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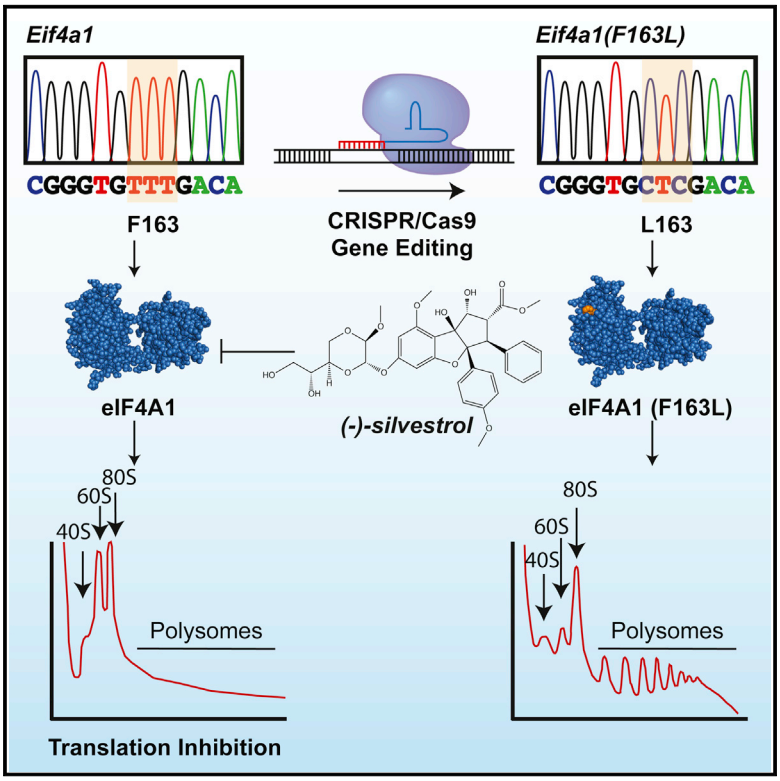
CRISPR-Mediated Drug-Target Validation Reveals Selective Pharmacological Inhibition of the RNA Helicase, eIF4A

Graphical Abstract



Highlights

- CRISPR/Cas9 gene editing is a powerful approach for in vivo drug-target validation
- Rocaglates interact with eIF4A1 in vitro and in vivo
- Anti-neoplastic activity of rocaglates is a consequence of eIF4A1 inhibition

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In Brief

Rocaglates are anti-neoplastic agents that are thought to inhibit the RNA helicase eIF4A, although alternative targets have also been proposed. Using a series of biochemical assays and CRISPR/Cas9 genome editing, Chu et al. provide genetic evidence that the anti-neoplastic activities of rocaglates are a consequence of eIF4A1 inhibition.

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CRISPR-Mediated Drug-Target Validation Reveals Selective Pharmacological Inhibition of the RNA Helicase, eIF4A

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SUMMARY

Targeting translation initiation is an emerging anti-neoplastic strategy that capitalizes on de-regulated upstream MAPK and PI3K-mTOR signaling pathways in cancers. A key regulator of translation that controls ribosome recruitment flux is eukaryotic initiation factor (eIF) 4F, a hetero-trimeric complex composed of the cap binding protein eIF4E, the scaffolding protein eIF4G, and the RNA helicase eIF4A. Small molecule inhibitors targeting eIF4F display promising anti-neoplastic activity in preclinical settings. Among these are some rocaglate family members that are well tolerated in vivo, deplete eIF4F of its eIF4A helicase subunit, have shown activity as single agents in several xenograft models, and can reverse acquired resistance to MAPK and PI3K-mTOR targeted therapies. Herein, we highlight the power of using genetic complementation approaches and CRISPR/Cas9-mediated editing for drug-target validation ex vivo and in vivo, linking the anti-tumor properties of rocaglates to eIF4A inhibition.

INTRODUCTION

Protein synthesis is a tightly controlled process that is deregulated in many human cancers and is required to sustain several cancer hallmarks (Bhat et al., 2015). In part, this is attributed to hyper-activation of the MAPK and PI3K-mTOR pathways, both of which impact on the activity of eukaryotic initiation factor (eIF) 4F. As well, resistance to targeted therapies aimed at inhibiting the PI3K-mTOR and MAPK signaling pathways in various cancers has been linked to elevated eIF4F activity (Bhat et al., 2015). Therefore, there is significant interest in developing eIF4F inhibitors as anti-neoplastic compounds (Bhat et al., 2015).

The eIF4F heterotrimeric complex binds to m^7GpppN mRNA cap structures through its eIF4E subunit, remodels proximal secondary structure via its eIF4A RNA helicase subunit, and recruits 40S ribosomes (with associated initiation factors) through its eIF4G subunit. The mammalian genome encodes two highly related (>90% identity) eIF4A isoforms, eIF4A1 and eIF4A2. These two isoforms were initially thought to be functionally redundant, but there is evidence suggesting they may also possess distinct biological properties (Galicia-Vázquez et al., 2012).

Strategies aimed at inhibiting eIF4F include blocking eIF4E:eIF4G and eIF4E-cap interaction, interfering with eIF4A1/2 activity, and suppressing eIF4E expression with antisense oligonucleotides (ASOs) (Bhat et al., 2015). The development of eIF4E ASOs has provided proof-of-concept validation for targeting eIF4F in xenograft models, as well as generating safety data profiling from phase I clinical trials (Graff et al., 2007; Hong et al., 2011). Transient inhibition of eIF4E (and hence eIF4F) is tolerated at the organismal level (Lin et al., 2012), despite its essential nature (Truitt et al., 2015). The most potent small molecule inhibitors of the eIF4F complex derive from a family of compounds referred to as rocaglates, which are characterized by a common cyclopenta[*b*]benzofuran skeleton. Extensive structure-activity relationship into the biological activity of these compounds has been obtained (Pan et al., 2014), with a few compounds capable of potently inhibiting translation (Bordeleau et al., 2008; Rodrigo et al., 2012).

Rocaglates decrease eIF4A1/2 levels present in the eIF4F complex (Bordeleau et al., 2008), exhibit anti-tumor activity in a number of pre-clinical models (Bordeleau et al., 2008; Cencic et al., 2009; Wolfe et al., 2014), and are thought to exert their effects by preferentially inhibiting the translation of key oncogenic mRNAs (e.g., MYC) (Cencic et al., 2009; Rubio et al., 2014; Wolfe et al., 2014). Chemogenomic profiling in yeast have identified the eIF4A orthologs, TIF1 and TIF2, as targets of rocaglates (Sadlish