Targeting the DCN1-UBC12 Protein-protein Interaction for Selective Modulation of Cullin Substrates

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Cullin-RING Ubiquitin Ligases (CRLs), the largest known family of ubiquitin ligases, are responsible for degradation of around 20% of cellular proteins [1]. Covalent modifications of CRLs with NEDD8 through the ATP-dependent enzymatic E1-E2-E3 cascade are essential for the CRL-mediated neddylation of downstream substrates [2]. Eight cullin family members (Cul1, Cul2, Cul3, Cul4A, Cul4B, Cul5, Cul7, and Cul9) have been found in mammalian cells, each of which is the central component of the respective CRL and modulates degradation of specific substrate [3]. Therefore, inhibition of CRL activity by blocking cullin neddylation has been pursued for the development of novel therapeutics [4]. To date, several natural and synthetic NEDD8-Activating Enzyme (NAE) inhibitors have been identified, of which pevonedistat (TAK-924/MLN4924) developed by Millennium Pharmaceuticals, a first-in-class NAE inhibitor, is currently being investigated in 25 clinical trials [5]. Pevonedistat covalently inhibits NAE by forming a NEDD8-MLN4924 adduct, therefore completely blocking activation of all CRLs and inducing accumulation of CRL substrates. However, pevonedistat has been found to be toxic at higher doses [6] and resistant toward cancer cells [7], which could be due to the inhibition of upstream NAE by pevonedistat.

Among the five DCN Isolforms (DCN1-5), DCN1 (Defective in cullin neddylation protein 1) is the most commonly dysregulated in many Squamous Cell Carcinomas (SCCs) [8] and has been reported to be essential in tumor formation, maintenance and regulation of the neddylation pathway [9]. In structure, DCN1 promotes formation of the multi-protein complex, while UBC12 (also known as UBE2M, NEDD8-conjugating enzyme) facilitates cullin neddylation by recruiting DCN1 [10-12]. Therefore, targeting the DCN1-UBC12 Protein-Protein Interaction (PPI) could achieve selective modulation of cullin substrates. Further computational analysis of the DCN1-UBC12 complex suggests that there are five hydrophobic hotspots at the UBC12 peptide binding site, which could serve as the structural basis for designing inhibitors targeting the DCN1-UBC12 Protein-Protein Interaction (PPI) [13,14]. Guy and colleagues identified the piperidinyl urea-based DCN1 inhibitor NAcM-OPT through the HTS and SBDD strategies, and further obtained an irreversible DCN1 inhibitor NAcM-COV by targeting Cys115 (Fig. 1) [14-16]. Both compounds effectively interrupted the DCN1-UBC12 interaction with an IC\textsubscript{50} value of 80 and 28 nM, respectively, selectively inhibiting neddylation of CUL1 /3 in DCN1-overexpressed HCC95 cells, and suppressed anchorage-independent growth of HCC95 cells.

\begin{figure}[ht]
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\includegraphics[width=\textwidth]{Fig_1.png}
\caption{Identification of NAcM-OPT and NAcM-COV targeting the DCN1-UBC12 PPI through the HTS and SBDD strategies.}
\end{figure}
Starting from a 12-residue UBC12 peptide, the Wang group recently successfully obtained the peptidomimetic inhibitor **DI-404** by sequential truncation strategy and small-molecule inhibitor **DI-591** through the SBDD strategy (Fig. 2). **DI-404** effectively blocked the DCN1-UBC12 interaction (DCN1 $K_{D}$ = 6.9 nM) and selectively inhibited CUL3 neddylation over other cullin members in H2170 and SK-MES-1 cells, and increased expression levels of cullin 3 CRL (CRL3) substrate NRF2 protein. **DI-591**, a high-affinity, cell-permeable small-molecule DCN1 inhibitor, bound to human DCN1 with a $K_{D}$ value of 12 nM, blocked the DCN1–UBC12 interaction in KYSE70 cells, and selectively inhibited CUL3 neddylation over other cullin members in a panel of cell lines [13].

These findings suggest that the DCN1-UBC12 PPI plays crucial roles in modulating neddylation of specific downstream Cul3/1 substrates, targeting this PPI may have applications in CUL3 dysregulated diseases. The development of inhibitors targeting DCN1-UBC12 interaction is currently undergoing worldwide, and the findings will be reported in due course.

**ACKNOWLEDGEMENTS**

This study was supported by the open fund of state key laboratory of Pharmaceutical Biotechnology, Nan-jing University, China (Grant no. KF-GN-201902). We are very grateful for the insightful discussion with Lei Yu from the School of Pharmaceutical Sciences, Gannan Medical University.

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