

**Multi-Targeted siRNA Against UbcH5, c-Src, and HSP90 Combined With Cetuximab Circumvented Oncogene Addiction, Transactivation, and Acquired Resistance Due to EGFR Ubiquitination, and Mutations/Deletions in the Kinase Domain of EGFR in mCRC**

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**Introduction:** Colorectal cancer (CRC) cells can develop resistance to cetuximab through insertion mutations at exon 20, ubiquitination, and c-Src, which activates epidermal growth factor receptor (EGFR) in the absence of EGFR ligand, despite treatment with the blocking anti-EGFR chimeric human mouse monoclonal antibody. We aim to circumvent these three types of acquired resistance.

**Materials and Methods:** CRC cells were obtained from patients with metastatic CRC (mCRC) who were resistant to cetuximab due to insertion mutations at exon 20, ubiquitination, and overexpressed c-Src.

Orthotopic mouse CRC models generated from the patients' tumor cells were injected with multi-targeted siRNA against HSP90, UbcH5, and c-Src.

**Results:** Multi-targeted siRNA inhibited expression of the E2 enzyme UbcH5, blocking the covalent attachment of ubiquitin to target protein EGFR, and neutralizing the multi-enzyme cascade. E1 deactivated ubiquitin, blocking transfer to the cysteine residue of E2 ubiquitin conjugating enzyme (UbcH5). This inhibited the E2 ligation of ubiquitin via its carboxy terminus to lysine residues of the protein substrate EGFR. Also, multi-targeted siRNA inhibited expression of HSP90, resulting in degradation of EGFR with kinase domain deletion type mutations in exon 19, substitutions in exon 21, and resistant

insertion mutations at exon 20. Simultaneous inhibition of c-Src circumvented transactivation, and inhibited EGFR-mediated signaling, inhibiting tumor proliferation and metastasis to the liver and peritoneum. Addiction to EGFR oncogene was circumvented. Inhibition of EGFR blocked the activation of downstream mediators including STAT3, AKT, Erk/MAPK and PI3K, while IRF-1 was upregulated. There was enhanced cell-to-cell adhesion, and membrane localization of  $\beta$ -catenin, while matrix metalloproteinase (MMP)-9 invasive activity was blocked. Furthermore, the HIF (hypoxia-inducible factor)-1 $\alpha$ /Met pathway was blocked, downregulating CAIX (carbonic anhydrase IX). Vascular endothelial growth factor receptor (VEGFR)-2 and VEGFR-3 were blocked, inhibiting vascularization and lymphangiogenesis, respectively. Finally, we observed type I, II, and III PCD in tumor cells.

**Conclusion:** These results indicate that systemic treatment of multi-targeted siRNA against UbcH5, c-Src, and HSP90 circumvented resistance to cetuximab, suppressing tumor growth and metastasis in an orthotopic mouse mCRC model.