

Letter to the Editor

The CTLH complex may be involved in myelomagenesis and serves as a new therapeutic target in multiple myeloma and related blood cancers

Dear Editor,

Multiple myeloma is a common blood cancer derived from terminally differentiated B-lymphocytes called plasma cells. Despite new insights into the genetic underpinnings of myeloma and recent advancements in treatment options, multiple myeloma remains incurable in the great majority of cases, with no more than half of all patients surviving past 5 years^[1–2]. The reasons for poor outcome include limitations in our knowledge base on genetic pathways that drive neoplastic plasma cell development from early progenitor stages, known as monoclonal gammopathy of undetermined significance and smoldering myeloma, to frank malignancy. To attack this knowledge gap, we hypothesized that unbiased genetic forward screening using proviral insertional mutagenesis in a transgenic mouse model of human myeloma may uncover new myeloma driver genes. Indeed, employing this approach, we recently discovered *WD repeat-containing protein 26* (*WDR26*) as a candidate myeloma gene. The protein encoded by this gene, WDR26, is a component of the mammalian CTLH (C-terminal to LisH) complex, which was first described in yeast as the glucose-induced deficient degradation, or GID complex. The multimeric CTLH protein complex may not only be involved in myelomagenesis, it may also yield new opportunities for the molecularly targeted myeloma treatment.

Our experimental strategy for detecting *WDR26* was described in greater depth elsewhere^[3]. Briefly, it began with a tumor induction study in iMyc^{ΔEμ} mice, a gene-insertion model of the chromosomal T(12;15) translocation that results in deregulated expression of *Myc* in B-lineage cells^[4]. The mice were infected with murine leukemia virus (MuLV) MOL4070LTR, which is a modified Moloney-MuLV that contains the long terminal repeat (LTR) U3 enhancer region from amphotropic MuLV 4070A^[5]. Tumor specimens harvested from iMyc^{ΔEμ} mice were analyzed for

common retroviral insertion sites (CIS) using a protocol that relied on: (1) digestion of genomic DNA with endonucleases; (2) ligation-mediated PCR to amplify fragments that contained viral sequences; (3) next generation sequencing (NGS) analysis of complementary junction libraries prepared from both sides of the virus. From nearly half a million mapped sequence reads, approximately 45 000 proviral integration events were extracted. A total of 171 CIS-tagged candidate genes were identified in rank order according to proviral insertion frequency. The list included many genes that one might expect to be found in a forward genetic screen of neoplastic plasma cell development, *e.g.*, *Ccnd2*, *Hras*, and *Myc*.

The next step in our research pipeline involved bioinformatics analyses of the top 100 genes using three different online tools: STRING (string-db.org), KEGG (www.kegg.jp), and GO (geneontology.org). Then, the MMRF CoMMpass dataset available at <https://research.themmr.org> was used to determine whether upregulation of the human ortholog of a given mouse gene was associated with inferior survival of patients with multiple myeloma. Next, online DepMap explorer (depmap.org/portal) was used to determine whether genes of interest may be important and functionally non-redundant in myeloma *in vitro*. This was followed by a rigorous PubMed analysis to search for "novelty" in order to exclude all genes for which evidence for involvement in MM and related diseases had already been published. Eight out of 100 genes passed the selection process described above. Interrogation at the functional level using shRNA-mediated knockdown of gene expression in myeloma cells demonstrated that significant ($P < 0.01$) and consistent inhibition (in 3 out of 3 independent cell lines) occurred in only 2 cases. One of these genes was *WDR26*.

To further strengthen the contention that WDR26 is a myeloma driver candidate and a promising molecular target of myeloma therapy, we gathered

additional clinical and biological data^[3]. Clinical results included findings that (1) *WDR26* expression was upregulated in smoldering and frank myeloma compared to normal bone marrow plasma cells; (2) in a DREAM Challenge study, *WDR26* message was elevated in high-risk relative to standard-risk myeloma; and (3) high levels of *WDR26* mRNA in myeloma cells of CoMMpass patients predicted inferior overall survival. The biological evidence of the significance of *WDR26* in myeloma included loss-of-function studies using CRISPR-Cas9 engineered gene knockouts in myeloma cells. *WDR26* deficiency compromised the growth of myeloma in bulk suspension culture and clonogenic soft-agar culture, and led to a significant increase in apoptotic cell death evaluated using flow cytometry. *In vivo* studies using myeloma xenografts in mice lent further credence to the *in vitro* results by demonstrating that *WDR26* deficient tumors grew more slowly than their normal counterparts and thus allowed host mice to live longer.

The significance of *WDR26* as a therapeutic target in cancer has been recognized for carcinomas^[6], but not yet for blood cancers, including multiple myeloma. *WDR26* is a component of the CTLH complex that is mutated or upregulated in many solid cancers^[7]. CTLH is a large and evolutionarily conserved multi-subunit RING E3 ligase (**Fig. 1**) that seems to play an important role in many fundamental biological processes, such as cell growth and proliferation, cell death and migration, and cell stemness, plasticity and differentiation^[7]. The principal function of the CTLH counterpart in yeast, the GID complex, is well understood: GID governs

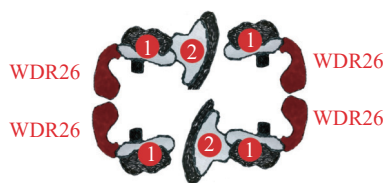


Fig. 1 Architecture of a human oligomeric CTLH (C-terminal to LisH) complex. Other supramolecular complexes of this kind were described. The complex depicted here is held together by 4 units of *WDR26*. The heterotetramer labeled "1" contains two GID complex subunit homologs, GID4 and GID8, RAN binding protein 9 (RANBP9) and armadillo repeat containing 8 (ARMC8). The heterodimer labeled "2" consists of macrophage erythroblast attacher (MAEA, E3 ubiquitin ligase) and RMND5A (required for meiotic nuclear division 5 homolog A). *WDR26* (red) functions as a clamp for the 4-unit ring-like structure. The figure was adapted from a scheme published in a recent paper by Maitland *et al.*^[9].

the signal-dependent ubiquitination of gluconeogenic enzymes. In contrast, we are just beginning to elucidate the role of CTLH in human blood cells. Chinese investigators are at the frontline, recently showing that *WDR26* regulates nuclear condensation in erythroblasts^[8]. Additional research is warranted to elucidate the mechanisms of *WDR26* and CTLH in myeloma. Maybe this letter will inspire more investigators from the readership of *Blood & Genomics* in China to get involved in these studies.

Yours Sincerely,
Siegfried Janz*

Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI 53226, USA.

***Corresponding to:** Siegfried Janz, Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI 53226, USA. E-mail: sjanz@mcw.edu.

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