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Chemoproteomic Profiling of a Pharmacophore-Focused Chemical Library(Abstract_要旨)

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論文題目	Chemoproteomic Profiling of a Pharmacophore-Focused Chemical Library （ファーマコフォアに焦点を当てたケミカルライブラリーのケモプロテオミクスプロファイリング）		

（論文内容の要旨）

Pharmacophore-focused chemical libraries are collections of molecules that contain biologically active scaffolds, which target a specific protein or family of proteins. These libraries are utilized in drug discovery programs to find seed molecules that could serve as chemical tools to modulate the function of a protein of interest or to elicit a specific biological response. Target-based screening and phenotypic screening are the traditional ways to discover bioactive ligands from chemical libraries. However, despite the success of these screening methods in generating valuable small molecule probes, a large fraction of the chemical libraries remains unexplored. In this study, an alternative screening strategy was explored to reutilize an in-house chemical library of 1,800 indole-containing molecules. Instead of performing another target-based screening in which indole structures are known to play an important role, a chemical proteomic approach was employed to identify unexpected targets of the indole structures.

To search for indole-based ligand-protein interactions, a competitive gel-based protein profiling of the library molecules was carried out using an indole-containing photo-affinity probe (**1**). Probe **1** consists of an indole scaffold equipped with a benzophenone for photo-crosslinking and an alkyne group for conjugation to an azide reporter tag. During the protein profiling, HEK293 cells were treated with probe **1** along with the indole-containing compounds, exposed to UV and lysed. The resulting cell lysates were reacted with sulforhodamine B-PEG3-azide via copper catalyzed azide-alkyne cycloaddition (CuAAC), and were characterized by SDS-PAGE and fluorescence imaging. In this context, a compound that has stronger affinity to a probe **1**-binding protein will compete with the probe resulting in the loss or decrease of fluorescence labeling of the protein in the gel profile. Of the 1,800 indole molecules examined, two compounds, 7oa3 and 7qc8, were found to abolish the probe labeling of a protein whose approximate molecular weight is 25 kDa. By biotin pulldown analysis, the ~25 kDa protein band was identified to be glyoxalase 1 (Glo1), an enzyme involved in the detoxification of the reactive methylglyoxal.

The ability of 7oa3 and 7qc8 to modulate Glo1 enzymatic activity was evaluated. The two Glo1 binders and the probe were found to exhibit marginal human Glo1 (hGlo1) inhibition. Structure optimization and protein profiling successfully generated a cell-permeable human and mouse Glo1 inhibitor (**9**). Molecule **9** was characterized to be an effective Glo1 inhibitor *in vitro*, which displays tight-binding and mixed-type of inhibition against hGlo1. Molecule **9** increased the cellular methylglyoxal levels in cultured human cells and suppressed the osteoclast formation of mouse bone marrow-derived macrophages, denoting that the molecule efficiently inhibits Glo1 function in cells. Characterization of the binding mode by X-ray structure analysis revealed that molecule **9** binds to a hydrophobic site abutting the substrate-binding site, which is consistent with the enzyme kinetic profile of **9**. The ligand-protein pair (**9**-Glo1) described here, uncovered a unique binding mode for Glo1 inhibition. These results showcase that a chemoproteomic way of screening may lead to the discovery of unknown small molecule binding modes and unpredicted allosteric sites for protein modulation. Taken together, the study demonstrated how chemical proteomics could be a valuable method for the identification of biologically active ligands from focused chemical libraries that have been fully exhausted in target and phenotypic-based screening.

（論文審査の結果の要旨）

化合物ライブラリーは多くの化合物のコレクションである。特定のタンパク質の活性や細胞表現型を指標にスクリーニングされ、優れた医薬品候補や薬理学ツールを生み出してきた。しかし、化合物ライブラリーに含まれるほとんどの化合物は利用されていない。本論文では、このような化合物ライブラリーの再利用法を提案している。

例として、インドール骨格をもつ1800個の化合物からなる化合物ライブラリーをとりあげ、ケモプロテオーム解析を行った。インドール含有光親和性プローブとプロテオームの相互作用を競合的に阻害する化合物を1800個の化合物から探索し、分子量約25 kDaのタンパク質と2種類の化合物7oa3と7qc8が相互作用することを見い出した。質量分析解析の結果、分子量約25 kDaのタンパク質はメチルグリオキサール代謝酵素であるグリオキサラーゼ 1（Glo1）と同定された。２種の化合物を構造最適化し、細胞内でGlo1活性を強く阻害し、マウス骨髄由来マクロファージの破骨細胞形成を効果的に抑制する化合物9を見出した。化合物9とGlo1のX線共結晶構造解析の結果、化合物9は基質結合部位に隣接する疎水性部位に結合し、酵素活性を非競合的に阻害することが明らかとなった。この結果は、ケモプロテオミクス解析によって、これまでに使い古された化合物ライブラリーから新たな化合物ツールを発掘できることを示唆する。

以上の研究は化合物ライブラリーの新しい利用法を提案し、医薬品の効果的な探索に寄与するところが多い。

したがって、本論文は博士（医学）の学位論文として価値あるものと認める。
なお、本学位授与申請者は、令和2年7月10日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。