Structure of the human DDB1– Cereblon – thalidomide complex

More than 50 years have passed since thalidomide was first prescribed as a sedative and antiemetic to provide effective relief from morning sickness during early pregnancy. Although the teratogenic effects associated with its use were soon discovered, more than 10,000 babies were born with severe birth defects such as phocomelia and amelia before thalidomide was withdrawn from the market. Pharmacological studies aimed at delineating the cause of thalidomide-induced teratogenicity led to the discovery of a number of unexpected pharmacological activities including anti-inflammatory, tumor necrosis factor (TNF)-α inhibitory and anti-angiogenic effects. Thalidomide and its derivatives are now widely used as potent immunomodulatory drugs (IMiDs) in the treatment of several diseases including multiple myeloma (MM) and leprosy (Hansen's disease). Furthermore, thalidomide has recently been employed in the treatment of vascular diseases. However, the precise mechanism by which thalidomide exerts its teratogenic and pharmacological effects remains unknown.

A breakthrough in thalidomide research was recently achieved with identification of its primary target cereblon (CRBN), which binds directly to thalidomide and was isolated as a thalidomide-binding protein from various cell types [1]. CRBN, originally identified as a cerebral protein associated with mild mental retardation, is a highly conserved protein that forms a CRL4-type E3 ubiquitin ligase complex with Cul4A and damaged DNA binding protein 1 (DDB1), and plays a key role in limb outgrowth and expression of fibroblast growth factor Fgf8 in zebrafish and chicks. Thalidomide initiates its teratogenic effects by binding to CRBN and inhibiting the associated ubiquitin ligase activity. Moreover, a human MM cell line with deletion of the CRBN gene is resistant to thalidomide derivatives, indicating that CRBN is involved in both the teratogenic and beneficial effects of thalidomide. We set out our collaboration of the structural works of human CRBN complexed with thalidomide (Thal) or its derivatives such as lenalidomide (Len) and pomalidomide (Pom) with the Prof. Handa group at Tokyo Medical University and the Celgene Corporation group at Saint Diego. Recently, we have reported a series of crystallographic and biochemical studies on the interaction between thalidomide and human DDB1-CRBN or mouse CRBN thalidomide-binding domain (TBD), and provide a structural basis of the binding specificity and stereospecific effects of thalidomide [2].

X-ray intensity data from our crystals were collected at the SPring-8 synchrotron facility for the provision of synchrotron data-collection facilities (beamlines **BL41XU** and **BL44XU**), the Advanced Photon Source supported by the US Department of Energy, the Advanced Light Source operated by the Lawrence Berkeley National Laboratory and the Canadian Light Source (beamline 08ID-1) supported by the Natural Sciences and Engineering Research Council of Canada. Using these synchrotron facilities, we determined structures of the human DDB1-CRBN-Len (3.01 Å resolution), the mouse CRBN TBD-Thal (1.88 Å) and the mouse CRBN TBD-Pom (2.0 Å) complexes and the free form of mouse CRBN TBD (2.0 Å). Our structures of the TBD-drug complexes are precise enough for discussion of the specific interactions between the drug and the target protein.

The overall structure of the ternary complex (Fig. 1) reveals that CRBN binds between the DDB1 β-propellers A and C in a similar location to other DDB1-cullin 4-associated factors (DCAFs), which play a roll of substrate receptors such as DDB2, DCAF9, Hbx and SV5V19. However, the nature of the interaction with DDB1 exhibits differences compared to those found in previously determined DDB1-DCAF complexes. CRBN made intimate interactions with both β-propeller A and C domains of DDB1 *via* a series

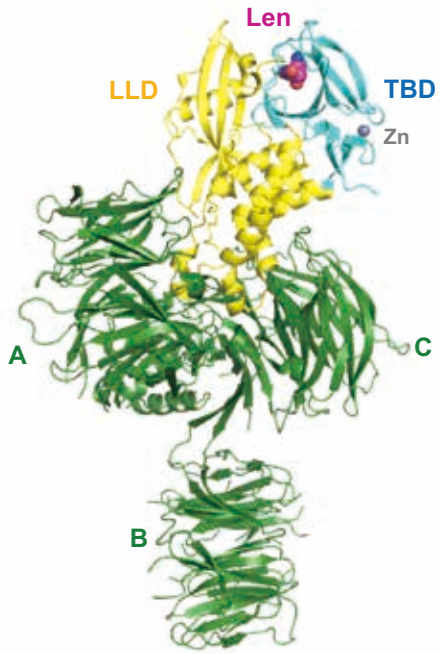

Fig. 1. Overall structure of the human DDB1–CRBN–Len complex. DDB1 (green) consists of three β-propeller domains A, B and C. CRBN has a Lon-like domain (LLD in yellow) directly bound to DDB1 and TBD (cyan) bound to Lon.

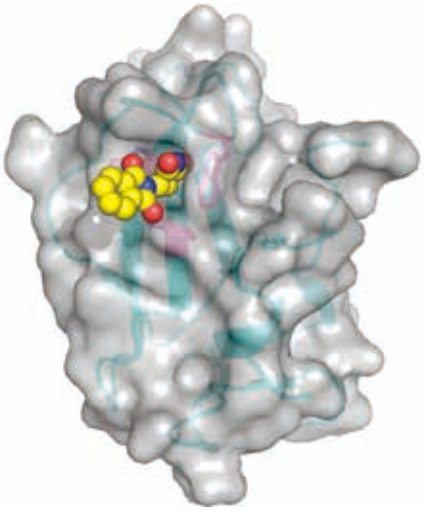

Fig. 2. Structure of TBD bound to Thal. The bound Thal molecule (a space-filling model) is docked into the binding pocket on the surface of TBD (transparency surface representation with a ribbon model).

of helices, whereas DCAFs generally position a helix-turn-helix motif in the DDB1 binding site predominantly formed by the β-propeller C domain. The structure of CRBN reveals that CRBN is a DCAF that does not exhibit WD-repeat architecture typical of the class and composed of two domains; an N-terminal Lon-like domain (LLD in yellow) directly bound to DDB1 and a C-terminal TBD (cyan) bound to Lon. The DDB1-binding motif exists in LLD and the thalidomide binding site exists in TBD. These sites are located at the opposite sides of the CRBN surface.

Interestingly, LLD and TBD are structurally distinct and in fact TBD isolated from LLD retains the binding affinity to Thal and its derivatives. High-resolution structures of TBD bound to the drugs reveal details of the specific interactions between the protein and the drug (Fig. 2). Thalidomide is a small synthetic compound, α-phthalimido-glutarimide (IUPAC systematic name, 3-(*RS*)-2-(2,6-dioxo-3-piperidyl) isoindole-1,3-dione). The phthalimido portion is largely nonpolar but glutarimide portion possesses imido group, which is capable of hydrogen bound formation. In our structures, Thal and its derivatives bind CRBN with the glutarimide portion inside of the binding pocket, which is formed by three imidazole rings from tryptophans (Fig. 3). Inside the pocket, the imido group of the glutarimide. This binding mode was also observed in the recently-reported structures of hybrid complexes of human DDB1-chick CRBN and Thal and its derivatives, while the structures were determined at a marginal resolution between 3.0 Å and 3.5 Å [3]. This binding mode is unusual since the nonpolar portion, phthalimido, of the drug is exposed to the solvent but the rather polar portion, glutarimide, is buried inside the pocket. This paradox could be solved by the recently-clarified fact that these drugs directly bound to CRBN promote the recruitment of substrates Ikaros (IKZF1) and Aiolos (IKZF3) to the E3 complex, thus leading to substrate ubiquitination and degradation [4,5]. Thus, Thal and its derivatives play a role of an interfacial drug, such as brefeldin A acting against Arf GEF. This specific substrate recruitment is thought to be responsible to potent immunomodulatory effects of Thal and its derivatives in the treatment of several diseases including MM. In contrast to the recruitment, Thal binding to CRBN should block binding of authentic or endogenous substrates to CRBN. This inhibition may cause teratogenic effects. Further experiments should be carried out to clarify these dual activity together with structure determination of ternary and quaternary complexes of the E3 substrate and Thal with CRBN or CRBN-DDB1.

Our structural study provides a structural framework for further investigations on the mechanisms of the pharmaceutical and teratogenic actions of this drug and for the development of more effective thalidomide derivatives.

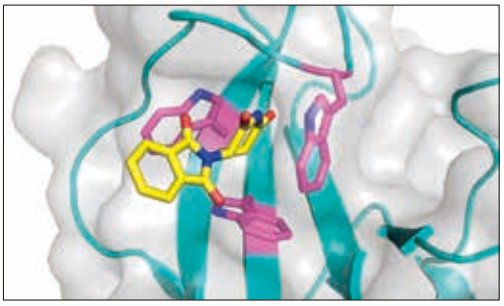

Fig. 3. A close-up view of Thal bound to the binding pocket on the TBD surface. The bound Thal molecule (a stick model in yellow) is sandwiched three side-chain imidazole rings from CRBN tryptophans (magenta).

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