Regulation of the cellular response to stress by NAT10

Hepatocellular carcinoma (HCC) is a malignant disease and the most common type of primary liver cancer. Risk factors of HCC, such as Hepatitis B and Cirrhosis, induce reactive oxygen species (ROS) in cells, facilitating chronic oxidative damage in the cells. These risk factors have become more common in recent years, changing the HCC landscape. While more than half of the global HCC cases can still be linked to HBV and its associated chronic stress of the liver, chronic hepatitis developed as a consequence of alcoholic cirrhosis, non-alcoholic fatty liver disease (NAFLD), or type two diabetes has become prevalent factors increasing patient susceptibility to develop HCC. Liver cancer patients often remain without disease symptoms, making diagnosis difficult despite available diagnostic tools. Once symptoms arise due to compromised liver function, cancer has often advanced to metastasis wherein it invades vascular tissues, leaving patients with poor chances of survival and highlighting the need for advanced therapeutic measures to increase survival.

Recent advancements in epitranscriptomics have led to the discovery of over 150 different RNA modifications while improving our understanding of these modifications' vital role in cellular processes. Such non-genetically encoded modifications can fine-tune gene expression by timing and regulating transcriptional processes (primarily through lncRNAs) and translation (mRNA, rRNA and tRNAs). A consequence of ROS is the direct oxidation of biomolecules, including RNA. RNAs are vulnerable to modifications, and while some modifications are enzymatically attached to RNAs, RNA damage does not require an enzymatic catalyst but results in the presence of free radicals in the RNA surroundings. While all RNA nucleotides can become the target of oxidation, adding oxygen at the C-8 of guanine, 8-oxo-guanine (8-oxoG, oxo8G) is the most abundant oxidized product because it is stable and relatively quickly formed. Therefore, studies investigating the effects of RNA oxidation are mainly focused on examining oxo8G. The presence of oxo8G on mRNA molecules can lead to stalling and termination of translation.

Furthermore, due to its ability to facilitate oxo8G-to-A base pairing, oxo8G alters RNA-RNA interactions, enabling redirection of posttranscriptional regulation and the translational apparatus. Because mRNAs are translated by multiple ribosomes per mRNA molecule, a single modified nucleotide can have detrimental effects. Remarkably, RNA oxidation is pervasively observed in chronic oxidative stress conditions such as neurodegenerative disease. While RNA oxidation has been linked to a variety of neurodegenerative diseases, its distinct role in cancer remains poorly understood. A major risk factor of HCC is the chronic production of ROS, which induce a constantly stressful environment that ultimately leads to liver cancer. Thus, we aim to understand how RNA oxidation contributes to HCC oncogenesis.

N-acetyltransferase 10 (NAT10) is the enzyme responsible for the acetylation of cytidines in RNA (ac4C). Current literature shows that NAT10 expression is elevated in numerous types of cancers, including HCC. Furthermore, elevated levels of NAT10 have been associated with poor patient survival. Of significant physiological relevance, NAT10 overexpression is an oncogenic driver in hepatocellular carcinoma (HCC). Importantly, acetylation of cytidine in mRNA control translation efficiency in a position-specific manner. Likewise, acetylation of cytidine in rRNA and tRNA affects ribosome biogenesis and tRNA maturation, ultimately affecting protein synthesis. Preliminary data in the Arango lab indicates that NAT10 expression is induced in response to ROS. Interestingly, NAT10 and ROS facilitate RNA modifications, with NAT10 promoting ac4C and ROS facilitating RNA oxidation, most commonly: oxidation of Guanosine (oxo8G). Based on the above observations, this study aimed to evaluate whether ROS influence the levels of RNA oxidation and RNA acetylation in liver cells.

I induced oxidative stress in HCC SNU-449 cells using chemical oxidants. After verifying ROS production using 2′,7′-dichlorofluorescein diacetate (DCFH-DA) and cellular viability, I measured the levels of RNA oxidation and acetylation using ImmunoNorthern blots. This antibody-based method identifies RNA modifications in different RNA species. I found an inverted correlation between the levels of RNA oxidation and RNA acetylation in 18S rRNA and tRNA. In other words, as oxo8G increases, ac4C decreases in 18S rRNA and tRNA. While this result was unexpected because the levels of NAT10 increase under oxidative stress, it opens a new avenue of studies to elucidate the physiological relevance of decreasing rRNA and tRNA acetylation in HCC cells under oxidative stress.

Although not evaluated in this study, it is still possible that mRNA acetylation, at least for some mRNAs, increases in the presence of oxidative stress, particularly because oxidative stress induces a change in the subcellular localization of NAT10. Focusing on the latter, I assessed how the presence of ac4C and oxo8G in mRNAs impacts protein expression. For this purpose, I generated synthetic mRNA reporters carrying acetylated and oxidized ribonucleobases. The mRNA reporters were encoding a red fluorescent protein (RFP) and a green fluorescent protein (eGFP) protein from two alternative translation initiation sites. It is worth noting that the RFP is an upstream Open Reading Frame (uORF), which is poorly expressed and works as an inhibitor of GFP expression. The synthetic mRNA reporters were transfected into cells, and protein expression was measured using flow cytometry 24 hours after transfection. My results indicate that the combined presence of both modifications on mRNA molecules significantly increases protein expression in a fluorescent reporter carrying an inhibitory upstream open reading frame. It is noteworthy that one of the characteristics of cancer is dysregulated protein expression. Here we identified that two RNA modifications, which rely on intermediate molecules that are upregulated in cancer, induce changes in protein translation levels.

Though these studies require orthogonal validations and further investigation into the physiological relevance of RNA acetylation and oxidation, they have significant implications in cancer biology. HCC is a malignant disease that is becoming increasingly prevalent in the developed world. While cases have tripled over the past 50 years, the therapeutic landscape to treat HCC has not improved significantly, with patient deaths doubling over the same time. The increasing number of HCC cases combined with the poor patient prognosis highlight why investigating this field is so important. Our findings suggest that RNA oxidation and acetylation may play a role in the etiology of HCC.