Horizon Discovery X-MAN™ cell lines reveal how PI3K mutation can result in EMT switch and increased invasiveness

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INTRODUCTION

A major challenge facing the development of PI3K-targeted pharmaceutical agents is the lack of patient-relevant, *in vitro* model systems. Studies into the function of oncogenic mutations are commonly carried out in cell line panels that are mutant or wild-type for the gene of interest. Interpretation of these studies can be confounded by additional genetic differences between each cell line background, calling into question the reliability of the results. An alternative approach is to over-express the protein of interest. However, generating a significantly higher protein level than is normally found in the cell can lead to results that do not reflect the true biology of the disease.

Horizon Discovery's proprietary rAAV GENESIS™ technology directly addresses this problem through the development of X-MAN™ isogenic cell lines. Isogenic cell lines are genetically identical except for the mutation status of an endogenous gene, thereby permitting evaluation of a single genetic alteration expressed at the endogenous level. The technology permits not only knock-out of existing mutations, but also introduction of single or multiple mutations, deletions, amplifications and translocations. Therefore, isogenic cell line models allow the interrogation of protein function without the problems associated with protein over-expression studies or genetically diverse cell line panels.

In order to investigate the effect that a PI3K mutation might have on tumour development, we employed isogenic cell lines derived from a 'normal' background (MCF10A mammary epithelial cells). A PI3Kα (H1047R) mutation (commonly found in breast cancer patients), was knocked-in to produce a pair of MCF10A cell lines; parental cells that express wild-type PI3K or PI3Kα (H1047R) cells that express mutant PI3K[1].

CELL LINE GENERATION

Figure 1 shows the generation of the MCF10A PI3Kα (H1047R) isogenic cell line using Horizon Discovery's GENESIS™ genome editing technology.

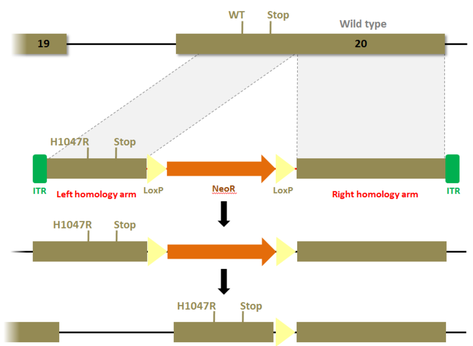

Figure 1. An overview of the MCF10A isogenic cell line generation process.

RESULTS

Parental and PI3Kα (H1047R) cells were profiled in 2D proliferation assays using a PI3K inhibitor, GDC-0941. Surprisingly, the parental and PI3Kα (H1047R) cell lines displayed similar sensitivities to the inhibitor in this 2D assay format (Figure 2A). Consistent with the principle that PI3K mutations activate AKT and other downstream signalling molecules, the phosphorylation levels of several downstream pathway components were increased in PI3Kα (H1047R) cells in comparison with parental cells (Figure 2B). As expected treatment with the PI3K inhibitor resulted in reduced phosphorylation of the downstream pathway proteins.

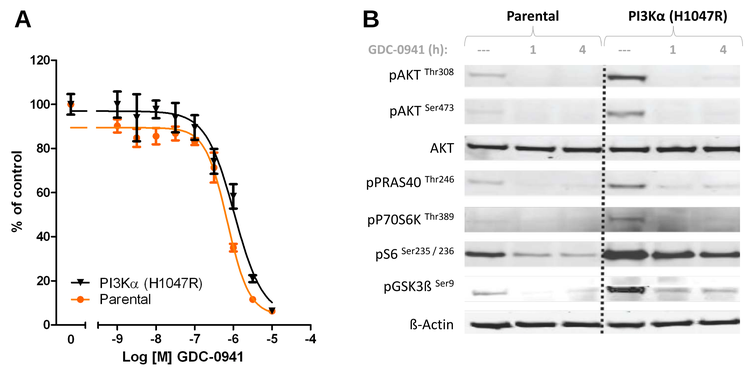

Figure 2. PI3Kα (H1047R) cells have an activated PI3K pathway but are not more sensitive to GDC-0941 when tested in a 2D proliferation assay. A. 96h proliferation assay using the PI3K inhibitor GDC-0941 in parental and PI3Kα (H1047R) MCF10A cell lines. B. Western blot analysis of PI3K pathway components.

Since a PI3K inhibitor did not selectively inhibit the growth of PI3Kα (H1047R) cells, the consequence of introducing the PI3K mutation was further investigated using a microarray study to compare the gene expression profiles of parental and PI3Kα (H1047R) MCF10A cells (Figure 3). This analysis revealed that the PI3Kα (H1047R) cells have up-regulated expression of mesenchymal genes and down-regulated expression of epithelial genes – a profile that is suggestive of the PI3Kα (H1047R) cells undergoing epithelial to mesenchymal transition (EMT).

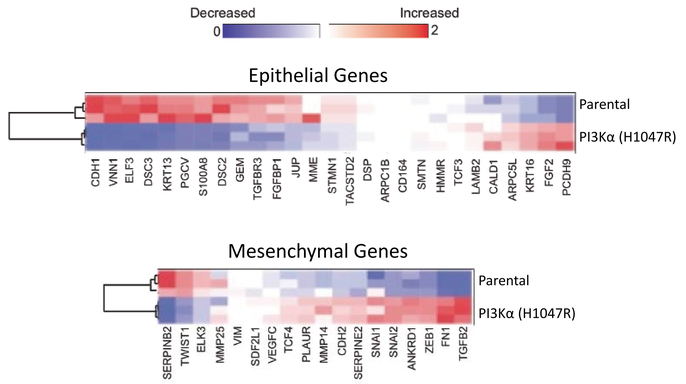

Figure 3. PI3Kα (H1047R) cells have a gene expression signature indicative of EMT switch. Parental and PI3Kα (H1047R) gene expression signatures were investigated by microarray analysis.

Increased invasive potential is commonly associated with EMT and oncogenic PI3K is believed to play a critical role in driving metastasis[2]. Therefore, the phenotype of parental and PI3Kα (H1047R) cells grown in 3D was investigated. In this format, PI3Kα (H1047R) cells formed a distinct networked morphology, suggestive of an invasive phenotype, while parental MCF10A cells expressing wild-type PI3Kα grew as small rounded cells (Figure 4). Treatment with the PI3K inhibitor GDC-0941 reversed this morphology but treatment with an AKT inhibitor (AKT1/2i) did not.

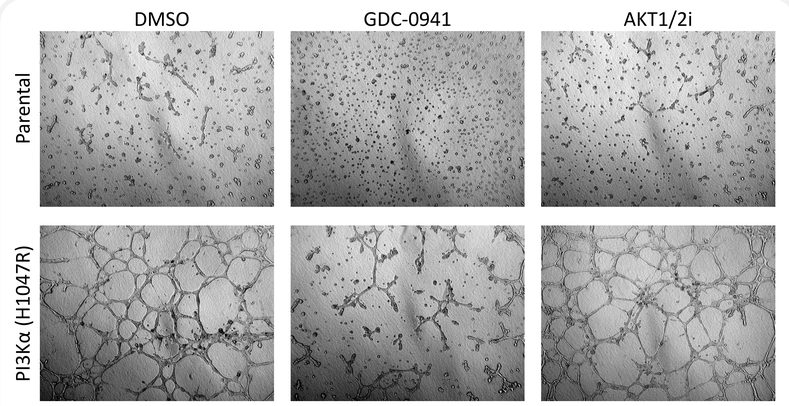

Figure 4. PI3K mutant cells have an invasive phenotype. Cells were seeded onto Geltrex BME and treated with DMSO, the PI3K inhibitor GDC-0941 or the AKT inhibitor AKT1/2i for 24h.

To investigate if the morphology displayed when the cells are grown in 3D is linked to increased invasiveness, invasion assays were performed with parental and PI3Kα (H1047R) cells. The PI3Kα (H1047R) cells invaded approximately 5-fold faster than the parental cells over 24h (Figure 5). Furthermore, treatment with GDC-0941 reduced this invasive phenotype, suggesting that the increased invasive capacity is due to the PI3Kα (H1047R) mutation. Interestingly, use of the AKT inhibitor increased the invasive phenotype of the PI3Kα (H1047R) cell line, suggesting that inhibition of AKT can actually result in feedback activation of this pathway.

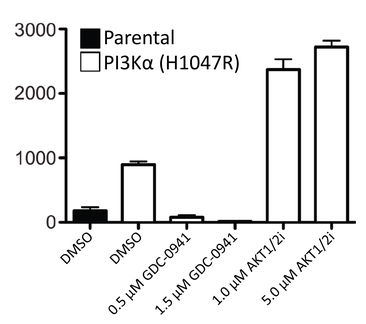

Figure 5. PI3Kα (H1047R) cells exhibit a more invasive phenotype than parental cells. Parental and PI3Kα (H1047R) cells were seeded into Boyden chambers coated with matrigel and treated with DMSO, GDC-0941 (PI3K inhibitor) or AKT1/2i (AKT inhibitor). The number of cells/well that invaded through the matrigel were counted 24h post-drug treatment.

CONCLUSION

- By using Horizon's rAAV GENESIS™ technology, it has been possible to demonstrate that introduction of a PI3K mutation results in EMT switch and increased cell invasiveness.
- This phenotype was revealed by the ability to engineer expression of the mutation at the endogenous level.

REFERENCES

1. Di Nicolantonio *et al.*, PNAS. 2008. 105(52):20864-9.
2. Samuels *et al.*, Cancer Cell. 2005. 7(6):561-73.