Liquid–Liquid Phase Separation in Cancer Signaling

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The process of LLPS(liquid–liquid phase separation (LLPS) is favored by multivalent interactions between specific protein domains, intrinsically disordered regions in proteins (IDR) and nucleic acids.

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1. Phase Separation and Mutant Oncoproteins

Several mutant oncoproteins for stable biomolecular condensates that confer new signaling abilities to cancer cells. Signaling scaffolds facilitate protein–protein interactions and regulate crosstalk with other signaling pathways [1]. Phase separation must be conferring additional advantages over scaffolding for these signaling modules to play a role in the transformation. In this sense, it is worth considering that aberrantly high activation of the ERK pathway induces senescence rather than oncogenic transformation [2][3]. Phase-separated signaling complexes may allow for a moderate level of signaling output, stimulating proliferation while avoiding the aberrantly high activation levels triggering senescence. One possibility is that phase separation may exclude phosphatases from signaling complexes, as reported for T cell receptor signaling [4]. The competition of phosphatases with kinases in signaling complexes confers ultra-sensitivity in signaling pathways [5]. Ultra-sensitivity amplifies the signal and can mediate changes in cell fate by allowing a higher level of signaling output [6]. Phase-separated MAPK signaling complexes organized by mutant SHP2 or EML4-ALK may exclude phosphatases and avoid ultra-sensitivity in ERK signaling, preventing the levels of signal amplification required for senescence.

Cancer-associated mutations can also drive the formation of phase-separated transcription complexes that drive the expression of oncogenic transcription programs. Mutation in the YEATS domain of ENL (Eleven-Nineteen Leukemia) in Wilm's tumors enhances the ability of this protein to seed biomolecular condensates at the loci with acetylated chromatin, recruiting transcription complexes to activate gene expression [7]. ENL-mutant condensates recovered quickly after photobleaching, revealing their dynamic nature. Similarly, the fusion proteins between the N-terminal phenylalanine-glycine rich (FG)-repeat domain of the nuclear pore protein NUP98 with the chromatin binding domains of HOXA9, KDM5A or NSD1 also increase the transcription of oncogenic programs by promoting the formation of biomolecular condensates and super-enhancers that recruit transcriptional coactivators [8]. Finally, the EWS-FLI1 fusion protein present in Ewing Sarcoma recruits the chromatin-remodeling complex BAF to tumor-specific enhancers activating gene expression via LLPS [9]. EWSR1, one of the partners in the EWS-FLI1 fusion proteins, requires RNA to interact with the BAF complex. However, the EWS-FLI1 fusion protein forms transcriptional activating biomolecular condensates that do not require RNA [9]. One important question is why phase separation is needed for transformation mediated by these mutant oncoproteins. One can speculate that cancer mutations in biomolecular condensates change the biophysical properties of condensates, making them more stable. As a consequence, cells expressing these oncoproteins cannot reverse the activation of gene expression programs that maintain the cancerous stage, which are similar to programs that characterize embryonic or stem cells [10][11][12][13]. This idea suggests that drugs capable of dissolving condensates of mutant oncoproteins could inhibit tumorigenesis.

2. Phase Separation and Tumor Suppressors

Cancer mutation can also disrupt the normal functioning of biomolecular condensates that prevent tumorigenesis. The functions of p53, the most frequently altered tumor suppressor in human cancer, are inactivated in phase-separated amyloid structures for which formation is facilitated by mutations in the DNA-binding domain of p53 [14]. The tumor suppressor E3 ligase SPOP forms phase-separated bodies in the nucleus with its substrates proteins such as DAXX. Cancer-associated mutations of SPOP disrupted the SPOP/DAXX nuclear bodies, driving DAXX accumulation and cancer progression [15]. cAMP compartmentation, a process that depends on the phase separation of RIα and PKA, is disrupted by mutations of RIα present in liver fibrolamellar carcinoma (FLC). RIα is the regulatory subunit of PKA, a protein kinase

that transduce cAMP signaling. The consequence of this RIα mutation is an aberrant cAMP signaling that increases cell proliferation and induces malignant properties in non-cancerous liver cells [16].

The promyelocytic leukemia protein PML acts as tumor suppressor, regulating apoptosis and cellular senescence [17]. PML forms phase-separated nuclear bodies, for which composition is regulated by multivalent interactions mediated by SUMOylation [18][19]. PML bodies organize the repression of the E2F target genes required for cell proliferation by organizing repression compartments with the retinoblastoma tumor suppressor [20][21]. In acute promyelocytic leukemia, the fusion protein PML-RARA disrupts the PML body's function, promoting transformation [22]. Another tumor suppressor that requires phase separation for its functions is the histone demethylase KDM6A, also known as UTX. The most frequent cancer mutations in UTX affect its IDR, abolishing the ability of the protein to form biomolecular condensates and regulate high-order chromatin interactions [23]. Intriguingly, the tumor suppressor functions of UTX do not depend on its demethylase activity but on phase separation, but it is known very little about how UTX condensates actually block tumorigenesis. The enigmatic functions of both PML and UTX suggest that their tumor suppressor activities could be linked to the disruption of oncogenic biomolecular condensates.

3. RNA-Binding Proteins and Non-Coding RNAs That Affect LLPS in Cancer

Biological condensates driving tumorigenesis can also be generated by an increased expression in proteins or RNA capable of undergoing LLPS. LLPS often depends on multivalent interactions that involve RNA molecules and/or RNA-binding proteins [24][25][26][27]. The RNA-binding protein AKAP8 (A-Kinase Anchoring Protein 8), also known as AKAP95, is overexpressed in ovarian and colorectal cancers and is required to inhibit oncogene-induced senescence. The oncogenic activity of AKAP8 involves LLPS and correlates with its ability to regulate RNA splicing [28]. For example, AKAP8 expression and LLPS leads to intron retention during the splicing of the cyclin A2 transcript. The resulting cyclin A2 isoform is resistant to degradation, driving oncogenic transformation [28]. Intriguingly, RNA binding reduces the mobility of AKAP8 in condensates [28]. Of note, AKAP8 prevents metastasis in breast cancer, indicating that the pro-tumorigenic effect is context-dependent [29].

The RNA-binding protein YBX1 (Y-Box Binding Protein 1) has emerged as an important driver in multiple cancers. YBX1 forms biomolecular condensates that recruit miR-223, sorting this miRNA to exosomes [30]. YBX1 also regulates transcription, splicing and translation, and is required for viability in cells that persist after targeted therapy against Jak2 [31][32]. Persistence is a non-genetic mechanism of the adaptation to stress that could be mediated by the formation of stable biomolecular condensates [33]. In sarcomas, YBX1 increases the translation of the RNA-binding protein G3BP1, which, via LLPS, initiates stress granule formation that enables cancer cells to sustain stress [34].

SNHG9 (Small Nucleolar RNA Host Gene 9) is a lipid-binding lncRNA highly expressed in breast cancers. Its oncogenic activity was demonstrated to be dependent on the sequestration of the tumor suppressor LATS1 via LLPS. LATS1 is a member of the Hippo pathway deregulated in multiple cancers [35]. The RNA-binding protein, YTHDC1, drives tumorigenesis in acute myeloid leukemia by protecting oncogenic mRNAs from degradation. This protein is a reader of the epigenetic RNA modification N^6-methyladenosine. The modification allows YTHDC1 to form biomolecular condensates called YACs (YTHDC1-m^6A condensates), which are increased in leukemia cells [36].

4. Phase Separation and Cancer Metabolism

Enzymes involved in specific metabolic pathways are often organized into multi-enzymatic complexes or metabolons. This strategy can increase the activity of enzymes with poor specificity or low substrate affinity, or prevent the accumulation of toxic metabolites for which their only role is to act as intermediates in a metabolic pathway [37]. The compartmentalization of metabolic pathways also allows substrate channeling where the metabolic intermediates are not in equilibrium with the bulk solution and the reactions do not depend on the free diffusion of these metabolites from long distances [38].

The enzyme ribulose bis-phosphate carboxylase oxygenase (RuBisCO) is found in 100–200 nm polyhedral bodies known as carboxysomes in bacteria [39] or in non-membrane organelles called pyrenoids in green alga [40],. In this way, carbon fixation is optimized perhaps by shielding RuBisCO from the actions of oxygen [39]. Both in bacteria and eukaryotes, RuBisCO adopts a liquid-like state, the assembly of which is mediated by multivalent interactions mediated by accessory proteins [40][41][42]. In yeast, a large number of metabolic enzymes form punctate cytoplasmic foci upon nutrient depletion [43]. Therefore, biological condensates may play key roles in stressed cells that require specific metabolic pathways to survive.

Consistent with the idea that metabolic stress leads to increased formation in biomolecular condensates, mammalian cells under purine-depleted conditions form purinosomes, where all enzymes for de novo purine biosynthesis cluster in puncta [44]. Intriguingly, hypoxia triggers the formation of purinosomes in a process that requires the activity of the transcription factor HIF1α [45]. Hypoxia also triggers the formation of discrete bodies of glycolytic enzymes (G bodies) [46]. The formation of G bodies requires RNA and multivalent interactions, suggesting they form via LLPS [47]. Importantly, G bodies also form in human liver cancer cells in response to hypoxia [46]. Additionally, human PFK (Phosphofructokinase), which catalyzes the rate-limiting reaction in glycolysis, localizes in discrete cytosolic clusters in liver cancer cells together with other enzymes of glucose metabolism. Of note, these foci were not present in non-cancerous cells, suggesting that they play a role in cancer metabolism [48]. Hypoxia also triggers the formation of puncta containing pyruvate carboxylase, malate dehydrogenase-1 and malic enzyme-1. These enzymes form a complex called HTC (hydride transfer complex) that catalyzes the transfer of hydride ions from NADH and NADP, regenerating NAD+, and forming NADPH. HTC assists the metabolism of cells with mitochondrial dysfunction and contributes to malignant transformation by inhibiting the process of oncogene-induced senescence. HTC puncta are dissolved by 1,6 hexanediol, suggesting that the complex is formed via LLPS [49]. Finally, amino acid starvation triggers LLPS of the proteasome, a process that decreases cell survival during p53-mediated apoptosis [50].

5. Phase Separation in Cancer Therapy

Since phase separation is often mediated by IDRs, compounds that bind these domains may help to modulate the process. However, few compounds have been identified that bind IDRs and their ability to disrupt biomolecular condensates is poorly explored [51]. Drugs may localize or be excluded from biomolecular condensates, affecting their ability to reach their targets. For example, cisplatin, mitoxantrone, the CDK7 inhibitor THZ1 and tamoxifen were found to selectively partition into condensates formed by MED1, a component of transcriptional condensates. Moreover, mitoxantrone concentrated in condensates formed by the nucleolar proteins FIB1 and NPM1. On the other hand, the BRD4 inhibitor concentrated in condensates of BRD4, ED1 and NPM1.

The ability of platinum drugs to concentrate in condensates of MED1, a component of super-enhancers, may explain why platinum drugs are efficient in many cancers [52]. Intriguingly, MED1 overexpression confers resistance to tamoxifen in breast cancer and this was explained by an increase in the size of transcriptional condensates and an inability of tamoxifen to prevent the partitioning of ERα in these condensates [52]. The importance of intracellular localization for anticancer drug action is well illustrated using multiplexed ion beam imaging (MIBI) and Nano SIMS (nanoscale secondary ion mass spectrometry) instruments in cells that develop resistance to cisplatin. In sensitive cells, cisplatin is enriched in nuclear speckles and excluded from closed chromatin. However, in resistant cells the drug is totally excluded from the nucleus [53]. Another potentially useful technique to konw the effects of drugs at the single-cell level is Raman spectroscopy. The Raman spectral signature of cells changes after treatment with drugs such as tamoxifen, and some Raman peaks correlate with the tamoxifen abundance in the same cells as measured using mass spectrometry [54].

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