HORIZON DISCOVERY

Application Note

Pathway activation analysis in X-MAN® SW48 K-Ras Cell Lines: A tool for predicting patient response

Jessica Hunt, Annette Little, John Goodall and Zhiqiang Chen

Introduction

K-Ras is a small GTP-binding protein, which is the common upstream activator of several signaling pathways including RAF-MEK-ERK, PI3K/AKT and RAL-GEF/RAL. Hyper-activation of these pathways can result in several of the key hallmarks of cancer such as growth factor independence and aberrant cell proliferation, survival and motility. Ras mutations are extremely frequent in human cancer and mutations in K-Ras G12 and G13 are the most common.

Horizon Discovery has a suite of X-MAN® Cell Lines which are ideal for modeling the effects of K-Ras mutations. The gene editing platform was used to introduce one of seven different K-Ras G12 or G13 mutations into the SW48 cell line. Using this cell panel we have shown that K-Ras variants display diverse pathway activation and do not respond equally to MEK inhibition. Understanding the role of mutant K-Ras in modulating key cellular processes is critical to enable the successful development and use of novel cancer therapeutics.

The isogenic system uses endogenous promoters and enables panels of cell lines to be studied which differ only by the point mutation of interest, providing patient relevant *in vitro* model systems and allowing the effect of the mutation to be studied in isolation.

Cell Lines Used

Cell Line	Genotype	Cat. No.
SW48	K-Ras (G12A/+)	HD 103-009
SW48	K-Ras (G12C/+)	HD 103-006
SW48	K-Ras (G12D/+)	HD 103-011
SW48	K-Ras (G12R/+)	HD 103-010
SW48	K-Ras (G12S/+)	HD 103-013
SW48	K-Ras (G12V/+)	HD 103-007
SW48	K-Ras (G13D/+)	HD 103-002
SW48	K-Ras (G12V^{HaloTag}/+)	HD103-019

Results and Discussion

The levels of GTP-bound (active) Ras in the panel of SW48 K-Ras isogenic cell lines was assessed. All seven K-Ras mutant lines displayed increased levels of active Ras, as compared to the parental (P) SW48 cells (WT for K-Ras). Active Ras was below the level of detection in the parental cells.

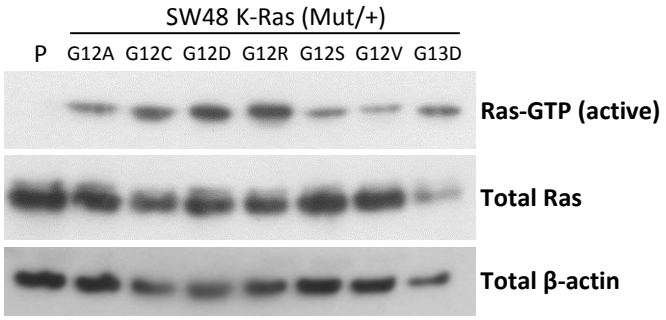

Figure 1. Ras activation assay (Cytoskeleton Inc, Cat#BK008). Western blots of total cell lysates were run to ensure that equal amounts of Ras were assayed.

The activation of downstream signaling pathways across the panel of SW48 K-Ras cell lines was characterized using Western blotting. A diverse pattern of pathway activation across the suite was observed. A broad increase in EGFR expression was seen and in some cases up-regulation of pAKT. Interestingly, only minimal effects were seen on pMEK/pERK in these cell-types.

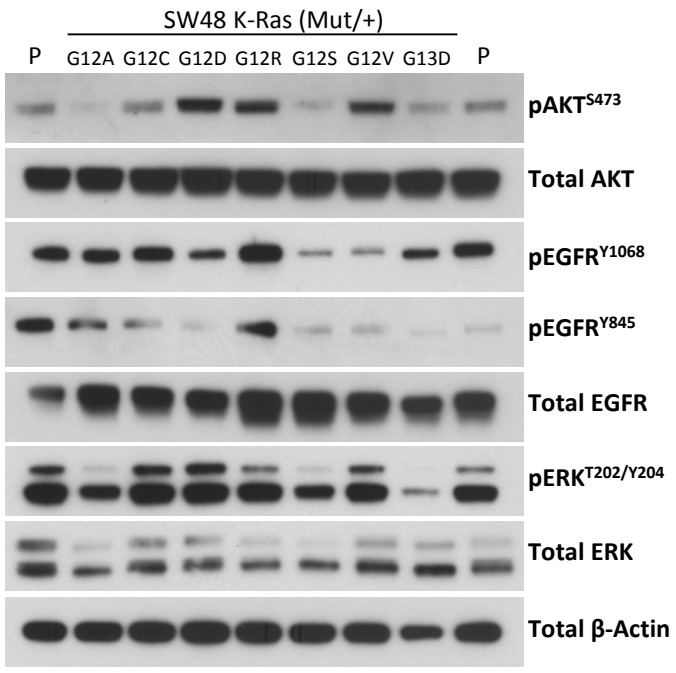

Figure 2. Basal pathway activation of AKT, EGFR and ERK was assessed by Western blotting in the SW48 K-Ras suite.

The anti-proliferative activity of a panel of over 30 therapeutic agents was profiled. This revealed a striking pattern of resistance towards five chemically distinct classes of MEK1/2 inhibitors across the K-Ras suite, highlighting the variation in response to MEK inhibition between K-Ras variants *in vitro*, and exemplifying the need for rational patient stratification in the clinic.

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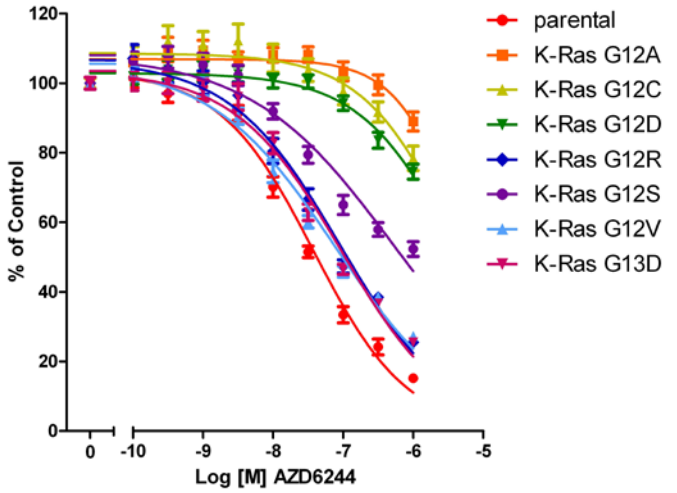

Figure 3. SW48 K-Ras mutant cells show a pattern of resistance to MEK inhibitors.

Resistance to the MEK inhibitor AZD6244 could be overcome by concentrations of the epidermal growth factor receptor inhibitor Cetuximab that had minimal anti-proliferative effects alone.

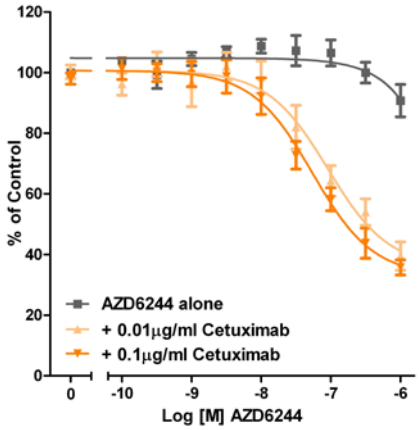

Figure 4. Resistance can be overcome by the addition of Cetuximab; example data from K-Ras (G12A/+) cells is shown.

A suite of reporter disease models have also been generated, combining the SW48 X-MAN® K-Ras mutant cell lines with HaloTag® reporter technology (available through partnership with Promega) providing additional resources for pathway activation studies. HaloTag® technology is based on the formation of a covalent bond between the protein tag and a synthetic ligand, and can be used for multiple applications including protein purification, protein interaction and imaging studies.

In these cell lines, HaloTag® is fused to the K-Ras Variant, expressed at the endogenous level. Protein interactions and localization can therefore be studied under physiologically relevant conditions.

We have shown by confocal microscopy that HaloTag® signal localization is consistent with the expected biology of K-Ras in the suite of K-Ras HaloTag® cell lines, and provides sufficient signal for imaging and protein interaction studies.

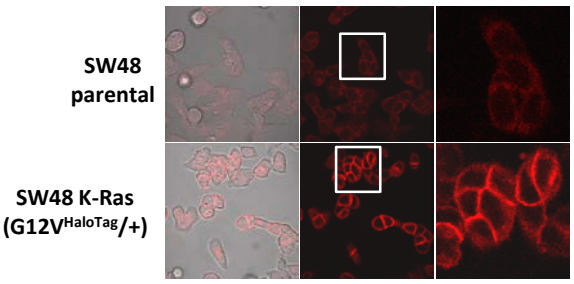

Figure 5. Confocal images of live cells labeled with TMR ligand show the K-Ras (G12VHaloTag/+) fusion protein localized to the plasma membrane, and low background in the parental cells.

Conclusion

X-MAN® K-Ras isogenic cells are a powerful and relevant tool for drug discovery and development. The K-Ras isogenic SW48 cell panel clearly demonstrates increased Ras activity and differential activation of downstream signaling pathways. In addition, this cell line suite exemplifies the significance of genetic background when determining the sensitivity of cells to clinically relevant inhibitors and highlights the need for personalized medicine and rational therapeutic combinations.

Horizon Support

Horizon supplies genetically-defined cell lines, custom cell line generation, *in vivo* models, reporter gene assay kits, molecular reference standards and assay development and screening services to organizations engaged in academic research; drug discovery and development; clinical diagnostics; and biopharmaceutical process optimization. Please contact us to learn more about how Horizon can support your work.

X-MAN® Reporter kits similar to those listed in this Application Note include:

Cell Line	Genotype
SW48	K-Ras (+HaloTag/+)
SW48	K-Ras (G12CHaloTag/+)
SW48	K-Ras (G12DHaloTag/+)
SW48	K-Ras (G13DHaloTag/+)
SW48	K-Ras (G12A/+); Luciferase over-expression
SW48	K-Ras (G12D/+); Luciferase over-expression
SW48	K-Ras (G12R/+); Luciferase over-expression

Please contact Horizon or got to: **www.horizondiscovery.com** for more information

t + 44 (0)1223 655580
f + 44 (0)1223 655581
e info@horizondiscovery.com
w www.horizondiscovery.com
Horizon Discovery, 7100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom