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Research Advance

Multifaceted function of YAP/TEAD on chromatin: *prospects of 'A non-canonical role of YAP/TEAD is required for activation of estrogen-regulated enhancers in breast cancer'*

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Cellular function and behavior are controlled by various signals during development and disease progression in mammals. It is important to understand how signals ultimately alter the function and structure of DNA regulatory elements, especially enhancers, causing the changes of gene expression patterns. On average, each mature human cell harbors at least ~100000 enhancers, but only a small percent of them have the function to activate gene expression. For instance, only ~12%–18% of ERα-bound enhancers are active enhancers. How enhancers become functionally active is critical for our overall understanding of enhancer biology and gene regulation. Enhancers are recognized by signaling-dependent DNA binding transcription factors (TFs), resulting in the recruitment of different enhancer activation components that allow distal enhancers to interact with their target promoters through chromatin looping (Plank and Dean, 2014). Increasing evidence indicates that the recruitment and switching of enhancer components underlie enhancer activation and the deregulation in enhancer component or architecture profoundly alters signal-regulated transcriptional machineries, leading to developmental defects or diseases.

In a recent study, we used ERα-bound enhancers as a model signaling-regulated enhancer system to understand the molecular mechanisms for enhancer activation, particularly focusing on TF/TF or TF/cofactor interaction on enhancers (Zhu et al., 2019). ERα is a ligand-dependent nuclear receptor that binds to estrogen response element (ERE) on enhancers to regulate enhancer activity (Carroll et al., 2006; Li et al., 2013). We have previously identified a category of ERα TF 'co-activators', termed as MegaTrans TFs (Liu et al., 2014). To capture additional ERα co-regulators on enhancers, including the weak and transient cofactors, we applied the BioID technique in MCF7 breast cancer cells and identified YAP1 and TEAD4, the two key effectors of Hippo pathway, as novel ERα cofactors. Our ChIP-seq data indicated that YAP1 and TEAD4 co-occupied ERα active enhancers in addition to the canonical DNA regulatory elements associated with the Hippo pathway targets. This non-canonical binding on ERα enhancers was elevated in response to E_2 treatment and was critical for the regulation of E_2-induced transcription and breast cancer cell growth. Loss of YAP1 or TEAD4 greatly abolished the E_2-induced eRNA transcription from YAP1/TEAD4/ERα co-bound enhancers, indicating their important role in enhancer activation. Our mechanistic studies revealed that YAP1 and TEAD4 facilitate the recruitment of enhancer activation machinery component MED1. Furthermore, we found that the binding of TEAD4 to ERα enhancers is mediated by ERα but independent of its DNA binding activity. Thus, our work provides the first evidence that Hippo and ER signaling pathways crosstalk at the chromatin level and converge on enhancers to control gene expression, highlighting a non-canonical function of YAP1 and TEAD4 in enhancer activation with potential therapeutic implications (Figure 1).

However, it remains unclear how YAP/TEAD is selectively recruited in trans to the active ERα enhancers. It has been previously speculated that the dual roles of the pioneer factor FOXA1, which is selectively recruited to the active enhancers and required for the binding of ERα. In addition, binding of FOXA1 may cause a conformational change in enhancer DNA architecture, which facilitates the recruitment of the MegaTrans complex. Future investigation

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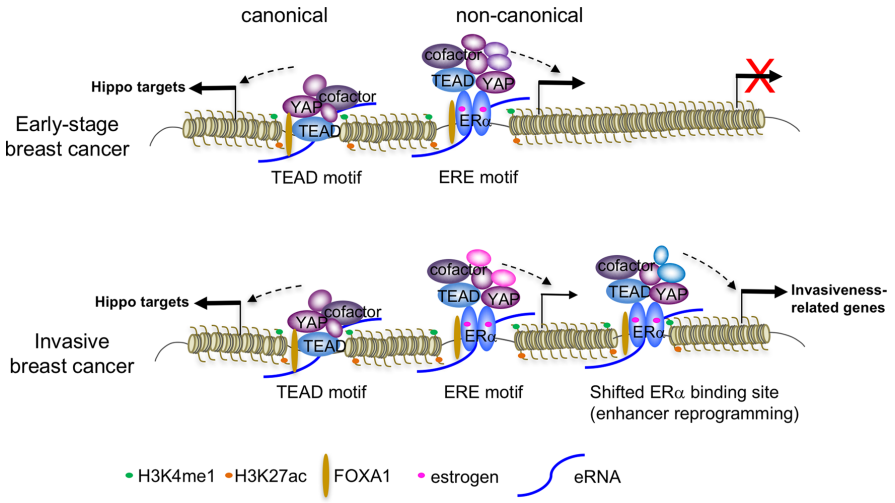

Figure 1 Illustration of the proposed working model. The canonical role of YAP/TEAD is well known to regulate Hippo targets. Our recent studies identified a non-canonical role of YAP/TEAD (Zhu et al., 2019), which is required for the activation of estrogen-regulated enhancers upon estrogen stimulation (top panel) and might contribute to context-specific changes in enhancer landscape and enhancer components (i.e. enhancer reprogramming) during cancer progression, such as metastasis or therapy resistance (bottom panel).

will be required to test these possibilities. Phase separation provides a means to compartmentalize and concentrate biochemical reactions within cells by forming membraneless organelles (Banani et al., 2017). Recent studies have shown that activation domains (ADs) of various TFs, including ERα, form phase-separated condensates with the mediator coactivator to activate gene expression. Moreover, it has been suggested that these condensates facilitate the compartmentalization and concentration of transcriptional components at specific sites (Sabari et al., 2018). ADs and coactivators generally consist of low-complexity amino acid sequences that are classified as intrinsically disordered regions (IDRs). IDR–IDR interactions are believed to facilitate the formation of phase-separation condensates. ERα consists of a central DNA binding domain, an N-terminal ligand-independent AD, and a C-terminal ligand-dependent AD (also called ligand-binding domain). It has been shown that ERα can form either homotypic droplets or heterotypic droplets with IDR of MED1 and that estrogen stimulates the formation of phase-separated ERα–MED1 droplets in vitro (Boija et al., 2018). Since both YAP1 and TEAD4 proteins seem to consist of IDRs, it will be interesting to test whether YAP/TEAD can form phase-separated droplets with ERα and whether they can be recruited to ERα enhancers through the phase-separating properties of their IDRs.

The role of YAP/TEAD as ERα co-regulators on estrogen-regulated enhancers adds further complexity and potentially context-dependent specificity to the regulation of these enhancers and their target genes. Numerous studies have shown that multitude intracellular and extracellular mechanisms, including ligands/growth factors and cellular signaling transduction, can regulate Hippo pathway and YAP/TEAD activity. Given that estrogen and ERα play important roles in breast cancer development and progression, which is also empowered by signaling networks, it is thus tempting to speculate that YAP/TEAD may cooperate with ERα to modulate cancer progression in response to various signaling (Figure 1). To explore this notion, Chen/Liu labs have recently started to use a culture model of hormone therapy resistance in breast cancer to test whether changes in YAP/TEAD expression or genome-wide occupancy on enhancers are associated with hormone resistance and whether and how YAP/TEAD cooperate with ERα to orchestrate enhancer reprogramming during the acquisition of hormone resistance. The non-canonical role of YAP/TEAD in ERα enhancers provides new therapeutic strategies for targeting ERα-regulated cancers. However, given the biological significance of the Hippo signaling network in a broad range of human carcinomas, it could be challenging to decouple the canonical and non-canonical functions in regulating cancer development and progression. A drug screen to identify small molecules that target specifically either the canonical or the non-canonical function of YAP/TEAD will be our future effort to address this challenge. It is also notable that cancer is a dynamic and heterogeneous disease and that heterogeneity provides the fuel for therapy resistance. Therefore, it is important to apply single-cell technologies, such as scRNA-seq, scATAC-seq, and CyTOF, to access the heterogeneous molecular signatures, including enhancer landscape and enhanceosome dynamics. We believe that these ongoing studies in our labs will help us to understand the dynamic interactions of YAP/TEAD and ERα at single-cell level during cancer development and progression and provide insights for the development of effective therapies.

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