**Understanding the role of alternative RNA splicing in Osimertinib resistance in lung adenocarcinoma**

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The third generation of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors, especially Osimertinib, has achieved remarkable clinical outcomes in the treatment of lung adenocarcinomas with EGFR mutations. However, resistance eventually emerges in most patients and the underlying molecular mechanisms are not yet fully understood. Using matched-pair Osimertinib-sensitive and -resistant previously published patient data and isogenic cell lines, we found that that Osimertinib-resistant (OR) lung adenocarcinomas have altered splicing of cassette exons, which are retained or excluded in Osimertinib-resistant conditions based on nucleotide sequence. In particular, these altered exons localize to specific chromosomal hotspots. We next sought to discover if alternative splicing was having a biological impact on gene expression in OR cells by inducing nonsense-mediated decay (NMD) of select transcripts. We found that alternative splicing-mediated NMD (AS-NMD) targets 82 genes in Osimertinib-resistant vs. -sensitive cells, and that these genes are enriched for double-strand DNA damage repair pathways. We also found increased DNA damage signal and increased NMD signal, as well as decreasing expression of specific AS-NMD targets as a function of Osimertinib resistance. These results suggest a possible mechanism in which OR cells bypass DNA repair and/or apoptosis by crippling the DNA damage repair response through altered pre-mRNA splicing and NMD. Current experiments continue to explore the cause of this resistance-correlated AS-NMD behavior. These findings shed new light on the mechanisms of Osimertinib resistance with regard to DNA repair and provide a rationale for targeting altered RNA splicing and NMD as therapeutic strategies to overcome acquired Osimertinib resistance in lung adenocarcinomas.