京都大学	博士（医科学）	氏　名	Ado Genyir
論文題目	Discovery of a Phase-Separating Small Molecule That Selectively Sequesters Tubulin in Cells. （細胞内でチュブリンを凝集させる相分離小分子化合物の発見）		

（論文内容の要旨）

Cells organize their contents into organelles, which have classically been considered as membrane-separated architectures. However, increasing reports have demonstrated the presence of membraneless organelles which can demix into distinct phases within the cells that form via liquid-liquid phase separation. Synthetic mimics of biomolecular membraneless organelles exploiting nucleic acids, peptides or small proteins has been reported. To date, only very limited success has been achieved using small molecules in formulating organelle-like structures that are capable of controlling biological processes. In the present study, a library of chemical compounds was screened for the discovery of small organic molecules that form artificial organelle-like condensates.

In order to search for phase-separating small molecules that specifically interact with cellular proteins, this study exploited a previously reported in-house chemical library of self-assembling small molecules. A cell-lysate-based co-precipitation screening of 843 library molecules led to the discovery of a self-assembling molecule, which was named R-huezole, that co-precipitated with a ~50-kDa protein selectively in the cell lysate. LC-MS analysis identified the co-precipitated protein as tubulin. In-depth analysis of R-huezole, including confocal microscopy and fluorescence recovery after photobleaching (FRAP) experiments, indicated that R-huezole gradually forms phase-separated liquid-like droplets in an aqueous solution to interact selectively with tubulin. Extended incubation of the R-huezole-tubulin condensates under a highly polymerization-inducing condition nucleated microtubules to form a centrosome-like microtubule aster similar to those previously observed in vitro with spindle-forming protein condensates.

Tubulin is a building block for microtubules, a protein assembly that drives cell division and intracellular transport. Cell culture experiments suggested that R-huezole prevents cell mitosis by forming a large number of tubulin-concentrating, phase-separated condensates in cells. Although the huezole condensates are not perfect mimetics of centrosome, huezole may represent the small organic molecules that exert biological activity by capturing characteristics of cellular biomolecular condensates.

The structure of huezole comprises of two features that mimic naturally occurring proteins in membraneless organelles. Namely a flexible tail for condensation and a more ordered structure for association with other proteins. Structure-activity relationship, NMR, and molecular dynamics experiments showed that the piperazine amide tail of huezole is required for self-assembly. Fluorescence polarization experiments suggested that the benzylphenyl triazole moiety engages in the interaction with tubulin.

The present study demonstrates the feasibility of producing a synthetic condensate out of a non-peptidic small molecule for exogenous control of cellular processes. Such self-assembling small molecules, with their cell permeability, stability, and chemical tractability, may open new possibilities for organelle-emulating molecules that complement the more established peptide-or protein-based artificial organelles.

（論文審査の結果の要旨）

近年、細胞内で液ー液相分離する非膜オルガネラの存在が多数報告されている。しかし、低分子化合物でオルガネラ様構造体を模倣した例はほとんど報告されていない。本研究では、非膜オルガネラ様の凝縮体を形成する有機低分子化合物を探索し、その細胞への影響を解析した。

細胞抽出液を用いた化合物ライブラリーのスクリーニングにより、細胞内タンパク質と選択的に相互作用する相分離性低分子化合物を発見し、R-フエゾールと名付けた。詳細な解析により、R-フエゾールは水溶液中で相分離した液滴を形成し、チュブリンと選択的に相互作用することが示唆された。

細胞内において、R-フエゾールはチュブリンの凝縮体を多数形成し、細胞の有糸分裂を阻害した。また、R-フエゾールのピペラジンアミド部位が自己集合に必要であり、ベンジルフェニルトリアゾール部分がチュブリンとの相互作用に関与していることが構造活性相関研究により示唆された。

以上の研究は、非ペプチド性低分子化合物によって人工的な凝集体を生成し、細胞の挙動を外因的に制御することが可能であることを例証し、化合物による新たな細胞制御法の開発に寄与する。

したがって、本論文は博士（医科学）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　4 年　11 月　15 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。

要旨公開可能日：　　年　　月　　日　以降