Cancer, a global health concern, is one of the top two leading causes of death worldwide. Dr. Lu’s laboratory at Tulane University has been studying the signaling pathways of cancer biology, focused on the molecular and biochemical mechanisms for cell proliferation and tumorigenesis involving the p53 and c-Myc pathways. We were specifically interested in understanding the role of unique post-translational modifications of proteins such as p53, a tumor suppressor, and c-Myc, an oncoprotein, in the regulation of the transcription factors’ functions and cancer biology. Short-chain fatty acids (SCFSs) are produced by the gut microbiome and have been shown to have anti-cancer effects due to their ability to suppress tumor growth and cancer cell metastasis.

Post-translational modifications (PTM) are processes that involve covalently modifying the amino acid chain of a protein by adding a chemical group to it after the protein is synthesized. Since the amino acids are very rapidly removed and taken off to respond to stimulus, they are relatively unexplored. Protein crotonylation is an important PTM of lysine. Histone or non-histone lysine can be crotonylated in the presence of crotonic acid in vitro cell culture. Crotonic acid (CA) has been shown to modify histone proteins and some non-histone proteins like p53.

Crotonylation of c-Myc was identified by our lab using a combination of western blots with a pan-crotonylation antibody, a technique that separates and identifies specific proteins, and high-resolution mass-spectrometry (HR-MS), which allows for the detection of analytes and determination of elemental and isotopic compositions of a sample. PCR generated K to R mutants identified two sites that ablated the western signal (2R mutant). HR-MS identified six additional sites, and the 8R mutant was generated for this as well.

The addition of CA to cancer cells caused a decrease in c-Myc activity and cellular growth, increased chemosensitivity, and decreased stem-cell formation. The mutant, however, has increased proliferation and colony formation capacity. Quantitative chain polymerase reaction (q-PCR) used to measure gene expression of downstream cell-cycle related c-Myc targets also shows increased activation by the mutant. Mechanisms to explain this phenotype are being investigated by Immunoprecipitation (IP), a process used to purify antigens using specific antibodies. Lentivirus is also being generated for c-Myc and its mutants to generate stable-cell lines to further investigate phenotypes, such as proliferation, colony formation, migration, and spheroid formation. In conclusion, we identified a novel post-translational modification on the oncoprotein c-Myc and early work shows a modulatory effect on the protein’s cancer-promoting activity.

References: