

Protein Phase Separation and Regulatory Mechanisms of Transcriptional Programming in Tumor Microenvironment in Stress Response

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Abstract. Malignant tumor evolution is governed by its symbiotic relationship with the tumor microenvironment, which features steep oxygen and acidity gradients that drive malignant progression. Traditional biochemical frameworks fail to adequately explain the rapid, coordinated genomic alterations under such stresses. This review focuses on liquid-liquid phase separation (LLPS), which acts as a biophysical switch regulating transcriptional programming. The study demonstrates that tumor microenvironmental stress directly modulates the phase behavior of intrinsically disordered proteins, forming transcriptional condensates. These condensates partition transcriptional machinery, condense super-enhancer regions, and amplify oncogenic expression. Key factors analyzed include c-Myc and YAP, establishing a physical environment–LLPS–transcriptional program axis that mediates tumor adaptation. Additionally, condensed-state physical properties induce non-genetic heterogeneity and chemotherapy resistance. This work introduces a novel therapeutic strategy combining molecular antagonism with physical state intervention, offering new approaches for previously intractable transcriptional drivers. It also underscores the value of measuring protein aggregate dynamics in improving prognosis for advanced malignancies.

Keywords: LLPS, tumor microenvironment, transcriptional condensates, malignant tumor evolution.

1. Introduction

Essentially, tumor progression relies on the formation of a supportive TME, which is shaped by sustained interactions between malignant cells and surrounding biological niches. Recent studies have proven the TME is not a passive growth environment, but a dynamic ecosystem driven by distinct physical and chemical fields, typified by chronic hypoxia, severe local acidosis and elevated interstitial fluid pressure[1]. Classical molecular theories define signal transduction as a rigid ligand-receptor lock-and-key mechanism and linear kinase cascades, yet this framework fails to explain how thousands of gene loci are rapidly activated synchronously for transcriptional reprogramming under stress, highlighting an urgent need for a unified biophysical mechanism linking macroenvironmental changes to microgenomic regulation [2].

Global research has increasingly focused on membrane-less organelles formed via Liquid-Liquid Phase Separation (LLPS), which describes the spontaneous assembly of biomolecules into liquid-like condensates in dilute solutions. These nuclear transcriptional condensates enable efficient, specific biochemical reactions, clustering transcription factors, coactivators and RNA polymerase II at genomic loci to boost gene expression; relevant studies have also explored their roles in disease-related transcriptional enhancement [3,4]. However, the specific TME triggers driving pathological LLPS and subsequent oncogenic transcriptional alterations remain poorly studied.

Multivalent weak interactions between proteins with intrinsically disordered regions (IDRs) – which lack fixed 3D structures but provide dynamic network elasticity – control condensate regulatory logic [5]. Briefly, this study systematically explores TME physicochemical stress-triggered protein phase transition and its transcriptional regulatory effects, verifying a brand-new tumor adaptation mechanism beyond traditional genetic mutation theories [6].

2. Physicochemical triggers of phase separation in the TME

2.1. Acidosis-dependent modulation of LLPS

There exists some evidence that an acidosis-dependent modulation of LLPS takes place. Acidic conditions of the TME constitute one of the most widespread stressors that the cancerous cells have to conquer to survive. The pH levels in solid tumors often plummet to a range of 6.5 to 6.8 because of the high rate of glycolysis and the deficient lymphatic drainage. This paper will examine the operation of these pH changes as a direct biophysical switch to control the distribution of charges of proteins [1]. Most of the transcriptional controllers have intrinsically disordered regions (IDRs) that are highly concentrated in acid-base delicate residues like histidine. The increase of protons leads to the reduction of the net charge of these residues, which in turn reduces intermolecular repulsion, which charges the electrostatic profile of the protein fundamentally. This electrostatic re-orientation, in a sense, reduces the concentration of these proteins before the coalescence into liquid droplets. Thus, the fusion point of LLPS decreases to make it possible to construct condensates quickly even with lower protein concentrations.

Histidine residues are highly sensitive to subtle pH fluctuations, acting as molecular pumps for tumor cells to detect environmental acidification. This protonation triggers the electrostatic network reorganization of IDRs, and can either promote or inhibit droplet formation based on sequence composition. Experiments have verified that acidic environments can induce tumor suppressive proteins to form condensates and inactivate their functions, and these pH-sensitive phase boundaries need to be quantified to clarify how tumors evade normal regulatory checkpoints via physical chemistry. Additionally, locally enriched ions in the TME can further stabilize these condensates, representing a rapid tumor cell stress adaptation response that is much faster than de novo protein synthesis.

2.2. Hypoxia, ATP depletion and LLPS

The other typical feature of the TME is hypoxia, which significantly affects the biophysics and metabolism of cells. When oxygen is in short supply, the ATP generated metabolically is minimal and this has extensive implications outside of the mere lack of energy. ATP acts as a cytoplasmic and nuclear biological hydrotrope that maintains hydrophobic proteins at millimolar levels [7]. This study examines whether the ATP depletion due to hypoxic stress is a precipitant to protein phase separation. On dropping below a given critical level, it is unable to prevent the aggregation of IDR-containing proteins. This leads to a shortage of ATP synthesis, which provokes the onset of a biophysical shift leading to the formation of stress-sensitive transcriptional hubs to steer through hypoxic stress.

High-affinity transcriptional hubs become stabilized by hypoxia because of reduced hydro-solubility as well as induced stress signaling. Reduction of ATP causes a decrease in the entropic penalty of protein aggregation, which favors the formation of dense liquid phases. This is usually accompanied by recruiting Hypoxia Inducible Factors into such heavy droplets to produce as much transcription as possible. These assemblies are further stabilized by the synergistic effect of reduced PH and low ATP, forming a strong physical shield exploiting the cancer cell. That is how this mechanism underlies the tendency of hypoxic portions of tumors to become highly resistant to common treatment options and demonstrate a significant degree of plasticity. The low ATP-induced biophysical transition conditions effectively convert the nucleus into a highly compartmentalized environment with a bias toward survival genes.

2.3. Molecular crowding and mechanical forces

Chemical gradients are not only seen in the TME, but there also exists considerable molecular crowding and interstitial pressure. The presence of high levels of macromolecules, including collagen and hyaluronic acid, in the extracellular matrix conditions the ambience and restricts the necessary volume in which the cellular processes take place. This paper discusses the effect of the excluded volume effect on increasing the propensity of proteins to phase separate. Inside the cell, the rise of

the protein and nucleic acid density of stress facilitates the multivalent interactions. The interactions are regulated by short linear motifs and aromatic residues present in the disordered regions of transcriptional co-activators. The crowding effect reduces the phase transition critical concentration, and the cell becomes hypersensitive to the slightest change in the environmental conditions.

The mechanical forces that are passed on by the tumor stroma to the stiffened nucleus are also very important in the regulation of LLPS. This paper examines the effect of the use of pressure to cause the deformation of the nuclei and therefore change the density of the chromatin and the proteins that are associated with it. The resultant rearrangements in molecular crowding can initiate the collection of condensates to certain genomic sites. Mechanosensitive transcription factors are usually recruited to these droplets caused by pressure. The cell provides a universal integrator of TME information by incorporating mechanical and chemical inputs into it via the use of LLPS. The school of thought places the emphasis on the signaling pathways of an individual cell on the overall corporeal view of the cell interior. This is because of the strength of these condensates under pressure that guarantees the stability of transcriptional programs amid the turbulent mechanical environment of the growing tumor.

3. Functional principles of transcriptional condensates

3.1. Compartmentalization and molecular diffusion kinetics

These described experimental approaches were compartmentalization and Molecular Diffusion Kinetics. Transcriptional condensates form a novel and separate microenvironment, radically changing the dynamics of gene expression. Local concentration of transcription factors and co-activators can be increased by several orders of magnitude in these droplets over a surrounding nucleoplasm container [3]. This work determines the effects of such compartmentalization on the rate of assembly of the pre-initiation complex. The time spent on molecules to randomly search and locate the target DNA sequences is shortened significantly by having the requisites in only one physical site in the form of stochastic diffusion. Cis diffusion kinetics of the condensate are very high in fluidity and exchange. The high dynamics are permitting the transcriptional outputs to assimilate environmental signals rapidly.

The selectivity in the permeability of these condensates will guarantee that particular regulatory proteins are recruited and rest denied. The presence of this exclusion of the inhibitory factors or competing enzymes also increases the effectiveness of the transcriptional process. It concludes that the viscosity of such droplets is a critical variable that determines the rate of molecular movement and reaction kinetics. The physical characteristics of these condensates are frequently modified such that the exchange rates in cancer cells can be even faster. This kinetic benefit enables the oncogenes to be upregulated quickly to changing TME conditions. The fact that the condensate serves as a specific filter is a more advanced form of gene regulation. Use of diffusion coefficient measurements in these droplets gives a new parameter to measure the transcriptional state of the tumor.

3.2. Super enhancer physical collapse and chromatin topology

Another vital regulatory role of transcriptional condensates is the physical restructuring of topographical chromatin on the scale of mesoscopes. Large clusters of enhancers are termed super enhancers, and they play a critical role in the process of cell identity maintenance and occurring oncogenic expression [8]. This study reveals that co-activator phase segregation at super enhancers generates sufficient physical force to reshape local chromatin structures, pulling distal enhancer elements close to promoters and establishing a stable, highly active transcriptional state with durable, robust output. Comparative analyses show super enhancers have a far lower phase transition threshold than ordinary enhancers under LLPS conditions. The surface tension and capillary forces of liquid droplets drive chromatin physical remodeling, overcoming DNA polymer elastic repulsion to form compact, highly transcriptionally active structures; this topological alteration is a core feature of pathological oncogene (e.g., c-Myc) activation, and the stability of these remodeled chromatin

structures sustains oncogenic transcriptional programs even after the initial stimulus fades. This study also demonstrates that chromatin acts not only as an information carrier but also a mechanical component of the transcriptional machinery, with protein phase states directly determining genome spatial organization, and disrupting related biophysical forces can alter cancer cell hereditary regulatory circuits.

3.3. Dynamic feedback and RNA-mediated regulation

Transcriptional condensates are regulated by a complex feedback mechanism between the nascent RNA transcripts and transcriptional condensates. This paper examines the nature of RNA as a building block and as a regulatory ligand that can be used to regulate phase behavior [4]. When the concentration of RNA is high, it may facilitate the breaking of condensates by raising the negative charge and creating a repulsive force. This negative feedback mechanism is used to maintain transcriptional activity within a physiological range so as to avoid excess production. However, in tumour cells, these feedback loops are impaired, causing abnormally large and stable condensates. Such pathological assemblies cause the oncogenes keep on being expressed, which adds to the malignant phenotype.

The association among RNA-binding proteins and transcriptional machines in the condensate produces a very sensitive regulatory node. The study shows how a possible mutation in these proteins may alter the RNA-sensing capacity of the condensate. The loss of the capacity to dissolve condensates in response to elevated levels of RNA results in a runaway transcriptional program when it occurs in the cell. This is one of the imperative aspects of the dysregulation of gene expression in metastatic cancers. Moreover, tumor cells incorporate specific non-coding RNAs in the TME and use them as a platform to assemble *de novo* condensates. This finding hints at the possibility of the external genetic material manipulating the actual physical state of the nucleus. RNA biology can be incorporated into the LLPS framework to give a clearer understanding of the regulation of genes.

4. Case study analysis of c-Myc and YAP condensation

The overall concepts of the regulation by LLPS are demonstrated by the activity of such important oncogenic drivers as c-Myc and YAP. The c-Myc protein is characterized by its capacity to control the programs of transcription in general and the activation domain is intrinsically disordered and is highly vulnerable to phase separation [9]. The analysis reveals that in the TME stress-induced environment, c-Myc is aggregated into high-mobility formations that permit it to quickly translocate to other sites in the genome as the conditions vary. This great motility is critical to allow the tumor to travel through the heterogeneous environment of the solid tumor bulge. Fluorescence recovery after photobleaching is then used in the paper to illustrate that these droplets do not lose much of the liquid-like fluidity. This enables c-Myc to traverse the genome and express pro-survival genes with a high degree of efficiency.

On the same note, the YAP protein reacts to the tumor stiffness of the stroma through the creation of nuclear condensates. These YAP droplets have the capability of reconciling the TEAD transcription factors as well as circumventing the conventional antagonistic signaling of the Hippo pathway. Continued growth signaling under the influence of tumor suppressive signals is possible in this physical bypass process. Mechanical tension of the cytoskeleton, in particular, is sensitive to the condensation of YAP. This correlation of extracellular rigidity and nuclear partitioning will give direct mechanical routes of tumor progression. The c-Myc and YAP case studies give an actual connection between the biophysical phase transitions and classical oncogenic signaling pathways. These observations indicate that mutational status, together with the physical state of such proteins, holds equal significance in clinical prognosis.

5. Non-genetic heterogeneity and evolution of resistance

Such flexibility of the LLPS mechanism makes its contribution to the non-genetic heterogeneity of clinical tumor samples [6]. In contrast to genetic mutations, which are quite long-lasting, physical state changes are reversible and highly responsive to localized micro-environmental gradients. This paper will examine how changes in pH and oxygen throughout the tumor tissue would lead to a diverse topography of transcriptional condensates. The cells can be in a highly condensed form that brings about dormant survival and some might be in a liquid-like form that facilitates high proliferation of the cells. This physical plasticity enables the tumor population to withstand various selective forces, such as chemotherapy and radiation-induced forces.

When a drug induces the death of most cells in a given transcriptional state, the population that has survived can readily change its biophysical profile to respond. It is emphasized in this study that resistance does not necessarily occur after a DNA damage sheath and a genetic selection mechanism, but as a consequence of biophysical adaptation. The effective concentration of drugs at the target sites can also be minimized by partitioning the drugs into the same condensates [10]. To give an instance, some molecules that are hydrophobic are isolated in the dense phase, and they cannot interrelate with their preferred receptors. This is a new mechanism of drug resistance that is independent of efflux pumps and is a physical sequestration mechanism. It is important to know the regulations that can be applied when it comes to these transitions to formulate measures that can be used to ensure that multidrug resistance does not develop. Physical tumor landscape is therefore a significant factor that has contributed to the therapeutic failure.

6. Conclusion

This paper establishes a complete biophysical model clarifying how protein phase transitions drive transcriptional reprogramming in response to tumor microenvironment (TME) stress. It demonstrates that pathological transcriptional condensates are formed via the extreme physicochemical gradients in the TME, mainly localized acidosis and chronic hypoxia. As highly efficient membrane-less micro-reactors independent of classical signaling cascades, these condensates compartmentalize transcriptional machinery to reshape chromatin topology and drastically amplify oncogenic signals. Further analysis of core drivers including c-Myc and YAP, reveals that Liquid-Liquid Phase Separation (LLPS) endows cancer cells with mechanical and chemical plasticity to bypass conventional regulatory constraints. Notably, this dynamic biophysical state underpins non-genetic heterogeneity and adaptive therapy resistance in advanced malignancies. This research redirects precision oncology toward biophysical state regulation, offering a novel direction for clinical development. Future clinical strategies should target the physical dynamics of such condensates, providing a feasible paradigm to overcome drug resistance in previously untreatable cancer pathways.

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