Review

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Unravelling the Interplay between Biomolecular Condensates and RNA in Cancer and Diseases

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Biomolecular condensates (BCs), including membraneless organelles, are basically Ribonucleic acid (RNA)-protein complexes that are emerging as a potential candidate because of its paramount significance in revealing fundamentals of cell biology and pathology including cancer. Aiming to understand its importance, scientists are dealing with it in an interdisciplinary manner—from polymer chemistry to genetics. Over time the mechanism of liquid-liquid phase separation (LLPS) and the model of 'Stickers and Spacers' have widened our understanding and broadened our scope of approaches to get insights into these molecular entities. RNA molecules and its specific partner proteins, certain ribonucleoproteins (RNPs), play pivotal roles in different trajectories of BCs, i.e., in localization, compartmentalization, epigenetic regulation, and dynamics. However, several aspects need to be made clearer to tackle human diseases including cancer. In addition to the RNAs, in this review specific aspects of the BCs are presented in the context of neurodegenerative diseases, viral infections and cancer, and their link with stress granules (SG), P bodies, paraspeckles, and host encoded and viral encoded non-coding RNAs, discussing cancer associated proteins, tumour suppressors and repressors, dysregulation of cancer signals, and role of super enhancers. A special focus is reserved to oncolytic viruses and epidemic viruses, through the intervention on stress granule protein partners, virus-formed intracellular condensates, and viral RNAs. The review covers not only the basics of BCs but also encompasses the emerging context of condensate-targeted drug discovery and cancer therapeutics by means of Proteolysis-targeting chimera (PROTAC), Ribonuclease-targeting chimera (RIBOTAC), small molecule inhibitors of protein-RNA interactions, Antisense oligonucleotides (ASO) and compounds targeting non-coding RNA (ncRNA) triple helix, determining RNA degradation.

Keywords: BCs; LLPS; RNA-protein interactions; nanoRNAs; nanodelivery; therapeutics

Introduction

Biomolecular condensates (BCs), crucial for intricate cellular biochemical reactions, are membraneless organelles, some of them are nuclear, as the nucleolus, speckles, paraspeckles, DNA-damage foci, and others are cytoplasmic, i.e., ribonucleoprotein (RNP) granules, stress granules (SG), P bodies, signalosomes, and the pyrenoid: in the most simple case, a single protein interacts with multiple copies of itself driving the homotypic phase separation, while the interaction with several other biomolecules leads to heterotypic phase separation, as in Ribonucleic acid (RNA)-protein complexes [1]. Liquid-liquid phase separation (LLPS) is named the physical process for both the types of BCs. Biomolecular condensates have been classified in BCs formed by proteins aggregating due to their intrinsically disordered regions (IDRs), or a prion-like structure, and those of heterotypic phase separation, containing RNAs (https://rps.renlab.org/#/Home) [2] which are the main focus of the review.

Existing throughout the cell, BCs lack a lipid bilayer, and form via liquid-liquid phase separation (LLPS) also known as liquid-gel phase separation changes, influenced by environmental factors [3]. Certain amino acids contribute to BC formation, revealing a "molecular grammar" [4]. Dysregulation of LLPS is closely associated with a variety of diseases: amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), cancer, and viral diseases.

RNA's involvement in BCs, influenced by its diverse features, actively controls condensate phase boundaries, compositions, and material properties [5]. RNA structure plays a pivotal role in determining dense phase identity within condensates [4]. Several parameters concern both RNAs and proteins, with an interplay in regulating condensate properties [6]. Noteworthy, RNAs and poly-ADP-ribose (PAR), with a DNA-like structure, regulate Fused in Sarcoma (FUS) self-assembly: their involvement in condensate formation near enhancers or promoters suggest regulatory roles of RNAs [7].

Condensates are regulated by physical and chemical properties: concentration threshold, type of chemical modification, structure of non-coding RNAs (ncRNAs), and partitioning between liquid and gel phase, events that may be

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deregulated and lead to dysregulation in cancer cells [8]. Epigenetic mechanisms, including histone modifications and altered ncRNA expression, are crucial in pathological states including cancer-related deregulated gene expression [9]. RNA molecules play regulatory roles in various condensates, influencing their behaviour [10]. Stress granules (SGs) are located in the cytoplasm, contain structural proteins, messenger RNAs (mRNAs) and ncRNAs, and assemble rapidly in cells in response to a variety of stresses, i.e., oxidative stress, heat stress, and viral infection. Viruses that replicate in the cytoplasm, with recruitment of RNA polymerase into SGs, exploit SGs for transcription of their viral RNAs to produce viral proteins at the expenses of the cell, the most relevant for the potential of application of inhibitors are macrodomain ADP-ribose hydrolases [11] able to counteract poly-ADP-ribosylation signalling required for cell-directed SG formation [12].

Biomolecular Condensate (BC) Intracellular Functions

Stress Granules

SG essential components are Ras-GTPase-activating protein (GAP)-Src homology 3 (SH3) domain-binding protein 1 and 2, GTPase-activating protein (SH3 domain)-binding proteins (G3BP)1 and G3BP2, playing a pivotal role in cell behaviour and in cancer [13]. RNA-dependent ATPase of the Asp-Glu-Ala-Asp (DEAD)-box family 3 (DDX3) is an RNA binding protein and RNA helicase, that together with DDX6 localizes to stress granules with the mRNA export factor Gle1A, a DDX regulator, and the RNA binding protein Caprin1. DDX3 is required for SG assembly. SGs are formed by a nucleation process involving the RNA decay factor G3BP1, which prevents the localisation of ribosome and initiation factors in silenced SG foci, DDX3, (DEAD)-box RNA helicase 6 (DDX6) (Rck/p54), the translational suppressor T-cell intracellular antigen 1 (TIA1), TIA1-related protein (TIAR), Caprin1, Ubiquitin specific peptidase 10 (USP10), poly-A binding protein (PAB or PABP) and U6 snRNA-associated Sm-like protein (Lsm1) belonging to the Lsm family of RNA-binding proteins.

During arsenite administration, oxidative stress, or eukaryotic initiation factor 2 (eIF2) phosphorylation, cells undergo a translation arrest, and the RNAs are stalled in the ternary complex that include eIF2/GTP/Met-tRNA. SGs have a role in spatial and temporal inhibition of mRNAs, until recovery from the stress through the processing of the mRNAs, or non- recovery by RNA degradation. SGs contain enzymes of the RNA silencing pathway, including Argonaute 2, trans-acting factors, Hsp90 complexes and RNA binding proteins, acting in the small RNA-mediated repression of RNA targets. Various viruses use the binding to protein scaffolds to avoid SG formation, or assemble their RNA into SGs exploiting the transcriptional machinery for their own replication [14,15].

Stress granules (SGs) become important in cancer development as well as in neurodegenerative diseases. Alterations in biomolecular condensates are pervasive in cancer, driven by diverse stresses within the tumour microenvironment. Oncogenes and tumour suppressors involve dysregulated condensate compartments in malignant cells [16]. Cancer development involves phase separation of cancer-associated proteins, influencing various cellular processes. The emerging concept of condensates and aggregation mediated by gene fusion and mutation genes deepens our understanding of intricate connections in cancer biology [17]. Various scientists emphasised the challenge of targeting biomolecular condensates in cancer [18]. Recent insights reveal connections between intracellular phase separation, disease, and drug actions [19].

RNA's role in condensates holds therapeutic potential, suggesting creative applications like active ribozymes [20]. Systematically, mutations have been studied to analyse the functional role in altering phase separation, particularly in cancer [21]. Future genetic systems and personalized medicine may leverage synthetic peptides and study mutant p53 (anti-oncogene and guardian of genome) dynamics for drug discovery targeting abnormal phase transition and aggregation [22]. Condensates are targeted in cancer therapy to address condensatopathies, impair aberrant formation, prevent target function, and deliver drugs directly [23,24]. In this review, the RNA's significance and associated epigenetic regulation of biomolecular condensate formation will be discussed in cancer and in human pathologies. Besides, scientists have incorporated certain relevant mechanisms of condensate assembly, and target candidates of BCs including some plausible experimental avenues to tackle down the drug discovery approaches.

Stress Granule Components Hijacked by Viruses

In the case of RNA viruses, for instance in Hepatitis C virus (HCV) infected cells, SG and P-body components are re-localized to the periphery of lipid droplets, and cells oscillate between SG assembly and disassembly upon treatment with interferon I, depending on the inhibition of protein kinase RNA dependent (PKR). West Nile Virus (WNV) inhibits SG formation by scavenging Reactive Oxygen Species (ROS), and re-localizes the SG scaffolding proteins into perinuclear foci, exploiting cell translation machinery for WNV replication. Human immunodeficiency virus (HIV)-1 inhibits SG assembly by Gag protein, through its interaction with host eukaryotic initiation factor 2 (eIF2) and G3BP1. Influenza A virus (IAV) proteins inhibit SG formation: IAV polymerase complexes in the nucleus generate mRNAs with a 5′ cap and polyA-tail, transferred into the cytoplasm for translation. Non-structural protein 1 (NS1) inactivates PKR, and prevents eIF2 phosphorylation; nucleoprotein (NP) inhibits SG formation through mechanisms independent from elongation initiation factor eIF; the host shut-off protein polymerase-acidic protein X (PA-X)

is required to inhibit SG formation. In picornaviride group, enterovirus proteinases are responsible for SG disassembly; in kobuviruses, small leader peptides are involved in SG inhibition. Human T cell leukemia virus 1 (HTLV-1), causing adult T-cell leukaemia (ATL), maintains ATL transformation through the function of the antisense protein and its RNA, the HTLV-1 basic leucine zipper factor (HBZ). Many virus families produce a large set of microRNAs, in addition to host microRNA (miRNA)-mimics. The family of human herpes virus includes the pathogenic species herpes simplex virus (HSV) and human cytomegalovirus (HCMV), as also the tumor-forming Epstein-Barr virus (EBV), Kaposi Sarcoma human virus (KSHV), and for murine tumours the herpesvirus 68 (MHV68). Viral ncRNAs come in all shapes and forms, associating with cellular proteins required for their functions or antagonistic to their infectivity. Viral ncRNAs support viral control of cell physiology and processes. Viruses utilise ncRNAs to regulate viral replication, viral latency or reactivation, and in host immune evasion [25]. Viruses produce ncRNAs like Adenovirus VA_I and VA_{II} RNAs, for host immune-response inhibition, PKR inhibition, and nuclear transport inhibition. Epstein-Barr virus produces EBV-encoded RNA (*EBER*) 1 and 2, consisting of 167 and 172 nucleotides in length, transcribed by RNA pol III, exerting its oncolytic effect. Mice expressing *EBER1* in lymphoid cells develop lymphoid hyperplasia that progresses into B cell tumours [26]. In Herpesviruses, *EBER* RNA inhibits the protein kinase RNA dependent (PKR) in infected cells.

Physiology and Pathology of BCs

Overview on Biomolecular Condensates (BCs)

Researchers try to understand the organization of tightly packed biomolecular structures in the cell, showing a tight control of such complex biochemical reactions [17–19]. Among these complexes, membraneless organelles (MLO) show their importance in regulating the reaction cascades in living entities. These MLO are formed by RNA-protein complexes, often referred to as biomolecular condensates (BCs) [1]. Sometimes these may also be constituted of DNA and other types of biomolecules [18]. These unique structures are scattered both within and beyond the nucleus (Fig. 1). For example, nucleoli, Cajal bodies, and promyelocytic leukaemia protein (PML) are found in the nucleus, whereas SG and germ granules are seen in the cytoplasm [2]. BCs lack a lipid bilayer and show non-stoichiometric ratios among their components [20].

Mechanism of Condensate Formation

Biomolecular condensate formation is crucial for understanding deadly diseases and cell biology basics. Recent focus areas include their characteristics, formation principles, and genetic control. Various established viewpoints elucidate BC assembly and interactions, often through polymer and physical chemistry lenses. Condensates are formed through LLPS, associating in micron-scale droplets with various molecules impacting their assembly, composition, and cellular functions. The intracellular environment can also influence protein structure and phase transitions, elucidating biochemical principles [20]. The process of phase separation is dynamic in nature and often shows reversibility [21–24,27]. For better understanding of the chemical mechanisms of BCs assembly, scientists also investigated the contribution of RNA or protein molecules in purified form *in vitro*. For example, in the context of protein domains, determination of the amino acid specific contribution to the construction of BCs has been performed, taking into account the amino acid tendency to be modified by post-translational modifications (PTM) [28]. In LLPS driving proteins, multiple non-covalent interactions between basic, acidic, and aromatic residues contribute to condensate formation and behaviour. The interaction of various amino acids influencing the formation and behaviour of basic protein condensates, often referred to as the “molecular grammar”, has been predominantly elucidated through findings on the protein Fused in Sarcoma (FUS), a member of Fused in sarcoma-Ewing sarcoma RNA binding protein-TATA box protein associated factor 15 (FET) family, which is often fused with Ewing Sarcoma/ TATA box protein associated factor 15 (TAF15)-TATA-binding protein-associated factor 15 (EWS). FUS, similarly to TAR DNA binding protein 43 (TDP-43) shows intrinsically-disordered regions and prion-like domains (PrLD) that determine protein aggregation in neurodegenerative diseases [28,29].

At low micromolar concentrations, FUS orchestrates multimolecular condensates driven by transient and multivalent interactions along with its arginine and tyrosine residues. In consequence, the amino acid sequences and compositions of FET family proteins provide valuable insights into the orchestration of condensate behaviour [3]. A model named “stickers and spacers” has widened the understanding of BCs by enhancing the prediction of the sequence by interpreting experiments incorporating model proteins and nucleic acids [30]. In Fused in Sarcoma (FUS) protein, spacer regions are made of glycine, serine, and glutamine, and occur between blocks of stickers with strongly interacting domains. In spacer regions, a higher content of glycine residues confers a more dynamic and liquid-like condensate, whereas a higher glutamine content confers a more solid-like condensate [28]. As per the model, polymers contain domains both with strong interaction ability and weak interaction ability. The potency of interactions between stickers, their frequency, and the positioning of stickers and spacers has been demonstrated in studies involving FET-family and other proteins, taking the saturation concentration of a condensate into account. Beyond this critical concentration, a network of associative polymers undergoes a phase transition, giving rise to dense phase in the presence of a dilute phase [31].

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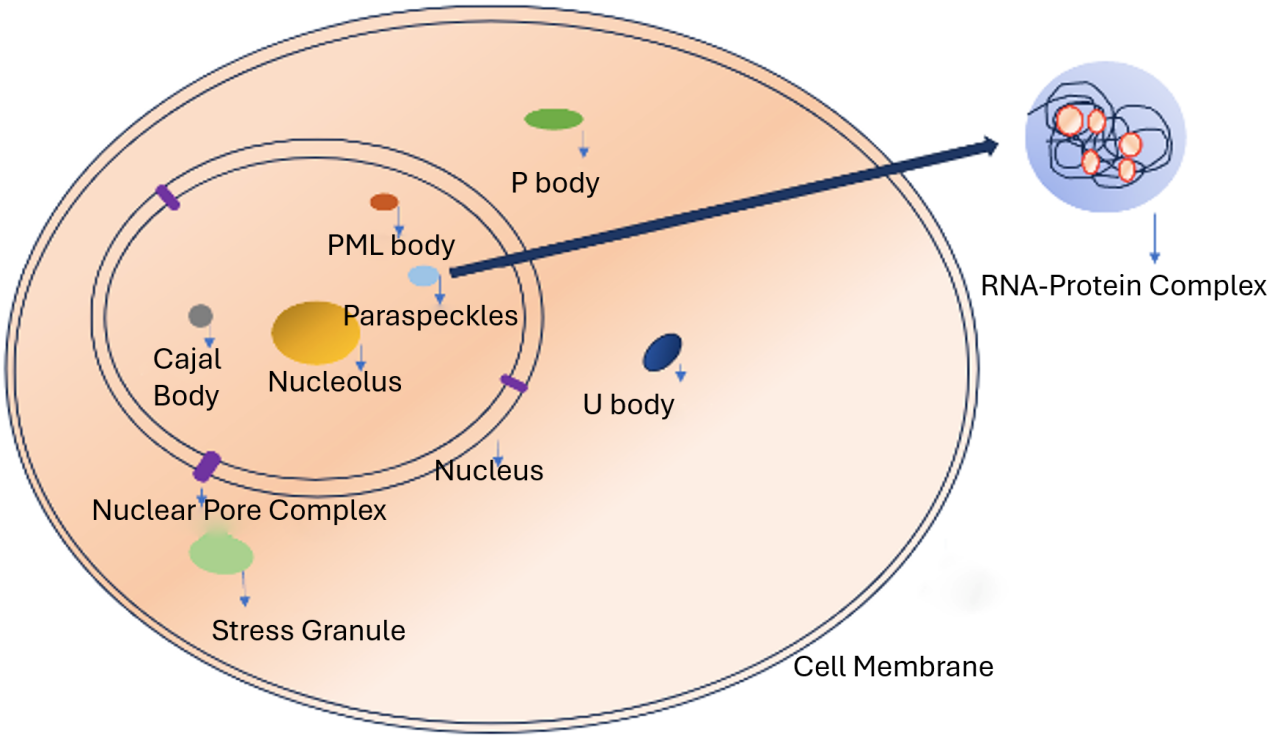

Fig. 1. Occurrence of different biomolecular condensates inside (promyelocytic leukaemia nuclear bodies, paraspeckles, Cajal body) and outside of the nucleus (stress granules, P bodies, U body). As an example, a single paraspeckle is marked separately to emphasize the components such as RNAs and proteins. The image was made using MS Office 365 (700 Bellevue Way NE, Microsoft Corporation, Bellevue, WA, USA) and converted to '.png' file format. RNA, Ribonucleic acid.

Dense phases are characterized by a great number of favourable sticker-sticker interactions [31], while dilute phases present fewer interactions. Recently, thermodynamic intervention, including the Flory-Huggins hypothesis, enabled to define behavioural aspects of BCs as the utilization of free energy of the polymers mixing in the solution, relevant dynamic interactions between molecules even with weak character, salt bridges, pi-cation and pi-pi bonds, and hydrophobic contacts, and the separation of biomolecules into condensates [28].

Localization

There are five ways of messenger RNA (mRNA) localization, (1) diffusion and entrapment locally, (2) stabilization and regulated degradation, (3) active transport by molecular motors, (4) endosomal vesicular trafficking, and (5) integration into BCs [17]. A ribonucleoprotein (RNP) particle floats through various ways throughout its lifetime, from transcription sites, for mRNA splicing in nuclear speckles and paraspeckles, through the nuclear pore, and in specific cells into cytoplasmic granules, including transport granules, P-bodies for degradation, and stress granules under stressed situations [32]. Collectively, these LLPS structures have been referred to as BCs [33–36]. Localized condensates consist of components that anchor the structure to a specific region within the cell. For instance, nuclear condensates can be observed with proteins binding specific DNA or RNA sequences, while in the cytoplasm, condensates can be found at the plasma membrane [36–38].

Compartmentalization

Compartmentalization is a crucial aspect in BCs, when locally high concentrations of biomolecules and their substrates occur, and other functionally irrelevant molecules are excluded [36]. Another instance is the transcription burst where a condensate located in the promoter region of a gene is capable of recruiting many RNA polymerase molecules, justifying its nonstoichiometric nature [21,37–39]. In brief time frames, condensates can swiftly form and dissolve, offering the cell a mechanism to generate temporary compartments. This, in turn, allows the release of biomolecules to other locations when they are no longer required at a particular site [3]. Furthermore, the compartmentalization within condensates participates in stabilizing protein concentrations in cells, acting as a buffer against the inherent stochasticity in gene expression [40,41].

RNA: The Key Member

RNAs are very chemically sensitive and diverse in terms of sequence, structure, and length. RNAs have been classified as protein-coding and non-coding RNAs (ncRNAs). Overall, RNAs include mRNA, rRNA, tRNA, mi-

croRNA (miRNA), Small Interfering RNA (siRNA), Trans-acting Small Interfering RNA (tasi-RNA), small nucleolar RNA (sno-RNA), circular-RNA, precursor messenger RNA (pre-mRNA), primary messenger RNA (pri-mRNA), and other types.

How RNA Sets the Bed for Condensation?

To determine the nature of these BCs, insights into the associated RNAs are essential, as both pre-and mRNAs combine with themselves as well as specific proteins while forming BCs [17,33]. RNA polymers, through their ability to act as scaffolds by forming weak multivalent interactions, are considered as building blocks for biomolecular condensates [37]. Within an *in vitro* complex environment, these molecules form base pairs with another RNA or with partner proteins leading to the formation of BCs, the existence of which, in turn, enhances to determine the factors of such an environment where it is born [24]. Described as tuneable viscoelastic fluids, these molecules exhibit a range of viscosity, varying from low to high, dependent on the composition, sequence, and structure of proteins and RNA, as well as environmental conditions [4,37,42].

Eukaryotic proteins display a wide size range, from micropeptides up to small proteins. In comparison, an mRNA is roughly nine times larger in mass than the protein it codes for, with the average mRNA length of 1–2 kilobases (kb) or higher. Introns in pre-mRNAs can be up to five times larger than the processed mRNA [43]. Furthermore, non-coding RNAs (ncRNAs) have an average size of around 1 kb [44], while small nuclear and nucleolar RNAs (snRNAs and snoRNAs) range from 60 to 300 bases [45] microRNAs (miRNAs) and Piwi-interacting RNAs (piRNAs) are 21–22 to 35 nucleotides (nt) in size [46]. RNAs are processed with a 5′ cap (i.e., mRNAs), while non-capped RNAs (nap-RNAs) function in regulation of gene expression [47]. For instance, a novel nap-RNA (DINAP RNA, with a composite box C/D-H/ACA) is involved with dyskerin pseudouridine synthase 1 (DKC1) in maintaining protein stability and promoting proliferation of hepatoblastoma cells.

Beyond the mere number of residues or bases, the physical dimensions of these molecules are contingent on their structure conformation, particularly evident in the case of stable, globular, and packed proteins [36].

The active involvement of RNA in the formation of membraneless bodies is now widely acknowledged, with the human genome extensively transcribed into processed, regulated non-protein-coding transcripts. The noncoding genome is transcribed into ncRNAs, whose number sums up to more than 80,000 transcripts. Notably, primate-specific ncRNAs exhibit low and cancer-restricted expression. This primate and cancer cell specificity are associated with the enrichment of transposable elements at ncRNA loci, containing cis-regulatory sequences acting as promoters and enhancers [37,38,48,49]. RNA endowed with its diverse features actively controls the phase boundary, composition, and physical properties of condensates. Re-entrant phase separation, characterized by the dynamic formation and dissolution of condensates, adds a layer of complexity to this regulation. The viscosity of these molecules, from eukaryotic proteins to various types of RNAs, varies based on their structural conformations and environmental conditions, contributing to the dynamic nature of condensates [4,5].

In the nucleus, these chromatin subdomains can undergo liquid-liquid phase separation, driven by the intrinsically disordered regions (IDRs) of histone tails, resulting in the formation of dense and dynamic droplets. Chromatin acetylation, is recognized by Bromodomain-containing protein 4 (BRD4), a protein binding acetylated histones at actively transcribed genes, induces a distinct phase-separated state with droplets exhibiting unique physical properties. The acetylated chromatin is not miscible with heterochromatin formed by non-acetylated chromatin, mirroring the separation of chromatin subdomains within the cell [37,38].

Oppositely charged species regulate condensate formation based on RNA levels: lower levels induce formation, excess leads to dissolution. Transcription and condensates create a feedback mechanism, where short-lived RNA enhances formation, and high RNA density later induces dissolution [28,39,40]. RNA structure determines dense phase identity in BCs, with Whi3 forming distinct RNA granules based on RNA structure differences, highlighting RNA specificity's role [3]. Simulations show phase separation of RNA-binding proteins depends on RNA length [5].

Lysine's role in condensation was studied in Alzheimer's-related Tau protein, showing a distinct phase separation mechanism compared to Tau/RNA coacervation [41,42]. Ukmar-Godec *et al*. [50] found lysine in disordered protein regions in P-bodies drives phase separation to form lysine/RNA coacervates. Other amino acids, like arginine, behave differently in protein condensates. Cation-π interactions occur between tyrosine and arginine, and Coulomb interactions involve nucleic acid phosphate groups. Glycine enhances fluidity, glutamine and serine promote hardening. PTMs like tyrosine and serine phosphorylation and arginine methylation orchestrate LLPS. Lysine acetylation reverses LLPS, reducing Tau localization in SGs. Lysine ubiquitination increases Tau's propensity to form BCs, weakening Tau/RNA interactions. Heterogeneous ubiquitination stabilizes droplets against dissolution [51]. Tau undergoes LLPS with DNA, mono-nucleosomes, and nucleosome arrays under low salt conditions. Tau interacts with linker and nucleosomal DNA, and localizes in droplets formed by nucleosomes and phosphorylated heterochromatin protein 1α (HP1α). Aberrant Tau hyperphosphorylation disrupts chromatin interactions and LLPS formation [52].

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RNA Interference and Condensates

By means of RNA Interference (RNAi), it was shown that mRNA deadenylation, the process of degradation of mRNAs, occurs within cytoplasmic condensates (RNA granules) in the presence of Argonaute 2 (AGO2) endonuclease. Super-resolution imaging studies allowed the observation of condensates, whose dimensions are below the standard microscopy resolution, addressing the search function of the role of condensation in RNA Interference *in vivo* [5,36].

RNAs, Condensates and Cancer

In recent times, certain ncRNAs have been reported that are directly connected to cancer cell biology, forming condensates: among them, X-inactive specific transcript (Xist), controlling chromosome X imprinting, Metastasis associated lung adenocarcinoma transcript 1 (*MALAT1*), an RNA necessary for the nuclear speckles, HOX transcript antisense RNA (*HOTAIR*), a structured RNA assembling the Polycomb repressive complex 2 (PRC2) on chromatin, Survival associated mitochondrial melanoma specific oncogenic ncRNA (*SAMMSON*), ncRNA activated by DNA damage (*NORAD*) and the Nuclear enreached abundant transcript 1 (*NEAT1*), assembling the paraspeckle, are only the tip of the iceberg of ten of thousand ncRNAs, many of which are associated to cancer development [53–55] (see Table 1). The issue of assigning to ncRNAs a specific role in tumorigenesis is made complex by the various roles they have in the interaction with other proteins and other RNAs, in particular with microRNAs: thus, ncRNAs may behave not only as oncogenes but also as a tumour suppressors, depending on the cell type and the specific function. This may vary from competing endogenous RNAs, able to sponge miRNAs, to the interaction with proteins, often acting as decoys for their processing, to the production of miRNAs from their sequence, or giving origin to micropeptides.

Nuclear Speckles and Paraspeckles

The speckles are nuclear compartments containing components of the splicing machinery: Spliceosomal subunits are constituted by small nuclear ribonucleoproteins (snRNPs), serine/arginine-rich (SR) proteins, and the ncRNA *MALAT1*. *MALAT1* is recruited to speckles by means of direct interactions with multiple splicing-associated proteins, but it is not necessarily required for speckle formation [56–59]. *MALAT1* (also named *NEAT2*), is a ~8 kb highly abundant ncRNA conserved across vertebrates and is considered an oncogene [9,60]. High expression of *MALAT1* is associated with poor prognosis and metastasis in lung cancer and other tumour types.

One of the well-studied condensates linked to cancer is the paraspeckle. Nuclear paraspeckles, membrane-less condensates deriving from a gel-like phase separation, are chains of spheroids each of which is endowed with a core and shell structure. Out of the known members, *NEAT1* recruits and keeps together proteins for paraspeckle formation, drawing special attention because of its appearance in the form of variants [46]. *NEAT1_2*, one of its variants, is essential for paraspeckle formation. *NEAT1_2* is a reliable marker for paraspeckles and is expressed in specific conditions, absent in normal neurons but overexpressed in degenerative diseases [61–65]. Its involvement in thyroid cancer has been explained with the property to sponge microRNAs with a role in cell control [66]. These structures involve over 60 proteins, with the critical ones, like Non-POU domain-containing octamer-binding protein-Splicing factor (NONO), splicing factor proline- and glutamine-rich (SFPQ) [67,68], Fused in Sarcoma (FUS), and Paraspeckle component 1 (PSPC1) are located in the core, while the RNA binding protein RNA binding protein 14 (RBM14) and Brahma homolog component of SWI/SNF (BRG1) are located in periphery. Several paraspeckle RNA-binding proteins (RBPs) possessing IDR domains with gel-forming property, as SFPQ, FUS, Ewing sarcoma RNA binding protein 1 (EWSR1), TATA box protein associated factor 15 (TAF15), TDP-43, SS18L1 and Heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1) are mutated in familial cases of amyotrophic lateral sclerosis (ALS), and this has been linked to defects in paraspeckle function [69].

The number of paraspeckles in a cell depends on cell types: HeLa cells contain 13–17 foci/nucleus, while NIH3T3 mouse fibroblast cells contain 5–10 foci/nucleus [67]. Eight proteins are necessary in paraspeckle nucleation: the list includes NONO, SFPQ, HNRNPA1, HNRNPA2, HNRNPK and HNRNPH3, FUS, the spicing activator DAZ associated protein 1 (DAZAP1), BRG1 and RNA binding protein 14 (RBM14). Nascent 2300-nucleotide long Nuclear enreached abundant transcript 1 (*NEAT1*) transcripts recruit nuclear RNA-binding proteins towards the construction of a paraspeckle. There are two variants originating from *NEAT1*, NEAT1_1 (3.7 kb in length) and NEAT1_2 (23 kb in length) encoded by the *NEAT1* gene. NEAT1_2 RNA is expressed and present in up to 50 copies per paraspeckle. Two closely related proteins, NONO and SFPQ are required for paraspeckle formation. Recently the crystal structure of NONO-SFPQ and FUS has been reported. In HeLa cells, the knockdown of any of these proteins results in paraspeckle ablation. Paraspeckle RNA and protein components have been involved in cancer development by expression analyses as well as using knock-out and RNA silencing experiments.

As soon as NEAT1_2 is generated, NONO and SFPQ rapidly bind and get stabilized by NEAT1_2, forming a NEAT1_2 ribonucleoprotein (RNP) complex. Experiments based on plasmid rescue showed NEAT1_2 as the RNA required to form the paraspeckle, while SFPQ or NONO silencing leads to disintegration of paraspeckles due to NEAT1_2 destabilization [62]. The number and dimension

Table 1. Examples of human ncRNAs associated with cancer development (https://rps.renlab.org/#/Home) with info on associated cancers from the LncRNA Disease database.

BC type	ncRNA	Interact with	Type of cancer
Pro-Myelocytic Leukemia body	double homeobox A pseudogene 10 (*DUXAP10*)	Phosphatase and tensin homolog (PTEN)	prostate, ovary, bladder, breast, pancreas, esophageal squamous cell carcinoma (ESCC)
Promyelocytic leukaemia protein (PML) body	*PTENP*	Asp-Glu-Ala-Asp (DEAD)- box family 54 (DDX54), PTEN, Fused in Sarcoma (FUS), fragile mental retardation proteins (FMR1, FXR1), HNRNPA2, HNRNPB1, MOV10 RNA helicase, insulin growth factor 2 binding proteins (IGF2BP2, IGF2BP3)	colon, non-small cell lung cancer (NSCLC), hepatocellular cancer (HCC), endometrium, glioma, prostate, colorectal cancer (CRC), multiple myeloma
PML body	Urothelial carcinoma associated 1 (*UCA1*)	PTEN	Acute Myeloid Leukemia, Bladder Carcinoma, glioma, breast, colon, astrocytoma, melanoma, pancreas, HCC, osteosarcoma
PML body	TP53 target 1 (*TP53TG1*)	p53	NSCLC, triple negative breast cancer, colorectal, gastric, ovarian, glioblastoma
Histone locus body	Myc-N opposite strand (*MYCNOS*)	CCCTC-Binding Factor (CTCF)	neuroblastoma, thyroid, HCC, lung kidney, colon, urinary bladder
Histone locus body	*Y3 RNA*	cleavage and polyadenylation specific factor (CPSF)	cervical, colon, kidney, prostate, lung, urinary bladder
P body, SG	*RP11-435F13.2*	ELAVL1/Human antigen R (HuR) RNA binding protein	pancreatic ductal cancer
SG, P body	*RP11-22P4.1, RP4-694A7.2*	ELAVL1 (HuR)	esophageal cancer
P body, SG	*AF131215.3*	Dicer endonuclease, Fat mass obesity (FTO), HNRNPU	prostate adenocarcinoma
P body	*MIR7-3HG*	Sponges miR-27a-3p	small cell lung cancer, prostate cancer
SG	*NORAD*	ALKB Homolog 5 (ALKBH5), Argonaute (AGO), (DEAD)-box RNA helicase 6 (DDX6)	many cancers

HNRNPA2, Heterogeneous nuclear ribonucleoprotein A2; BC, biomolecular condensate; SG, stress granules.

of paraspeckles is directly proportional to NEAT1_2 transcription levels. A scheme of NEAT1_2 activity in organization of paraspeckles is described in Fig. 2.

Transmission electron microscopy (EM) of cells using paraspeckle markers evidenced the localization of labels into distinct nuclear structures rich in *NEAT1_2* RNA [70–72]. Immunoprecipitation by cross-linking experiments showed the binding of proteins all along the length of *NEAT1_2* [73]. In hepatocellular cancer (HCC) progression, either *NEAT1_2* as well as NONO, RBM14 and PSPC1 are induced and associated with poor survival [74]. Using cross-linking and immunoprecipitation (CLIP) studies, NONO was shown to bind the 5' end of pre-mRNAs and to modulate pre-mRNA processing, depending on its RNA-binding activity. NONO forms RNA- and DNA-tethered condensates throughout the nucleus and undergoes phase separation *in vitro*, modulated by nucleic acid binding. Inhibiting NONO binding to RNA, or phase separation activity, results in decreased expression of super-enhancer-associated genes Heart and neural crest derivatives expressed 2 (*HAND2*) and GATA binding protein 2 (*GATA2*). Thus, novel compounds and agents targeting NONO RNA-binding activity may be used as therapeutics in neuroblastoma [75].

The complex Drosophila behaviour human splicing (DBHS) is formed by RNA-binding proteins NONO, SFPQ and PSPC1, and have numerous roles in genome stability and transcriptional and posttranscriptional regulation, dependent on their heterodimerization ability and subcellular compartmentalization, in particular in paraspeckles [76].

PSPC1 promotes the expression of insulin-like growth factor 1 receptor (IGF1R), a tyrosine kinase receptor, and IGF1R regulates cell adhesion and motility. It was shown by knockdown of Paraspeckle components that every component of paraspeckles PSPC1, including NONO, FUS, SFPQ and *NEAT1_2,* abolished IGF1R expression. IGF1R

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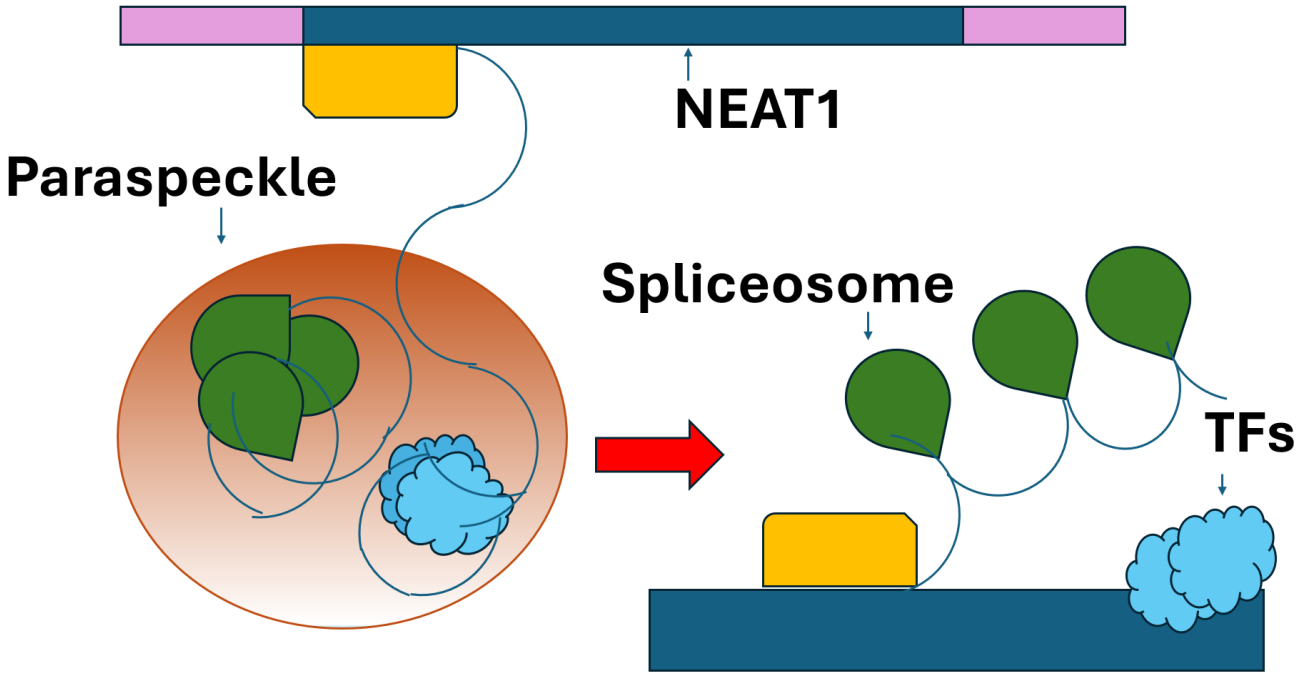

Fig. 2. *NEAT1_2* transcript, expressed from *NEAT1* gene, assembles with RNA binding proteins. Non-POU domain-containing octamer-binding protein-Splicing factor (NONO) and splicing factor proline- and glutamine-rich (SFPQ), Paraspeckle component 1 (PSPC1), bind and get stabilized by NEAT1_2, which associates with HNRNPK, HNRNPA1, Fused in Sarcoma (FUS) and RNA binding protein 14 (RBM14), forming the NEAT1_2 ribonucleoprotein (RNP) complex. As a throughput of the activity of the complex, transcription factors are co-opted and assembled on genes and transcription is activated, while spliceosome processes are regulated. The image was designed using MS Office 365 (700 Bellevue Way NE, Microsoft Corporation, Bellevue, WA, USA) and converted to '.png' file format. *NEAT1*, Nuclear enreached abundant transcript 1.

overexpression promotes cancer cells by formation of stress fibres, and activation of focal adhesion kinase (FAK) and Src signalling. In fact, an enhanced cell adhesion and motility toward extracellular matrix (ECM) is observed in HCC. IGF1R is a candidate therapeutic target due to its overexpression in tumour metastasis, drug resistance, and poor prognosis in multiple cancer types [77].

SFPQ promotes lung cancer malignancy [78], favouring cell proliferation, migration and invasion in HCC [79]; SFPQ condensates sequester SMAD4, preventing transforming growth factor β (TGF-β) tumour-suppressive signalling [80]; *MALAT1* binding to SFPQ releases polypyrimidine binding protein 2 (PTBP2) oncogene from the SFPQ/PTBP2 complex in colorectal cancer (CRC) [81], while in the same cancers SFPQ depletion is synthetically lethal with $BRAF_{V600E}$ mutations [82] in B-Raf kinase, homolog of murine sarcoma v-Raf.

PSCP1 is a regulatory subunit of ALKB Homolog 5 (ALKBH5) demethylase, one of the cellular erasers of 6mA modification on RNAs in the epitranscriptome. PSPC1 interacts with acetylated K235 in ALKBH5, promoting ALKBH5 erasing activity on N6-methyladenosine (m^6A), due to ALKBH5 recognition of m^6A-modified RNAs. K235 acetylation of ALKBH5 is upregulated in cancers and promotes tumorigenesis [83]. Paraspeckle component 1 (PSPC1) also interacts with lysine demethylase 5C (KDM5C), so these proteins are suggested to be collaborating partners in prostate cancer. Specific and selective compounds for the chemical inhibition of KDM5C and PSPC1 might be developed for the treatment of prostate cancer [84]. In sporadic ALS, either NONO as well as PSPC1 are still localised in motor neuron nuclei, while SFPQ and FUS exhibit nuclear loss and TDP-43 show cytoplasmic localization, in sporadic ALS [85].

RNA-binding proteins (RBPs) are the most important component of the chromatin proteome in embryonic stem cells (ESCs), many of them are localized with RNA polymerase (Pol) II at promoters and enhancers. The paraspeckle protein PSPC1 inhibits the premature release of Pol II, RNA-induced, and exploits RNAs to increase the formation of transcription condensates followed by phosphorylation and release of Pol II [86].

PSPC1 can undergo phase separation, due to a prion-like domains (PrLD) enriched in uncharged polar amino acids, such as asparagine, glutamine, tyrosine and glycine, required for phase separation. The loss of PSPC1 PrLD weakens its phase separation ability. In the maturation of mouse oocytes, checkpoint kinase 1 (CHK1) plays important roles in the germinal vesicle (GV) stage of mouse oocytes. Co-immunoprecipitation experiment showed that

PSPC1 interacts with phosphatase serine/threonine-protein phosphatase 5 (PPP5C), which regulates CHK1 phosphorylation [87].

PSPC1 is the master activator of transcription factors in epithelial-mesenchymal transition (EMT) and stemness. PSPC1 is required after c-Myc activation in facilitating tumour growth in breast and prostate cancer. PSPC1 increases TGF-β1 secretion through an interaction with phosphorylated SMAD2/3 and potentiates TGF-β1 autocrine signalling. Finally, PSPC1 acts as a determinant of the TGF-β1 pro-metastatic switch from transcription of tumour-suppressor genes to that of pro-metastatic genes [88].

In CRC, the expression of LOC105369504 is downregulated and is required to block proliferation, invasion, migration and epithelial-mesenchymal transition (EMT). LOC105369504 binding to PSPC1 regulates PSPC1 stability through the ubiquitin-proteasome pathway [89].

The pro-oncogenic activities of PSPC1 are linked to those of the orphan nuclear receptor 4A1 (NR4A1, Nur77): NR4A1 knock-down decreased expression of PSPC1 in MDA-MB-231 breast cancer cells, H1299 lung cancer cells, and SNU449 liver cancer cells. Similar results were reported in these cell lines with treatment with bisindole-derived (CDIMs) NR4A1 antagonists. Chromatin immunoprecipitation (ChIP) study showed that NR4A1 regulates *PSPC1* gene through interaction with a nuclear receptor binding responsive element (NBRE) sequence in the PSPC1 promoter. Thus, NR4A1 antagonists inhibit breast tumour growth and downregulate PSPC1 [90].

Roles of BCs in Chromatin

Condensates exhibit regulation at multiple levels, with alterations in properties influencing their formation, viscoelasticity, dissolution, and other physicochemical aspects, consequently modifying their function. Certain features are crucial for condensate regulation (concentration, chemical modification, involvement of non-coding RNAs (ncRNAs), and selective partitioning) since these are recognized or likely to be dysregulated in cancer cells [22]. The concentration of biomolecules stands out as a pivotal parameter in condensate dynamics. Condensation occurs at a critical threshold concentration, achievable through protein biosynthesis, protein degradation inhibition, regulation of transport into a membrane-bound compartment, or substrate binding. Notably, the loss of the nuclear membrane during mitosis leads to a reduction in the concentration of nuclear components and is linked to the dissolution of nuclear condensates [7,50]. The reformation of nuclear condensates occurs when the nuclear envelope is re-established, a process facilitated by the level of concentration of components, enabling the diffusion and the transport of proteins into the nucleus. Chemical modification, exemplified by the post-translational modifications (PTM) of histone proteins, induces alterations in the physicochemical properties of proteins and consequently influences the condensates they associate with [51,52]. Chromatin exhibits phase-separated condensates, and the behaviour of chromatin in unmodified and modified states serves as an illustrative example of this regulatory mechanism. Repressed genes are typically linked to non-acetylated nucleosomes, the heterochromatin subdomain, while active genes in euchromatin are marked by nucleosome acetylation. These two types of chromatins, organized into separate subdomains within the nucleus, maintain high compactness yet remain dynamically accessible to regulation by various modifying enzymes and proteins binding to modified nucleosomes [51–54].

Epigenetic Regulation of Condensate Formation

Epigenetic mechanisms are involved in regulation of cell function, such as chromatin accessibility, gene transcription, mRNA splicing, transcript stability, mRNA translation, RNA folding state and RNA-protein interaction, even with enzymes that impact chromatin modifying complexes [53]. Diverse chemical modifications of histone tails impart changes to the physicochemical properties of chromatin, thereby offering each modification the potential to influence the behaviour of chromatin condensates. RNA molecules serve regulatory functions across various biomolecular condensates, including the nucleolus, transcriptional condensates, co-transcriptional splicing condensates, nuclear speckles, paraspeckles, and stress granules [54–56]. Condensates, formed by a network of low-affinity molecular interactions, including electrostatic interactions, are subject to powerful regulation by RNA. RNA's ability to influence condensates formed and maintained by these forces is well-documented [2,15,57,58]. While the functions of most non-coding RNA (ncRNA) species expressed in cells remain unknown, as we progress into the RNA world more transcripts will be discovered comprising regulatory roles in diverse condensates. The partitioning of biomolecules into specific BCs supports high local concentrations of functional components within these nuclear compartments [59]. The phosphorylation of RNA polymerase II during transcription initiation induces a switch between transcriptional and splicing condensates: therefore, RNA pol II is a prototype of modification of intrinsically disordered regions (IDRs) that demonstrate the partitioning behaviour of a macromolecule [42,54].

Nuclear RNA plays a role in preventing the spontaneous self-assembly of FUS. Heterochromatin protein 1α (HP1α) is a highly conserved protein from fission yeast to humans, participating in the formation of heterochromatin—an extensively condensed chromosomal domain throughout the cell cycle, maintaining transcriptional inactivity. Phosphorylation of the N-terminus promotes the assembly of HP1α in the nucleus, and mitotic phosphorylation of the hinge region further enhances the formation of nuclear assemblies.

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In FUS and HP1α, the RNA was found to have an opposite contribution for the assembly of BCs formed by these proteins. Reducing nuclear RNA by inhibiting the transcription, triggers the assembly of FUS that was distributed in the nucleoplasm, but disperses the assembly of spontaneously formed HP1α. Noteworthy, substitutions in HP1α mimicking its cell cycle-dependent phosphorylation promoted the formation of condensates. The transcription inhibition studies were effective in understanding the various roles of nuclear RNA in regulating biomolecular condensates [90], underscoring the contribution of nuclear RNA in facilitating the assembly of HP1α throughout the cell cycle. However, both chromatin compaction and HP1α dimerization contributes to HP1α condensation [91,92]. In conditions of disruption of HP1α dimerization, the mobility of the droplets increases, preventing the maturation of heterochromatin formation [93,94].

Specific enhancer and promoter elements become the loci for the formation of transcriptional condensates through the selective binding of transcription factors (TFs) [95]. Transcription factors, being bifunctional proteins equipped with both a structured DNA binding domain and an intrinsically disordered region (IDR), engage in condensation with coactivator proteins [96]. The crowded environment of enhancer and promoter elements, housing multiple TF binding sites, induces the assembly of TFs and coactivators [97].

Constitutive heterochromatin condensates form at methylated satellite repeats, primarily due to the binding of methylated DNA by Methyl-CpG-binding protein 2 (MeCP2) and methylated histone modification known as lysine 9 methylation on histone H3 (histone H3K9) by HP1 proteins [38,92]. In parallel, facultative heterochromatin condensates emerge at sites featuring trimethylated H3K27, driven by the phase separation of Polycomb-repressive complexes [62]. Similarly, ligand binding to cell-surface signalling receptors can increase signalling component concentration at the plasma membrane, thereby triggering the formation of condensates with signalling molecules [63,64]. The most prevalent RNA modification, N6-methyladenosine (m^6A), holds the potential to influence RNA-driven phase separation by altering RNA–RNA and RNA-protein interactions. For example, the loading of m^6A readers facilitates phase separation [98]. It has been recently reported that Zinc finger MYND-type containing protein 11 (ZMYND11) acts as an epigenetic reader of histone methylation, serving as a transcriptional corepressor. Its downregulation is prevalent in cancers, particularly in prostate cancer, correlating with adverse patient outcomes. Depletion of ZMYND11 enhances tumour cell growth, migration, and invasion, both *in vitro* and *in vivo*, and its tumour-suppressive role involves binding to the methylated arginine194 in HNRNPA1 with its MYND domain (Myeloid-Nervy-DEAF-1), causing HNRPNA1 sequestration within the nuclei, with block of stress granule formation. Additionally, ZMYND11 counters the HNRNPA1-driven high Pyruvate kinase muscle isozyme (PKM) 2/PKM1 ratio [99].

RNA binding proteins are commonly associated with enhancers and promoters, playing a role in shaping condensate behaviours. For instance, the methyl-adenosine reader protein YTH Domain Containing protein 1 (YTHDC1) facilitates condensate formation at enhancers by binding enhancer RNAs, which are rich in m^6A methylation [97]. Some transcription factors exhibit the capability to bind both RNA and DNA, and the presence of nascent RNA molecules enhances their occupancy of gene regulatory elements [98]. In this context, it has been argued that DNA elements collectively influence transcription factors and formation of condensates, by concentrating these factors and additional transcriptional molecules [100]. On the other side, poly-ADP-ribosylation (PARylation) of HNRNPA1 at Lys298 regulates its nucleocytoplasmic transport and association with stress granules, through the binding to the poly-ADP-ribose (PAR) chains by protein partners by means of their PAR-binding motifs (PBM) [101].

RNA molecules transcribed from regulatory regions can play a role in facilitating condensate formation, controlling initiation, and contributing to the fusion or pairing of enhancer condensates with promoter sites, leading to a burst in transcription. RNA-mediated feedback may contribute to the termination of this transcriptional burst, with potential implications for the roles of ncRNAs near enhancers or promoters [102]. The interactions of such RNAs with enhancers could impact the rate and size of associated transcriptional bursts, while the accumulation of lncRNA near a gene might influence condensate size or dissolution through feedback processes. Additionally, ncRNAs could influence transcription by interacting with CCCTC-Binding Factor (CTCF) bound to insulators, contributing to boundary stabilization, and delimiting a genomic territory over which enhancers and super-enhancers operate [103].

RNA splicing, secondary structure, and modifications were shown to possess the ability to modulate the assembly and composition of cellular condensates. Furthermore, protein phosphorylation, ubiquitination, and methylation of arginine and lysine, could impart the assembly of condensates by fine-tuning multivalent weak interactions. Therefore, the driving force at the base of assembly of BCs lies in multiple interactions of protein-protein and protein-RNA [12]. Suppressing or inhibiting the primary enzymes responsible for PAR synthesis, poly ADP-ribose polymerases (PARP) 5a (tankyrase) and/or PARP1, reduces FUS condensation within cells [104]. Although PAR and RNA share structural similarities, their co-condensation with FUS is influenced by different modes of interaction [6,104].

Targeting Stress Granules

Exploiting the Stress Granules for Anticancer Strategies

Stress granules (SGs) are exploited by cancer cells to reversibly sequester and protect critical cellular components during stress. Highly expressed G3BP1, a stress granule nucleation protein, promotes proliferation, migration, and invasion in various cancers [3,105]. The mechanism of upregulation of SG formation has not been yet understood, but G3BP1 expression depends on the SG protein Y-Box binding protein 1 (YB1), a nucleocytoplasmic shuttle protein, that binds to the 5′ UTR of *G3BP1* mRNA, promoting its translation; noteworthy, YB1 is highly expressed in sarcomas [16]. SG formation can also be triggered by the release of lipid signalling molecules [105], and SG formation can be inhibited by two small molecule G3BP inhibitors (G3Ia and G3Ib) [106]. Independent of SGs, G3BP1 may lead to carcinogenesis by participating in growth-related signalling pathways and promoting epithelial-to-mesenchymal transition in tumors [107].

Ras-GTPase-activating protein (SH3 domain)-binding proteins (G3BPs) can regulate the expression of mRNAs even outside of SGs, through interactions with RNA. G3BPs are linked with several diseases, and in cancer progression, invasion, metastasis, as well as in viral infections. G3BPs regulate SGs, RNA stability, interaction with viruses, and tumorigenesis [106]. Therefore, compounds that target G3BPs, or block SG formation or promote SG dissolution have potential application as therapeutics. G3BPs may be useful as an antiviral and as anticancer therapeutic target [108–110].

Melanoma-associated antigen B2 (MAGE-B2) reduces the levels of G3BP below the concentration critical for phase separation, suppressing SG initiation [111].

G3BP1 is a central key player of SG dynamics. G3BP1 RNA binding is impaired by K376 acetylation. The lysine 376 of G3BP1, in the RRM RNA binding domain, can be acetylated [112]. A mutation, K376Q, mimicking K376 acetylation, impairs G3BP1 interaction with poly-A binding protein 1 (PABP1), while the RNA-independent interactions of G3BP with caprin-1 and USP10 are not affected. The formation of SGs depends on G3BP1 RNA binding ability; the substitution of G3BP1 with its K376Q mutant or the RNA binding-deficient F380L/F382L mutant blocked the formation of SGs. During SG resolution, K376-acetylated G3BP1 was observed outside SGs [113].

Four genes, karyopherin subunit alpha 2 (*KPNA2)*, Mex-3 RNA binding family member A/ring finger protein 162 (*MEX3A* or *RNF162*), WD repeat domain 62 (*WDR62*), and stratifin (*SFN*), are highly expressed in human HCC tissues. By knocking down each one of these four genes, HCC cell proliferation was reduced by disrupting the SG formation process. G3BP2B, a cell specific form of G3BPs, induces condensates and mRNA expression changes under endoplasmic reticulum (ER) stress [114]. Key residues for G3BP condensation (i.e., V11) are required for formation of the G3BP-Caprin-1 complex, thus promoting SG assembly [115].

G3BP1 and G3BP2 knocked-down cells cannot form SGs in response to eIF2α phosphorylation or eIF4A inhibition. However, the cells are still SG-competent when challenged with heat or osmotic stress. Using G3BP1 mutants, rescue experiments showed that phosphomimetic G3BP1-S149E failed to rescue SG formation, while a mutant unable to bind G3BP partner proteins Caprin1 or USP10, G3BP1-F33W, rescues SG formation. Caprin1 binding to G3BP promotes SG, on the opposite USP10 binding inhibits SG formation. G3BP through its RGG motif interacts with 40S ribosomal subunits, and RGG is required for G3BP-mediated SG formation. In synthesis, G3BP facilitates SGs condensation by shuttling between two different states controlled by S149 phosphorylation and by binding to Caprin1 or USP10 [115].

Exploiting the Stress Granules for Antiviral Strategies

Viruses pose great challenges to their containment, especially after the new epidemics of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2). Novel stains may suddenly develop by trans-species transfer. Epstein-Barr virus (EBV), Kaposi Sarcoma human virus (KSHV), papillomaviruses are oncogenic, and other RNA viruses are epidemic, i.e. HIV, influenza A virus (IAV), SARS-CoV2. In addition, the interventions do not obtain the recovery of patients due to disease states or low response to the medicines. Therefore, many papers have been published to develop novel therapeutic approaches. Most viruses produce ncRNAs that may behave as antagonistic to the immune response, or help viruses for their replication. Several microRNAs have been widely described, and also some miRNA mimic that substitute or sustain the host microRNAs to subvert cellular responses. A list of noteworthy viral ncRNAs is listed in Table 2. In the future, it may be possible to set up approaches to antagonize viral RNAs using antisense sequences or target viral RNA-host protein interactions. It is worth stressing that a role of viral RNAs in biomolecular condensates may be deleterious for the host, since they are formed and delivered to antagonize cellular functions. Stress granules are hijacked by cytoplasmic viruses to carry on viral replication, sequestering host proteins and modifying stress granule PTM signals.

Seneca Valley Virus (SVV) is an oncolytic RNA virus of the Picornaviridae family. SVV causes vesicular disease (VD) similar to foot-and-mouth disease (FMD). Wen and colleagues [109] showed that SVV induce formation of SGs in the early stage of infection, in a PKR-eIF2α dependent manner, with recruitment of G3BP1 and eIF4GI. Interestingly, inhibition of SG formation had no effect on SVV replication, on the contrary G3BP1 depletion blocked

Table 2. List of viral ncRNAs affecting the host proteins, signals and physiology.

Virus	RNA	Host partner	Effect	Other action
Adenovirus	*VA RNA$_I$*, *VA RNA$_{II}$*	protein kinase RNA dependent (PKR)-binding by VA RNA$_I$, autoantigen La, ribosomal protein L22 (RlpL22)	block PKR and the immune response	Dicer/RNA induced silencing complex (RISC) processing
Epstein-Barr virus (EBV)	EBV-encoded RNA 1 (*EBER1*)	HNRNPD, AU-rich element-binding factor 1 (AUF1), nucleolin		
Epstein-Barr virus (EBV)	*EBER2*	paired box protein 5 (PAX5)		
Herpesvirus	*EBNA-LP v-snoRNA1 ebv-sisRNA-1*	fibrillarin, nucleolar protein 56 (Nop56)		
Flavivirus, West Nile v., Dengue v.	subgenomic flavivirus RNA *sfRNA*	5′3′ exoribonuclease (XRN1), tripartite Motif Containing 25 (TRIM25)	inhibits interferon activation	decoy substrate for Dicer
Herpesvirus HSV1	*LAT* latency associated transcript	(syntaxin-binding protein gene 1 (STXBP1), gamma aminobutyric acid type B receptor subunit 2 (GABBR2), small mother against decapentaplegic family protein 3, 4 (SMAD3, SMAD4)	downregulation	virus encoded *niR-H3 miR-H4*
HVS Saimiri in primates	*HSUR 1-7*	Spliceosomal (Sm) proteins	block *miR-142-3p* and *miR-16*	
Kaposi Sarcoma human virus (KSHV)	*miR-K12-9*	p53 upregulated modulator of apoptosis (PUMA), Translocase of outer mitochondrial membrane 22 (TOMM22), Bcl2 associated transcription factor 1 (BCLAF1), caspase 3	downregulation	
KSHV Marek disease 1 Simian foamy v.	*miR-M4* homolog of cellular miR-155	support host overexpression of *miR-155*	increased degradation of mir-155 targets	*MiR-155* mimic
KSHV	polyadenylated nuclear (*PAN*) RNA	demethylases JMJD3, X chromosome (UTX), poly A binding protein C1 (PABC1)	shuttle proteins to the nuclei	
Human immunodeficiency virus (HIV)	*Antisense protein* (*ASP*) RNA	Polycomb repressive complex 2 (PRC2)	trimethylated histone 3 lysine 37 (H3K27me3) repressive epigenetic mark on 5′ long terminal repeats (LTR)	viral latency, *MALAT1* releases PRC2
HIV	*Psi packaging RNA*	Nuclear enreached abundant transcript 1 (*NEAT1*)	paraspeckles	upregulates *miR-28-5p, miR-150, miR-223, miR-382* pivotal in HIV-1 latency/reservoir
HSV	Latency-associated transcript *LAT*, *miR-H3 miR-H4, sncRNA1*	STXBP1, GABBR2		
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2)	*miR-D7-5p*, *miR-P1-3p*, *miR-rL1-5-3p* *miR-R1* to *R6, miR-D1* to *D13*, *miR-385* *MR147*, *MD-241*	target host *miRNAs MiR-574–5p*, *miR-214*, *miR-17, miR-98*, *miR-223*, *miR-148a*	*miR-155*, *miR-148a-3p*, *miR-486-5p, miR-451a*, *miR-369-3p*, growth arrest specific 5 (GAS5), RP11-773H22.4	disease severity, Regulating Anexelekto (AXL) tyrosine kinase
SARS-CoV2	*3′UTR* of *SARS-CoV-2* Small RNAs	sponging host miRNAs down-regulate *miR-296, miR-520h, miR-602*	alters cell cycle, reduces apoptosis, enhances IL6, TNF-α, induce cytokine storm	Acute respiratory distress syndrome (ARDS), cell proliferation

IL6, Interleukin 6; TNF, Tumor necrosis factor; JMJD3, Jumonji domain-containing protein-3; *HSUR 1-7*, Herpesvirus saimiri U RNAs 1-7.

nuclear factor-κB (NF-κB) pathway activation. Furthermore, SVV inhibited SG formation at the late stage of infection, with a role for 3C protease, that blocked the SG formation by disrupting the eIF4GI-G3BP1 interaction.

Viruses form condensates *de novo* as part of their replication programmes. Viral ribonucleoproteins (vRNPs) and the host Rab11a protein drive the formation of liquid condensates called viral inclusions [116]. It is thought that hardening these viral inclusions so that they become stiffer and less round will make it more difficult for viruses to replicate and assemble their genomes. Etibor *et al*. [117] studied how various factors, i.e., temperature changes, concentration of driving factors, and the valency (i.e., the number/types of interactions among the vRNPs) affected the properties of influenza A virus (IAV) inclusions [117]. Liquid IAV inclusions can be hardened by targeting vRNP interactions by means of nucleozin, a known nucleoprotein (NP) oligomerising molecule, without affecting host proteome abundance or solubility. Drugs strengthening the bonds between molecules in condensates are effective in hardening. This study showed that pharmacologically active compounds may modulate the material properties of IAV inclusions.

Studying HIV interplay with the nuclear environment, it was found that HIV involves the protein cleavage and polyadenylation specific factor 6 (CPSF6). This involvement leads to the formation of nuclear HIV-induced membraneless organelles (HIV-1 MLOs). Viral BCs regulate key steps of retroviral replication. Reverse transcription occurs in the nuclei in the HIV-MLOs. During the late replication steps, retroviral nucleocapsid acts as a scaffold recruiting client viral components to aid the assembly of progeny virions [118–121].

For several viruses, including human respiratory syncytial virus (RSV), replication occurs in virus-induced compartments called inclusion bodies (IBs) or viroplasm. IBs of negative-strand RNA viruses were identified as BCs that form by means of phase separation [122,123]. Cyclopamine (CPM), a steroidal alkaloid, and A3E, its chemical analogue, blocked RSV replication through disorganization and hardening of IB condensates [124]. The assembly of IBs is driven by LLPS through the interactions between the viral nucleoprotein N and the phosphoprotein P. The activity of cyclopamine and A3E were disrupted by a point mutation in the RSV transcription factor M2-1, an RNA binding protein. A single mutation in the viral transcription factor M2-1 or in P induced resistance to CPM. Viral inclusion bodies (IBs) are membrane-less organelles concentrating the viral RNA polymerase complex. CPM activity depends on the presence of M2-1 together with N and P. The CPM effect was a rapid IB disorganization of IB, and A3E molecules are supposed to act on the IBs liquid properties. A3E and cyclopamine inhibited RSV in the lungs of infected mice. It is possible that CPM affects the dynamics of the M2-1-P interaction, thereby influencing the relative mobility of the proteins contained in RSV IBs. Therefore, condensate-targeting drug-like small molecules have been taken into account as inhibitors of viral activity. These condensate-hardening drugs may be effective in modulating undruggable targets in viral replication but can also target BCs in transcription factor complexes at cancer-driving super-enhancers.

Condensates and Cancer

Modifications in BCs are a pervasive feature of malignancies and tumour metastasis. The tumour microenvironment presents diverse stresses, namely: nutrient starvation, hypoxia, hyperosmolarity, Reactive Oxygen Species, response to chemotherapeutics, activating the integrated stress response (ISR) triggering stress granule formation [10].

Caprin1 is an RNA-binding protein with a role in stress granules, P bodies, and granules transporting mRNAs, and is highly expressed in cancers. Caprin1 is a phase-separating protein that mediates LLPS and phase separation within tumour cells. In addition, the formation of Caprin1-mediated phase separation is regulated by ncRNAs and circular RNAs (circRNA). Various ncRNAs participate in the regulation of SGs formation by Caprin1-mediated LLPS. *CircVAMP3* expression was correlated to the formation of droplets by Caprin1 and G3BP1: the ability to form droplets was linked to circVAMP3 concentration and timing of activity [125]. Caprin1 plays a role in metabolic adaptation. Therefore, chemical modification of cell adaptation to an adverse metabolic environment could be feasible by targeting condensates with drugs and chemical modifiers.

In ovarian cancer, upregulation of small nucleolar RNA host gene 8 (*SNHG8)* RNA was linked to its binding with Caprin1, positively regulating its expression, and promoting the stability of catenin beta 1 (CTNNB1) and Axin 1 [126]. This was followed by the regulation of the wingless and mouse Int-1 (Wnt)/β-catenin pathway by CTNNB1 and Axin1, therefore affecting cell migration and EMT transition while maintaining cell stemness of ovarian cancer.

In addition, upregulation of ncRNA *SNHG9* regulated Caprin1 expression positively and promoted cisplatin resistance in lung cancer [127,128]. After binding to *SNHG9*, Caprin1 promoted the proliferation and metastasis, maintaining cellular stemness and improving the survival ability of tumours. Glutamine, as a major nitrogen source in tumour cell growth, plays a central role in tumor cell survival. In condition of glutamine deprivation, Glutamine Insufficiency Regulator Of Glutaminase (*GIRGL*) RNA promoted the formation of LLPS by assembling the trimeric complex *GIRGL*-Caprin1-Glutaminase 1 (GLS1) mRNA and suppressed glutamine synthase translation, induced the formation of SGs enhancing the viability of cancer cells [127,128] and adapting cells to an adverse glutamine-deficient environment. When cells live in the glutamine-deficient environment, c-JUN-induced and stress-activated *GIRGL* is ex-

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pressed. Moreover, the ncRNAs *MA301* [129], *Xist* [130], and Zinc Finger NFX1-Type Containing 1-antisense RNA 1 (*ZNFX1-AS1*) [131] also affected cancer cells by linking their expression with Caprin1 activity.

Stress granule-associated Caprin1 is involved in cancer progression of nasopharyngeal carcinoma and can be targeted for treatment with small molecules, or oligonucleotides targeting the domains that bind these RNAs, disrupting the interactions with RNA [132].

Cancer-Associated Proteins can Undergo Phase Separation

At a concentration and affinity threshold, these biomolecules (BCs) have the capability to amalgamate into liquid-like droplets, forming compartments that orchestrate specific biochemical reactions. Recently, it has been revealed that many deregulated processes occur within BCs in cancer, prompting investigators to explore how pro-tumoral alterations impact BC biology and cooperate to develop the malignancies [12]. Moreover, new findings indicating that BCs affect the pharmacodynamic behaviour of drugs and small-molecules may provide clues for new therapeutic approaches in tumour treatment [107,133].

Due to chromosome instability, disruptions occur in genes, causing oncogene or anti-oncogene dysregulation, leading to cell proliferation in an uncontrolled manner. Among the targets for clinical intervention, drugs active on proteins and RNAs responsible for LLPS association and turn over may be effective in cancer treatment [133]. For instance, in pancreatic cancer, the inhibitor ZW-115 targets the stress-inducible nuclear protein 1 (NUPR1), an intrinsically disordered protein, and triggers apoptosis of PDAC cells dependent on K-RAS expression [134]. Small conjugates of SG inhibitory peptides (SIPs) derived from SG proteins Caprin1 and USP10 fused with cell-penetrating peptides produced the active cancer inhibitors TAT-SIP-C1/2 and SIP-U1-Antp. SIPs increased the sensitivity of cancer cells to sorafenib via the disruption of SGs [135–138]. Pancreatic cancer cells were found sensitive to 1,6 hexanediol, an LLPS inhibitor that also affects kinase and phosphatase activities. 1,6-hexanediol reduced pancreatic cancer growth in an atopic BxPC-3 xenograft model, using the BXPC-3 human pancreatic adenocarcinoma cell line. By means of transcriptional studies, 1,6-hexanediol was shown to downregulate *myc* expression of that of other genes involved in cytokine-cytokine receptor interactions, Wnt signalling pathway, interaction of extracellular matrix with integrin/cadherin receptor, Mitogen-activated protein kinase (MAPK) signals, focal adhesion pathways [139].

In the attempts to find therapeutic targets for neuronal diseases, a multi-omic approach was performed to study protein-phase-separation (PPS) in animal models of neurodegenerative diseases. Mutant Speckle-type POZ protein (SPOP), SHP2, and AKAP95 interfere with the physiological activity of MLO. Researchers found differential phase separation behaviours of three predicted Alzheimer's disease (AD) targets (CAMKK2, MARCKS and p62) in two cell models of AD. The result of the study was identification of 8 protein targets (MARCKS, CAMKK2, SYN1, SQSTM1, MAP4K4, APC, YAP1 and NECTIN2) that may be targeted to decrease their expression or to modulate the cytoplasmic localization and formation of MLO [140].

The Tumour Suppressor Scenario

Cell compartments can undergo dysregulation in tumour cells [11]. Cancer cell sequencing has unveiled recurring mutations affecting epigenetic enzymes and regulatory proteins, potentially reshaping the landscape of chromatin condensates. Notably, the histone mark rimethylated histone 2 lysine 37 (H3K27me2) or trimethylated histone 3 lysine 37 (H3K27me3), with lysine methylation, is found in juvenile brain-stem gliomas, altering the structure of euchromatin domains, and gene promoters silenced, leading to dysregulated gene expression. Dysregulated compartmentalization of condensates is also evident in the context of tumour suppressors. The tumour suppressor speckle-type POZ (Poxvirus and Zinc finger domain) protein (SPOP), implicated in a broad spectrum of solid tumours, forms substrate-dependent granules and membraneless structures. SPOP mutations, linked to cancer development, sequester the protein from condensing with its substrate, leading to a reduction in its enzymatic activity [141]. A central player in this process is SPOP, serving as a substrate adaptor for cullin 3 Really interesting new gene (*RING*) *E3* ubiquitin ligase. SPOP assembles oncogenic proteins with the E3 ligase for their ubiquitination followed by proteasomal turn-over, providing an antiproliferative effect in cells. Solid tumours, including breast and prostate tumours, often exhibit SPOP mutations. Localised in nuclear speckles, a form of biomolecular condensates, SPOP's oligomerization and interaction with substrates induces the formation of SPOP-containing nuclear phase-separated droplets, where ubiquitination of death domain-associated protein (DAXX) contributes to its degradation. DAXX is localized in heterochromatin and in promyelocytic leukaemia protein nuclear bodies (PML-NBs) and has been involved in nuclear processes, including transcription. Later through the work of Bouchard *et al*. [141], published in 2018, this mechanism has been demonstrated by co-expression of the SPOP substrate DAXX together with SPOP, forming a condensate enriched in SPOP/DAXX, different from nuclear speckles, which facilitated the ubiquitination of DAXX, leading to its turnover. Cancer-causing SPOP mutations block the recruitment of DAXX, and prevent SPOP/DAXX bodies nucleation, resulting in DAXX stability. Since DAXX facilitates cell proliferation and tumour development by repressing the activity of tumour suppressors like p53, the cytoplasmic/nuclear accumulation of DAXX in SPOP mutants may contribute to malignancy [80,95].

NEAT1 and *MALAT1* are associated with actively transcribed genes [142] suggesting that these ncRNAs localize their subnuclear bodies in proximity to nascent transcripts. Thus, *NEAT1* and *MALAT1* might contribute structurally to the arrangement of nuclear bodies at transcribed sites, as *NEAT1* has previously been observed to organize paraspeckle formation around its transcription site [89].

The tumour suppressor promyelocytic leukaemia protein (PML) forms nuclear membrane-less structures, considered biomolecular condensates. These PML bodies compartmentalize diverse proteins, among them p53 and DNA repair protein complexes, regulating cellular functions, apoptotic pathways and stability of the genome. PML mutations lead to loss-of-function and are linked to tumour formation and unfavourable prognosis [11].

In the DNA repair pathways discussed earlier phase-separated DNA repair foci include the DNA damage-response factor p53-binding protein 1 (53BP1) [143]. Studies have revealed that these repair foci also concentrate p53, and disruptions of phase-separated foci impairs 53BP1-dependent induction of p53, reducing p53-dependent expression of target genes. Since p53 is a tumour suppressor, decreasing p53 concentration and signalling at DNA repair foci may trigger events promoting tumour malignancy [95]. Turning on p53 expression, either through drugs or when stress from cancer-causing genes occurs, was shown to cause paraspeckles formation in both mouse and human cells.

NEAT1 levels go out of balance in various human cancers and are seen as a helpful indicator of prognosis, especially in triple negative breast cancer, although the exact way it works in this case is not known. Additionally, *NEAT1* contributes to resistance to chemotherapy and promotes cancer stemness [64,65,98,144]. NEAT1 is overexpressed and dysregulated in various forms of cancer cell lines including prostate, breast and ovarian cancers [99,145]. *NEAT1* is upregulated in TNBC, where its expression is linked to cell proliferation, cell migration, and invasion.

NEAT1 RNA is regulated by p53, as a p53-direct target, induced under stress, DNA damage and oncogene-induced replication stress [97,146]. In mice, blocking *NEAT1* expression, which stops paraspeckles from forming, made pre-cancerous cells more vulnerable to cell death caused by DNA damage and reduced the formation of skin tumours [96]. Furthermore, *NEAT1* mediates cisplatin/taxol resistance, so that its downregulation may sensitize cancer cells to chemotherapy. *NEAT1* upregulation contributes to the pathogenesis of liver diseases such as fibrosis. Considering the mechanism of action, various molecular mechanisms can be taken into account, such as competition for miRNA sequences, and by this multitasking activity *NEAT1* contributes to liver-related disorders such as acute liver failure, non-alcoholic fatty liver disease (NAFLD), and liver cancer [147].

Mechanistic Target of Rapamycin (mTOR) activation reduced NEAT1_2 transcription, diminished paraspeckles, and released NONO, SFPQ, and RBP14, which are important for RNA splicing [148].

Over- and Under-Expression of RNAs

In cancer cells, ncRNAs exhibit diverse expression patterns: overexpressed *MALAT1*, *HOTAIR*, Steroid receptor RNA activator (*SRA*), Colon cancer associated transcript 2 (*CCAT2*), Regulator of reprogramming (*ROR*), Activated by TGF-β (*ATB*), T cell factor 7 (*TCF7*), Second chromosome locus associated with prostate-1 (*SCHLAP1*), Testis-derived ncRNA regulating Epithelial-Mesenchymal Transition (*treRNA*), Zinc finger E-box binding Homeobox 2 antisense RNA 1 (*ZEB2-AS1*), Urothelial carcinoma associated 1 (*UCA1*); under-expressed Low expression in tumor (*LET*), Down-regulated in atherosclerosis regulator of p21 (*DRAIC*), EGFR-coamplified and overexpressed transcript (*EGOT*), growth arrest specific 5 (*GAS5*), Maternally expressed gene 3 (*MEG3*), Neuroblastoma associated transcript 1 (*NBAT-1*), NF-κB interacting ncRNA (*NKILA*), Prostate cancer associated transcript (*PCAT*); and many of them are post-transcriptionally modified [87,88]. *MALAT1*, a constituent of nuclear speckles, is notably increased in cervical, colorectal, lung, breast, liver and bladder tumours. Cells exhibiting improper expression of these ncRNAs display various dysregulated functions, with mechanisms of action showing inconsistencies and sometimes conflicting views. It is plausible that the deregulation of oncogenic RNAs influences the function of condensates [50].

A newly established method, Capture Hybridization Analysis of RNA Targets (CHART) [143] showed the binding of *NEAT1* and *MALAT1* to active genes. Engreitz *et al.* [144] performing RNA-RNA interactions (RAP-RNA) showed that MALAT1 is associated with actively transcribed gene *BCs*, through interactions with proteins guiding this ncRNA to nascent pre-mRNAs. It has been proposed that *MALAT1* acts as a scaffold facilitating nuclear speckles positioning at active gene loci.

Zacco and colleagues [149] as well as Roden and Gladfelter [150] reviewed the function of various RNAs involved in LLPS structures, not only oncogenic RNA such as *NEAT1*, but also the imprinting regulator of X chromosome Xist, describing their requirement in the formation of phase-separated assemblies and their influence on the cellular landscape.

Dysregulation of Cellular Events Linked to Condensates in Cancer

In healthy cells, precise control over cell growth is the norm to maintain tissue homeostasis. Nevertheless, a distinctive characteristic of cancer cells lies in their remarkable ability to circumvent these regulatory mechanisms, persistently undergoing division [91]. This often involves activation of receptor tyrosine kinases (RTKs), often due to

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single amino acid substitutions, that cause cell growth and cell migration through downstream Ras protein signalling. Typically located at the plasma membrane, RTKs are bound by extracellular ligands, leading to RTK dimerization and transduction of signals through Ras proteins or GTP-GDP exchange factors. Recent findings indicate the role of adaptor proteins in RTK and Ras signalling, such as Linker for activation of T cells (LAT), growth factor receptor-bound protein 2 (GRB2), and Son of Sevenless (SOS), which undergo phase separation stimulated by RTK activation. This phase separation allows to extend the interaction time between SOS and RTK/Ras, acting as a proofreading mechanism for RTK signalling [92–94]. This proofreading control prevents spontaneous localization of SOS to membranes, inhibiting the inadvertent activation of Ras and its downstream pathways, while also modulating Ras signalling downstream to RTKs [92]. Cancer-inducing RTKs mutations may disrupt this proofreading checkup by modifying membrane-association and phase separation driven by SOS and other Ras signalling molecules. In alternative, RTK mutations interfere with the recruitment of cytoskeletal factors crucial for Ras signalling, including actin, whose nucleation by Actin-related protein 2/3 (ARP2/3) complexes is dependent on phase-separation and LLPS formation [95]. In connection to this, RTK condensates have been calculated to perform an exchange of growth factor receptor-bound protein 2 (GRB2) at a slow rate, when compared to membrane-bound GRB2 in the canonical pathway, which undergoes rapid cycling on and off membranes. The formation of RTKs associated granules may facilitate the concentration of key downstream signalling molecules, enabling the constitutive activation of RAS and Mitogen-activated protein kinase (MAPK) and downstream signals in cancer cells [95,96].

Furthermore, recent research has unveiled that gain-of-function mutations in miRNA-140 seed sequence, driven by chondrocyte-specific super-enhancers, result in a skeletal dysplasia ("spondyloepiphyseal dysplasia MIR140 type Nishimura"), expanding the role of super-enhancers as causes of disease. While the in-depth exploration of the correlation between super-enhancers and ncRNAs in cell identity control is lacking, it has been proposed that super-enhancer-driven ncRNAs (SE-ncRNAs) are linked to tumour development and pathogenesis. A crosstalk between ncRNAs and super-enhancers, determining gene control in cis and trans, as well as super-enhancer regulation by ncRNAs, has also been proposed. Non-coding mutations contribute to super-enhancers dysregulation in specific cancers. For instance, in approximately 5% of T-cell acute lymphoblastic leukaemia (T-ALL), small insertions near the *TAL1* gene coding for the BHLH transcription factor Erythroid Differentiation Factor, induce the creation of ectopic super-enhancers, providing a working hypothesis requiring studies to understand how non-coding mutations trigger the establishment of ectopic super-enhancers [151].

PML bodies, dynamic nuclear compartments, participate in various cellular activities, including responding to DNA damage, regulating transcription, controlling the cell cycle, defending against viruses, and promoting apoptosis. The N-terminal Ring, B-box, Coiled-Coil (RBCC) domain facilitates self-oligomerization of the protein scaffold, PML, while the C-terminus, containing Small ubiquitin-like modifier (SUMOylation) sites and a SUMO-interacting motif, enhances the assembly of granules through SUMO and SUMO-interacting motif (SIM) arrays, promoting liquid-like condensate formation. In cancer, PML bodies exhibit varied roles, with acute PML linked to chromosomal translocation resulting in the fusion protein PML-RARa lacking the C-terminal SIM and SUMOylation sites. This alteration reduces PML phase separation, causing dispersed microspeckles and concentrating transcriptional machinery at oncogenes, potentially impacting RNA splicing and contributing to cancer.

WD40-encoding RNA antisense to p53 (WRAP53) locus, implicated in cancer development, encodes both an RNA transcript, antisense to p53, and a WD40 repeat-containing protein that promotes Cajal body formation. While the antisense transcript is important in regulating p53 activity, the connection to formation of phase separated Cajal bodies awaits more in-depth investigation [152]. In addition to accumulation of phase separated p53 and its post-translational modifications in cancer, also WRAP53 overexpression contributes to carcinogenesis.

HNRNPA1 is implicated in the control of alternative splicing; among the activities assigned to HNRNPA1 are: interaction with ncRNAs, translation of specific mRNAs, and processing of miRNAs. Furthermore, HNRNPA1 by controlling the packaging into exosomes of miRNAs and ncRNAs, may regulate molecular processes in other cells [153]. A growing body of evidence indicates that the phase separation of proteins involved in cancers, influencing epigenetic modifications, signal transduction, transcription, translation, and protein degradation, plays crucial roles in tumour development.

Condensates and Drug Discovery for Cancer Treatment

In the context of targeting the BCs, scientists have stated the necessity to get insights on their importance in normal and disease states [13].

The properties of the condensate may be altered chemically, and this may affect the concentration and activity of drugs [79]. Over time, various approaches have emerged in the forefront, including tuning valency. For instance, replacing multivalent interactions with monovalent ones can modify condensates, termed condensate-modifying therapeutics (c-mods), aimed at preventing or reversing diseases. Such discoveries typically focus on repairing condensatopathies, disrupting normal condensate functions in diseases, or rendering specific targets inactive within their

condensates. These strategies involve screening and validation models correlating condensate optical phenotypes with disease-relevant outputs. Another approach involves directly blocking or stabilizing molecular interactions contributing to scaffolding [107]. For instance, TDP-43 mutations as well as HNRNPA1 mutations are involved in SG dysregulation in amyotrophic lateral sclerosis (ALS). Mutations (P288A and D262V) in HNRNPA1, linked to ALS and MSP, were studied in patients with myopathy, ALS, and hereditary motor neuropathy. The mutations were shown to affect differently either HNRNPA1 fibrillization, liquid-liquid phase separation, and SG dynamics. P288A accelerated fibrillization and decelerated SG disassembly, whereas a truncated HNRNPA1 had no effect on fibrillization but decelerated SG disassembly and other truncated variants reduced the time of fibrillization and blocked LLPS. Short-bait RNAs have been shown to prevent the formation of TDP-43 inclusions in an optogenetic cell model, possibly through a mechanism that outcompetes TDP-43/TDP-43 self-interaction. Another drug used is mitoxantrone, the topoisomerase inhibitor and nucleic acid intercalator, able to inhibit SG formation in a phenotypic screen using different cell lines.

In frontotemporal degeneration (FT) and amyotrophic lateral sclerosis (ALS), the LLPS forming TDP-43 binds to PAR non-covalently by means of PAR-binding motifs (PBM) residing in its nuclear-localization sequence. PAR binding promotes TDP-43 LLPS and is necessary for TDP-43 accumulation in stress granules in mammalian cells and neurons. SG localization protects TDP-43 from disease-associated phosphorylation, but upon long-term stress, SGs dissociate, but phosphorylated TDP-43 aggregates remain stable in the cells. As a therapeutic approach, small-molecule inhibition of Tankyrase, one of the enzymes performing PARylation, blocks the formation of cytoplasmic TDP-43 foci. Tankyrase inhibition attenuates TDP-43-associated pathology and neurodegeneration without affecting stress-granule assembly [108]. Several PARPs, including the tankyrase PARP5a, and Mono-ADP-ribose (MAR) producing PARP12, PARP14, and PARP15, PARP13, inactive in ADP-ribose synthesis but possessing antiviral property, and two PAR glycohydrolases are localized to SGs in the cytoplasm. PAR degrading enzymes are required for neuronal development, since defective cells with mutated ARH3 showed to present neurogenetic disorders and SG-linked stress dependent epileptic ataxia.

Various forms of stress were demonstrated to inhibit the RNA-dependent enlistment of RNA-binding proteins, such as TDP-43 [109].

New findings have shown the relationship between BCs, disease, and the activity of drugs for disease treatment. Traditional drug development involves small molecules binding to enzyme active sites. Proteins and peptide-based biomolecules targeted by Proteolysis-targeting chimeras (PROTACs) broaden the scope to target non-enzyme proteins if a ligandable pocket exists [110]. In a similar way, Ribonuclease-targeting chimera (RIBOTAC) exploits an RNAse tag to degrade RNA targets. However, identifying ligandable proteins and RNA remains challenging [154]. Phase modulators, whether small molecules or RNA, offer a novel approach by targeting proteins and RNA based on their phase separation propensity, addressing druggability gaps. These modulators can impact phase separation pathways or become condensate-resident, utilizing transient contacts within the condensate. Drugging condensates doesn't necessarily require new approaches; covalent modifications and enzymes involved in these modifications can be targeted conventionally. While small molecule drugging of condensates is feasible, rules governing specific condensate partitioning remain largely unknown, with challenges in understanding the role of small molecules in this context. Despite these challenges, research in phase modulators with drug-like properties is coming up to light [14].

The biology of biomolecular condensates requires great attention by the developers of drugs and chemotherapeutics. As a first case, castration-resistant prostate cancer (CRPC) is a tumour developing in ineffective androgen receptor (AR) therapies caused by low AR expression [111]. CRPC cells show exceptionally high expression of death domain-associated protein 3 (DDX3), that regulates RNA metabolism, such as transcription, pre-mRNA splicing, RNA export and translation. DDX3 localizes, together with DDX6, to stress granules in association with Gle1A, a DDX protein regulator, and the RNA binding protein Caprin1. DDX3 is required for SG assembly, leading to rapid SG nucleation [112]. AR expression is due to mRNA sequestration into the SGs [110]. Furthermore, *AR* mRNA can be freed and AR protein is reported to normal levels by DDX3 inhibition. Although chemotherapies may lead to cancer cell death, a large number of drugs (e.g., bortezomib, lapatinib, morusin, 5-fluorouracil (5-FU), 5-azacytidine, 6-thioguanine, sodium selenite) show a paradoxical effect through the induction of SG formation, they enhance cancer cell survival [113].

It is known that few drugs may concentrate in specific condensates by physico-chemical interactions that are not linked to the drug's affinity for its target [108]. Moreover, certain medications seem to selectively interfere with condensates, providing a chance to regulate compartments that have a role in disease development [113]. In addition, certain drugs that inhibit enzymes involved in post-translational modifications (PTM), may affect condensate function and can modify oncogenic activities localized in condensates [114]. The processes leading to drug resistance offer insights into drug efficacy in clinical scenarios. Persistent clinical challenges like endocrine therapy and tamoxifen resistance are linked to diverse mechanisms, encompassing Estrogen receptor α (ERα) mutation and elevated Mediator 1 (MED1) expression [79].

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Future Perspectives and Therapeutics

Scientists specialised in LLPS structures, expressed their support in the perspectives and challenges about this field, stressing on the wide range of unknown aspects that await to be studied [13].

BCs are classified by the ability to concentrate proteins and RNAs at discrete cellular sites. Since there are no cellular barriers delimiting these cellular compartments and isolating their interior from the surrounding environment, for numerous years, it remained undetermined how the molecules concentrate in the BC, which mechanisms orchestrates to support and govern their structures, manage their compositions, and adjust biochemical activities in the interior. Awaiting additional investigation, the potential of RNA to adopt diverse structures within or outside condensates could be utilized for innovative therapeutic purposes. This includes enzymatic RNA molecules, called ribozymes, exhibiting activity exclusively within condensates. The structure also plays a role in RNA-RNA interactions. This influences the material properties of RNA-seeded condensates and impacts RNA sorting during development of phase-separated assemblies [64,73,115]. In the same context, RNA is a component in many BCs and contributes to phase separation for its scaffolding property, via RNA/RNA and protein/RNA interactions. Analysing RNA structures in dense phases and the supramolecular formations within them poses significant challenges, resulting in limited available data on RNA structure within biomolecular condensates [15]. For example, structural studies on *NEAT1_2* are essential for RNA-based drug discovery [116]. But it is essential to acknowledge the obstacle to isolate this specific RNA from a quantitative point of view. Recently reported needle-shear method is effective for RNA extraction in this aspect [117]. RNA structural biology is crucial to deepen the knowledge, with cryogenic electron microscopy (cryo-EM) SPA revealing protein-free RNA structures. 'Ribosolve' workflow can aid in macromolecular complex validation [118]. Implementing this workflow on ncRNA modules may uncover new structural elements [119]. RNA structure determination applying cryo-EM methods are still yet to progress [130]. Questions persist about the resolution of cryo-EM for RNA-only systems [121]. Studying *NEAT1_2* using cryo-EM can provide insights into RNA-based drug discovery [95].

Additional research is required to address RTK granules in tumours. For instance, studies may focus on factors that regulate the nucleation and BC stabilization, to use these factors as a platform for activating RTKs and Ras GTPase. These findings may show how the disruption of BCs (i.e., BC containing SPOP and its substrates) may cause cancer. Further research on the molecular determinants and cell signals localizing proteins to SPOP nuclear bodies may help to understand the mechanism and may be useful in testing therapeutic applications [94]. To investigate the molecules, determining factors and cellular signals for proteins involved in SPOP nuclear bodies, researchers used immunofluorescence or immunohistochemistry to visualize protein localization [121] followed by co-immunoprecipitation to identify interacting proteins [123]. To evaluate the genetic manipulation the employment of knockout/knockdown systems and monitoring the dynamic changes through live cell imaging are promising techniques [124,125]. On the contrary, Clustered regularly iinterspersed short palindromic repeats (CRISPR)-Cas9 screening, mass-spectrometry and functional assays can be implemented to uncover regulatory genes to analyse protein composition [126].

Many mRNA transcripts separate within SGs when cells experience stress (oxidative, hypoxic, chemical). It is noteworthy that mRNAs with cell survival roles remain outside the SGs and are selectively translated in the cytoplasm. We need to understand the mechanisms at the basis of this orchestration, in normal and in cancer cells, while the disruption of this process may be effective to prevent cancer. Lastly, another research target is the development of strategies able to reverse SG formation in tumours, through the targeting of SG assembling proteins with small molecule compounds, such as G3BP1 and/or its regulators. This may render cancer cells more sensitive to stressors [68]. Stress induction, viability assessments, molecular analyses, and functional assays can be deployed to elucidate the impact of targeting G3BP1 and its regulators on SG formation and cancer cell response to stressors.

Since normal cells utilize super-enhancer transcriptional programs, similarly to cancer cells, we need to understand what makes cancer cells super-sensitive to perturbation of translation; in addition, it is required to modulate the means to target specifically "transcriptional dependency" [101]. Identification of transcriptional dependencies can be performed conducting RNA sequencing on cancer cells to identify key transcription factors overexpressed or uniquely regulated. Utilization of CRISPR/Cas9 screens to systematically knockdown or knockout individual transcription factors is preferable [126]. Functional genomics approaches incorporating CRISPR interference (CRISPRi) or CRISPR activation (CRISPRa) to modulate transcription factor activity without permanent knockout can be performed to validate dependencies. To validate the impact on downstream target genes and pathways through gene expression analysis can be considered [128,129]. Eventually, for drug screening for targeted inhibition, performing high-throughput small molecule screening against identified transcriptional dependencies, validation of hits using cell-based assays to confirm specificity and efficacy is necessary. Employing combination therapy strategies to enhance effectiveness and minimize resistance cannot be neglected [130,131]. To get mechanistic insights and novel biomarker discovery investigating the molecular mechanisms underlying transcriptional dependency by studying

chromatin dynamics and epigenetic modifications is feasible. Simultaneously, single-cell RNA sequencing is essential to dissect heterogeneity in response within the cancer cell population [132,133].

For *in vivo* validation, establishing xenograft or patient-derived xenograft (PDX) models can be included to assess the efficacy of transcriptional dependency-targeting drugs. To monitor tumour growth, metastasis, and overall survival we can perform pharmacokinetic and pharmacodynamic analyses [134,135]. We must call up to adapt the experimental design based on the specific characteristics of the cancer type under investigation and the identified transcriptional dependencies. Ethical considerations should be prioritized and regulatory compliance in experimental design and execution should not be overlooked.

In addition, we need to understand the difference between neurons and cancer cells in the function of assembling and regulation of BCs composition, leading to a variety of modular effects on condensate-associated processes (nucleocytoplasmic transport, protein aggregation, etc.) in neuropathies and in tumours [136]. To address this from an experimental point of view, certain approaches can be suggested, such as characterization of condensates, functional analysis of nucleocytoplasmic transport, protein aggregation dynamics, quantitative proteomics and RNA sequencing, comparative pathophysiological studies, and finally computational modelling [2,26,137,138]. This multifaceted approach will contribute to understanding how BC assembly and composition differ in neurons and tumoral cells, shedding light on the vast array of effects on condensate-associated processes in these diseases.

The systematic evaluation of mutations in influencing phase separation, particularly in cancer, is crucial. Cancer gene fusions may significantly impact phase separation, potentially driving cancer initiation and progression through this property. Understanding this mechanism requires systematic efforts, as cancer mutations can lead to either the gain or loss of BCs and LLPS structures that contribute to the set up of disease states. Notably, disease alleles, especially gene mutations, could be dominant in tumours, forming condensates by sequestering wild-type protein counterparts. This characteristic requires further studies, offering insights into fundamental principles of genetic inheritance [102]. Future identification of cancer gene fusions through Whole-Genome Sequencing (WGS), especially by hybrid sequencing, through Isoform Detection and Prediction (IDP) to quantify gene isoforms, holds significant promise. Additionally, *in vitro* phase separation assays, including droplet formation and turbidity measurements, can provide added value. Exploring functional consequences on cancer initiation and progression, along with proteomic analysis of phase-separated condensates in patient-derived samples, correlated with clinical data and pharmacological intervention, represents a promising avenue [57,139–141].

This systematic approach will provide critical insights into how mutations within cancer gene fusions alter phase separation properties, potentially driving cancer initiation and progression. Understanding this mechanism can guide the design and application of therapies for cancers associated with aberrant phase separation. The genetic systems can be applied to deliver proteins capable of dissolving p53 aggregates, based on DAXX degradation, or to develop crosslinked mimic condensates for drug selective delivery. Therapies may benefit from using synthetic peptides and nucleic acid-based oligonucleotides (RNA mimics, RNA sponges) to target mutant phenotypes. Studying the dynamics of mutant p53 may support the understanding of mechanisms of protein misfolding and aggregation. It can be assumed that novel drugs targeting the abnormal phase transition and aggregation of p53 can be translated into clinical use in oncology [129].

For drug delivery, engineering crosslinked mimetic condensates that selectively incorporate therapeutic drugs is an important requirement [58,142]. In accordance with personalized medicine protocols, scientists can develop synthetic peptides and RNA analogues designed to specifically target mutant p53 phenotypes followed by patient-derived cell lines or xenograft models. Personalized therapeutics try to identify optimal sequences for blocking mutant p53 function [143–145] and to study mutant p53 dynamics and dehydrons and investigate the role of dehydrons in protein misfolding and aggregation. This study plans to harness genetic systems and synthetic molecules for targeted protein dissolution, drug delivery, and personalized medicine, with a specific focus on addressing mutant p53-associated misfolding and aggregation.

A role of phase separation has been acknowledged in different biological processes, such as signal transduction, transcriptional activation, and ribosome biogenesis. It highlights the potential significance of stress-induced biomolecular condensates, particularly in cancer development. The field of phase separation in tumours is under development, with opportunities to predict specific domains targeted by drugs and to assess the role of phase separation in tumoral tissue. The paragraph emphasizes the need for further research to understand the dynamics of MLO, aiming for therapies of human disease, and for early diagnosis, and prevention of tumours [12].

Biomolecular condensates in the viral replication cycle induce scientists to test novel strategies to challenge BC biology for antiviral drug discovery. BCs may also provide an opportunity to develop antivirals that are broad-spectrum or less prone to acquired drug resistance [116].

Viruses form condensates *de novo* as part of their replication programmes. Viral ribonucleoproteins (vRNPs) viral inclusions have been shown to be sensitive to nucleozin [117], cyclopamine and A3E [124]. Other small compounds can be discovered for inhibition of viral inclusions and virus replication.

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Antisense oligonucleotides (ASO), Small Interfering RNA (siRNA), and nanoRNAs are sequence specific gene-silencing approaches that can be used for suppressing or inhibiting gene expression. Finally, catalytic nucleic acids such as DNAzymes and ribozymes can suppress viral replication by repeatedly cleaving viral mRNAs and template RNAs [153]. It was shown that Kirsten rat sarcoma (KRAS)-dependent tumours upregulate stress granules [155]. Therefore, methods to inhibit or reduce the SGs and their function may be beneficial.

Researchers designed photo-switchable small molecules that reversibly regulate RNA splicing [156], or bind reversibly other RNAs, for application in RNA therapeutics [157].

Researchers showed the efficacy of a TNBC therapy based on nanoparticle-mediated RNAi of the oncogenic lncRNA Differentiation Antagonizing Non-Protein Coding RNA (DANCR) using tumour-targeting arginine-glycine-aspartate (RGD) (cell targeting signal) -PEG-(1-aminoethyl)iminobis[N-(oleicylcysteinyl-1-amino-ethyl)propionamide] (ECO)/siDANCR nanoparticles using the multifunctional lipid (ECO). In other reports, several approaches have been developed for the treatment of epigenetic dysfunctions through regulation of ncRNAs [158]. It was shown that single wall carbon nanotubes (SWCNT) may deliver nucleic-acid drugs stably and efficiently with good tolerability and minimal toxicity [159].

Systemic delivery of tumour targeting siRNA nanoparticles against an oncogenic ncRNA [160,161] showed its efficacy and potential for applicative uses. Small molecule libraries have been selected for efficient RNA binding, that may target dysregulated ncRNAs [160,162].

The European Medicines Agency reported the guidelines on RNA-based medicines [163], making the point on the progress in chemical modifications of ASO, engineering the sequences, and methods for delivery for an efficient targeting to the tumours and cell specific delivery [164–169].

Benhamou designed an oncogenic non-coding RNA inhibitor by selection of best-behaving sequences from an RNA library and comparison with a DNA library [168]. Defective or dysregulated proteins can be targeted by PROTAC methods, or modulated by intercalating molecules able to induce changes in their structure and functions. Proteolysis-targeting chimeras (PROTACs) [170,171] is a novel strategy to knock down proteins of interest, showing efficiency at nanomolar levels on B-Raf inhibitors (BRAFi)-resistant cell lines and advantages over conventional small molecule inhibitors [171].

Oncogenic RNAs may be targeted by RIBOTAC [155], RNA silencing with Antisense oligonucleotides (ASO), and RNA interacting domains of RNA binding proteins targeted by small molecules, pointing toward potential breakthroughs in therapeutic interventions [172–175].

RNA nanoparticles are produced by ExonanoRNA company and patented as NanoRNAs, are designed for cancer therapeutics research applications. A series of customizable RNA nanoparticles contains a targeting ligand and a therapeutic modality to assay the therapeutic efficacy of novel targeting or therapeutic modalities. The particles are ideal to test cell uptake, endosome release, and therapeutic efficacy of a therapeutic drug.

Patents have been already applied for targeting miR-22 in cancer and metabolism (Resalis Therapeutics). Two drugs based on ASO technology have been tested for possible application in therapy, such as oligonucleotides antisense to protein coding genes, such as B-cell leukemia/lymphoma 2 (*bcl-2*), vascular endothelial growth factor (*VEGF*), activator protein 1 (*AP1*), while two drug antagomiRs target small RNAs, anti-mir-155 in cutaneous T-cell lymphomas, and miR-122, Miravirsen, tested in primates for liver disease; other oligonucleotides are miRNA mimics [176].

Liquid nanoparticles (LNP) have been optimized for self-amplifying RNA (saRNA) using the FDA-approved ionizable lipids (NC3, ALC-0315, SM-102) [177] and applied to production of RNA medicines, such as mRNA vaccines and Small Interfering RNA (siRNA)-LNP therapeutics like patisiran, treating polyneuropathies induced by hereditary transthyretin amyloidosis [178].

Highly expressed MALAT1 is linked to poor prognosis and metastasis in many tumours. Small molecule ligands have been used as base for backbones and scaffolds to provide modified skeletons able to interact with RNA domains and structures within the RNA: this is the case of the diphenylfuran (DPF) group of MALAT1 interactors, which bind to *MALAT1* triple helix thus inducing degradation of the RNA [179]. Administrating MALAT1 ASOs resulted in slower growing, more differentiated tumours and a significant reduction in metastases without overt toxic effects in a mouse breast cancer model [180]. It is unclear which pathway targeted by the ASO, either the disruption of the role of *MALAT1* within nuclear speckles or other additional *MALAT1* functions (i.e., through miRNA sponging regions, or producing miRNAs or micropeptides) are involved. Further studies are required for a better understanding of the molecular basis for *MALAT1* oncogenic activity, which may reveal new approaches for targeted anti-metastatic therapeutics.

Traditional medicine expresses plant bioactives such as stilbenes that are known regulators of stress granules [181]. It is possible to engineer nanostructures for cell delivery preserving their bioactivity, such as nanoparticles or self-nano-emulsifying drug delivery systems [182].

Conclusions

The function of biomolecular condensates (BCs) shows their relevance in the set-up of human pathologies,

viral infection and cancers. The interdisciplinary approach, from polymer chemistry to genetics, has expanded our understanding of the mechanisms of liquid-liquid phase separation (LLPS) through the model of 'Stickers and Spacers'.

The roles played by RNA molecules, ribonucleoproteins (RNPs), and their specific protein partners have been discussed, orchestrating the diverse functioning of BCs, from precise localization and compartmentalization to epigenetic regulation and dynamic properties. The involvement of BCs in cancer cell biology has emerged, encompassing stress granules, cancer-associated proteins, tumour suppressors and corepressors, and the dysregulation within cancer development and progression.

Furthermore, this review sheds light on the frontier of condensate-targeted drug development, such as oncogenic RNA targeting by RIBOTAC, RNA silencing with Antisense oligonucleotides (ASO), and RNA interacting domains of RNA binding proteins targeted by small molecules, pointing toward potential breakthroughs in therapeutic interventions. In the future, the BC research holds promise of a deeper comprehension of diseases and cancers, but also paving the way for advancements at the intersection of molecular studies and in clinical applications.

Availability of Data and Materials

All experimental data included in this study can be obtained by contacting the corresponding author if needed.

Author Contributions

SJ wrote the first draft, by selecting relevnt bibliography, and making the experiment of cell compartments by microscopy applications. PP contributed by drafting the paper and data analysis to the paper. Both authors contributed to important editorial changes in the manuscript. Authors read and approved the final manuscript. Authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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