


Regulation of tumorigenic splicing by protein condensates with specific biophysical properties

Wei Li and Hao Jiang 

Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, UVA Cancer Center, Charlottesville, VA, USA

ABSTRACT

The role of biophysical properties of protein condensates in regulating gene expression and tumorigenesis remains unclear. We recently discovered that A-kinase anchoring protein 8 (AKAP8, also known as AKAP95), a RNA splicing regulator, supports tumorigenesis by forming liquid-like condensates, and that perturbing the biophysical properties of the condensates impairs its activity in regulating splicing and tumorigenesis.

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Cancer arises from genetic alterations that almost always elicit major reprogramming of gene expression. Moreover, cancer cells can also become exceptionally dependent on the altered gene expression programs to cope with stresses not encountered in normal cells. It remains largely unclear how gene expression is spatiotemporally controlled in normal or cancer cells to meet biological needs.

It is now established that liquid–liquid phase separation is a fundamental principle in organizing cellular space and biochemistry,^{1,2} including the spatiotemporal regulation of gene expression.^{3–5} While the impact of phase separation on human health is well established in neurodegenerative diseases,² very few studies have directly demonstrated if phase separation underlies cancer development, especially through control of gene expression.⁶ Biomolecular condensates formed by phase separation are known to adopt a gradient of different material properties ranging from liquid-like to more solid-like states with different molecular dynamics,¹ but our understanding of the consequences of the different material properties in health is again rather limited to neurodegenerative diseases involving pathological protein aggregates. It has not been demonstrated that the varying material properties, including liquidity and dynamicity, may have functional impact on gene regulation with biological consequences in a major disease type such as cancer.

Our previous studies identified A-kinase anchoring protein 8 (AKAP8, also known as AKAP95), a nuclear zinc-finger protein, as a novel factor that integrates regulation of transcription and RNA splicing.^{7,8} In our following studies of its physiological roles, we showed that AKAP95 overexpression is associated with human cancer.⁹ AKAP95 directly binds to a number of cancer-related gene transcripts and facilitates their splicing to ensure proper expression of these genes. Using cultured cancer cells and generating a knockout mouse model, we showed that AKAP95 is dispensable for normal cell and animal physiology but important for tumorigenesis

through promoting cell proliferation and overcoming the senescence barrier to cancer. Compared to normal cells, the survival and growth of cancer cells appear to be particularly dependent on AKAP95-mediated splice regulation. AKAP95 may thus be a good target for cancer treatment. We are currently studying how AKAP95 inhibition may affect tumorigenesis in more physiologically relevant cancer models.

We were most interested in the fundamental properties of AKAP95 that underlie its activities in splice and cancer regulation. Intrigued by its propensity in self-association, we took a biochemical approach by studying the purified AKAP95 protein and its truncation fragments. These studies showed that AKAP95 forms dynamic and liquid-like condensates *in vitro*, suggesting its phase separation property. AKAP95 also forms dynamic foci in the cell nucleus. Mutations of key amino acids (tyrosine to alanine or serine) abolish AKAP95 condensation *in vitro* and *in vivo*, and also inactivates AKAP95 in regulating reporter splicing. Moreover, chimeric proteins that replaced the key domains of AKAP95 with condensation-domains from irrelevant proteins can often retain splice regulation activity. Therefore, the condensation property of AKAP95 is crucial for its activity in splice regulation. Most interestingly, we identified a mutation (tyrosine to phenylalanine) that enhances AKAP95 condensation but surprisingly also significantly impairs its splicing activity. Our detailed analysis shows that this mutant has enhanced propensity to phase separate, but the condensates formed by this mutant have altered material properties in that they are less liquid-like, with reduced molecular dynamics and diffusion rate, both *in vitro* and in the cell nucleus (Figure 1). The reduced molecular diffusion thus conceivably reduces efficiency of molecular interactions involved in splice reaction, thereby reducing splicing reaction rate. Finally, we showed that the mutants that disrupt AKAP95 condensates abolish, and the mutant that solidifies AKAP95 condensates significantly impairs, its

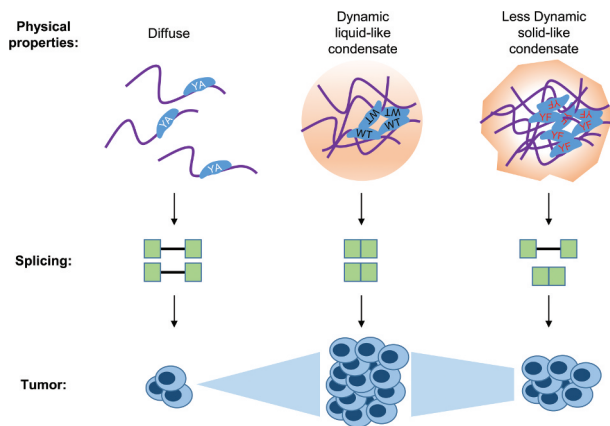


Figure 1. Regulation of splicing and tumorigenesis by AKAP95 molecules in different biophysical states. A-kinase anchoring protein 8 (AKAP8, also known as AKAP95) and its associated RNA (purple thread) are normally in phase-separated and liquid-like condensates with high molecular dynamics, as shown in the middle for the wild type (WT) AKAP95. These condensates support efficient pre-mRNA splicing reactions, which are important for tumorigenesis initiated by oncogenic processes. The tyrosine to alanine mutant (YA) of AKAP95, shown on the left, loses ability in phase separation and thus unable to support splicing for tumorigenesis. The tyrosine to phenylalanine mutant (YF) of AKAP95, on the right, forms condensates with in a more solid-like state with reduced molecular dynamics, and thereby reducing the efficiency of splicing and tumorigenesis.

activity in regulating endogenous gene expression and splicing as well as tumorigenesis (Figure 1).⁹

Our work thus experimentally demonstrates a crucial role of not only forming condensates but also a previously unrecognized role of material properties of the condensates, in cancer. Our work highlights that, instead of two simple states (diffuse or condensate) that act as an on/off switch for activity, a gene regulator can be in a gradient of varying condensate material states, in which only a proper window confers molecular activity in gene regulation and biological function in cancer (Figure 1). It is of general interest if we can tune the material properties of condensates to produce a biological outcome. Our results raise the possibility that perturbing the material properties of key regulators out of an appropriate window in either direction, i.e., disrupting or hardening condensates, may open an unconventional avenue for cancer intervention. Our work should prompt us to pay attention to condensate-hardening molecules too, which would be otherwise neglected since previous studies have mainly shown that aggregates are bad in neurodegenerative diseases. More studies will be needed to understand how the quantitative variations of the material properties of protein condensates regulate gene expression and disease.

Disclosure of potential conflict of interest

No potential conflicts of interest were disclosed.

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ORCID

Hao Jiang  <http://orcid.org/0000-0003-1890-9813>

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