Poster No. 401

Developing KRAS G12C Inhibitor-resistant Tumor Models for Efficacy Evaluation of Next-generation Anticancer Therapies

Jun Zhou[1], Zi Ye[1], Ning Bao[1], Dan Zhang[1], Aaron Hua[1], Chenpan Nie[1], Jessie (Jingjing) Wang[1], Rajendra Kumari[1], Leo Price[2], Xiaoxi Xu[1]

[1] Crown Bioscience Inc., 16550 West Bernardo Drive, Building 5, Suite 525, San Diego, CA, USA

[2] Crown Bioscience Inc., J.H. Oortweg 21, 2333 CH Leiden, The Netherlands

Introduction

AMG510, a novel KRAS G12C mutation-specific inhibitor approved in 2021 by the FDA, was the first therapy to directly target the KRAS oncoprotein in KRAS-mutant cancers, with MRTX849 as the second drug therapy approved in 2022. However, the emergence of resistance in patients who initially were responsive is a challenge. Uncovering the underlying mechanism of resistance would support the identification and development of novel therapies to overcome drug resistance. Several studies show that diverse genomic and histologic mechanism can confer resistance to covalent KRAS G12C inhibitors, including KRAS mutation. In this study, a panel of KRAS G12C inhibitor-resistant tumor models were generated by introducing a secondary KRAS mutation on top of G12C for use as *in vitro* and *in vivo* tools to develop possible strategies to overcome such resistance.

Methods

- CRISPR/Cas9 was used to introduce a secondary KRAS mutation, including Y96C, Y96D and Y96S was introduced into MIA PaCa-2, a human pancreatic carcinoma cell line, which harbors a homozygous KRAS G12C mutation. Point mutation knock-in was validated by sanger sequencing and cell identity was confirmed by SNP.
- The cell viability was measured by CellTiter-Glo after five days incubation with AMG510 and MRTX849.
- RAS–MAPK pathway activity was assessed in the parental and mutant cell lines Y96D by western blot.
- A xenograft of MIA PaCa KRAS G12C/Y96D cells was also established.

Results

- Successful homozygous point mutation knock-in was achieved as confirmed by Sanger sequencing **(Figure 1B)**. The double mutant cells displayed a similar growth rate **(Figure 1D)** as well as cell morphology **(Figure 1C)**.
- In cell viability assays, relative to KRAS G12C parental cells, cells expressing double-mutant alleles showed marked resistance to AMG510 and MRTX849 **(Figure 2A)**.
- Consistent with the effects on cell viability, persistent phosphorylated ERK (p-ERK) and p-RSK levels were also observed in KRAS G12C/Y96D expressing cells even at high concentrations of MRTX849, indicating sustained RAS-MAPK activity **(Figure 2B,C).**
- In addition, MIA PaCa-2-KRAS G12C/Y96D was able to grow *in vivo* **(Figure 3A,B)** and will be used for pharmacological evaluation of KRAS inhibitors.

References

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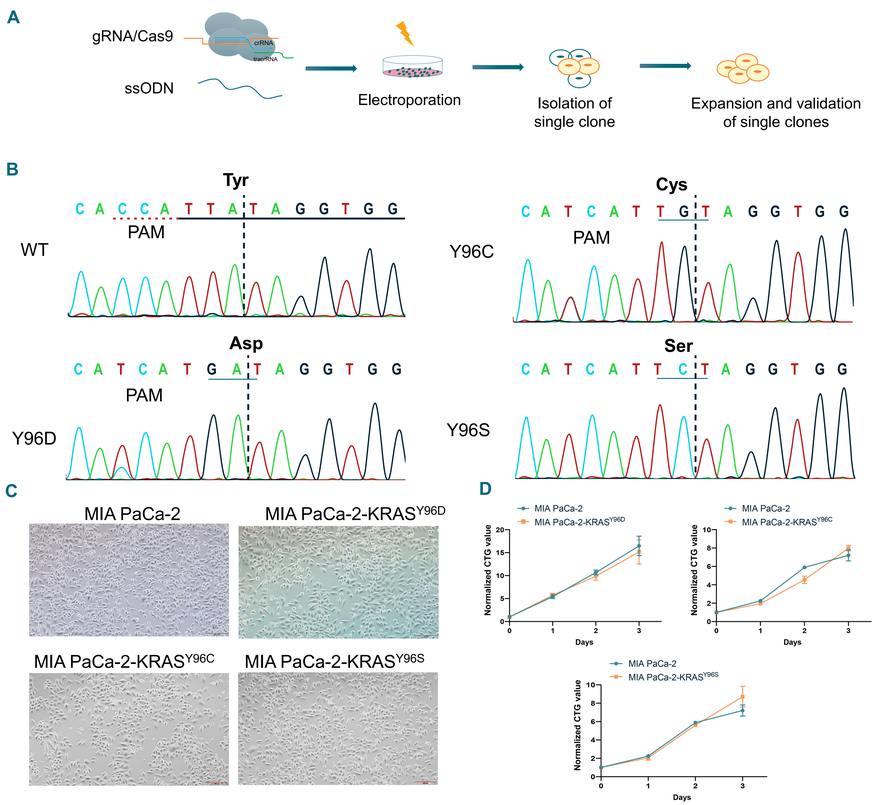
Results

Figure 1. Generation of KRAS Y96D, Y96C and Y96S point mutation in MIA PaCa-2 via CRISPR/Cas9.

1A. Scheme of developing a stable cell line with point mutation knock-ins by CRISPR/Cas9.
1B. Genomic DNA was extracted from MIA PaCa-2 and engineered clones of KRAS^{Y96D}, KRAS^{Y96C} and KRAS^{Y96S}. The target region was PCR-amplified followed by Sanger sequencing.
1C. Cell morphology of MIA PaCa-2 and the mutant cell lines was observed under microscopy and images were taken via digital camera (scale bar=200μm).
1D. Cells were seeded in 96-well plate at 2000 cells per well and cell growth kinetics were monitored on day one to day three by CellTiter-Glo. N=3, error bars=standard deviation.



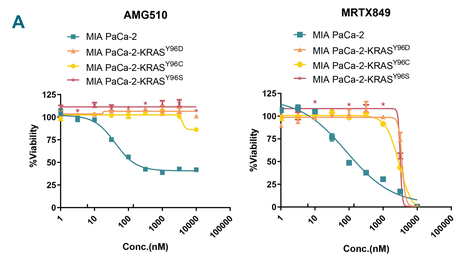
Conclusion

In summary, the KRAS double-mutant cells generated via CRISPR/Cas9 showed remarkable resistance to KRAS G12C inhibitors *in vitro*, consistent with persistent ERK activity, and can be used to assess preclinical *in vitro* / *in vivo* efficacy of novel KRAS-targeted therapeutics aimed to overcome resistance in the clinic. Crown Bioscience is developing a series of KRAS G12C inhibitor-resistant cell lines and patient-derived organoids to better facilitate efficacy testing of KRAS-targeted therapeutics.

Results Cont.

Figure 2. Cellular characterization of MIA PaCa-2-KRAS mutant cells *in vitro*.

2A. Cell viability assays performed in MIA PaCa-2, MIA PaCa-2-KRAS^{Y96D}, KRAS^{Y96C} or KRAS^{Y96S} mutant cell lines. Cells were treated with indicated drugs and the viabilities were measured with CellTiter-Glo. IC_{50} was summarized in the table. N = 3, error bars = standard deviation.
2B. Western blot analysis was performed after treating MIA PaCa-2 cells or MIA PaCa-2 KRAS^{Y96D} with vehicle or indicated concentration of AMG510 or MRTX849 for 4 hours.
2C. Quantification of phosphorylation of ERK and RSK levels in western blot analysis.



Summary of IC_{50} (nM)

Cell line	AMG510	MRTX849
MIA PaCa-2	138.04	151.36
MIA PaCa-2-KRAS^{Y96D}	NA	3595.66
MIA PaCa-2-KRAS^{Y96C}	NA	2829.02
MIA PaCa-2-KRAS^{Y96S}	NA	3214.48

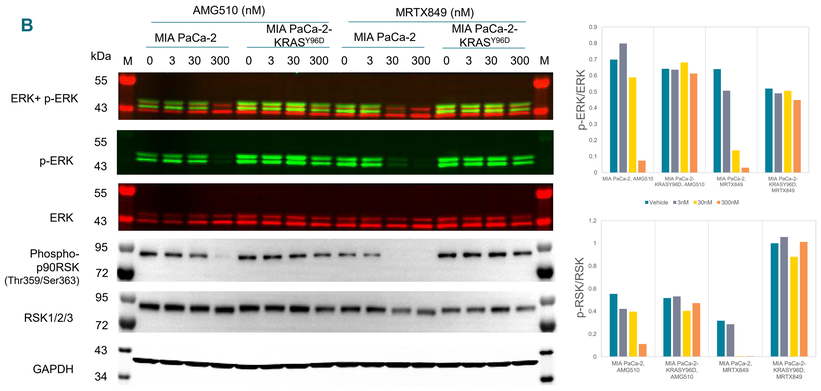
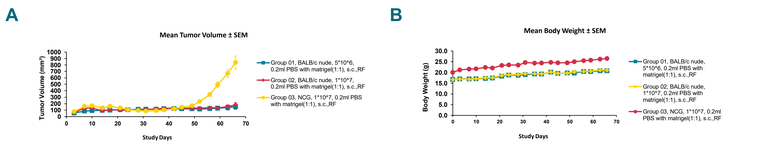


Figure 3. Model validation of MIA PaCa-2-KRAS^{Y96D} *in vivo*.

3A. MIA PaCa-2-KRAS^{Y96D} was inoculated into BALB/c nude mice or NCG mice and tumor volume was measured every three or four days. N=8, error bars=standard error mean.
3B. Body weight change was also monitored. N=8, error bars=standard error mean.



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