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Opinion

The emerging role of receptor tyrosine kinase phase separation in cancer

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Receptor tyrosine kinase (RTK)-mediated signal transduction is fundamental to cell function and drives important cellular outcomes which, when dysregulated, can lead to malignant tumour growth and metastasis. The initiation of signals from plasma membrane-bound RTKs is subjected to multiple regulatory mechanisms that control downstream effector protein recruitment and function. The high propensity of RTKs to condense via liquid–liquid phase separation (LLPS) into membraneless organelles with downstream effector proteins provides a further fundamental mechanism for signal regulation. Herein we highlight how this phenomenon contributes to cancer signalling and consider the potential impact of LLPS on outcomes for cancer patients.

Highlights

Liquid–liquid phase separation (LLPS) of biomolecules into condensed phases is associated with multiple functions in cells. The importance of this in receptor tyrosine kinase (RTK)-mediated cancer signalling is explored.

The condensation of RTKs and associated downstream effector molecules via LLPS results in elevated local concentrations, increasing the propagation of signal transduction, including that associated with cancer.

A subset of RTKs have a high propensity to be included in LLPS based on their intrinsic structural features.

Observations from patient data which indicate high concentrations of RTKs and downstream effector proteins, and hence an elevated probability of RTK-mediated LLPS might provide diagnostic/prognostic indicators.

RTK signal transduction in cancer

RTKs are transmembrane proteins that mediate intracellular signals resulting in discrete and enduring cellular outcomes such as proliferation, cell growth, differentiation, motility, metabolism, and survival. RTKs typically exist in a dynamic equilibrium between monomers, dimers, and higher-order oligomers. Activation generally requires self-association which initiates phosphorylation of one RTK by another. Normally this is promoted by the binding of extracellular stimuli, such as growth factors, that can sustain RTK dimerization or higher oligomer formation; however, other mechanisms that drive clustering of RTKs can also produce a signalling response. The transduction of a signal from an RTK involves the recruitment of effector proteins to phosphorylated tyrosine residues on the C terminal (CT) region of the receptor. These residues provide docking sites for downstream effector proteins that possess cognate binding domains. Effector proteins initiate further activation of downstream enzymes along a prescribed pathway. As might be expected for such a fundamental signal initiation platform, other regulatory control functions that affect signalling outputs are present, including; multidomain linkage through concentration-dependent adaptor protein recruitment [1–3], phosphatase action on RTK phosphorylation [4,5], clustering of RTKs based on membrane lipid composition and microdomain formation [6,7], receptor internalisation [8], and generation of receptor or signalling protein splice variants [9–11]. Importantly, RTK-mediated signal transduction requires that proteins associated with downstream pathways be localised and available at sufficient concentration to interact with the appropriate receptor.

Pathological signalling outcomes such as cancer often develop when RTK activation is abnormal. Overwhelmingly, this oncogenic activation is the result of hyperactivation of the kinase resulting from genetic amplification, mutation, overexpression, autocrine activation, and/or chromosomal rearrangements resulting in fusion proteins and truncation of the receptor proteins. This leads to the uncontrolled transduction of signals and/or the recruitment of aberrant signalling proteins. Most of the hallmarks of cancer can be correlated at the molecular level with abnormal RTK activity [12], and hence targeting of RTKs has been a major focus of therapeutic intervention.

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However, promising efficacy observed preclinically and in early clinical trials often disappoints on patient treatment. Understanding failure of directed RTK therapies is a major challenge, and it is clear that our knowledge of the underlying mechanisms is only partial.

Recently, LLPS of proteins has been observed intracellularly and is implicated in a multitude of cellular functions. LLPS represents the state whereby biomolecules condense out of the bulk cellular milieu to form distinct membraneless phases [13]. Upon phase separation, local protein concentrations within the condensates are elevated dramatically, which raises the apparent affinity for a given binding event and serves to exclude competing interactions. The biophysical basis of phase separation is the topic of many excellent reviews [14–16]; however, in all cases it is mediated by multivalent, moderate/weak, transient interactions which are driven by local fluctuations in protein/biomolecular concentrations. Through spatial compartmentalisation of a subset of effector proteins, LLPS has been shown to be involved in a range of signal transduction pathways [17–20].

Accumulating evidence shows that phase separation is implicated in several human diseases, including cancer [21–28]. However, the connection between phase separation and RTK-driven tumorigenesis is only now beginning to be appreciated. In this opinion article we focus on recent studies linking LLPS and RTK signalling. We provide insight into the possibility of LLPS playing a general role in RTK-driven signalling, especially in cancer, and introduce the prognostic opportunity represented by identification of features of LLPS in patients.

RTK architecture forms a platform for LLPS

The involvement of RTKs in LLPS is a unique example of intracellular protein condensation because of the particular environment proximal to the plasma membrane. Self-association or clustering of RTKs provides a platform for recruitment of multiple downstream effector proteins and hence initiation of LLPS on the inner leaflet of the plasma membrane. Since RTK-mediated signal transduction is predominantly based on equilibrium interactions described by affinity constants, the respective concentrations of interacting proteins will dictate the likelihood of one interaction prevailing over another. In other words, proteins that are within a phase-separated droplet have a dramatically increased local concentration, which elevates the probability of molecular interactions with cognate ligands. This amplifies a given signal initiation event and the transduction mechanism, and ultimately increases the probability of the prescribed cellular outcome. Therefore, if these interactions are associated with oncogenic pathways, RTK-mediated LLPS will drive tumorigenesis and/or metastatic outcomes [29].

Even before the recent findings that RTKs can undergo phase separation, an examination of RTK architecture would strongly suggest that these molecules could be components of biomolecular condensates [18] (Figure 1). RTK-associated LLPS is highly dependent on three defining features: (i) multivalent interactions mediated by intrinsically disordered regions (IDRs) of the incorporated proteins [30,31], (ii) post-translational modifications (PTMs) such as tyrosine phosphorylation [32–35], and (iii) the elevation of protein expression levels of component signalling proteins, particularly RTKs and their cognate downstream effectors. These molecular determinants control the assembly or disassembly of condensates, as well as the constituent nature of the condensed state [32–34]. There are many sites on a given RTK that can be invoked in moderate/weak interactions associated with sustaining LLPS (typically K_d >0.1 μM). Most RTKs consist of three distinct regions: an extracellular region, a single-pass transmembrane domain, and a cytoplasmic region. Extracellular interactions induce clustering of RTKs [36–38] which can include interactions mediated by ligands such as growth factors or cytokines, as well as inter-RTK association. Interactions between transmembrane domains can be influential in receptor clustering in lipid

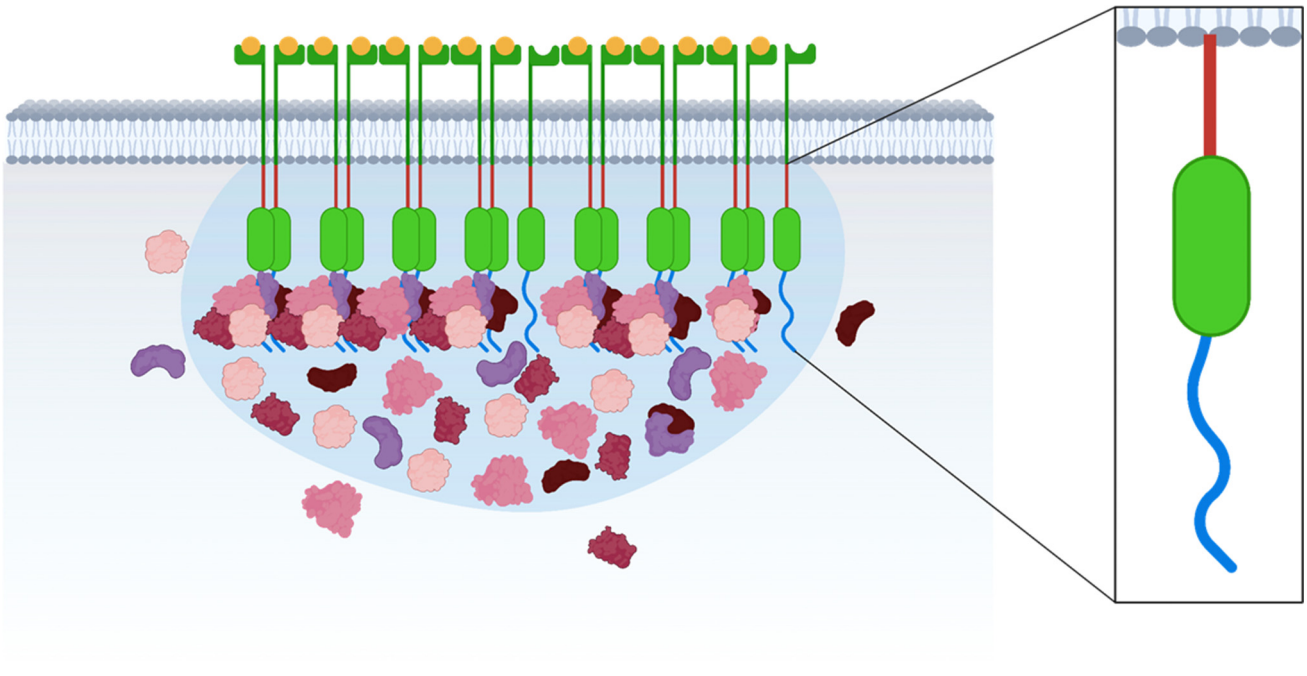

Figure 1. Schematic diagrams of membrane-bound receptor tyrosine kinases (RTKs) at the liquid–liquid phase separation (LLPS) state: a schematic of a condensed droplet (light blue) sustained by RTKs at the plasma membrane. The RTKs are brought together as a cluster via interactions with extracellular ligands (e.g., growth factors, orange) and/or lipid microdomain formation. These form multiple interactions with downstream signalling molecules (purple, pink, salmon, and brown) which are at high concentration within the droplet. Signalling molecules are exchanging with the bulk cytoplasm in and out of the droplet. Right-hand side: expanded view of an RTK showing the juxtamembrane region (red), the kinase domain (green), and the C terminal domain (blue). The C terminal region often consists of intrinsically disordered regions (IDRs) and can include sequences of hydrophobic or charged/polar residues as well as proline-rich motifs and phosphorylated tyrosines. These structural features can be used for protein recruitment and hence sustain phase-separated condensates. Schematic diagrams were created with BioRender.

microdomains, which are a common feature in the plasma membrane [39–41]. The cytoplasmic region includes the catalytically required kinase domain which is sandwiched between juxtamembrane (JM) and CT regions. Following ligand binding, the kinase domains of RTKs are activated. This leads to initiation of tyrosine phosphorylation events in the JM, kinase, and CT regions. These phosphorylated tyrosine residues become docking sites for proteins that possess cognate binding domains. JM regions have been shown to be capable of self-association, which can act as a regulatory mechanism for kinase activity [6]. CT regions range considerably in size; however, they frequently possess the characteristics of IDRs. Sites required for downstream protein recruitment provide multiple binding regions required for multivalent protein condensation. For example, phosphorylated tyrosine residues provide cognate sites for moderate-affinity interactions with SH2 and phosphotyrosine-binding (PTB) domains on binding proteins. In addition, proline-rich motifs (PRMs) on CTs are common (apparent on >40% of RTK CTs), forming moderate/weak affinity interaction sites for SH3, WW, or EVH1 domain-containing signalling proteins [42]. Through interactions of these different regions, receptors provide a platform for condensation of intracellular signalling proteins.

The likelihood of LLPS being mediated by any of the 58 known RTKs and the propensity of their CT regions to phase separate was analysed using prediction software programs: PSPredictor[i] [43] and FuzDrop[ii] [44]. The analysis shows the correlation between the predictive datasets for the propensity score for phase separation (using two independent predictive algorithms) (Figure 2 and Table 1). This analysis reveals that more than half of RTKs have at least one feature

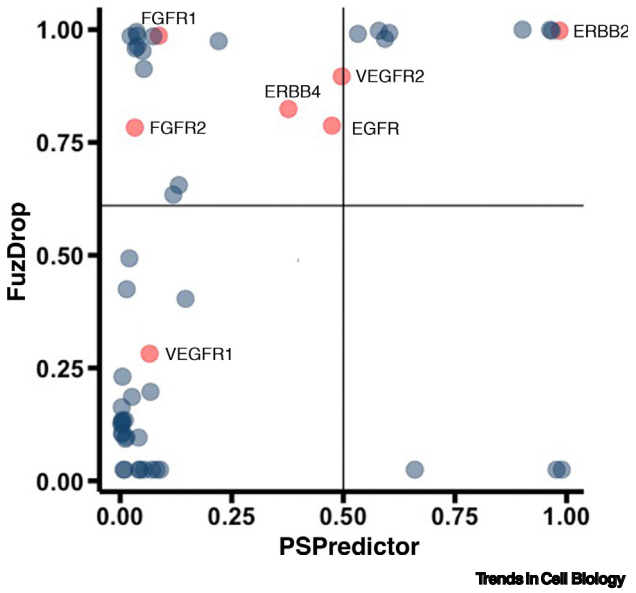

Figure 2. Phase separation potential of receptor tyrosine kinase (RTK) C terminal tails derived from software programs, PSPredictor and FuzDrop. C terminal tail sequences of RTKs were analysed using PSPredictor [43] or FuzDrop [44]. The probability scores are listed in Table 1 in the main text. Scores higher than 0.5 in PSPredictor or 0.61 in FuzDrop (represented by black lines) indicate the ability of RTKs to phase separate. RTKs in the top right-hand quadrant are predicted by both programs to phase separate. RTKs that have been experimentally shown to form condensates are highlighted in pink [45,50].

that is conducive to multivalent interactions required for LLPS. Eight RTKs score highly for both datasets. All but one of the RTKs that have to date been experimentally validated as forming LLPS condensates with downstream effector proteins, appear with a high score from at least one of the algorithms (i.e., EGFR, ERBB2, ERBB4, FGFR1, FGFR2, and VEGFR2 [45]), and all of these are implicated in cancer pathogenic signalling.

Evidence for RTK-mediated LLPS in cancer signalling

Phase separation has been described in several plasma membrane receptor systems such as TCR/LAT [46], Nephrin [47], WNT [48], and integrin [49]. All these LLPS systems are driven by the availability of multiple interaction sites on IDRs present on the receptors with a range of component downstream proteins. Despite the structural properties of RTKs being conducive to sustaining LLPS, there has been only limited investigation of phase separation based on this class of receptors.

The physiological importance of RTK-mediated LLPS at the plasma membrane was exemplified with FGFR2. FGFR2 forms a ternary complex with the phosphatase SHP2 and the phospholipase PLCγ1, maintained by specific binding events with a range of affinities which sustain assembly of phase separation into droplets both *in vitro* and in cells [45]. The impact of LLPS on RTK signal regulation is further underscored in the case of EGFR-mediated LLPS which modulates GRB2 recruitment and RAS signalling outcomes [50]. These two exemplar LLPS-mediating receptors have independently been shown to be involved in cancer-related signal transduction in numerous malignancies [51,52]. The insulin receptor also mediates the formation of dynamic protein clusters, and their physicomechanical features contribute to insulin resistance [53].

Chromosomal rearrangements found in cancers lead various RTKs to form fusion proteins. These oncogenic fusions can be found in either the N terminal or the CT of the RTK; in both cases, the fusion preserves the tyrosine kinase activity. The N terminal fusions lack the extracellular ligand binding and transmembrane domains, and therefore reside in the cytosol. Reported examples include anaplastic lymphoma kinase (ALK), C-Ros oncogene 1 (ROS1), neurotrophic tyrosine

Table 1. Phase separation potential of RTK C terminal tails derived from software programs, PSPredictor and FuzDrop

RTK	PSPredictor	FuzDrop	RTK	PSPredictor	FuzDrop
ALK	0.221	0.974	IGF1R	0.533	0.991
AXL	0.593	0.979	INSR	0.023	0.985
CSF1R	0.603	0.992	INSRR	0.660	0.025
DDR1	0.042	0.025	KIT	0.053	0.025
EGFR	0.474	0.787	LMTK1	0.901	1.000
EPHA1	0.005	0.135	LMTK2	0.963	1.000
EPHA10	0.004	0.163	LTK	0.037	0.995
EPHA2	0.003	0.125	MER	0.068	0.198
EPHA3	0.004	0.104	MET	0.132	0.655
EPHA4	0.041	0.096	MST1R	0.039	0.963
EPHA5	0.003	0.129	MUSK	0.071	0.025
EPHA6	0.014	0.097	PDGFRα	0.035	0.958
EPHA7	0.005	0.106	PDGFRβ	0.074	0.985
EPHA8	0.005	0.231	RET	0.015	0.425
EPHB1	0.005	0.132	RON	0.050	0.952
EPHB2	0.146	0.403	ROR1/ROR2	0.967	0.997
EPHB3	0.012	0.093	ROS	0.119	0.634
EPHB4	0.021	0.493	STYK	0.010	0.025
EPHB6	0.011	0.134	TIE1	0.082	0.025
ERBB2	0.984	0.998	TIE2	0.044	0.025
ERBB3	0.579	0.998	TRKA	0.089	0.025
ERBB4	0.377	0.824	TRKB	0.007	0.025
FGFR1	0.087	0.986	TYRO3	0.038	0.987
FGFR2	0.033	0.783	VEGFR1	0.066	0.282
FGFR3	0.977	0.025	VEGFR2	0.496	0.896
FGFR4	0.989	0.025	VEGFR3	0.053	0.913
FLT3	0.026	0.186			

kinase (NTRK), and rearranged during transfection (RET) receptors. The fusion partners are often proteins with coiled-coil oligomerisation domains which promote ligand-independent activation of the RTK kinase domain. This can lead to constitutive kinase activity of the RTK, which in turn can result in effector protein recruitment. Despite their cytosolic location several of these fusion proteins have been shown to separate into condensed phases and be involved in oncogenic signalling across multiple cancer subtypes. A good example is EML4-ALK, an oncogenic RTK fusion involved in human lung adenocarcinoma [19,54,55]. The presence of EML4-ALK condensates correlates with elevated ERK, PI3K/AKT, and STAT activity which are common downstream effectors in RTK signalling associated with oncogenic potential. This is underscored in a mouse xenograft model whereby tumours formed from an EML4-ALK phase-separation-deficient mutant are significantly smaller than those expressing the wild-type EML4-ALK fusion protein [54]. Condensation of the CCDC6-RET oncogenic fusion has also been identified in lung cancer [19].

While examples of N terminal fusions of RTKs have been shown to form condensates, a high proportion of structural rearrangement of RTK genes result in CT fusion proteins, whereby the

CT disordered regions are replaced by partner genes. These CT fusions will still function as a membrane-bound receptor, but are activated in a ligand-independent manner; CT fused RTKs can be dimerised/oligomerised through their fusion partners, resulting in constitutive autophosphorylation of the kinase domain and aberrant oncogenic activation of downstream signalling pathways. Examples are EGFR-RAD51 fusion protein, and FGFR fusions (e.g., FGFR2-CCDC6, FGFR2-CASP7, FGFR3-TACC3, and FGFR3-BAIAP2L1) where a kinase domain of the RTK fuses with the dimerised/oligomerised partner. In these proteins the RTKs lack their flexible CT tail that contains the IDRs and PTM sites for protein recruitment mediated by multivalent interactions. However, further investigation is required to assess how these C-terminally fused RTKs could also undergo phase separation through their fusion partners as a general mechanism for oncogenic RTK signalling.

A further cancer-related structural rearrangement of RTK genes results in CT truncation. How this might impact the ability of RTKs to phase separate remains to be assessed. However, given that the CT tail of an RTK is predicted to contribute to LLPS due to its capacity for multivalent interactions, its truncation would decrease the LLPS potential of RTKs. This might suggest that, in some cases, the loss of LLPS can drive cancer outcomes. An important example is the FGFR2 E18 truncation oncogenic mutants [56] which contain a severely truncated CT. Interestingly, while these types of truncation would predict a lower level of LLPS, and therefore of LLPS-dependent signalling, it would also, in theory, be more susceptible to small-molecule inhibitor binding, suggesting a potential connection between phase separation and small-molecule inhibitor pharmaceutic intervention.

How might phase separation augment cancer signalling pathways? The number of reported downstream effector enzymes and adaptor proteins involved in RTK-mediated LLPS is growing and currently includes a subset of prevalent oncoproteins (Table 2). Incorporation of these proteins within the LLPS state elevates their apparent concentrations proximal to the RTK and enhances both their ability to induce downstream signalling and their oncogenic potency.

Within the condensed phase, enzymes and substrates exist at elevated local concentrations which can enhance substrate turnover and amplify signals [64,65], as demonstrated by upregulation of FGFR2 transphosphorylation under condensate-forming conditions *in vitro* [45]. Formation of phase-separated condensates can also restrict the presence of molecules capable of

Table 2. Accessory proteins: proteins found in RTK condensates or in other transmembrane receptor systems that are known to have oncogenic potential

Gene symbol	Refs	Gene symbol	Refs
AKT1	[57]	PDK1	[57]
CNKSR1	[19]	PIK3CB	[19,57]
CNKSR3	[19]	PIK3R1	[19,57,60]
GAB1	[19]	PIK3R2	[55]
GRAP2	[46,58]	PLCG1	[19,45,58]
GRB2	[19,46,50,55,58,59,60]	PLCG2	[55]
IRS1	[57,60]	PTPN11	[19,45,63]
LAT	[46,58,59]	SHC1	[19,45]
LCK	[46]	SOS1	[19,46,50,55,58,59]
LCP2	[46]	WASL	[61,62]
NCK1	[46,58,61,62]	ZAP70	[46]

downregulating a signal. For example, GAP proteins, the inactivators of RAS, were not enriched in the ELM4-ALK condensates [19,54,55]. This mechanism has also been observed with the T cell receptor whereby the phosphatase CD45 is excluded from the LAT-mediated condensate [46].

Prediction of RTK-LLPS involvement in cancer

RTK-mediated signal transduction is implicated in the majority of cancer pathologies. Analysis indicated that >50% of RTKs exhibit features capable of sustaining LLPS (Figure 2). Since current available studies suggest a role of LLPS in the regulation of RTK-mediated signalling, it could be hypothesised that phase separation may play an important role in cancer. Based on this assumption, it might be possible to predict the extent of RTK-mediated LLPS in cancer signalling. To do this we need to address a number of key questions, one of which is: are patients more susceptible to forming LLPS as a result of elevated RTK and oncogenic accessory signalling protein gene copy number?

The expression level of RTKs and accessory proteins in various cancer types can be obtained from patient data derived from The Cancer Genome Atlas (TCGA) program (Figure S1 in the supplemental information online). It is proposed that coexpression of high levels of RTKs and downstream cytosolic proteins, which can be recruited to condensates and contribute to signalling, could be potential markers for LLPS-mediated cancer. Comparison of highly expressed RTKs from patient data with those with predicted high propensity to sustain LLPS (Figure S2 in the supplemental information online) suggests that RTK-mediated protein condensation at the plasma membrane could be prognostic of a subset of cancer signalling. For example, overexpression of ERBB2 has been demonstrated as an adverse prognostic factor in human breast cancer [66,67]. High expression levels of the adaptor proteins SHC1 and GRB2 also play an important role in breast cancer initiation and invasive progression [68,69], as well as in several other cancer types (Figure S1). In addition, ERBB2 scores highly on the phase-separation prediction software (Figure 2) and it has been demonstrated that ERBB2 and SHC form LLPS condensates *in vitro* [45]. Therefore, it can be predicted with some confidence that these proteins, which drive cancer progression, will phase separate in a subset of cancer cells (Figure S2). The resulting elevated local concentrations of these proteins will enhance cancer-related signal transduction. Another example is in oesophageal carcinoma, where a relatively high percentage of patients harbour high expression of EGFR which recruits GRB2 for downstream proliferative signalling. EGFR undergoes LLPS with GRB2 and can promote RAS/SOS signalling in an *in vitro* system [50].

The potential role of RTK-mediated LLPS in cancer could have a significant impact on the efficacy of small-molecule directed therapeutic development. RTKs and their downstream signalling partners represent a major class of targets for numerous tyrosine kinase inhibitors (TKIs). Despite significant progress using TKIs to target RTKs in cancer, substantial challenges have limited the development of efficacious therapies. Currently, it is not clear how LLPS might affect drug delivery and therapeutic response; however, it has been shown that some antineoplastic drugs can be concentrated in condensates via physicochemical interactions, whereas others can be excluded entirely [70]. Thus, specific compartmentalisation and small-molecule concentration in LLPS can influence the pharmacodynamic properties of RTK-mediated cancer treatment. Further examination of the ability to predict RTK-mediated LLPS in cancer patients might provide valuable input to future therapeutic intervention.

Concluding remarks

As many RTKs are directly involved in cancer development and progression, it will be essential to expand research to other RTKs and identify those capable of sustaining LLPS condensates and examine their impact on cancer progression. We should appreciate that this field of study is still in

Outstanding questions

How do RTK oncogenic mutations (e.g., mutation hotspots that lead to gain- or loss-of-function mutants, N-terminal and CT RTK fusions, CT truncations) affect LLPS?

What features distinguish cancer-related RTK-mediated LLPS?

Can the intrinsic features of RTK-mediated LLPS constitute a marker for specific cancers?

Can knowledge of LLPS propensity enable better therapeutic targeting in cancer patients?

Can we use the gene/protein expression analysis of individual cancer patients to identify novel prognostic markers and facilitate the development of personalised treatment by targeting LLPS?

its infancy, and there are many questions that need to be addressed to fill gaps in our knowledge (see Outstanding questions). For example, is LLPS the cause or the consequence of a given dysregulated, cancer-related RTK pathway? Can we correlate the degree of RTK LLPS condensates with cancer progression? Does the formation of LLPS condensates affect our current cancer treatment methods? What are the mechanisms of dissolving LLPS to terminate signalling? What is the efficiency of kinase/phosphatase-mediated RTK phosphotyrosine turnover and complex dissociation in an LLPS environment? How does LLPS connect to RTK endocytosis and internalisation of ligand–RTK complexes to lysosomes for degradation? Would it be possible to target specific LLPS condensates to treat cancer?

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Declaration of interests

The authors have no competing interests to declare.

Supplemental information

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Resources

[i]www.pkumdl.cn:8000/PSPredictor/

[ii]https://fuzdrop.bio.unipd.it/predictor

References

1. Ahmed, Z. *et al.* (2010) Direct binding of Grb2 SH3 domain to FGFR2 regulates SHP2 function. *Cell. Signal.* 22, 23–33
2. Ahmed, Z. *et al.* (2013) Grb2 controls phosphorylation of FGFR2 by inhibiting receptor kinase and Shp2 phosphatase activity. *J. Cell Biol.* 200, 493–504
3. Lin, C.-C. *et al.* (2012) Inhibition of basal FGF receptor signaling by dimeric Grb2. *Cell* 149, 1514–1524
4. Yao, Z. *et al.* (2017) A global analysis of the receptor tyrosine kinase–protein phosphatase interactome. *Mol. Cell* 65, 347–360
5. Yao, Z. and Stagljar, I. (2017) Multiple functions of protein phosphatases in receptor tyrosine kinase signaling revealed by interactome analysis. *Mol. Cell Oncol.* 4, e1297101
6. Hedger, G. *et al.* (2015) The juxtamembrane regions of human receptor tyrosine kinases exhibit conserved interaction sites with anionic lipids. *Sci. Rep.* 5, 9198
7. Rohwedder, A. *et al.* (2021) Composition of receptor tyrosine kinase-mediated lipid micro-domains controlled by adaptor protein interaction. *Sci. Rep.* 11, 6160
8. Auciello, G. *et al.* (2013) Regulation of fibroblast growth factor receptor signalling and trafficking by Src and Eps8. *J. Cell Sci.* 126, 613–624
9. Abou-Fayçal, C. *et al.* (2017) Splice variants of the RTK family: their role in tumour progression and response to targeted therapy. *Int. J. Mol. Sci.* 18, 383
10. Holzmann, K. *et al.* (2012) Alternative splicing of fibroblast growth factor receptor IgIII loops in cancer. *J. Nucleic Acids* 2012, 950508
11. Seiler, C. *et al.* (2022) The Grb2 splice variant, Grb3-3, is a negative regulator of RAS activation. *Commun. Biol.* 5, 1029
12. Casaletto, J.B. and McClatchey, A.I. (2012) Spatial regulation of receptor tyrosine kinases in development and cancer. *Nat. Rev. Cancer* 12, 387–400
13. Alberti, S. *et al.* (2019) Considerations and challenges in studying liquid–liquid phase separation and biomolecular condensates. *Cell* 176, 419–434
14. Joshi, A. and Mukhopadhyay, S. (2023) Biophysics of biomolecular condensates. *Biophys. J.* 122, 737–740
15. Hazra, M.K. and Levy, Y. (2021) Biophysics of phase separation of disordered proteins is governed by balance between short- and long-range interactions. *J. Phys. Chem. B* 125, 2202–2211
16. Yoshizawa, T. *et al.* (2020) Biological phase separation: cell biology meets biophysics. *Biophys. Rev.* 12, 519–539
17. Chong, P.A. and Forman-Kay, J.D. (2016) Liquid–liquid phase separation in cellular signaling systems. *Curr. Opin. Struct. Biol.* 41, 180–186
18. Case, L.B. *et al.* (2019) Regulation of transmembrane signaling by phase separation. *Annu. Rev. Biophys.* 48, 465–494
19. Tulpule, A. *et al.* (2021) Kinase-mediated RAS signaling via membraneless cytoplasmic protein granules. *Cell* 184, 2649–2664.e18
20. Boeynaems, S. *et al.* (2018) Protein phase separation: a new phase in cell biology. *Trends Cell Biol.* 28, 420–435
21. Alberti, S. and Dormann, D. (2019) Liquid–liquid phase separation in disease. *Annu. Rev. Genet.* 53, 171–194
22. Jiang, S. *et al.* (2020) Protein phase separation and its role in tumorigenesis. *eLife* 9, e60264
23. Spannl, S. *et al.* (2019) Biomolecular condensates in neurodegeneration and cancer. *Traffic* 20, 890–911
24. Wang, B. *et al.* (2021) Liquid–liquid phase separation in human health and diseases. *Signal Transduct. Target. Ther.* 6, 290
25. Taniue, K. and Akimitsu, N. (2022) Aberrant phase separation and cancer. *FEBS J.* 289, 17–39
26. Verdile, V. *et al.* (2019) Aberrant phase transitions: side effects and novel therapeutic strategies in human disease. *Front. Genet.* 10, 173
27. Shin, Y. and Brangwynne, C.P. (2017) Liquid phase condensation in cell physiology and disease. *Science* 357, eaaf4382
28. Cai, D. *et al.* (2021) Biomolecular condensates and their links to cancer progression. *Trends Biochem. Sci.* 46, 535–549
29. Ladbury, J.E. *et al.* (2023) Phase separation enhances probability of receptor signalling and drug targeting. *Trends Biochem. Sci.* 48, 428–436
30. Dignon, G.L. *et al.* (2020) Biomolecular phase separation: from molecular driving forces to macroscopic properties. *Annu. Rev. Phys. Chem.* 71, 53–75

31. Tsang, B. *et al.* (2020) Phase separation as a missing mechanism for interpretation of disease mutations. *Cell* 183, 1742–1756
32. Hofweber, M. and Dormann, D. (2019) Friend or foe – post-translational modifications as regulators of phase separation and RNP granule dynamics. *J. Biol. Chem.* 294, 7137–7150
33. Owen, I. and Shewmaker, F. (2019) The role of post-translational modifications in the phase transitions of intrinsically disordered proteins. *Int. J. Mol. Sci.* 20, 5501
34. Snead, W.T. and Gladfelter, A.S. (2019) The control centers of biomolecular phase separation: how membrane surfaces, PTMs, and active processes regulate condensation. *Mol. Cell* 76, 295–305
35. Li, J. *et al.* (2022) Post-translational modifications in liquid–liquid phase separation: a comprehensive review. *Mol. Biomed.* 3, 13
36. Du, Z. and Lovly, C.M. (2018) Mechanisms of receptor tyrosine kinase activation in cancer. *Mol. Cancer* 17, 58
37. Hastings, J.F. *et al.* (2019) The extracellular matrix as a key regulator of intracellular signalling networks. *Br. J. Pharmacol.* 176, 82–92
38. Schlessinger, J. (2000) Cell signaling by receptor tyrosine kinases. *Cell* 103, 211–225
39. Sezgin, E. *et al.* (2017) The mystery of membrane organization: composition, regulation and roles of lipid rafts. *Nat. Rev. Mol. Cell Biol.* 18, 361–374
40. Nikolov, D.B. *et al.* (2013) Eph/ephrin recognition and the role of Eph/ephrin clusters in signaling initiation. *Biochim. Biophys. Acta* 1834, 2160–2165
41. Ojosnegros, S. *et al.* (2017) Eph-ephrin signaling modulated by polymerization and condensation of receptors. *Proc. Natl. Acad. Sci. U. S. A.* 114, 13188–13193
42. Li, S.S. (2005) Specificity and versatility of SH3 and other proline-recognition domains: structural basis and implications for cellular signal transduction. *Biochem. J.* 390, 641–653
43. Chu, X. *et al.* (2022) Prediction of liquid–liquid phase separating proteins using machine learning. *BMC Bioinforma.* 23, 72
44. Hardenberg, M. *et al.* (2020) Widespread occurrence of the droplet state of proteins in the human proteome. *Proc. Natl. Acad. Sci. U. S. A.* 117, 33254–33262
45. Lin, C.-C. *et al.* (2022) Receptor tyrosine kinases regulate signal transduction through a liquid–liquid phase separated state. *Mol. Cell* 82, 1089–1106.e12
46. Su, X. *et al.* (2016) Phase separation of signaling molecules promotes T cell receptor signal transduction. *Science* 352, 595–599
47. Case, L.B. *et al.* (2019) Stoichiometry controls activity of phase-separated clusters of actin signaling proteins. *Science* 363, 1093–1097
48. Schaefer, K.N. and Peifer, M. (2019) Wnt/beta-catenin signaling regulation and a role for biomolecular condensates. *Dev. Cell* 48, 429–444
49. Case, L.B. *et al.* (2022) Synergistic phase separation of two pathways promotes integrin clustering and nascent adhesion formation. *eLife* 11, e72588
50. Lin, C.W. *et al.* (2022) A two-component protein condensate of the EGFR cytoplasmic tail and Grb2 regulates Ras activation by SOS at the membrane. *Proc. Natl. Acad. Sci. U. S. A.* 119, e2122531119
51. Krook, M.A. *et al.* (2021) Fibroblast growth factor receptors in cancer: genetic alterations, diagnostics, therapeutic targets and mechanisms of resistance. *Br. J. Cancer* 124, 880–892
52. Sigismund, S. *et al.* (2017) Emerging functions of the EGFR in cancer. *Mol. Oncol.* 12, 3–20
53. Dall'Agnese, A. *et al.* (2022) The dynamic clustering of insulin receptor underlies its signaling and is disrupted in insulin resistance. *Nat. Commun.* 13, 7522
54. Qin, Z. *et al.* (2021) Phase separation of EML4-ALK in firing downstream signaling and promoting lung tumorigenesis. *Cell Discov.* 7, 33
55. Sampson, J. *et al.* (2021) Phase-separated foci of EML4-ALK facilitate signalling and depend upon an active kinase conformation. *EMBO Rep.* 22, e53693
56. Zingg, D. *et al.* (2022) Truncated FGFR2 is a clinically actionable oncogene in multiple cancers. *Nature* 608, 609–617
57. Zhou, K. *et al.* (2022) Spatiotemporal regulation of insulin signaling by liquid–liquid phase separation. *Cell Discov.* 8, 64
58. Zeng, L. *et al.* (2021) PLCγ1 promotes phase separation of T cell signaling components. *J. Cell Biol.* 220, e202009154
59. Huang, W.Y.C. *et al.* (2019) A molecular assembly phase transition and kinetic proofreading modulate Ras activation by SOS. *Science* 363, 1098–1103
60. Gao, X.K. *et al.* (2022) Phase separation of insulin receptor substrate 1 drives the formation of insulin/IGF-1 signalosomes. *Cell Discov.* 8, 60
61. Banjade, S. and Rosen, M.K. (2014) Phase transitions of multivalent proteins can promote clustering of membrane receptors. *eLife* 3, e04123
62. Li, P. *et al.* (2012) Phase transitions in the assembly of multivalent signalling proteins. *Nature* 483, 336–430
63. Zhu, G. *et al.* (2020) Phase separation of disease-associated SHP2 mutants underlies MAPK hyperactivation. *Cell* 183, 490–502.e18
64. O'Flynn, B.G. and Mittag, T. (2021) The role of liquid–liquid phase separation in regulating enzyme activity. *Curr. Opin. Cell Biol.* 69, 70–79
65. Peeples, W. and Rosen, M.K. (2021) Mechanistic dissection of increased enzymatic rate in a phase-separated compartment. *Nat. Chem. Biol.* 17, 693–702
66. Révillion, F. *et al.* (1998) ERBB2 oncogene in human breast cancer and its clinical significance. *Eur. J. Cancer* 34, 791–808
67. Yu, D. and Hung, M.C. (2000) Overexpression of ErbB2 in cancer and ErbB2-targeting strategies. *Oncogene* 19, 6115–6121
68. Haines, E. *et al.* (2014) The adaptor proteins p66Shc and Grb2 regulate the activation of the GTPases ARF1 and ARF6 in invasive breast cancer cells. *J. Biol. Chem.* 289, 5687–5703
69. Wright, K.D. *et al.* (2019) The p52 isoform of SHC1 is a key driver of breast cancer initiation. *Breast Cancer Res.* 21, 74
70. Klein, I.A. *et al.* (2020) Partitioning of cancer therapeutics in nuclear condensates. *Science* 368, 1386–1392