

2011 JMNMF RSA SUMMARY

John Kwon

University of Texas

MD Anderson Cancer Center

Skin cancer is the most common cancer and melanoma is one of the deadliest.

In the United States, the overall incidence of melanoma is increasing at a rate faster than any other cancer with recent estimates for the lifetime risk of developing invasive melanoma at 1/49. While early detection and surgical excision can cure melanoma, patients with advanced disseminated disease have a very poor prognosis due to its extremely aggressive growth characteristics in combination with high resistance to chemotherapy and radiation. Thus, there is an urgent need to better understand melanoma biology and develop innovated therapeutic strategies and explore new drug targets for the efficient treatment of this disease.

Our previous studies have confirmed that many melanoma patient's tumor cells express iNOS (inducible nitric oxide synthase), an enzyme that produces nitric oxide (NO), which is a free-radical molecule in the cell that plays various physiological roles in the human body. The expression of iNOS in melanoma tumor cells was shown to correlate with poor patient survival irrespective of treatment, and is now proposed as a molecular marker of poor prognosis and possibly as a target for therapy. Our published data provides strong circumstantial evidence that endogenous NO is produced by some human melanomas and that in these cases, melanoma cell survival appears dependent on NO. Molecular analysis supports the hypothesis that iNOS-produced NO can be responsible for driving proliferation as well as regulating resistance to apoptosis; and revealed that p53 is functionally inactivated by NO, as its function to induce apoptosis is restored when NO is quenched in vitro. Although p53 is the most commonly mutated gene in a broad spectrum of cancers, it is rarely mutated in melanoma, and increased expression is associated with tumor progression.

Therefore, in this current study we hypothesized that iNOS leads to intracellular NO production, which modifies various critical apoptosis controlling proteins, including p53 in melanoma. To determine whether p53 in human melanoma is under chemical redox regulation by a post-translational modification called S-Nitrosylation, the biotin switch assay was conducted. S-Nitrosylation is a modification in which a sulfhydryl group of a cysteine residue gets modified by NO. NO levels can be regulated using NO donors (SNAP) and scavengers (c-PTIO) that are commercially available. We have accumulated evidence showing that p53 is S-Nitrosylated at residue 277, and the effects of this modification lead to functional inactivation of p53, a key tumor suppressor protein. This was shown by p53 binding assays (ChIP) with key p53 targeting promoters. Furthermore, molecular modeling analysis shows that S-Nitrosylation of p53 at 277 shows a conformational change in the p53 protein structure.

This is a novel finding of p53 post-translational modification. Since the discovery of NO, the field of NO has had tremendous impact in a variety of different fields but not much is known about the association of NO and the pro-inflammatory state of some cancers. Findings from this project will unravel some of these mysteries and give clues to better therapeutic options.