An antisense molecule to HER3 sustains growth inhibitory effects in gefitinib resistant cells that are independent of MET overexpression

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INTRODUCTION

Treatments with selective tyrosine kinase inhibitors, e.g. gefitinib and erlotinib, in advanced NSCLC patients harboring EGFR mutations have given promising results. However, after initial response to the treatment, acquired drug-resistance cancers eventually develop, due to additional mutations in EGFR or other mechanisms independent of EGFR. Although HER2 is not typically amplified or overexpressed in many tumor cell lines like EGFR or HER2, it is emerging as a critical family member since 3) it is a key link to the PI3K/AKT signaling pathway for HER family members, 2) it can heterodimerize with EGFR and HER2, and 3) it can be activated via autocrine signaling by binding its cognate ligand, heregulin, or other receptor tyrosine kinases (e.g. MET). These features help explain why increased activation of HER3 can mediate resistance to EGFR and HER2 inhibitors such as gefitinib, erlotinib, lapatinib, or Herceptin.

We have been attempting to understand the basis of unusual sensitivity of the lung carcinoma cell line, HCC827 to gefitinib ([IC50] ~10 nM) and acquired resistance mechanisms after the cells were chronically exposed to increasing concentrations of gefitinib in vitro. We have focused on inhibition of HER3 by using LNA-antisense technology. We report here the biological activities of a HER3 mRNA antagonist, EZN-3920, against HCC827 parental and the gefitinib-resistant cells.

METHODS

• Gene targeting and cell growth assay: The ability of EZN-3920 to down modulate its direct target HER3 mRNA, protein, and to inhibit cell growth was evaluated by qRT-PCR, immunofluorescence, and MTT, respectively. Cell treatment was conducted by addition of EZN-3920 to the cell culture medium at designated concentrations without transfection reagent.

• Genomic DNA analysis. EGFR copy number in HCC722 and GB clones were determined by qPCR using TaqMan gene copy number assay. Purified genomic DNA: cDNA was used as templates.

• Drug combination study. The gefitinib-resistant clone and parent HCC827 were treated with varying concentrations of EZN-3920 ([1-4 uM]) or EZN-4150 ([0-20 uM]) alone, or in combination (a ratio of EZN-3920: EZN-4150 1:5), for 8 days. Cell proliferation was determined by MTT assay. A formal quantitative analysis of the data was used to calculate the combination index (CI) based on a multiple drug-effect equation (Reynolds 2005 Mol Med).

LNA TECHNOLOGY & ACTION

Locked Nucleic Acid

• 3rd generation LNA-based antisense technology
• Very high mRna affinity
• Excellent plasma stability
• Long tissue residence time (days)
• Short half-life (14-16 hrs)
• IV administration without delivery vehicle (unlike siRNA)

REFERENCES


CONCLUSIONS

• Diverse mechanisms exist for cells become resistant to selective TKI agents.
• Gefitinib resistance could be the result of tumor cells with loss of EGFR amplification and acquisition of wild-type EGFR escaping from gefitinib treatment.
• Gefitinib hyperactivity may indicate that cells are dependent on HER3 and will be abolished by an anti-HER3 agent.
• Pharmacological manipulation to down-regulate HER3 by EZN-3920 could prove to be a translational approach to controlling HER3-mediated tumor growth in cancer patients.
• Down-regulation of both HER3 and PIK3CA with antisense LNA combination could be an effective approach to treat certain type of drug-resistant tumors.

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