Received: 14 January 2024 | Accepted: 2 June 2024

DOI: 10.1002/cai2.144

CANCER INNOVATION

REVIEW

Role of liquid–liquid phase separation in cancer: Mechanisms and therapeutic implications

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Funding information
National Natural Scientific Foundation of China, Grant/Award Number: 82172566; Beijing Science and Technology Innovation Medical Development Foundation, Grant/Award Number: KC2021-JX-0186-101; Tsinghua University Initiative Scientific Research Program of Precision Medicine, Grant/Award Number: 2022ZLB004

Abstract

Liquid–liquid phase separation (LLPS) has emerged as a pivotal biological phenomenon involved in various cellular processes, including the formation of membrane-less organelles and the regulation of biomolecular condensates through precise spatiotemporal coordination of signaling pathways in cells. Dysregulation of LLPSs results in aberrant biomolecular condensates, which are widely implicated in tumorigenesis and cancer progression. Here, we comprehensively summarize the multifaceted roles of LLPS in tumor biology from the perspective of cancer hallmarks, including genomic stability, metabolic reprogramming progression, ferroptosis, and metastasis, to unveil the intricate mechanisms by which LLPS occurs in tumorigenesis. We discuss current discoveries related to therapeutic involvement and potential clinical applications of LLPS in cancer treatment, highlighting the potential of targeting LLPS-driven processes as novel therapeutic strategies. Additionally, we discuss the challenges associated with new approaches for cancer treatment based on LLPS. This in-depth discussion of the impact of LLPS on fundamental aspects of tumor biology provides new insights into overcoming cancer.

KEYWORDS
cancer, liquid–liquid phase separation, mechanism

1 | INTRODUCTION

Liquid–liquid phase separation (LLPS) primarily involves the formation of membraneless droplets in the cellular environment when proteins and nucleotides reach a certain concentration threshold [1]. In eukaryotic cells, multiple membrane-enclosed organelles and membraneless compartments are spatially and temporally separated to coordinate intricate biochemical reactions. These membraneless compartments, also termed biomolecular

Abbreviations: 53BP1, p53-binding protein 1; AR, androgen receptor; CPC, chromosome passenger complex; CPs, cationic polymers; CRC, colorectal cancer; DSBs, DNA double-strand breaks; FUS, fused in sarcoma; HCC, hepatocellular carcinoma; IDR, intrinsically disordered regions; IFI16, interferon-induced protein 16; IGF2BPs, insulin-like growth factor 2 mRNA-binding proteins; IRS, insulin receptor substrates; LC-core, low complexity core regions; LLPS, liquid–liquid phase separation; MED1, mediator complex subunit 1; MLL, mixed lineage leukemia; MRN, MRE11/RAD50/NBS1; NBR1, neighbor of BRCA1 gene 1; NHEJ, nonhomologous end joining; PABP1, poly(A) binding protein cytoplasmic 1; PD-1, programmed cell death protein-1; PD-L1, programmed cell death ligand 1; PML, promyelocytic leukemia protein; SG, stress granular.

condensates, result from reversible and dynamic LLPS [2]. Biomolecular condensates harbor different physical and chemical properties that can absorb and concentrate specific proteins and nucleic acids. In recent years, increasing evidence has suggested that LLPS is not only involved in regulating physiological processes but also plays an important role in cancer initiation and progression [3–5]. Furthermore, targeting LLPS and biomolecular condensates holds promise as a novel strategy for cancer treatment.

In this review, we aim to provide a comprehensive review of the current understanding of LLPS in cancer, summarizing the underlying mechanisms in tumorigenesis and progression, including genomic stability, metabolic reprogramming, ferroptosis, and metastasis. By focusing on the biophysical principles underlying LLPS, we also discuss the potential implications of LLPS in cancer treatment, including immunotherapy, targeted therapy, chemotherapy, endocrine therapy, and radiotherapy. Finally, we outline challenges and propose new applications that may pave the way for the development of innovative therapeutic approaches to overcome cancer.

2 | HALLMARKS OF LLSP

In 2009, Brangwyne and colleagues observed liquid-like P granules in *Caenorhabditis elegans* embryos, reported as the first recognition of the role of phase separation in biology [6]. Indeed, the core concept of phase separation of proteins involves the assembly of high-concentration condensates of molecules within specific cellular regions, driven by multivalent protein–protein or protein-RNA interactions. Such protein droplets exhibit distinct physical properties, including:

(1) LLSP proteins in vivo appear as point-like aggregates upon staining [7];
(2) LLSP proteins in vitro have a spherical shape [8];
(3) LLSP droplets can fuse with each other to form larger droplets.

Intrinsically disordered regions (IDRs), that is, peptides with folded modular domains, are characterized by heterogeneous conformational ensembles and are known to play important roles in forming biomolecular condensates, in which numerous signaling pathways and cancer-related proteins exist [9]. The dynamics of LLPS rely on the biophysical activity of proteins, with scaffold and client proteins serving as key mediators. Moreover, various external factors can modulate LLPS by regulating the concentration and affinity of scaffold proteins, as well as recruiting client proteins. Recently, various methods have been used to investigate LLPS behaviors through both in vitro and in vivo experiments. The structure of biomolecular condensates that have droplet-like characteristics can be directly observed by an ordinary optical microscope; however, their bioactivity remains challenging to evaluate [10]. Fluorescence recovery after photobleaching is a universally recognized method for detecting LLPS in cells [11]. A fluorescent protein initially fuses with the target protein, and the bleached condensate regains its fluorescence if the membraneless condensate undergoes frequent material exchange with the surrounding environment over a short period of time [12]. The OptoDroplet system is a frequently used method for in vitro experiments, relying on optogenetic techniques to induce phase transitions and construct membrane-free organelles. This system illustrates that concentrated phases can be driven by the IDRs of various RNA/protein body proteins, such as fused in sarcoma (FUS), dead-box helicase 4, and heterogeneous nuclear ribonucleoprotein A1 [13]. Additionally, Du et al. developed a live-cell super-resolution and multi-color 3D-imaging approach to directly observe condensates composed of Pol II and Mediator that spatially and temporally regulate gene transcription [14]. Databases that predict LLPS-related proteins have been established, such as PhaSePro [15], PhaSepDB [16], DrLLPS [17], PhaSePred [18], catGRANULE [19], PLAAC [20], PScore [21], LLPSDB [22], MLOsMetaDB [23], PSPire [24], IUPred2A [25], FuzDrop [26], D^2P^2 [27], PSPHunter [28], and LLPhyScore [29] (Table 1).

3 | LLSP IN PHYSIOLOGY

Phase separation is extensively implicated in various physiological processes, including proliferation, metabolism, and immunity. The fragile X-related gene 1 is an important translation activator that undergoes LLPS to stimulate mRNA translation stored in mouse sperm cells, contributing to spermatogenesis and the reproductive capacity of male mice [30]. Paraspeckle component 1 LLPS interacts with serine/threonine protein phosphatase 5 to regulate checkpoint kinase 1 phosphorylation, thereby promoting mouse oocyte maturation [31].

The LLPS of the nuclear mitotic apparatus protein can coordinate and regulate the assembly, structural dynamics, and function of the spindle apparatus during mitosis [32]. Insulin recruits insulin receptor substrates (IRSs) and PI3K to the cell membrane, initiating a series of downstream cascades. Research has revealed that insulin-driven IRS LLPS forms protein liquid droplets that recruit PI(4,5)P2, p85-PI3K, and PDK1, activating subsequent signaling pathways. Inhibition of insulin-induced IRS LLPS by

TABLE 1 Databases predicting liquid–liquid phase separation (LLPS)-related proteins.

Database	Website	Introduction	Year of construction	Origin	Reference
PhaSePro	https://phasepro.elte.hu	A database manually curated and solely based on experimentally verified cases of LLPS.	2019	Hungary	[15]
PhaSepDB	http://db.phasep.pro	A collection of manually curated phase separation and membraneless organelles-related proteins.	2019	China	[16]
DrLLPS	http://llps.biocuckoo.cn	A data resource containing known and computationally detected LLPS-associated proteins.	2019	China	[17]
PhaSePred	http://predict.phasep.pro	A centralized resource that provided self-assembling and partner-dependent phase-separating protein prediction.	2021	China	[18]
catGRANULE	http://s.tartaglialab.com/new_submission/catGRANULES	An algorithm to predict LLPS propensity.	2016	Spain	[19]
PLAAC	http://plaac.wi.mit.edu	A web application to analyze protein sequences and identify domains with prion-like amino acid composition.	2014	USA	[20]
PScore	https://github.com/haocai1992/PScore-online#pscore-online	A machine learning algorithm predicting phase separation based on propensity for long-range planar pi-pi contacts.	2018	Canada	[21]
LLPSDB	http://bio-comp.ucas.ac.cn/llpsdb	A database containing LLPS-related proteins together with the corresponding phase separation conditions.	2019	China	[22]
MLOsMetaDB	http://mlos.leloir.org.ar	A resource of information on membraneless organelles and LLPS-related proteins with biological visualizations.	2023	Argentina	[23]
PSPire	https://github.com/TongjiZhanglab/PSPire	A machine learning model based on integrated residue-level and structure-level features to predict phase-separating proteins.	2024	China	[24]
IUPred2A	http://iupred2a.elte.hu	A web to identify IDRs.	2018	Hungary	[25]
FuzDrop	https://fuzdrop.bio.unipd.it	A method predicting the probability of proteins to undergo LLPS.	2020	Italy	[26]
D^2P^2	http://d2p2.pro	A database of disordered protein predictions.	2012	UK	[27]
PSPHunter	http://psphunter.stemcellding.org	A machine learning algorithm to predict phase-separating proteins and their corresponding driving residues.	2024	China	[28]
LLPhyScore	https://github.com/julie-forman-kay-lab/LLPhyScore	A bioinformatic tool for the prediction of protein phase separation.	2022	Canada	[29]

palmitate salts leads to insulin resistance [33]. Hence, LLPS plays a key role in insulin signal transduction and is intricately involved in cellular metabolic processes. Moreover, the cGAS-STING signaling pathway participates in the monitoring of exogenous DNA and activating innate immune responses. DNA can induce LLPS of cGAS, promoting the production of cyclic GMP-AMP and activation of innate immune signaling [34]. Interferon-induced protein 16 (IFI16) is an important sensor that initiates innate immune signaling. A combination of multiple phosphorylation sites in an IDR activates the LLPS of IFI16, and phosphorylation of IDR provides a switch between active and inactive IFI16 [35].

4 | LLSP IN TUMORS

The onset and progression of cancer constitute a multifaceted process involving multiple genes, steps, and stages. Despite considerable advances in identifying cancer-associated mutations and pathways, the precise pathological mechanisms underlying tumors remain elusive [36]. Phase separation offers a new dimension to explore in cancer therapy that is unlike classical genetic models and may reveal novel pathways for the transformation of cancer. Phase separation plays a critical role in tumor signal transduction, DNA damage repair, epigenetic changes, metabolic reprogramming, autophagy, and blood vessel formation [37].

Although phase separation is common in both tumor cells and normal cells, it maintains cellular homeostasis in normal cells but becomes a pivotal step in the carcinogenesis of cancer cells, as exemplified by fusion proteins, such as EWS-FLI1 and FUS-CHOP in sarcomas [38]. Furthermore, wild-type p53 undergoes phase separation more frequently than mutant p53, exerting its normal anticancer function [39]. Peptide segments targeting the IDR of p53 can regulate its phase separation, enhancing the formation of p53 droplets [40].

4.1 | Genomic stability

The primary function of the cell is to maintain genomic stability. Genomic instability is a prevalent characteristic of cancer, primarily involving DNA damage, DNA replication stress, and chromosome segregation defects [41, 42]. Tumor cells exhibiting genomic instability demonstrate genetic heterogeneity, conferring potent survival properties such as evasion and resistance to death [43]. Epigenetic dysregulation may lead to the development of cancer [44]. In mammalian cells, mixed lineage leukemia (MLL) 1–4 enzymes, members of COMPASS-like complexes, serve as the dominant methylases of histone H3 lysine 4, with a particular focus on MLL1 activity [45]. Borealin, a subunit of the chromosome passenger complex (CPC), was initially identified as a nonhistone substrate of MLL1. Borealin K143 within IDR can undergo methylation, subsequently facilitating LLPS of CPC, which is essential for its inner-centromere localization and function [46]. The LLPS of CPC on centrioles leads to destabilization, resulting in premature sister chromatid separation during the metaphase of mitosis in cells expressing Borealin K143R mutants. When MLL1 was knocked down in different hepatocellular carcinoma (HCC) cell lines, abnormal mitosis, and increased aneuploidy were exclusively observed in HCC cells with high CPC expression, thus affecting the proliferation and tumorigenicity of cancer cells [47].

Genomic stability relies on the critical function of the DNA damage response. It is now recognized that DNA repair processes occur within specialized condensates, the formation of which depends on poly(ADP-ribosyl)ation of proteins and DNA [48]. Poly(ADP-ribose) undergoes phase separation within the cell nucleus, triggering the transient and reversible assembly of numerous IDRs at DNA break sites as the earliest cellular response to DNA breakage [49]. Oshidarit et al. reported that the DNA repair protein Rad52 within liquid droplets interacts with DNA damage-inducible intranuclear microtubule filaments, enhancing the aggregation of DNA damage sites, and leading to the maintenance of genome stability [50]. The MRE11/RAD50/NBS1 (MRN) complex serves as a DNA double-strand break (DSB) sensor and then activates the DNA damage response [51]. MRN complex interacting protein condensates aggregate the MRN complex into liquid-like droplets within the nucleus. Once DSB occurs, the MRN complex interacting protein droplets translocate to the sites of damaged DNA, facilitating the interaction of the damaged DNA with the MRN complex and expediting ataxia-telangiectasia mutated activation and DSB end resection [52].

The p53-binding protein 1 (53BP1) is a multifunctional protein primarily recognized as a crucial mediator within the nonhomologous end joining (NHEJ) DNA repair pathway [53]. The 53BP1 phase separation integrates DNA damage foci and protects break sites with the activation of effector proteins, such as stabilizing p53 [54, 55]. Ubiquitin E3 ligase RNF168 is crucial for orchestrating the assembly of various DNA repair proteins at damaged sites [56]. Sentrin/SUMO-specific protease 1 can be recruited to the damage site to destroy LLPS of RNF168 by removing SUMO modification where the key repair protein 53BP1 is encapsulated, abrogating restriction of RNF168 and 53BP1. Depletion of sentrin/SUMO-specific protease 1 reverses the resistance of

colorectal cancer (CRC) cells to chemotherapy [57]. In the absence of DNA damage, 53BP1 localizes to heterochromatin regions, where it interacts with heterochromatin protein to maintain transcriptional silencing through LLPS, thus preserving genomic stability [58].

4.2 | Metabolic reprogramming

Metabolic reprogramming, a hallmark of cancer, involves altered metabolic pathways such as amino acid, nucleotide, and carbohydrate metabolism, which exhibit flexible and context-specific properties that support cancer progression [59, 60]. A stress granule (SG) is a membraneless condensate formed by eukaryotic cells under external pressure, playing a crucial role in mRNA metabolism [61]. In conditions of glutamine deficiency, elevated levels of long noncoding RNA GIRGL interact with CAPRIN1 and glutaminase-1 mRNA, leading to the LLPS of CAPRIN1 and the formation of SGs. This interaction inhibits glutaminase-1 translation, thereby extending tumor survival time [62]. Circular RNA VAMP3 can induce LLPS of CAPRIN1, resulting in SGs formation, which impedes c-Myc translation and consequently hampers tumor proliferation and metastasis [63]. Furthermore, in obesity-associated pancreatic ductal adenocarcinoma, serine/arginine protein kinase 2 mediates SG formation through the IGF1/PI3K/mTOR/S6K1 signaling pathway. Inhibition of S6K1 selectively weakens the formation of SGs and suppresses the development of obesity-associated pancreatic ductal adenocarcinoma [64].

The emerging understanding of cancer glucose metabolism suggests that tumor cells may uptake glucose, storing it as glycogen for energy instead of metabolizing it immediately through anaerobic glycolysis. Importantly, the accumulated glycogen can undergo LLPS, contributing to inactivation of the Hippo signaling pathway and activation of the downstream proto-oncogene YAP, ultimately driving tumor initiation [65]. Purinosomes are metabolic compartments comprising six enzymes associated with the de novo purine synthesis pathway, which contribute to purine synthesis by interacting with other enzymes [66]. Phosphoribosylaminoimidazole carboxylase and phosphoribosylaminoimidazolesuccinocarboxamide synthase, the key enzymes in purine synthesis, undergo K6-linked polyubiquitination, mediated by the E3 ligase Cul5/ASB11. This process promotes the LLPS of purine bodies, subsequently enhancing purine synthesis and fostering tumor development [67]. A recent study has reported that ALKBH3 expression is intricately linked to lactic acid and histone lactylation, which facilitates the activation of oncogenes. Mechanically, histone lactylation upregulates ALKBH3 expression while concomitantly reducing the formation of tumor-suppressive promyelocytic leukemia protein (PML) condensates. Therefore, lactose-driven ALKBH3 is indispensable for the formation of PML nuclear condensates, and this finding provides a novel insight into the interplay between metabolic reprogramming and phase separation [68].

4.3 | Ferroptosis

Ferroptosis is a form of programmed cell death characterized by iron-dependent lipid peroxidation that effectively inhibits tumor development [69]. Ferroptosis suppressor protein 1 (FSP1) is a glutathione-independent inhibitor of ferroptosis, which can inhibit peroxidation and prevent ferroptosis by converting panquinone on cell membranes into its reduced panthenol [70]. Recently, a class of 3-phenylquinazolinone compounds, represented by icFSP1, was successfully screened as inhibitors of FSP1 through a small molecule library. icFSP1 induces ferroptosis in tumor cells by dislocating FSP1 from the cell membrane and forming condensates with droplet-like properties, suggesting a rationale for targeting the interaction between ferroptosis and LLPS. In sorafenib-resistant HCC, long noncoding RNA URB1-antisense RNA 1 induces phase separation of ferritin and inhibits ferritin autophagy, which leads to a marked reduction in cellular free iron content and disruption of sorafenib-induced ferroptosis [71]. Additionally, lncFASA directly binds to PRDX1, promoting the formation of droplets within the cytoplasm that impair the catalytic activity of PRDX1, disrupting ROS homeostasis by interruption of the SLC7A11-GPX4 signaling pathway and then inducing ferroptosis [72].

In conclusion, compartmental lncRNA in the regulation of intracellular homeostasis exhibits dynamic plasticity and functional diversity. Targeting phase separation to inhibit ferroptosis provides a theoretical basis for early intervention in malignant tumors.

4.4 | Metastasis

Tumor metastasis is a multifaceted and multistep biological process involving tumor cells and the tumor microenvironment (TME). Tumor budding exhibits stem-like characteristics and a partial epithelial-mesenchymal transition phenotype during the dynamic progression of head and neck squamous cell carcinoma, facilitated by the LLPS of FOSL1 protein, which activates the super-enhancer and regulates transcription of key genes [73]. DDX21, a representative RNA-binding protein, activates

epithelial-mesenchymal transition-associated signaling pathways by driving phase separation to enhance the metastatic ability of CRC [74].

Liu et al. have reported that nuclear circASH2 mediates LLPS of YBX1 and regulates the mRNA/pre-mRNA splicing process of a crucial cytoskeletal stabilizing protein. This ultimately alters the cytoskeletal structure of tumor cells, suppressing invasion and metastasis of HCC [75]. The extracellular matrix can influence cell behavior by changing the biochemical composition or mechanical properties. The communication between the extracellular matrix and the cell is mainly achieved through focal adhesions, a dynamic protein assembly responsible for the perception and transduction of mechanical signals [76]. Liang et al. found that autophagy-induced serine/threonine kinases ULK disrupts paxillin phase separation and impedes focal adhesion assembly to inhibit tumor cell adhesion and migration, implying a novel mechanism of tumor metastasis mediated by the interplay of phase separation with mechanotransduction [77]. Moreover, the lysosomal stress response initiates LLPS of QSTM1/p62 and neighbor of the BRCA1 gene 1 (NBR1) in response to tyrosine kinase inhibitors. In p62/NBR1 droplets, inhibition of apoptosis protein 1 accelerates the degradation of Ras-related C3 botulinum toxin substrate 1, thereby restricting the motility of cancer cells. While knockdown of p62 and NBR1 completely abrogates the antimetastatic effect of tyrosine kinase inhibitors in tumor cells [78]. HOXB8 and FOSL1 are core transcription factors of regulatory circuitry in highly metastatic osteosarcoma. Disruption of HOXB8 and FOSL1 LLPS decreases chromatin accessibility at super-enhancer loci and inhibits RNA polymerase II release in the promoters of oncogenes, reducing the growth and metastasis of osteosarcoma [79].

4.5 | Immunotherapy

Inhibition of programmed cell death protein-1 (PD-1)/programmed cell death ligand 1 (PD-L1) to hinder T cell exhaustion and enhance immune surveillance has been recognized as a promising strategy in antitumor immune therapy for solid tumors [80]. IFNγ, primarily released by T cells within TME, can up-regulate PD-L1 expression in tumor cells and modulate complex immune responses [81]. IFNγ-induced condensates of KAT8-IRF1 facilitate PD-L1 expression in tumor cells by enhancing condensates and promoting tumor activities. Mechanistically, KAT8 acetylated IRF1 at the K78 site enhances the DNA binding activity of IRF1 and facilitates the recruitment of IRF1 to the PD-L1 promoter, thereby activating PD-L1 mRNA transcription [82]. In PD-1-resistant lung adenocarcinoma, activation of IFN-γ triggers the nuclear separation and aggregation of YAP. Subsequently, YAP recruits TAZ, TEAD4, histone acetyltransferase EP300, and the transcriptional intermediary complex mediator, forming a central hub of transcriptional activity that activates downstream immunosuppressive genes. Inhibiting the phase separation of YAP slows tumor growth, promotes the immune response, and restores the sensitivity of tumor cells to PD-1 antibodies [83].

Cationic polymers (CPs) exhibit a robust ability to induce RNA phase separation. In a mouse breast cancer model, treatment with CPs enhances overall immune response, resulting in increased levels of CD4+ and CD8+ T cells in the TME. This effect is mediated by the phase separation of RNA, which encapsulates TGF-β mRNA, markedly inhibiting its translation and expression. Consequently, this process diminishes the immunosuppressive function of the TME and augments the efficacy of tumor immunotherapy. Furthermore, the combined use of PD-1 inhibitors with CPs markedly enhances tumor-killing effects, surpassing those observed with either PD-1 inhibitors or CPs alone [84].

4.6 | Targeted therapy

In recent years, targeted kinase therapy has rapidly developed and yielded considerable clinical benefits. However, IDRs such as MYC lack conventional “protein pockets”, rendering them devoid of drug targets [85]. Recent studies have reported that lncRNA MTAR1 facilitates the recruitment of insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs) into condensates mediated by poly(A) binding protein cytoplasmic 1 (PABP1), promoting the binding of IGF2BPs with PABP1. This process promotes the interaction between IGF2BPs and PABP1, enhancing the stability and translation of MYC mRNA [86]. Additionally, the upregulation of lncRNA MNX1-AS1 expression in non-small cell lung cancer correlates with MYC-mediated transcriptional activation. LncRNA MNX1-AS1 induces the phase separation of IGF2BP1, enhancing its interactions among IGF2BP1, MYC mRNA, and transcription factor E2F1 mRNA. Consequently, the positive feedback loop involving c-Myc/MNX1-AS1/IGF2BP1 continuously promotes the proliferation of non-small cell lung cancer cells [87]. Targeting the lncRNA/MYC signaling pathway may constitute a novel antitumor therapeutic strategy. The PML nuclear body is a liquid-protein condensate formed through LLPS via intricate mechanisms that play an essential role in both its formation and functional activities [88]. In leukemia patients, mutations at key sites on the

PML disrupt the normal LLPS process, ultimately resulting in resistance to arsenic-targeted therapy, thus offering a new theoretical basis for overcoming leukemia resistance [89].

4.7 | Chemotherapy

The intracellular distribution of drugs markedly impacts their activity [90]. However, traditional pharmacological approaches typically do not assess the intracellular distribution of drugs [37]. Mediator complex subunit 1 (MED1) can interact with most nuclear receptors and transcription factors to regulate cell proliferation, differentiation, and metabolism [91, 92]. Researchers have discovered that cisplatin can selectively accumulate in MED1 condensates owing to its physicochemical properties, irrespective of the drug's target. Cisplatin exhibits a high level of enrichment within MED1 condensates, leading to platinization of the DNA residing within these condensates. Conversely, the disruption of the MED1 structure results in a dramatic reduction of DNA platinization [93]. Consequently, chemotherapeutic agents can accumulate within particular condensates in tumor cells, potentially exerting a marked influence on their efficacy and concentration, thereby contributing to the future development of treatments.

4.8 | Endocrine therapy

LLPS of androgen receptors (ARs) occurs in transcription induced by dihydrotestosterone. Enzalutamide inhibits phase separation in wild-type AR but paradoxically promotes phase separation in androgen-deprived conditions, enhancing transcriptional activity and reinforcing the signaling pathway in resistant mutant AR. The small molecule compound ET516 can directly bind to the N-terminal domain of the AR, selectively disrupting AR condensates and suppressing the growth of prostate cancer cells with AR-resistant mutants [94]. Another study found that OCT-4 underwent phase separation to induce resistance to cabazitaxel in prostate cancer cells lacking AR expression [95]. Estrogen receptors selectively accumulate within MED1 transcription condensates, and tamoxifen can enter those condensates to facilitate estrogen receptor extrusion. However, high expression of MED1 expands the volume of transcription condensates, diluting the concentration of tamoxifen, and leading to drug resistance [93]. Therefore, selective delivery of drugs into biomolecular condensates may enhance target efficacy. However, high expression of the condensates owing to adverse conditions will dilute internal drugs and weaken their efficacy.

4.9 | Radiotherapy

Radiotherapy is widely used in treating solid tumors [96]. Radiation kills cancer cells by generating large amounts of cytotoxic DSBs [97]. However, radiation-resistant cancer cells primarily repair DSBs through NHEJ and homologous recombination pathways, accelerating the repair of DNA damage [98, 99]. In radiation-resistant cancer cells, LLPS of NONO facilitate the recruitment of nuclear EGFR and DNA-PK, increasing damage-induced DNA-PK phosphorylation at T2609 DNA levels and promoting NHEJ-mediated DNA repair. While inhibition of NONO droplets hinders NHEJ approach-based DNA repair and sensitizes tumor cells to radiation [100]. Additionally, NOP53, a nucleolar protein with crucial functions in DNA damage repair, effectively inhibits irradiation-induced p53 activation, thereby promoting radio-resistance of tumor cells through LLPS (Figure 1) [101].

5 | FACTORS AFFECTING LLPS

LLPS is influenced by ATP levels, salt concentration, and pH within the microenvironment. ATP is indispensable for the occurrence of phase separation by providing the energy required for various proteins and enzymes undergoing phase separation [102]. The interplay between the low complexity core regions (LC-core) of RNA-binding protein FUS is necessary for the formation of droplets. It has been found that the adenine groups of ATP frequently contact FUS-LC-cores, and the phosphate groups of ATP are exposed to the external solvent, facilitating the hydration and solvation of FUS [103]. More importantly, the stability of the FUS particles depends on the concentration of ATP in the cell [104].

LLPS of RNA-binding motif protein 15 promotes tumor cell proliferation in a manner dependent on salt concentration, whereas the phase separation of RNA-binding motif protein 15 is inhibited at higher salt concentrations [105]. Under pressure, changes in pH facilitate the transition of condensates into a gel state, which can be reversed by increasing pH to restore the translational function of the condensates [106]. Additionally, research has quantified variations in the protein concentration of the FUS-LC-core. Minor changes were observed in protein concentration with variations in pH and salt concentration, indicating that under conditions unfavorable for droplet formation, the protein concentration within the droplets is lowered [107]. However, the ability to achieve precise control of phase separation through the microenvironment requires further exploration.

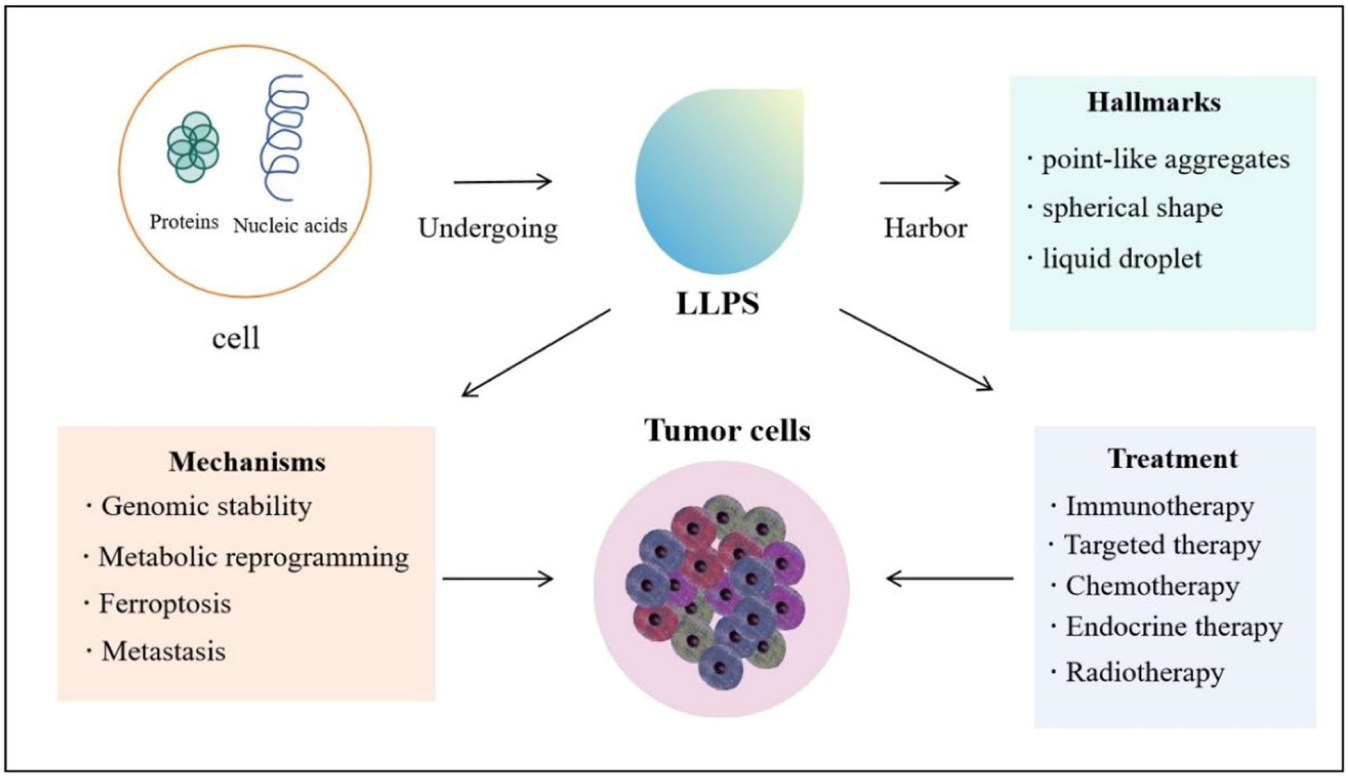

FIGURE 1 Mechanisms and clinical implications of liquid–liquid phase separation (LLPS) in cancer.

6 | PERSPECTIVES AND CHALLENGES

Novel approaches to cancer treatment based on LLPS have focused on targeting the aberrant dynamics of phase separation identified within cancer cells, such as the formation, modulation, or function of condensates implicated in cancer pathogenesis. Several specific aspects of LLPS that could be targeted for cancer therapy include:

(1) Intervention in the formation of oncogenic condensates;
(2) Disruption of upstream regulation and downstream modulation of condensates;
(3) Alteration of the properties and microenvironment of condensates;
(4) Activation of tumor-suppressor condensates.

Targeting LLPS directly addresses dysregulated molecular organization. Owing to its focus on the specific biochemical characteristics of cancer cells, targeting LLPS may exhibit higher specificity and precision, reducing adverse effects during treatment. Targeting LLPS is a relatively novel strategy that may have the potential to reduce treatment resistance. Moreover, LLPS is a complex process involving multiple protein interactions and assemblies. Targeting LLPS could simultaneously interfere with multiple key molecular targets, comprehensively blocking cancer cell survival pathways.

Although research on LLPS in cancer is expanding, cancer treatment based on phase separation is just the tip of the iceberg and is fraught with numerous challenges. For example, how can specific small molecules be designed to precisely regulate phase separation, and how can phase separation in the microenvironment be controlled? Current techniques for exploring LLPS remain limited, warranting the development of more sophisticated tools for observing and analyzing dynamic LLPS. The identification and confirmation of molecular targets remain poorly understood. More research is needed to reveal which protein interactions play a crucial role in tumor growth and metastasis. Further research is also necessary to uncover the function of condensates that participate in tumor development. Whereas most phase separation studies have been conducted in vitro, more in vivo studies should be performed in the future, given the complexity of the interactions. Translating LLPS therapy from the bench to clinical application faces challenges. Clinical trials and validation are needed to determine the safety and efficacy of treatment protocols. Recent research has found that phase separation is also relevant to bacteria in the mammalian gut, suggesting that

manipulating the gut microbiota through phase separation modulation could offer promising targets for cancer treatment [108].

7 | CONCLUSIONS

LLPS in cancer represents a burgeoning area of research and elucidation of the pathogenesis of cancer, and continuous exploration of LLPS is required. The regulation of cancer-related LLPS should be a key focus for future research. The exploration of LLPS could provide novel prospects for antitumor therapy, but the realization of this goal requires ongoing innovation.

AUTHOR CONTRIBUTIONS

Xuesong Li: Writing—original draft (lead); writing—review and editing (lead). **Zhuo Yu**: Conceptualization (lead); funding acquisition (lead); supervision (lead).

ACKNOWLEDGMENTS

None.

CONFLICTS OF INTEREST STATEMENT

Professor Zhuo Yu is a member of the Cancer Innovation Editorial Board. To minimize bias, he was excluded from all editorial decision-making related to the acceptance of this article for publication. The remaining authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

No new data are generated, or the article describes entirely theoretical research.

ETHICS STATEMENT

Not applicable.

INFORMED CONSENT

Not applicable.

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How to cite this article: Li X, Yu Z. Role of liquid–liquid phase separation in cancer: Mechanisms and therapeutic implications. Cancer Innov. 2024;3:e144. https://doi.org/10.1002/cai2.144