishi L, Hannon M, Salome M, Hasemann M, Frank A-K, Campos J, et al. (2014) Regulation of Trib2 by an E2F1-C/EBPalpha feedback loop in AML cell proliferation. Blood. **123**, 2389–2400.

[11] Mancini E, Sanjuan-Pla A, Luciani L, Moore S, Grover A, Zay A, et al. (2011) FOG-1 and GATA-1 act sequentially to specify definitive megakaryocytic and erythroid progenitors. The EMBO Journal. 1–15.

[12] Nagel S, Venturini L, Przybylski GK, Grabarczyk P, Schneider B, Meyer C, et al. (2011) Activation of Paired-homeobox gene PITX1 by del(5)(q31) in T-cell acute lymphoblastic leukemia. Leuk Lymphoma. **52**, 1348–1359.

[13] Zhang C, Chi YL, Wang PY, Wang YQ, Zhang YX, Deng J, et al. (2012) miR-511 and miR-1297 inhibit human lung adenocarcinoma cell proliferation by targeting oncogene TRIB2. PLoS ONE. **7**, e46090–e46090.

[14] Zhang L, Li X, Ke Z, Huang L, Liang Y, Wu J, et al. (2013) MiR-99a may serve as a potential oncogene in pediatric myeloid leukemia. Cancer Cell Int. **13**, 110.

[15] Xin J-X, Yue Z, Zhang S, Jiang Z-H, Wang PY, Li Y-J, et al. (2013) miR-99 inhibits cervical carcinoma cell proliferation by targeting TRIB2. Oncol Lett. **6**, 1025–1030.

[16] Palma CA, Sheikha Al D, Lim TK, Bryant A, Vu TT, Jayaswal V, et al. (2014) MicroRNA-155 as an inducer of apoptosis and cell differentiation in Acute Myeloid Leukaemia. Mol Cancer. **13**, 79.

[17] Wang PY, Sun Y-X, Zhang S, Pang M, Zhang H-H, Gao S-Y, et al. (2013) Let-7c inhibits A549 cell proliferation through oncogenic TRIB2 related factors. FEBS Letters. **587**, 2675–2681.

[18] Komander D. (2009) The emerging complexity of protein ubiquitination. Biochem. Soc. Trans. **37**, 937–953.

[19] Niewerth D, Franke NE, Jansen G, Assaraf YG, van Meerloo J, Kirk CJ, et al. (2013) Higher ratio immune versus constitutive proteasome level as novel indicator of sensitivity of pediatric acute leukemia cells to proteasome inhibitors. Haematologica. **98**, 1896–1904.

[20] Dobens LL Jr., Bouyain S. (2012) Developmental roles of tribbles protein family members. Dev. Dyn. **241**, 1239–1248.

[21] Keeshan K, Santilli G, Corradini F, Perrotti D, Calabretta B. (2003) Transcription activation function of C/EBPalpha is required for induction of granulocytic differentiation. Blood. **102**, 1267–1275.

[22] Porse BT. (2005) Loss of C/EBPα cell cycle control increases myeloid progenitor proliferation and transforms the neutrophil granulocyte lineage. Journal of Experimental Medicine. **202**, 85–96.

[23] Dedhia PH, Keeshan K, Uljon S, Xu L, Vega ME, Shestova O, et al. (2010) Differential ability of Tribbles family members to promote degradation of C/EBPα and induce acute myelogenous leukemia. Blood. **116**, 1321–1328.

[24] Yokoyama T, Kanno Y, Yamazaki Y, Takahara T, Miyata S, Nakamura T. (2010) Trib1 links the MEK1/ERK pathway in myeloid leukemogenesis. Blood. **116**, 2768–2775.

[25] Keeshan K, Bailis W, Dedhia PH, Vega ME, Shestova O, Xu L, et al. (2010) Transformation by Tribbles homolog 2 (Trib2) requires both the Trib2 kinase domain and COP1 binding. Blood. **116**, 4948–4957.

[26] Qi L, Heredia JE, Altarejos JY, Screaton R, Goebel N, Niessen S, et al. (2006) TRB3 links the E3 ubiquitin ligase COP1 to lipid metabolism. Science. **312**, 1763–1766.

[27] Naiki T, Saijou E, Miyaoka Y, Sekine K, Miyajima A. (2007) TRB2, a mouse Tribbles ortholog, suppresses adipocyte differentiation by inhibiting AKT and C/EBPbeta. J Biol Chem. **282**, 24075–24082.

[28] Bowers AJ, Scully S, Boylan JF. (2003) SKIP3, a novel Drosophila tribbles ortholog, is overexpressed in human tumors and is regulated by hypoxia. Oncogene. **22**, 2823–2835.

[29] Örd D, Örd T. (2003) Mouse NIPK interacts with ATF4 and affects its transcriptional activity. Exp Cell Res. **286**, 308–320.

[30] Wang J, Zhang Y, Weng W, Qiao Y, Ma L, Xiao W, et al. (2013) Impaired phosphorylation and ubiquitination by P70 S6 kinase (P70S6K) and Smad ubiquitination

regulatory factor 1 (Smurf1) promotes Tribbles homolog 2 (TRIB2) stability and carcinogenic property in liver cancer. Journal of Biological Chemistry.

[31] Xu S, Tong M, Huang J, Zhang Y, Qiao Y, Weng W, et al. (2014) TRIB2 inhibits Wnt/beta-Catenin/TCF4 signaling through its associated ubiquitin E3 ligases, beta-TrCP, COP1 and Smurf1, in liver cancer cells. FEBS Letters. **588**, 4334–4341.

[32] Qiao Y, Zhang Y, Wang J. (2013) Ubiquitin E3 ligase SCF(β-TRCP) regulates TRIB2 stability in liver cancer cells. Biochemical and Biophysical Research Communications. **441**, 555–559.

[33] Kales SC, Ryan PE, Nau MM, Lipkowitz S. (2010) Cbl and human myeloid neoplasms: the Cbl oncogene comes of age. Cancer Research. **70**, 4789–4794.

[34] Thompson BJ, Buonamici S, Sulis ML, Palomero T, Vilimas T, Basso G, et al. (2007) The SCFFBW7 ubiquitin ligase complex as a tumor suppressor in T cell leukemia. J. Exp. Med. **204**, 1825–1835.

[35] Huether R, Dong L, Chen X, Wu G, Parker M, Wei L, et al. (2014) The landscape of somatic mutations in epigenetic regulators across 1,000 paediatric cancer genomes. Nature Communications. **5**, 3630.

[36] Fernandes A, Azevedo MM, Pereira O, Sampaio-Marques B, Paiva A, Correia-Neves M, et al. (2014) Proteolytic systems and AMP-activated protein kinase are critical targets of acute myeloid leukemia therapeutic approaches. Oncotarget. 1-13

[37] Ma W, Kantarjian H, Zhang X, Wang X, Estrov Z, O'Brien S, et al. (2011) Ubiquitin-proteasome system profiling in acute leukemias and its clinical relevance. Leukemia Research. **35**, 526–533.

[38] Kumatori A, Tanaka K, Inamura N, Sone S, Ogura T, Matsumoto T, et al. (1990) Abnormally high expression of proteasomes in human leukemic cells. Proc. Natl. Acad. Sci. U.S.A. **87**, 7071–7075.

[39] Orlowski RZ, Kuhn DJ. (2008) Proteasome inhibitors in cancer therapy: lessons from the first decade. Clin. Cancer Res. **14**, 1649–1657.

[40] Nencioni A, Grünebach F, Patrone F, Ballestrero A, Brossart P. (2007) Proteasome inhibitors: antitumor effects and beyond. Leukemia. **21**, 30–36.

[41] Niewerth D, Jansen G, Assaraf YG, Zweegman S, Kaspers GJL, Cloos J. (2015) Molecular basis of resistance to proteasome inhibitors in hematological malignancies. Drug Resistance Updates. **18**, 18–35.

[42] Attar EC, Johnson JL, Amrein PC, Lozanski G, Wadleigh M, DeAngelo DJ, et al. (2013) Bortezomib Added to Daunorubicin and Cytarabine During Induction Therapy and to Intermediate-Dose Cytarabine for Consolidation in Patients With Previously Untreated Acute Myeloid Leukemia Age 60 to 75 Years: CALGB (Alliance) Study 10502. Journal of Clinical Oncology. **31**, 923–929.

[43] Katsnelson A. (2012) Next-generation proteasome inhibitor approved in multiple myeloma. Nat Biotechnol. **30**, 1011–1012.

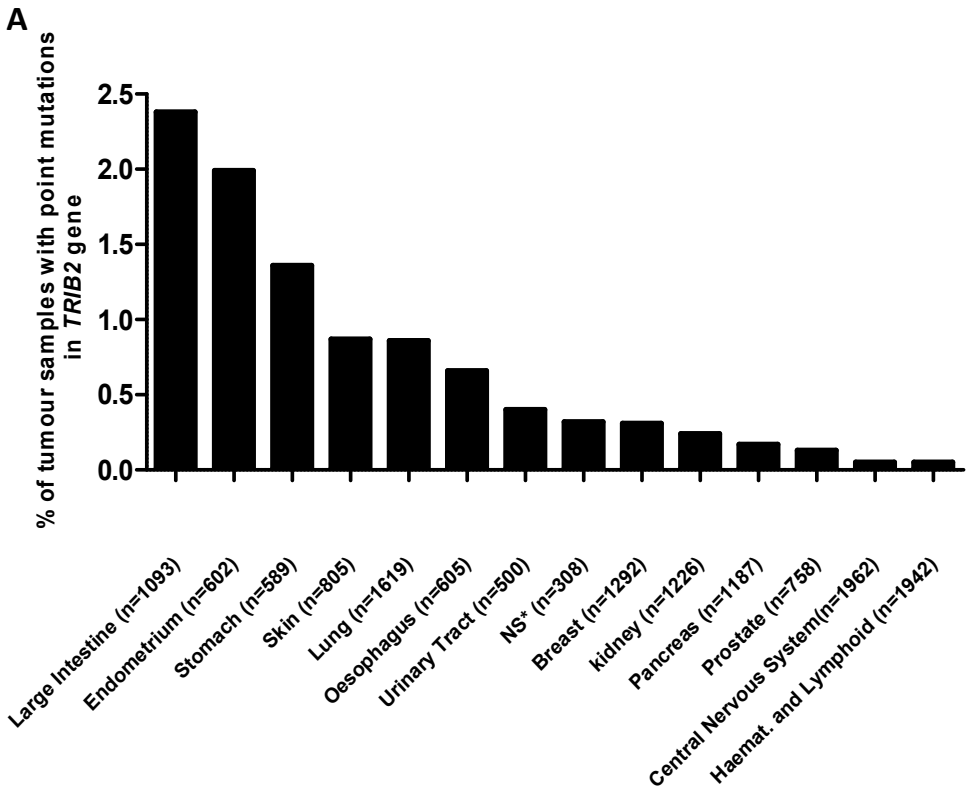
[44] Crawford LJ, Irvine AE. (2013) Targeting the ubiquitin proteasome system in haematological malignancies. Blood Rev. **27**, 297–304.

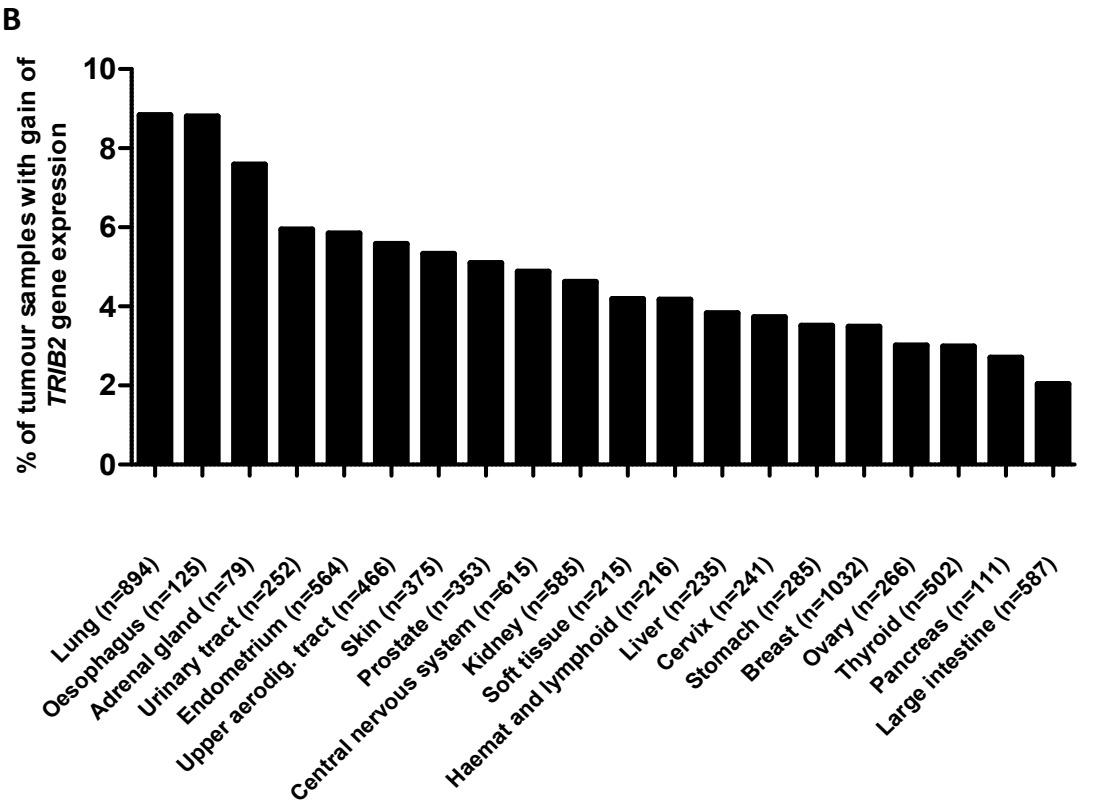
[45] Swords RT, Erba HP, DeAngelo DJ, Bixby DL, Altman JK, Maris M, et al. (2015) Pevonedistat (MLN4924), a First-in-Class NEDD8-activating enzyme inhibitor, in patients with acute myeloid leukaemia and myelodysplastic syndromes: a phase 1 study. Br. J. Haematol. **169**, 534–543.

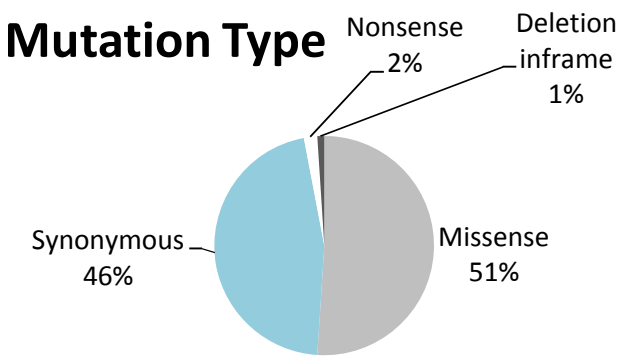
[46] Zhao Y, Aguilar A, Bernard D, Wang S. (2015) Small-molecule inhibitors of the MDM2-p53 protein-protein interaction (MDM2 Inhibitors) in clinical trials for cancer treatment. J. Med. Chem. **58**, 1038–1052.

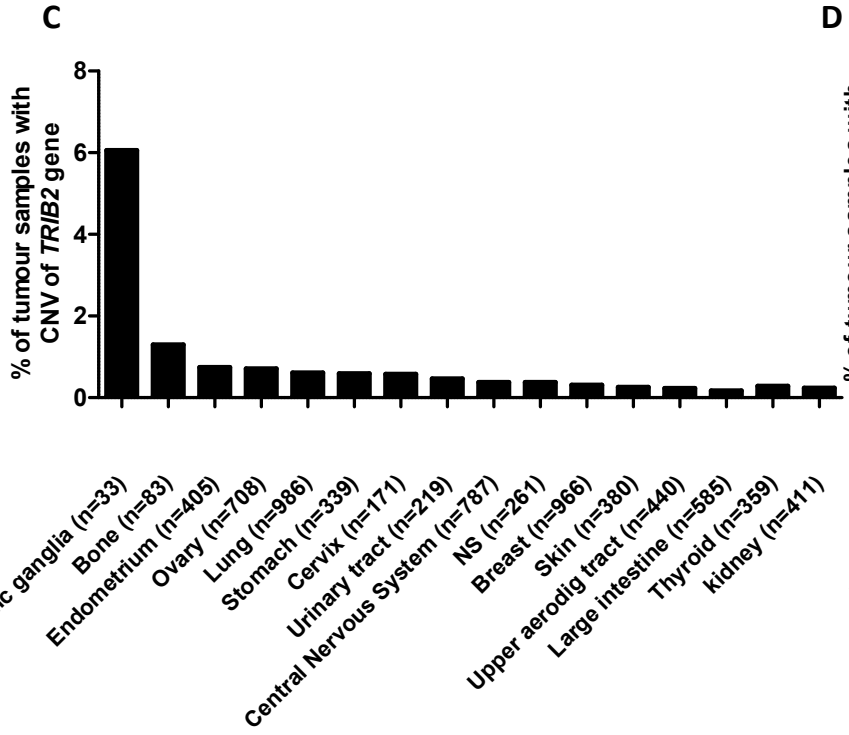
[47] Chan C-H, Morrow JK, Li C-F, Gao Y, Jin G, Moten A, et al. (2013) Pharmacological inactivation of Skp2 SCF ubiquitin ligase restricts cancer stem cell traits and cancer progression. Cell. **154**, 556–568.

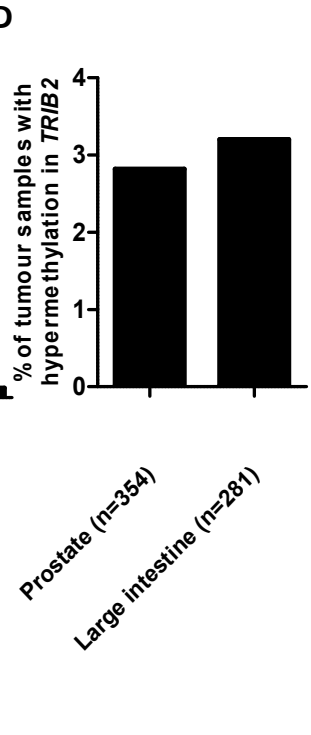
[48] Hill R, Kalathur RKR, Colaco L, Brandao R, Ugurel S, Futschik M, et al. (2015) TRIB2 as a biomarker for diagnosis and progression of melanoma. Carcinogenesis. **36**, 469–477.


A
% of tumour samples with point mutations in TRIB2 gene
2.5
2.0
1.5
1.0
0.5
0.0
Large Intestine (n=1093)
Endometrium (n=602)
Stomach (n=589)
Skin (n=805)
Lung (n=1619)
Oesophagus (n=605)
Urinary Tract (n=500)
NS* (n=308)
Breast (n=1292)
kidney (n=1226)
Pancreas (n=1187)
Prostate (n=758)
Central Nervous System(n=1962)
Haemat. and Lymphoid (n=1942)


B
% of tumour samples with gain of TRIB2 gene expression
10
8
6
4
2
0
Lung (n=894)
Oesophagus (n=125)
Adrenal gland (n=79)
Urinary tract (n=252)
Endometrium (n=564)
Upper aerodig. tract (n=466)
Skin (n=375)
Prostate (n=353)
Central nervous system (n=615)
Kidney (n=585)
Soft tissue (n=215)
Haemat and lymphoid (n=216)
Liver (n=235)
Cervix (n=241)
Stomach (n=285)
Breast (n=1032)
Ovary (n=266)
Thyroid (n=502)
Pancreas (n=111)
Large intestine (n=587)


Mutation Type
Nonsense
2%
Deletion
inframe
1%
Synonymous
46%
Missense
51%


C
% of tumour samples with CNV of TRIB2 gene
8
6
4
2
0
Autonomic ganglia (n=33)
Bone (n=83)
Endometrium (n=405)
Ovary (n=708)
Lung (n=986)
Stomach (n=339)
Cervix (n=171)
Urinary tract (n=219)
Central Nervous System (n=787)
NS (n=261)
Breast (n=966)
Skin (n=380)
Upper aerodig tract (n=440)
Large intestine (n=585)
Thyroid (n=359)
kidney (n=411)


D
% of tumour samples with hypermethylation in TRIB2
4
3
2
1
0
Prostate (n=354)
Large intestine (n=281)