Novel Epigenetic Targeting of the MYC Oncogene for the Treatment of NSCLC Using Programmable mRNA Therapeutics

#1114

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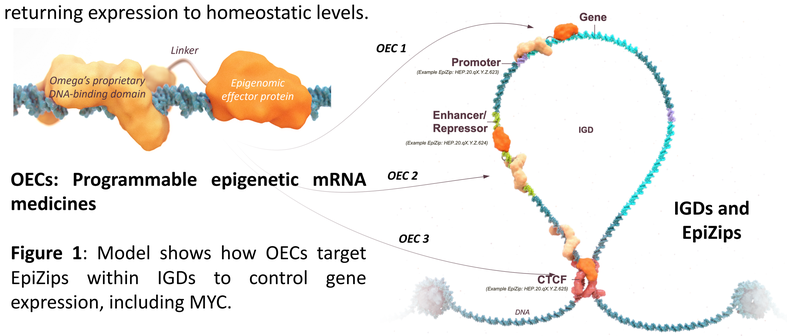
Background: The MYC oncogene is a master regulator which controls many critical physiologic processes in cellular metabolism and growth regulation. While MYC expression is normally tightly controlled in non-transformed cells, its activity is frequently dysregulated in cancers. In the genome, MYC and its regulatory elements are located inside an insulated genomic domain (IGD), a discrete chromatin-looping region within which gene expression is mediated by CTCF (CCTC binding factor), in concert with other regulatory factors. At Omega Therapeutics, we specifically and durably tune expression of genes via epigenomic programming to treat or cure diseases. Leveraging our OMEGA Epigenomic Programming™ platform, we have identified, mapped, and validated thousands of DNA sequences that can be epigenetically modified to alter gene expression in associated IGDs; we call these sequence intervention points epigenomic zip codes, or EpiZips™. We have rationally designed and engineered programmable mRNA therapeutics called Omega Epigenomic Controllers™ (OECs), that act pre-transcriptionally to target IGDs, including previously elusive targets like MYC, with unprecedented precision, returning expression to homeostatic levels.



OECs: Programmable epigenetic mRNA medicines

Figure 1: Model shows how OECs target EpiZips within IGDs to control gene expression, including MYC.

Non-small cell lung cancer (NSCLC) is the leading cause of global cancer-related mortality, making up almost 25% of all cancer deaths. NSCLCs often exhibit aberrant MYC activity, including genomic amplification and overexpression. Here, we describe a NSCLC-specific therapy that downregulates MYC expression levels utilizing a novel OEC targeting two sites within the MYC IGD.

On-target effect of a NSCLC OEC targeting two different EpiZips within MYC IGD

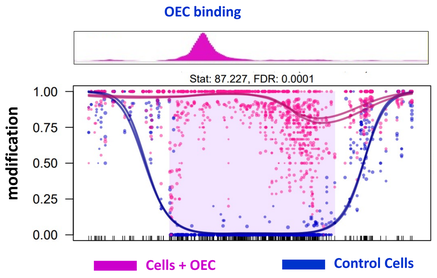


Figure 2: ChIP-seq shows the binding of NSCLC OEC module to its predicted genomic region and sequencing shows increased epigenetic mark at the target site after treatment.

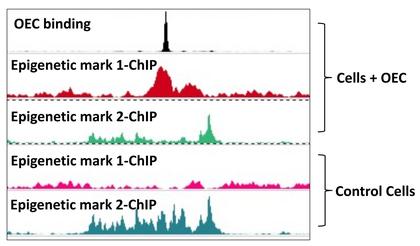


Figure 3: ChIP-seq shows the binding of NSCLC OEC module to its predicted genomic region and corresponding epigenetic mark changes after treatment.

NSCLC OEC treatment downregulates MYC mRNA levels across multiple NSCLC cell lines

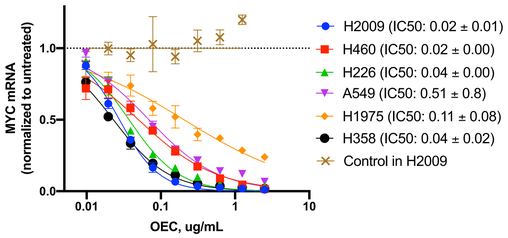
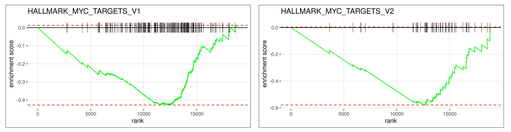


Figure 4: NSCLC OEC reduces MYC mRNA level in a panel of NSCLC cell lines, including different subtypes with varying levels of MYC expression. Representative dose-response curves shown. Average ± standard deviation of IC50 shown in legend.



NES= -1.81
padj=3.8x10^{-3}

NES= -2.02
padj=3.8x10^{-3}

Figure 5: GSEA analysis on RNA-seq data shows that MYC target hallmark gene sets are significantly downregulated in OEC-treated H2009 cells

MYC downregulation following NSCLC OEC treatment is rapid and durable

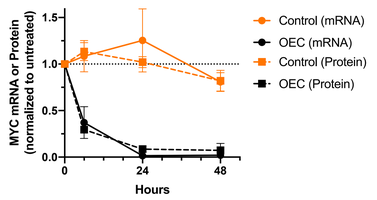


Figure 6: NSCLC OEC rapidly reduces MYC mRNA and myc protein levels in H2009 cells treated with OEC but not negative control RNA

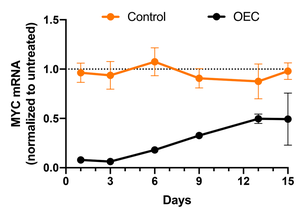


Figure 7: NSCLC OEC treated H2009 cells show durable MYC down- regulation (mRNA) after a single treatment on day 0

NSCLC OEC reduces viability of multiple NSCLC cell lines with limited effect on normal primary cells

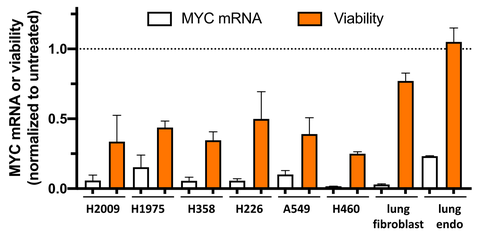


Figure 8: NSCLC OEC reduces viability in a panel of NSCLC cell lines, with limited effect on normal lung primary fibroblasts or endothelial (endo) cells.

NSCLC OEC treatment induces apoptosis

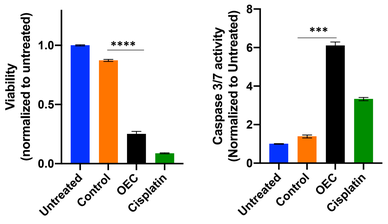


Figure 9: NSCLC OEC reduces viability due in part to induction of apoptosis shown by an increase in caspase 3/7 activity 72h after treatment. Cisplatin treatment was used as a positive control. Stats only shown for control and OEC comparison.

NSCLC OEC combination therapy with MEK or EGFR inhibitor enhances MYC protein downregulation and growth inhibition

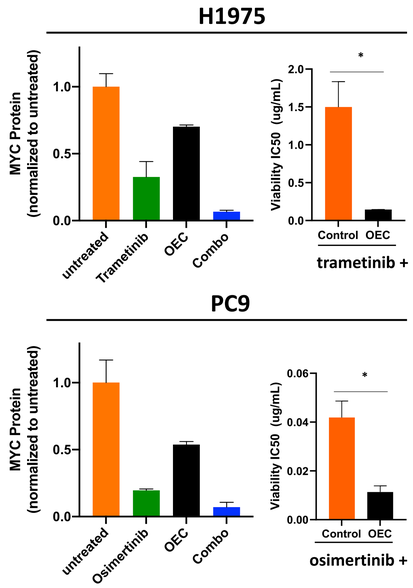


Figure 10: NSCLC OEC combination therapy with MEK inhibitor (trametinib, top panel) in H1975 cells or EGFR inhibitor (osimertinib, bottom panel) in PC9 enhances MYC protein downregulation (left graphs) as well as reduction of cell viability (right graphs) as compared to monotherapy. H1975 or PC9 cells were treated with 1uM inhibitor, 1ug/ml OEC or combination of both.

Treatment of NSCLC tumor-bearing mice with OEC as monotherapy significantly inhibits overall tumor growth *in vivo*

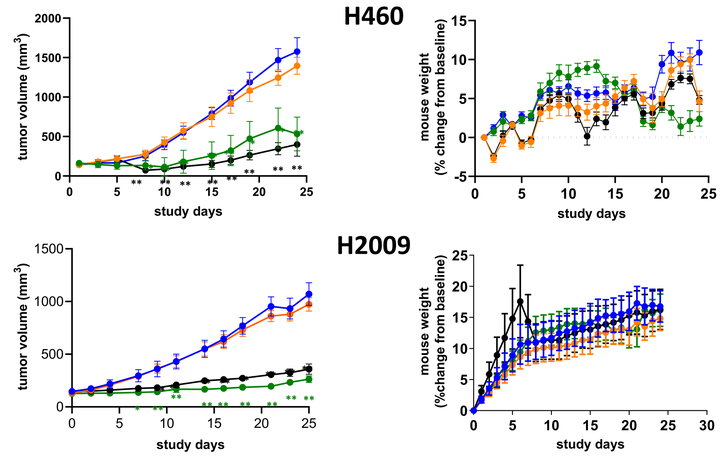


Figure 11: NSCLC OEC treatment reduces tumor growth (left) in vivo with minimal effects on body weights (right). H460 (top) and H2009 (bottom) subQ tumors were treated with PBS (blue), 3 mg/kg negative control (orange), 3 mg/kg NSCLC OEC (black), or SOC drug (green) every 5 days. Significant difference from tumor size in PBS-treated mice shown as '**' denotes p<0.01, '*' denotes p<0.05

In summary, our findings demonstrate the potential of a novel mRNA therapeutic, an OEC, for the treatment of NSCLC via epigenomic programming and control of MYC expression by targeting the MYC IGD. Modulating MYC pre-transcriptionally in this manner may represent a novel and differentiated therapeutic approach in patients with advanced NSCLC.