RNA m^5C modification upregulates E2F1 expression in a manner dependent on YBX1 phase separation and promotes tumor progression in ovarian cancer

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Background: Previous studies have found correlations between various RNA modifications and the progression of diseases, as well as their effects on posttranscriptional modulation of gene expression. 5-Methylcytosine (m^5C) is a reversible RNA methylation modification, catalyzed by NOP2/Sun RNA methyltransferase family members 1-7 (NSUN1-7) and DNA methyltransferase 2 (DNMT2). NSUN2 is found to be one of the primary controllers in m^5C modification of mRNAs. These modifications modulate RNA metabolism, such as nuclear export and translation. The RNA-binding protein (RBP) Y-box binding protein 1 (YBX1), which regulates RNA stability, is one of the RBPs that recognize m^5C and regulates these RNA metabolism processes. RNA m^5C modification has been uncovered to be one of the driving forces behind the progression and tumorigenesis in various cancers, however, the role of m^5C modification in ovarian cancer remains widely unknown.

Objective: To investigate the role of aberrant NSUN2-mediated m^5C modification of mRNAs in the tumorigenesis and progression of ovarian cancer and the molecular mechanism underlying it.

Results: Firstly, the authors determined that NSUN2 expression is significantly increased in ovarian cancer cells. Then, *in vitro* assays and *in vivo* xenograft models using NSUN2 knockdown ovarian cancer cell lines revealed that the loss of NSUN2 expression significantly suppressed cell growth and metastasis abilities. Next, they proved that NSUN2 promotes RNA m^5C modification in ovarian cancer cells using multiomics strategy. Dot blot assay and RNA bisulfite sequencing (RNA-BisSeq) also revealed that mRNA m^5C alterations were directly proportional to NSUN2 expression. RNA-seq, RNA immunoprecipitation sequencing (RIP-seq), and RNA-BisSeq strategies were applied to narrow down potential targets of NSUN2-mediated m^5C modification. Oncogenic factor E2F transcription factor 1 (E2F1) was found to be one of the targets of NSUN2. To further investigate the mechanism behind this, results of knockdown of m^5C readers and RIP assays uncovered that YBX1 possesses E2F1 mRNA binding capability, improving E2F1 mRNA stability by binding to the 3'-UTR m^5C. Furthermore, they investigated the liquid-liquid phase separation (LLPS) capability of YBX1 and confirmed regulation of the LLPS property of YBX1 directly proportional to m^5C level and the importance of YBX1 phase separation on E2F1 expression via m^5C. As a result, high YBX1 expression was correlated with poor survival in ovarian cancer patients. Finally, the authors confirmed that E2F1 binds to the NSUN2 promoter as well as promoters of oncogenic factors MYBL2 and RAD45L to promote their expression in ovarian cancer cell lines. Therefore, the regulation of E2F1 in ovarian cancer occurs in a positive loop fashion, in which E2F1 regulates NSUN2, which mediates YBX1 binding to E2F1 via m^5C.

Conclusion: YBX1 phase separation as an RBP plays an important role in the binding of m^5C-modified E2F1, which is mediated by NSUN2. By inhibiting the phase separation of YBX1, the aberrant expression of E2F1 and other oncogenic factors downstream of E2F1 can be inhibited, which in turn, reduces the expression of NSUN2, thereby reducing m^5C modifications of E2F1 and slowing down the progression and metastasis of cancer.