Exploring snRNP Biogenesis in Cancer with Oncogenic MYC Hyperactivation

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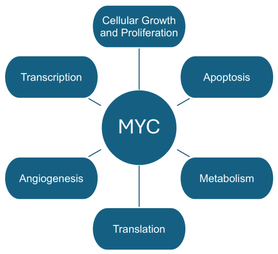
Abstract

MYC, a proto-oncogene, regulates cellular growth and proliferation by controlling the transcription and translation of core spliceosome components. The spliceosome is a macromolecular RNA-Protein complex that catalyzes splicing, a two-step reaction that converts precursor messenger RNA (pre-mRNA) into mature mRNA—an essential intermediate in gene-to-protein expression. Under normal conditions, MYC ensures a sufficient supply of small nuclear ribonucleoproteins (snRNPs), major components of the spliceosome. However, MYC hyperactivation increases pre-mRNA levels beyond the spliceosome's processing capacity, leading to pre-mRNA accumulation and deregulation of essential genes. This may create a bottleneck at the snRNP biogenesis step, where the production of mature snRNPs cannot keep pace. This imbalance could result in defective spliceosome assembly and aberrant mRNA splicing.

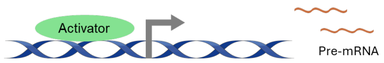
To investigate this vulnerability, we used mammary epithelial cell lines engineered with an estrogen receptor to induce MYC hyperactivation. We assessed the abundance of the SMN complex, a multi-component chaperone that assembles Sm rings on snRNAs, a critical step in snRNP stability and function. By evaluating this process, we aim to elucidate how MYC-driven dysregulation of snRNP biogenesis contributes to splicing stress. Our findings will provide insights into how MYC hyperactivation disrupts pre-mRNA processing, highlighting splicing stress as a therapeutic strategy in MYC-driven malignancies.

MYC hyperactivation leads to transcription amplification

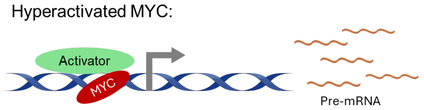
Physiological MYC regulates diverse fundamental biological processes



Physiological MYC:

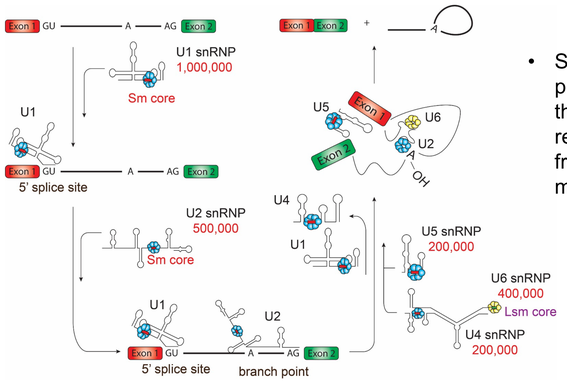


Hyperactivated MYC:



MYC hyperactivation increases gene transcription, leading to increased protein synthesis and cell activity — common in cancer due to MYC overexpression.

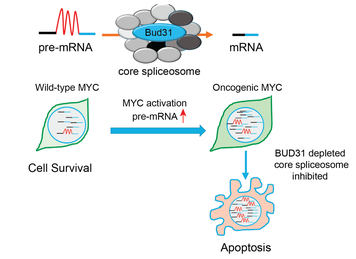
Spliceosome-mediated Pre-mRNA Splicing



- Spliceosome: RNA-protein complex that catalyzes the removal of introns from nuclear pre-mRNA.

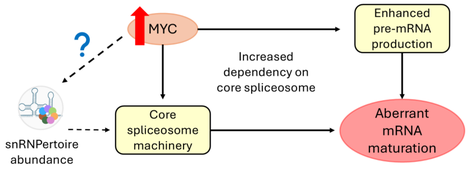
Adapted from Patel and Steitz Nat. Rev. Mol Cell Biol. (2003)

MYC hyperactivation exposes spliceosome vulnerabilities

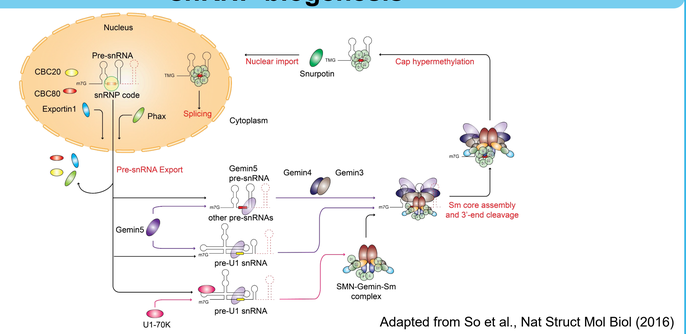


Stine and Dang, Cancer Cell (2015)

- MYC-driven transcription amplification increases the need for a functional spliceosome
- Its depletion induces vulnerability in MYC-driven tumors.
- Imbalance in the snRNP repertoire (snRNPertoire) may lead to aberrant mRNA splicing.



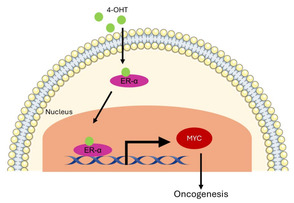
SMN complex-mediated Sm core Assembly is the key step in snRNP biogenesis

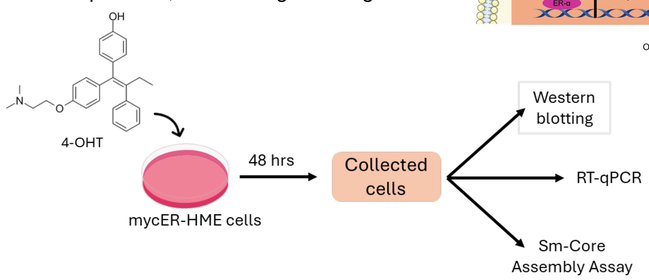


Adapted from So et al., Nat Struct Mol Biol (2016)

Methods

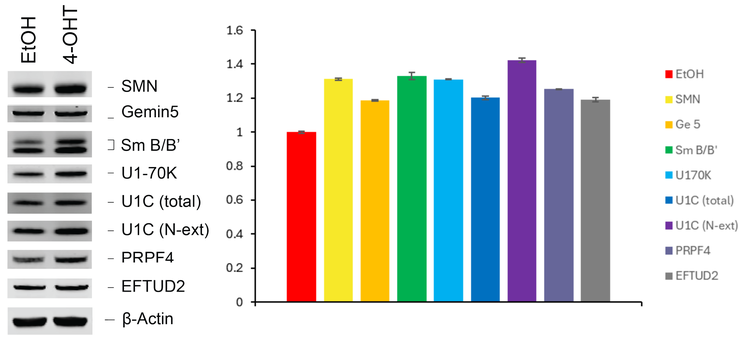
- 4-hydroxytamoxifen (4OHT), an estrogen agonist, is a selective estrogen receptor modulator.
- Estrogen receptor activation leads to increased MYC expression, contributing to oncogenesis.





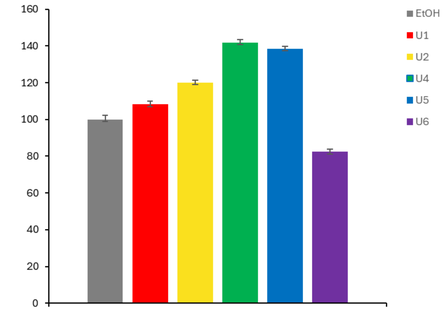
- After treating the cells with 4OHT, cells were collected 48 hours later.
- Cells were processed to isolate protein for Western Blotting. RNA was purified to synthesize cDNA for RT-qPCR analysis.
- Cytoplasmic extracts were isolated for Sm core assembly assays.

Myc hyperactivation increases snRNP associated proteins



The snRNP repertoire is altered in response to myc hyperactivation

- MYC hyperactivation, induced by 4-OHT, increases snRNP levels unevenly.
- This may disrupt the snRNP repertoire leading to defective snRNA splicing.



Future work

- Sm core assembly assays will evaluate how MYC hyperactivation alters Sm core formation and impacts snRNP assembly.
- Immoprecipitation with Sm antibody to assess snRNP levels by qPCR and western blotting following myc hyperactivation.
- Characterizing splicing isoforms of snRNP-specific proteins under myc-hyperactivation, focusing on the involvement of mammalian Target of Rapamycin (mTOR) signaling pathways.

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