The pathology and schizophrenia-associated CYFIP1 protein is required for pancreatic tumor growth and presents a potential therapeutic target

Kiley Couto, Matthew Strickland, Tiffany Liao, Mortada Najem, Ameya Apte, Aaron Fulgham, Shan Lou, Hongyue Dai, Pearl Huang, Jonathan Hurv and Alexandra Lantermann

kcuto@cygnalnx.com Cygnal Therapeutics 325 Vassar St, Suite 2B, Cambridge, MA 02139

Abstract

The pancreas is highly innervated with sensory, autonomic, and motoric neurons, and recent studies have highlighted the cross-talk between neurons and pancreatic cells and their potential role in driving tumourigenesis and promoting metastasis. Published data has shown that ablation of sensory neurons in models of pancreatic ductal adenocarcinoma (PDAC) results in delayed tumor initiation and progression. In addition, peripheral invasion (PIE), the invasion of tumor cells along the nerve, is a pathological characteristic frequently observed in PDAC. PIK3CA has been detected in early stages of pancreatic cancer and associated with a poor outcome. We hypothesized that PDAC tumor cells express neuronal genes which may be playing a role in enabling tumor-nerve interactions and promoting PDAC tumorigenesis.

Here we used pooled CRISPR screening to probe a library of 1500 sgRNAs targeting 1135 neuronal genes. We selected three established (MiaPaCa-2, Panc-1 and BxPC3) and two patient derived human PDAC cell lines (PAXF1657 and PAXF1997) and performed in vitro and in vivo CRISPR screens. sgRNAs targeting the CYFIP1 gene were negatively selected among multiple cell line models in vitro and in vivo. Cytosplastic FMR1 Interacting Protein 1 (CYFIP1) is a protein that has been described to regulate actin polymerisation and protein translation through two distinct protein complexes: CYFIP1 is a member of the heispermotaxic WAVE regulatory complex (WRC) which includes CYFIP1, WAVE, NIP1, NIP2, and NIP3, and CYFIP1 binds to WAVE in actin polymerization. Alternatively, CYFIP1 can interact with Fragile X mental retardation protein (FMRP) and eIF4E, acting as a translation repressor. CYFIP1 is highly expressed in breast carcinoma and is required for proper dendritic spine morphology. CYFIP1 has been linked to neurological and neuropsychiatric conditions such as autism, epilepsy and schizophrenia due to its location in a chromosomal region ([15q11.2]) with frequent copy number variations.

In order to validate the pooled CRISPR screen results we individually knocked out CYFIP1 in PDAC cancer cell lines. We were able to show knockdown of CYFIP1 reduced proliferation of PDAC cells in vitro and dramatically reduced tumor growth in vivo.

Summarization

- Pooled sgRNA CRISPR screens in pancreatic cancer cells lines revealed CYFIP1 as a negative selection hit in vitro and in vivo.
- Most high tumor burden models in PDAC and pancreatic adenocarcinoma patients correlates with an unfavorable patient survival.
- In vivo validation of CRISPR screens using individual sgRNA targeting CYFIP1 show reduced growth in colony formation and invasion assays. sgRNA reduced growth phenotypes were confirmed to be on target by rescuing with vector resistant CYFIP1 cDNA.
- CYFIP1 knockouts in PAXF1657 xenograft model results in a significant delay in tumor growth in vivo.
- Protease deactivation of WAVE1, WAVE2 and NIP1/NIP2 was observed upon CYFIP1 knockout in PAXF1657 cells.
- sgRNA knockout or treatment with peptide targeting the WAVE complex reduced wound closure in scratch wound assay, consistent with previously described role of CYFIP1 in migration of breast cancer cells.
- The role of CYFIP1 in driving cancer cell growth was expanded to additional PDAC cell lines suggesting CYFIP1 as a potential therapeutic target for the treatment of pancreatic cancer.

Summary

- Pooled sgRNA CRISPR screens in pancreatic cancer cell lines revealed CYFIP1 as a negative selection hit in vitro and in vivo. Moreover, high tumor burden models in PDAC and pancreatic adenocarcinoma patients correlates with an unfavorable patient survival.
- In vivo validation of CRISPR screens using individual sgRNA targeting CYFIP1 show reduced growth in colony formation and invasion assays. sgRNA reduced growth phenotypes were confirmed to be on target by rescuing with vector resistant CYFIP1 cDNA.
- CYFIP1 knockouts in PAXF1657 xenograft model results in a significant delay in tumor growth in vivo.
- Protease deactivation of WAVE1, WAVE2 and NIP1/NIP2 was observed upon CYFIP1 knockout in PAXF1657 cells.
- sgRNA knockout or treatment with peptide targeting the WAVE complex reduced wound closure in scratch wound assay, consistent with previously described role of CYFIP1 in migration of breast cancer cells.
- The role of CYFIP1 in driving cancer cell growth was expanded to additional PDAC cell lines suggesting CYFIP1 as a potential therapeutic target for the treatment of pancreatic cancer.