Partitioning of cancer therapeutics in nuclear condensates

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The nucleus contains diverse phase-separated condensates that compartmentalize and concentrate biomolecules with distinct physicochemical properties. Here, we investigated whether condensates concentrate small-molecule cancer therapeutics such that their pharmacodynamic properties are altered. We found that antineoplastic drugs become concentrated in specific protein condensates in vitro and that this occurs through physicochemical properties independent of the drug target. This behavior was also observed in tumor cells, where drug partitioning influenced drug activity. Altering the properties of the condensate was found to affect the concentration and activity of drugs. These results suggest that selective partitioning and concentration of small molecules within condensates contributes to drug pharmacodynamics and that further understanding of this phenomenon may facilitate advances in disease therapy.

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The five to 10 billion molecule contents of cells are compartmentalized into both membrane-bound and nonmembrane-bound organelles (1–3). Many non–membrane-bound organelles are phase-separated biomolecular condensates with distinct physicochemical properties that can absorb and concentrate specific proteins and nucleic acids (4–17). We reasoned that selective condensate partitioning might also occur with small-molecule targets with drugs that occur within condensates (Fig. 1A), and that the therapeutic index and efficacy of such compounds might therefore relate to their ability to partition into condensates that harbor their target. To test this idea, we focused our study on a collection of nuclear condensates previously reported in cell lines, demonstrated that they all occur in normal human cells and in tumor cells, and then developed in vitro droplet assays with key components of each of the nuclear condensates to enable testing of small molecules.

Nuclear condensates have been described in diverse cultured cell lines and contain one or more proteins that can serve both as markers of the condensate and as a scaffold for condensate formation in droplet assays in vitro (10–12, 18–31). Specifically, transcriptional condensates are marked by the condensate-forming proteins MED1 and BRD4 (10, 12, 19), splicing speckles by SRSP2 (11, 20), heterochromatin by HP1α (21, 22), and nucleoli by FIB1 and NPM1 (23–25) (fig. S1A). To determine whether such condensates can also be observed in the cells of healthy and malignant human tissue, we obtained biopsies of breast ductal epithelium, invasive ductal carcinoma, normal colon, and colon cancer (fig. S1, B and C). Immunofluorescence revealed nuclear bodies containing these marker proteins in both normal and transformed tissue (Fig. 1, B and C). There was a broad distribution of nuclear body sizes and numbers, as expected for dynamic biomolecular condensates, and no significant differences were observed between benign and malignant tissue (fig. S2, A to C). However, tumor cells acquire large superenhancers (SEs) at driver oncogenes (32) and these can form tumor-specific transcriptional condensates.

We developed an assay to model these nuclear condensates and study the behavior of small molecules within these droplets (Fig. 1D). We produced and purified recombinant, fluorescently labeled versions of MED1, BRD4, SRSP2, HP1α, FIB1, and NPM1 (fig. S3, A and B) and confirmed the ability of these proteins to form droplets in an in vitro assay (fig. S4, A and B). To investigate the partitioning behavior of small molecules, we added the dyes fluorescent (332 Da) and Hoechst (452 Da), as well as fluorescently labeled dextran (4.4 kDa), to solutions containing each of the six protein condensates. The dyes and dextrans appeared to diffuse through all the condensates without substantial partitioning (Fig. 1E and figs. S5 and S6, A to D). Small-molecule drugs are generally smaller than 1 kDa, so these results suggested that small molecules can freely diffuse through these nuclear condensates unless there are factors other than size that influence partitioning.

We next sought to determine whether diverse clinically important drugs with targets that reside in nuclear condensates also exhibit free diffusion across these condensates or if they display a different behavior. Cisplatin and mitoxantrone, members of a class of antineoplastic compounds that modify DNA through platination or intercalation, can either be modified to have fluorescent properties (cisplatin (33)) or are already inherently fluorescent (mitoxantrone). When added to droplet formation buffer with purified MED1, BRD4, SRSP2, HP1α, FIB1, or NPM1, cisplatin was found to be selectively concentrated in MED1 droplets (Fig. 2A and fig. S7A), with a partition coefficient of up to 600 (fig. S8, A to C). Fluorescent modification of cisplatin did not appear to contribute to this behavior in vitro, because the modified drug could be chased out of the condensate with unmodified cisplatin, and an isomer of cisplatin did not exhibit the same behavior (fig. S7, B to D). Mitoxantrone was also concentrated in MED1 condensates, as well as in FIB1 and NPM1 condensates (Fig. 2B and fig. S7A). Consistent with these results, mitoxantrone is known to concentrate in the nucleus, where FIB1 and NPM1 reside (34, 35). These results show that, in contrast to the dyes tested above, small-molecule drugs may concentrate in certain condensates even in the absence of the drug target.

We selected for further study antineoplastic drugs that target transcriptional regulators...
Fig. 1. Nuclear condensates in human tissue and in vitro. (A) Model illustrating the potential behaviors of small molecules in nuclear condensates. (B and C) Immunofluorescence of scaffold proteins of various nuclear condensates in tissue biopsies from benign and malignant human breast tissue (B) and from benign and malignant colon tissue (C) in nuclei stained with Hoechst and imaged at 100× on a fluorescent confocal microscope (see also figs. S1 and S2). (D) Schematic of in vitro droplet formation assay to measure small-molecule partitioning into nuclear condensates. (E) In vitro droplet assay showing the behavior of fluorescein dye in the presence of six protein condensates formed in 125 mM NaCl and 10% PEG with 10 μM protein and 5 μM fluorescein imaged at 350× on a confocal fluorescent microscope (see also figs. S3 to S6). Quantification of enrichment of the drug is shown on the right. Error bars represent SEM.
expected to be contained within transcriptional condensates in cells. These targets include: (i) the estrogen receptor (ER), a transcription factor and nuclear hormone receptor; (ii) CDK7, a cyclin-dependent kinase that functions in transcription initiation and cell cycle control; and (iii) BRD4, a bromodomain protein and coactivator involved in oncogene regulation (fig. S9, A and B). To monitor drug behavior with a confocal fluorescent microscope, we used a fluorescent tamoxifen analog (FLTX1) that targets ER and modified fluorescent THZ1 and JQ1, which target CDK7 and BRD4, respectively (36, 37). FLTX1 and THZ1 concentrated preferentially in MED1 droplets (Fig. 2, C and D, and fig. S7A), and this behavior was not attributable to the fluorescent moiety (fig. S7, B and D). JQ1 concentration presented a different pattern, being concentrated in MED1, BRD4, and NPM1 droplets (Fig. 2E and fig. S7, A and B). Reinforcing these results, we found that the small molecules that concentrated in MED1 condensates were also concentrated in condensates formed from purified whole Mediator complexes (fig. S10A) and in MED1 condensates formed in an alternative crowding agent (fig. S11A). The targets of these three compounds (ERα, CDK7, and the bromodomains of BRD4) are not present in these in vitro condensates but are present in the SEs that form condensates with transcription factors and Mediator in vivo (10, 12, 38) (fig. S9, A and B), suggesting that the ability of some small molecules to concentrate preferentially in the same condensate as their protein target may contribute to the pharmacological properties of these drugs.

To gain additional insight into the nature of interactions governing small-molecule enrichment in condensates, we focused on the MED1-IRD condensate. Fluorescence recovery after photobleaching (FRAP) experiments showed that cisplatin molecules were highly mobile in this condensate (fig. S12, A and B), suggesting that the condensate produces a physicochemical environment that facilitates drug concentration in a state of high dynamic mobility. To gain insights into the chemical features of small molecules that may contribute to selective association with MED1 in condensates, we used a fluorescent boron-dipyromethene (BODIPY) library of 81 compounds with various combinations of chemical side groups (fig. S13A). Molecules that contained aromatic rings were found to preferentially concentrate in MED1 condensates (figs. S13, A to D, and S14A). These data suggest that pi–pi or pi–cation interactions are among the physicochemical properties that favor small-molecule partitioning into MED1 condensates. Aromatic amino acids participate in pi–system interactions and are overrepresented in the MED1 IDR relative to the other condensate-forming proteins studied (fig. S3B). We generated a MED1 aromatic mutant protein (all 30 aromatic amino acids mutated to alanine) that retained the ability to form droplets in vitro, indicating that the aromatic amino acids are not required for droplet formation (fig. S14, A to D, and S14A). These data suggest that pi–pi or pi–cation interactions are among the physicochemical properties that favor small-molecule partitioning into MED1 condensates.

Fig. 2. Partitioning behavior of small-molecule drugs in nuclear condensates in a droplet assay.

Six nuclear condensates formed in 125 mM NaCl and 10% PEG with 10 μM protein treated with (A) 5 μM cisplatin-TMR, (B) 50 μM mitoxantrone, (C) 100 μM FLTX1, (D) 5 μM THZ1-TMR, or (E) 1 μM JQ1-ROX imaged at 150× on a confocal fluorescent microscope (see also figs. S7 to S11). Quantification of enrichment of the drug within droplets is shown on the right of each panel. Error bars represent SEM (see also figs. S12 to S14).
formed by the MED1 aromatic mutant protein (fig. S14, D to F). These results suggest that the aromatic residues of MED1 condensates contribute to the physicochemical properties that selectively concentrate these small molecules.

We anticipated that the ability of small molecules to concentrate in specific condensates would influence target engagement and thus drug pharmacodynamics. To investigate this, we took advantage of the ability of condensates to incorporate DNA (Fig. 3A and fig. S15A) and measured the relative efficiency of DNA platination by cisplatin in MED1 condensates, where cisplatin is concentrated, versus HP1α condensates, where cisplatin freely diffuses (Fig. 2A). DNA platination, visualized by size shift on a bioanalyzer, was more prevalent in MED1 condensates than in HP1α condensates (Fig. 3B), consistent with the expectation that elevated concentrations of cisplatin in the MED1 condensates yield enhanced target engagement and drug efficacy.
engagement. If cisplatin becomes concentrated in Mediator condensates in cells, then we would expect that DNA colocalized within Mediator condensates would be preferentially platinated. To test this idea, we performed coimmunofluorescence in cisplatin-treated HCT116 colon cancer cells using an antibody that specifically recognizes platinated DNA (fig. S16A) (39), together with antibodies specific for MED1, HP1α, or FIB1. Consistent with cisplatin’s preference for MED1 condensates in vitro, we found that platinated DNA frequently colocalized with MED1 condensates but not with HP1α or FIB1 condensates (Fig. 3C). To determine whether the ability of cisplatin to engage DNA is dependent on the presence of a MED1 condensate, we treated cells with JQ1, which caused a loss of MED1 condensates (fig. S16B), and observed a concomitant reduction in platinated DNA at the MYC oncogene (fig. S16, C and D). These results are consistent with the idea that the concentration of small molecules in specific condensates can influence the efficiency of target engagement.

In cells, the preferential modification of DNA in MED1-containing condensates might be expected to selectively disrupt these condensates with prolonged treatment. To test this, HCT116 colon cancer cells were engineered...
Mechanisms, including ERα mutation and MED1 overexpression (Fig. 4A and fig. S25) (50–54). To investigate whether ERα mutations alter ERα behavior in condensates, we produced four patient-derived ERα mutant proteins and tested their partitioning in the presence of tamoxifen. In contrast to wild-type ERα, condensates composed of patient-derived ERα mutants and MED1 were not disrupted upon tamoxifen treatment (Fig. 4B and fig. S26, A and B). The ERα point mutations reduce the affinity for tamoxifen by -10-fold (55), indicating that the drug concentration in the droplet is inadequate to evict these ER mutant proteins when this affinity is reduced.

MED1 overexpression is associated with tamoxifen resistance and poor prognosis in breast cancer (50), but it is not clear why overexpression of one subunit of the Mediator complex produces resistance. We considered the possibility that overexpressed MED1 is incorporated into transcriptional condensates, which contain clusters of Mediator molecules (38), thereby expanding their volumes and diluting the available tamoxifen (fig. S27A). We found that the tamoxifen-resistant breast cancer cell line TAMR7 (55), which was derived from the tamoxifen-sensitive cell line MCF7, produced fourfold elevated levels of the MED1 protein (fig. S27B). The volume of MED1-containing condensates was twofold larger in these cells (Fig. 4C and fig. S27C). When modeled in an in vitro droplet assay, we found that a fourfold increase in MED1 levels led to a commensurate increase in droplet size (fig. S28A, A and B). Furthermore, we found that 100 μM tamoxifen prevented ERα incorporation into MED1 condensates (Fig. 4, B and D), but was much less effective in preventing ERα incorporation into the larger MED1 condensates produced with higher MED1 levels (Fig. 4D). To confirm that the levels of tamoxifen in the larger droplets are more dilute, we measured the enrichment of the fluorescent tamoxifen analog FLTX1 in MED1 droplets and found that the larger condensates had lower concentrations of the drug (Fig. 4E). These results were mirrored in cells, where a collection of tethered ERα molecules formed a MED1 condensate that was eliminated by tamoxifen, but when MED1 was overexpressed, tamoxifen was unable to dissociate the ERα-MED1 condensate (fig. S29A). Similarly, knockdown of MED1 in tamoxifen-resistant breast cancer cells sensitizes cells to tamoxifen (50,54). These results support a model of tamoxifen resistance where MED1 overexpression causes the formation of larger transcriptional condensates in which tamoxifen is diluted and is thereby less effective at dissociating ER from the condensate (Fig. 4F).

Our results show that drugs partition selectively into condensates, that this can occur through physicochemical properties that exist independently of their molecular targets, and that cells can develop resistance to drugs through condensate-altering mechanisms. This may explain the surprising observation that inhibition of global gene regulators such as BRD4 or CDK7 can have selective effects on oncogenes that have acquired large SEs (45); selective partitioning of inhibitors such as JQ1 and THZ1 into SE condensates will preferentially disrupt transcription at those loci. These results also have implications for the future development of efficacious disease therapeutics; effective target engagement will depend on measurable factors such as drug partitioning in condensates (fig. S30, A to D). Condensate assays of the type described here may thus help to optimize condensate partitioning, target engagement, and the therapeutic index of small-molecule drugs.

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**Author contributions:**

**Competing interests:** R.A.Y. is a founder and shareholder of Syros Pharmaceuticals, Camp4 Therapeutics, Omega Therapeutics and Dewpoint Therapeutics. A.A.H. is a founder and shareholder of Syros Pharmaceuticals and a consultant of Camp4 Therapeutics. A.C. is on the Scientific Advisory Board of Dewpoint Therapeutics and Omega Therapeutics. P.A.S. is a shareholder and consultant of Dewpoint Therapeutics. Y.T.C. is an inventor on U.S. Patent US5513294 B2, China Patent ZL 201380067802.8, EP 2398639 B1, and Japan Patent 6300380 held by POSTECH University that cover diversity-oriented fluorescent library approaches. I.A.K., A.B., and R.A.Y. are inventors on patent application submitted by The Whitehead Institute that covers small molecule drug partitioning in and acting upon biomolecular condensates. All other authors declare no competing interests.

**Data and materials availability:** All data are available in the main text or the supplementary materials. High-throughput sequencing data sets are available in GEO (GSE149085).

**SUPPLEMENTARY MATERIALS**

science.sciencemag.org/content/368/6497/1386/suppl/DC1
Materials and Methods
Figs. S1 to S30
References (56–68)
MDAR Reproducibility Checklist
View/request a protocol for this paper from Bio-protocol.

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**References**

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Drug partitioning in nuclear condensates

There is increasing interest in the function of phase-separated biomolecular condensates in cells because of their distinct properties and expanding roles in important biological processes. Klein et al., considered the fate of small-molecule therapeutics in the context of nuclear condensates (see the Perspective by Viny and Levine). They show that certain antineoplastic drugs have physicochemical properties that cause them to concentrate preferentially in condensates, both in vitro and in cancer cells. This property influences drug activity, and protein mutations that alter condensate formation can lead to drug resistance. Optimizing condensate partitioning may be valuable in developing improved therapeutics.

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