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Spotlight

Partitioning of Chemotherapeutics into Nuclear Condensates—Opening the Door to New Approaches for Drug Development

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Klein et al. (2020) demonstrate for the first time that small-molecule cancer therapeutics are selectively partitioned and concentrated within phase-separated nuclear condensates, providing new insights to drug efficacy and creating the opportunity for enhanced control of therapeutic targeting.

A challenge in drug development is that known physiochemical properties are often insufficiently predictive of *in vivo* effects. For example, some compounds with sub-nanomolar dissociation constants (KDs) and good solubility prove ineffective, while others with KDs in the micromolar range work well. In the June 19th issue of *Science*, Klein et al. provide new insight through the discovery that phase-separated biomolecular condensates influence the distribution of small-molecule drugs, opening the door to the possibility of drugs being designed to concentrate in specific membraneless organelles (Klein et al., 2020).

The subdivision of cells into membrane-bound organelles is one of the most fundamental concepts in biology. Basic diagrams of cells often imply an otherwise aqueous environment with free diffusion of macromolecules and proteins within the cytosol or the interior of an organelle. However, the sheer number of protein molecules in a cell—on the order of tens of millions in even the simplest eukaryotic cells (Ho et al., 2018)—implies a dense and complex environment. In recent years, a growing body of research has revealed that proteins and other macromolecules aggregate into phase-separated biomolecular condensates, providing an additional level of compartmentalization that controls reactions by concentrating or excluding components (reviewed in Banani et al., 2017). These condensates have been implicated in myriad cellular structures and processes that are both well-characterized, such as nucleoli (Feric et al., 2016), Cajal bodies (Banani et al., 2017), stress granules (Protter and Parker, 2016), and splicing granules (Galganski et al., 2017), or more recently discovered, including autophagy (Fujioka et al., 2020) and super-enhancer transcriptomes (Hnisz et al., 2017).

Klein et al. ask a simple yet essential question: do condensates impact the concentrations, and therefore the efficacies, of small-molecule drugs? They first generate, in a cell-free context, condensates formed from six different fluorescently labeled nuclear proteins, including the transcriptional regulators MED1 and BRD4. By labeling chemotherapeutics with a second fluorophore, the authors are able to microscopically observe the distribution of drugs within condensates formed from different proteins. They asked whether diverse clinically important drugs with targets that reside in nuclear condensates exhibit free diffusion across these condensates. The drugs studied include classical antineoplastics cisplatin and mitoxantrone, estrogen receptor binding tamoxifen, and newly developed agents that bind transcriptional regulators BRD4 and CDK7. In each case Klein et al. find that these molecules do not freely diffuse but are instead concentrated, or excluded, from different classes of condensates (Figure 1A). While the specific properties of most of these interactions remain to be determined, the authors convincingly show that drugs with aromatic rings preferentially concentrate in MED1 condensates. To further explore the relationship, the authors generate a version of MED1 with all aromatic amino acids removed. While the modified MED1 still forms condensates, it no longer concentrates aromatic-containing drugs, thus providing insight into the underlying chemistry.

The authors find that these same properties apply to MED1 condensates observed in cells. Super-enhancers, potent facilitators of the transcription of lineage-determining genes, are defined by high concentrations of Mediator, of which MED1 is a key subunit (Whyte et al., 2013). Super-enhancers are often co-opted in oncogenesis to drive proliferative fates (Lovén et al., 2013). In studying cisplatin, a widely used chemotherapeutic, Klein et al. find that cisplatin is concentrated 600-fold in MED1 condensates. To evaluate whether the effects of cisplatin, previously considered an indiscriminate DNA crosslinking agent, are in fact preferentially targeted to these key regions of fate control, the authors pull down and sequence platinated DNA. They find that cisplatin preferentially damages super-enhancer DNA. These experiments open up the intriguing possibility that effective chemotherapeutic drugs often characterized as relatively nonspecific may instead be highly targeted.

A key finding from Klein et al.'s work is that the selective partitioning of drugs into specific condensates occurs through physiochemical properties independent of those that underlie engagement of the drug's target. Illustrating this, the authors show that tamoxifen concentrates in MED1 condensates whether or not its

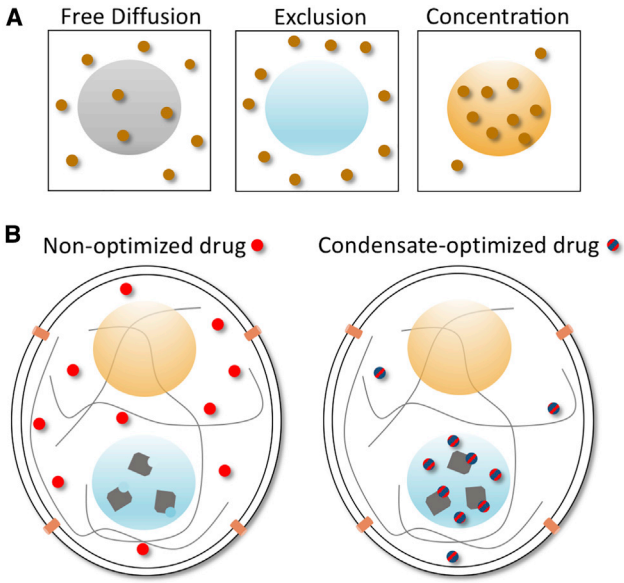

Figure 1. Physiochemical Properties of Drugs and Condensates Determine Their Relative Partitioning

(A) Based on the properties of the condensate, individual drugs may exhibit free diffusion, exclusion, or concentration within different types of condensates.

(B) By changing the physiochemical properties of a drug, it could be redesigned to concentrate within the appropriate condensate (blue) that contains its protein target.

Adapted from Klein et al. (2020), with permission from AAAS.

molecular target, estrogen receptor (ER), is present. Consequently, the chemical properties of tamoxifen that underlie its enrichment in MED1 condensates are key to its anti-cancer activity independent of its binding affinity for ER. Such properties may also be relevant for resistance mechanisms. The authors show that increased expression of MED1, which has been identified in breast cancers resistant to tamoxifen, results in an enlargement of MED1 condensates and thus a decrease in the relative concentration of both ER and tamoxifen. Using *in vitro* droplet assays, they proceed to demonstrate that the increase in MED1 levels impairs the efficacy of tamoxifen, providing new insights into potential resistance mechanisms.

There are a multitude of implications for drug design based on the work of Klein et al. It will now be important to decipher the physiochemical properties of compounds that enable them to concentrate within different condensates. Conversely, it may be informative to determine in which condensates, if any, important drug targets are enriched. Such information may enhance biological understanding of drug function, such as the suggestion that cisplatin activity is markedly enriched at DNA contained within active super-enhancers. Combining new knowledge of the chemistry underlying condensate class targeting with novel understanding of biological function seemingly has the potential to generate major advances in drug design and efficacy. While leaving the structures that underlie target engagement intact, other chemical aspects of drugs could be redesigned to enhance condensate enrichment or to explore the effects of targeting drugs to different condensates (Figure 1B). Additionally, rather than modifying the drug itself, it may also be possible to tether a drug to a chemical moiety that targets it to specific condensates. With continued work, this may even allow for a combinatorial library of inhibitors and localizers. Lastly, the authors speculate that disrupting a condensate without requiring a target may have therapeutic implications on its own. Thus, it may be possible to investigate the effects of targeting moiety-compound conjugates to explore capabilities to disrupt specific condensates and to determine the resultant biological and therapeutic consequences.

Like Frodo delivering the one ring to Mordor, many of our potential drugs may simply "not know the way." Now that Klein et al. have informed us of an additional level of precision required for drug targeting, future work may be able to push us into a new and improved era of drug discovery.

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