Drug resistance mechanisms in FGFR-driven cancers

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Introduction

Fibroblast growth factor (FGF) signalling regulates a plethora of cellular functions such as development, proliferation, survival, migration and differentiation. Due to its powerful effects on the cell, FGF signalling is often hijacked in cancer (Table 1). One relatively common aberration is activating mutation, seen in a variety of cancers. Many of these are highly sensitive to treatment with FGFR inhibitors. However, cancer cells are able to develop resistance to such therapies relatively readily – an Achilles heel of kinase inhibitors. Therefore, our aim is to delineate resistance mechanisms in cancer cells to ultimately improve treatment options.

In my project I focus on cancers harbouring FGFR1 and FGFR2 alterations (Table 1).

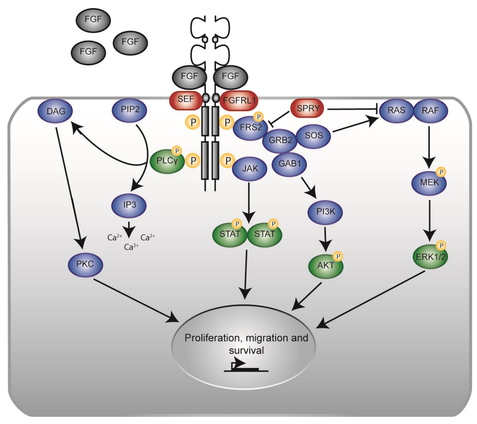


Figure 1 – FGFR signalling. Schematic representation of FGFR signalling. Upon ligand binding four key pathways are induced: MAPK, PI3K/AKT, PLCγ and JAK/STAT.

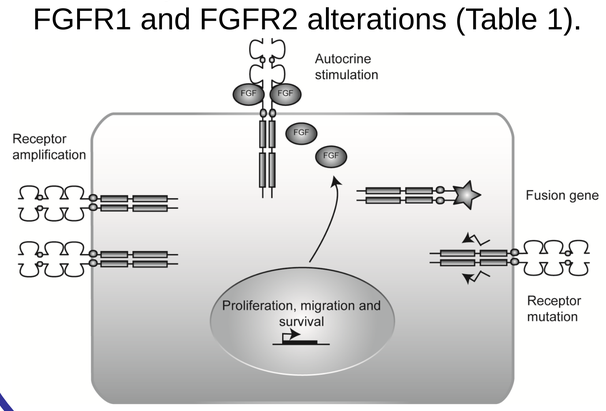


Figure 2 – Aberrant FGFR signalling in cancer.

Receptor	Related Cancer
FGFR1	
Amplification	Lung (16.3%) (Cancer Genome Atlas Research Network 2012) Breast (10.8%) (Cancer Genome Atlas Network 2012) Bladder (8.7%) (Cancer Genome Atlas Research Network 2014a)
Mutation	Stomach (4.2%) (Cancer Genome Atlas Research Network 2014c) Melanoma (4.1%) (Hodis et al. 2012) Lung (3.4%) (Peifer et al. 2012)
Deletion	Prostate (8.2%) (Grasso et al. 2012) Lung (3.3%) (Imielinski et al. 2012) Bladder (3.1%) (Iyer et al. 2013)
FGFR2	
Amplification	Stomach (5.2%) (Cancer Genome Atlas Research Network 2014c) Breast (1.9%) (Cancer Genome Atlas Network 2012) Ovary (1.9%) (Cancer Genome Atlas Research Network 2011)
Mutation	Endometrium (12.5%) (Cancer Genome Atlas Research Network et al. 2013) Cholangiocarcinoma (10%) (Jiao et al. 2013) Melanoma (4.4%) (Krauthammer et al. 2012)
Deletion	Prostate (1.6%) (Grasso et al. 2012) Lung (0.9%) (Cancer Genome Atlas Research Network 2014b) Glioblastoma (0.4%) (Brennan et al. 2013)

Table 1 – Aberrant FGFR1 and FGFR2 signalling and associated neoplastic diseases.

Current therapies

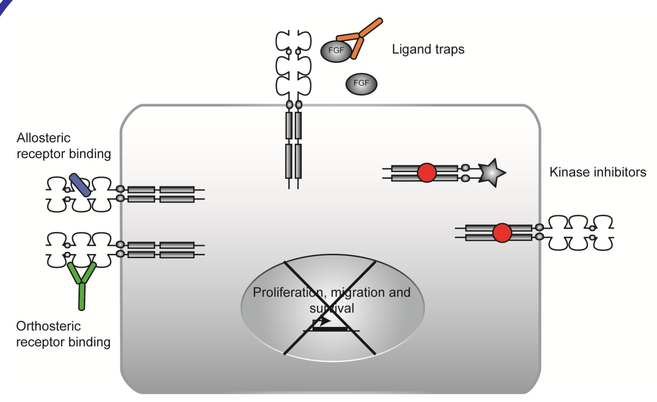


Figure 3 – Mechanisms of FGFR inhibition. Schematic representation of therapeutic options and their mechanism of action.

There has been a large motivation to develop FGFR-targeted therapeutics. Kinase inhibitors to target FGF signalling have been developed as shown in Table 2.

FGFR-specific TKI inhibitors				
AZD4547	Astra Zeneca	FGFR1-3	Phase II	Multiple solid tumours
BGJ398	Novartis	FGFR1-3	Phase II	Multiple solid tumours, melanoma
LY2874455	Eli Lilly	FGFR1-4	Phase I	Multiple solid tumours
Debio 1347	Debiopharm	FGFR1-3	Phase I	Solid tumours
TAS-120	Taiho Pharma	FGFR1-4	Phase I/II	Solid tumours, multiple myeloma
JNJ42756493	Astex pharma/ Janssen	FGFR1-4	Phase I	Solid cancers, lymphoma, urothelial

Table 2 – FGFR-targeted therapies in clinical trials.

Resistance

Current compound therapies have a limited utility due to compensatory and adaptative mechanisms of target nodes in the receptor tyrosine kinase network. This means cells can become resistant by re-wiring their signalling pathways. For our studies we are interested in a variety of cancers, however we are focusing on endometrial and gastric cancer in the first instance. From endometrial cancer studies, PHLDA1 was identified to underpin the development of resistance by an AKT-related compensatory mechanism. PHLDA1 is a negative regulator of AKT and was significantly down-regulated in resistant cells.

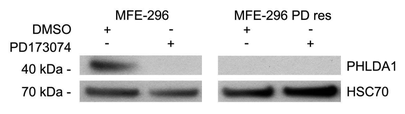


Figure 4 – PHLDA1 levels of MFE-296 cells and resistant MFE-296 upon PD173074 treatment.

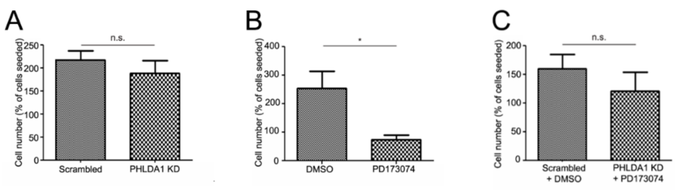


Figure 5 – PHLDA1 knockdown. AN3CA endometrial cells can be made resistant to FGFR inhibitors by knocking down PHLDA1.

3D Alvetex model

- Co-culture of resistant and parental cells with fibroblasts for a more physiological cell model system.
- Mimics the 3D structure of cancer cells and the interaction with stromal cells to create a more physiomimetic environment.

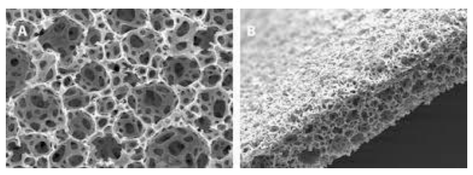
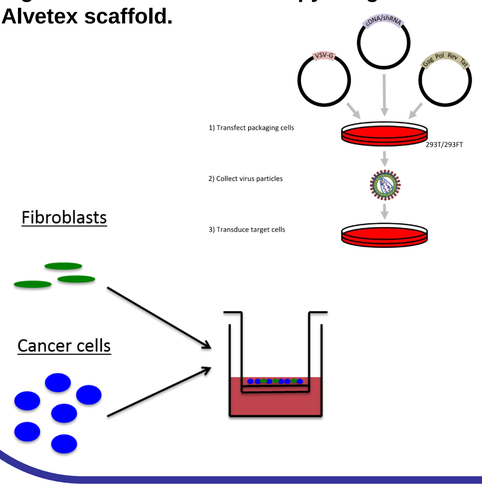


Figure 6 – Electron microscopy image of an Alvetex scaffold.

Feature	Benefits for 3D cell culture
Simple polystyrene	• Easy switch between 2D and 3D cell culture • Inert, no new experimental variables • Stable, does not degrade • Can be precoated with ECM proteins
Consisitent scaffold structure	• Reproducible, consistent results, low batch to bath variability
Scaffold is only 200µm thick	• No cell is ever more than 100µm away from each other – mimics in vivo conditions • Cells can feed and excrete via passive diffusion
Very high porosity (>90%)	• Cells can easily and move freely into the scaffold and around
Void dimension is 36-40µm	• Up to 75 cells can occupy a single void

Table 3 – Benefits of Alvetex for 3D cell culture.



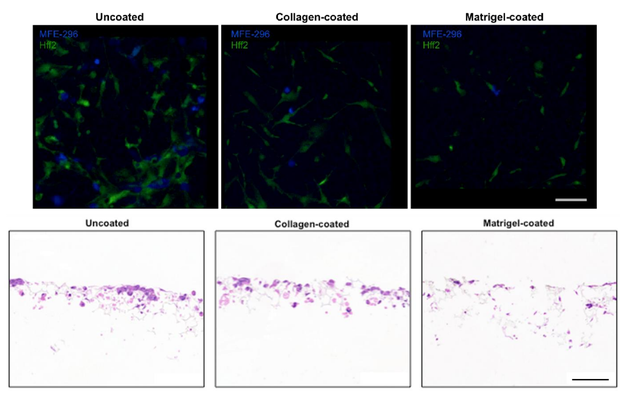
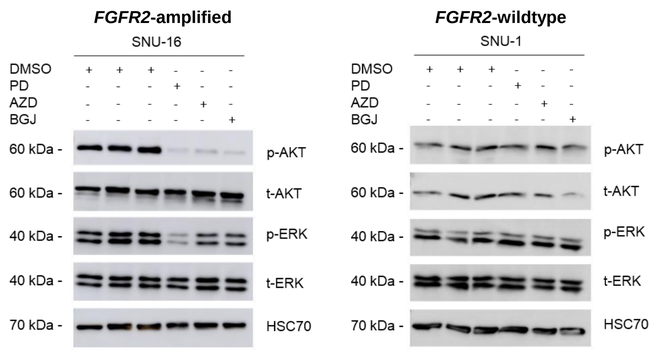


Figure 7 – Coating methods of Alvetex scaffolds showing uncoated collagen and Matrigel-coated scaffolds.



Mutations: *CDKN2A*, *TP53*

Mutations: *KRAS*, *MLH1*

Figure 8 – Cell signaling of *FGFR2*-amplified (SNU-16) and *FGFR2* wild-type (SNU-1) gastric cancer upon treatment with FGFR inhibitors.

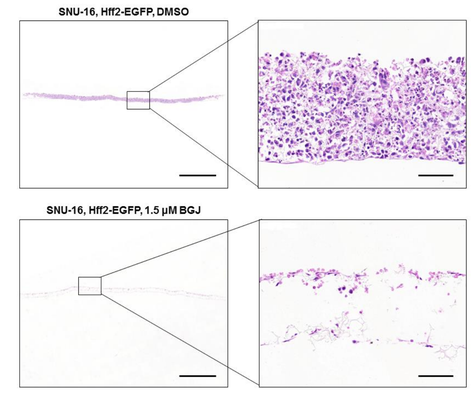


Figure 9 – SNU-16 cells are highly sensitive to FGFR inhibitor BGJ398.

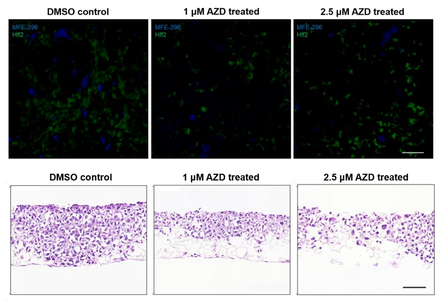


Figure 10 – AZD treatments of MFE-296-azurite, Hff2-EGFP co-culture model reduces cell number over a time range of 7 days.

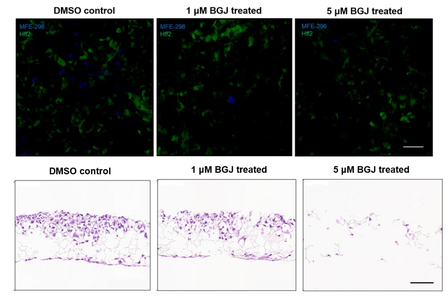


Figure 11 – BGJ treatments of MFE-296-azurite, Hff2-EGFP co-culture model reduces cell number over a time range of 7 days.

Future work & Ideas

- Generation of BGJ-resistant MFE-296 and SNU-16 with and without stromal support, comparing the acquisition of resistance in cells cultured in Alvetex and conventional plasticware.
- Microarray: Generate RNA samples for microarray of co-culture model of cancer models in 2D and 3D with an without stromal support.
- qPCR to check for expression levels of downstream targets of FGF signalling in gastric cancer.
- Fluorescent labelling of further cancer cell lines (SNU-16, SNU-1, H1299, H520) and fibroblasts (MRC-5).

Acknowledgments

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References

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Fearon et al. *submitted* PHLDA1 mediates drug resistance in receptor tyrosine kinase driven cancer.

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