

ADEOLUWA ADELUOLA, TIMOTHY E. LONG, & A. R. M. RUHUL AMIN, Dept. of Pharmaceutical Sciences and Research, Marshall University, Huntington, WV, 25755. Mechanism of apoptosis induced by actinomycin D in upper aerodigestive tract cancers.

Upper aerodigestive tract cancers (i.e., oral cavity, pharynx, larynx, esophagus, and lungs) are one of the most prevalent and leading causes of cancer-related deaths. Drug resistance is a big challenge for successfully treating these cancers. Recent studies have shown that combining low-dose actinomycin D with existing therapies is a promising strategy to overcome resistance. The development of these treatment strategies however requires an understanding of the molecular mechanisms of resistance and drug action.

This study aimed to identify the mechanism of actinomycin D-induced apoptosis in aerodigestive tract cancers. We determined the  $IC_{50}$  of actinomycin D which spanned between 0.027-3.72 ng/ml in a range of aerodigestive tract cancer cell lines using the SRB assay. Subsequently, we conducted apoptosis assays using the Annexin V-PE staining. FlowJo was used to quantify apoptosis. We found that actinomycin D induced apoptosis of all cell lines tested, but the sensitivity varies between cell lines. Mechanistic studies revealed that actinomycin D time- and dose-dependently increased the expression of p53 and its downstream targets p21 and Puma in cells expressing wild-type p53. Interestingly, ablation of p53 expression using shRNA decreased the expression of p21 and Puma in A549 cell line and p21 in H460 cell line. Moreover, ablation of p53 significantly protected cells from actinomycin D-induced apoptosis ( $p < 0.0001$ ). Summarily, our data revealed that actinomycin D induced p53-dependent apoptosis of cells expressing wild-type p53. Since actinomycin D also induced apoptosis of cells expressing mutant p53 or lacking p53, future studies will explore the mechanism of p53-independent apoptosis.