The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	**Molecular mechanisms of acquired chemotherapy resistance in colorectal cancer and their implications for novel therapeutics development**
Short Project Title:	Deciphering and targeting mechanisms of acquired chemotherapy resistance in colorectal cancer
SUPERVISORY TEAM	
Primary Supervisor(s):	Dr Marco Gerlinger
Associate Supervisor(s):	Dr Jyoti Chaudhary
Backup Supervisor:	Dr Anguraj Sadanandam
Lead contact person for the project:	Dr Gerlinger
DIVISIONAL AFFILIATION	
Primary Division:	Molecular Pathology
Primary Team:	Translational Oncogenomics Team

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Colorectal cancer (CRC) is the third commonest cause of cancer mortality[1]. Multiple trials of novel targeted- and immuno-therapies recently failed and chemotherapy remains the most effective therapeutic modality. Resistance to chemotherapy eventually develops in all patients but the molecular mechanisms driving resistance are very poorly understood. Detailed insights into drug resistance mechanisms in other tumour types enabled the development of innovative drugs that overcome resistance and increase survival[2,3]. However, the lack of mechanistic understanding precludes similar advances in CRC. The main reasons for the limited insights are a) the difficulties in obtaining biopsies from chemotherapy resistant CRCs and b) a lack of cancer cell lines from resistant CRCs for functional interrogation.

Recent patient derived organoid (PDO) culture technologies allow establishing long-term cancer cell cultures from CRCs[4]. We have generated 12 PDOs from chemotherapy resistant CRCs and also acquired PDOs from untreated CRCs. PDOs from treatment naïve CRCs were sensitive to 5FU, irinotecan and oxaliplatin in vitro whereas those from resistant tumours were refractory, demonstrating that chemotherapy resistance is maintained as a cell intrinsic property in PDOs. Exome- and RNA-sequencing of both PDO groups found no genetic alterations associated with resistance but identified 634 genes and multiple signalling pathway signatures that were significantly over- or underexpressed in resistant PDOs. This supports the notion that deregulated gene expression, potentially as a consequence of epigenetic alterations, is important for resistance.

PROJECT AIMS

We will use this PDO collection to dissect molecular and functional phenotypes of resistant vs sensitive PDOs. These will inform genetic and drug screens to determine the key mechanism of CRC chemotherapy resistance and

then evaluate opportunities to reverse these (Fig.1). The specific aims are to:

1. **define how resistant vs sensitive PDOs differ with respect to functional phenotypes with relevance for chemotherapy efficacy**
2. **apply proteomics and phospho-proteomics analysis to reveal differentially expressed proteins and signalling events in chemotherapy sensitive vs resistant PDOs**
3. **integrate cellular phenotypes, RNA-sequencing data and proteomics data to identify candidate genes and molecular pathways that are likely to drive drug resistance**
4. **perturb genes from Aim 3 by drug and genetic (CRISPR and cDNA) screens in PDOs to validate the exact genes/pathways that drive chemotherapy resistance.**
5. **assess whether resistance mechanisms can be reversed in order to restore drug sensitivity**

RESEARCH PROPOSAL

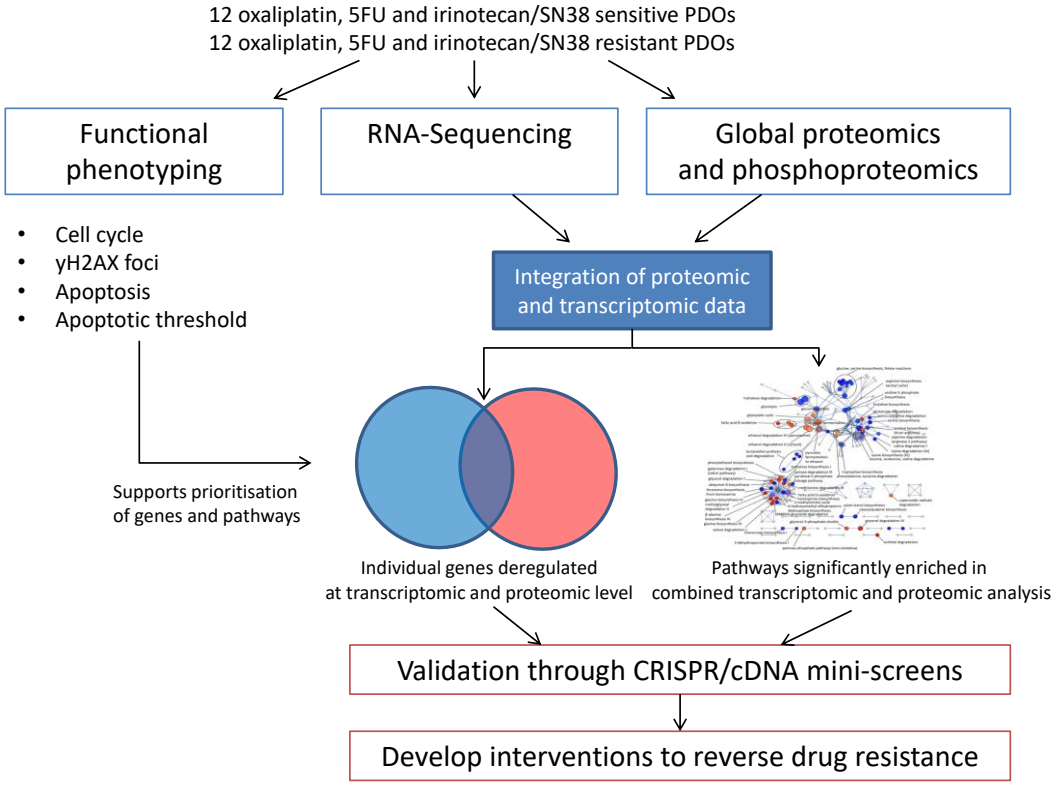

Figure 1: Schematic of the project indicating the different datasets that will be generated and their integration to prioritize genes for functional validation and subsequent identifications of approaches to reverse resistance.

Aim 1. Define functional phenotypes of resistant vs sensitive PDOs

Profiling of phenotypic responses to chemotherapy will help to dissect the mechanisms that contribute to chemotherapy resistance. In particular, we will investigate whether resistant PDOs differ from sensitive PDOs in their cell cycle response, apoptosis induction and DNA damage induction 48 hours after exposure to 5FU, irinotecan or oxaliplatin. Cell cycle responses (G1-arrest, G2-arrest, sub-G1 fraction) will be measured by propidium iodide staining and FACS, DNA damage by yH2AX foci quantification by FACS, apoptosis by cleaved PARP Western and the apoptotic threshold of the cells will be determined by BH3 profiling[5]. These results will for example reveal whether impaired arrest at cell cycle checkpoints contributes to resistance, whether DNA damage induction is lower in resistant lines or whether they are less likely to undergo apoptosis despite similar DNA damage levels. This will help prioritizing genes/pathways identified in Aims 2/3 for further study if they have known links to deregulated phenotypes.

Aim 2. Global proteomics and phospho-proteomics analysis

Differential gene expression analysis identified 634 candidate genes which are differentially expressed between chemo-resistant and -sensitive PDOs. However, RNA expression levels are poor surrogates of protein expression for many genes. Genes downregulated specifically at the protein are therefore overlooked by RNA-seq. Furthermore, any single large scale screening modality is likely to identify large numbers of false positives and negatives and integration with a second screening modality can significantly improve accuracy[6,7]. We will therefore apply shotgun proteomics using tandem mass spectrometry (MS) for quantitative protein and protein phosphorylation analysis in sensitive and resistant PDOs. MS will be applied to 12 resistant and 12 sensitive PDOs, untreated and after 6h of chemotherapy exposure (to identify early phosphorylation/signalling events that differ between sensitive and resistant PDOs). These datasets will be interrogated for differentially expressed proteins and differential phosphorylation using statistical approaches and correlation analysis[8].

Aim 3. Integration of RNA and protein/phosphoprotein expression data

Genes and proteins deregulated in resistant vs sensitive PDOs will be mapped onto cellular pathways and biological processes in a proteo-transcriptomics approach[8] to reveal genes and pathways/processes which are significantly differentially modulated for subsequent functional analysis. If a large number of pathways/genes are identified, we will prioritize those known to influence phenotypes that are altered in resistant cells (from Aim 1) for further study (e.g. prioritizing cell cycle regulatory genes if for example cell cycle regulation is disrupted).

Aim 4. Validation of resistance mechanisms through functional genetic and compound mini-screens in PDOs

Genes and key regulators of signalling pathways prioritized from 3. will be a) knocked out through CRIPSR in resistant PDOs if there was evidence that upregulation/activation associates with resistance or b) stably overexpressed by lentiviral cDNA transduction if downregulation was associated with resistance. The reverse approach (overexpression of genes upregulated at resistance and knock out of those downregulated will be taken in sensitive PDOs). We will assess if each of the perturbations influences the response to each of the three chemotherapy agents (5FU, irinotecan, oxaliplatin) using a 96 well plate high-throughput format with CellTiter-Glow as a viability readout. Signalling pathways or genes that show increased or decreased phosphorylation in resistant PDOs will also be targeted with suitable drugs (if available for this pathway) to validate their role in chemotherapy resistance. Once the role of specific genes or pathways in chemotherapy resistance has been validated, we will perform further mechanistic investigations.

Aim 5. Identification of therapeutic opportunities to reverse chemotherapy resistance

The robust validation of genes/pathways that regulate chemotherapy sensitivity will be followed by efforts to target and reverse these resistance mechanisms. Suitable drugs or chemical compounds will be identified (either those that target upregulated pathways directly or indirectly, by identifying compounds from the NIH LINCS database[9] that counter the gene-network perturbations identified in resistant PDOs). Genes/proteins that have been validated to drive resistance through overexpression or increased phosphorylation and for which no drugs are currently available will be discussed with the Cancer Therapeutics Division to assess the potential for drug development efforts.

Expected outcomes:

Mechanisms of chemotherapy resistance remain elusive in CRC but our living biobank of sensitive and resistant PDOs combined with large-scale RNA sequencing and proteomics should identify candidate mechanisms of resistance that will be thoroughly validated through subsequent mini-screens. This project should hence provide first insights into clinically relevant chemotherapy resistant mechanisms and reveal rational new approaches to target or re-sensitise chemotherapy resistant CRCs.

LITERATURE REFERENCES

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5 Deng, J. *et al.* BH3 profiling identifies three distinct classes of apoptotic blocks to predict response to ABT-737 and conventional chemotherapeutic agents. *Cancer cell* **12**, 171-185, doi:10.1016/j.ccr.2007.07.001 (2007).

6 Xie, Y. & Ahn, C. Statistical methods for integrating multiple types of high-throughput data. *Methods Mol Biol* **620**, 511-529, doi:10.1007/978-1-60761-580-4_19 (2010).

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8 Roumeliotis, T. I. *et al.* Genomic Determinants of Protein Abundance Variation in Colorectal Cancer Cells. *Cell reports* **20**, 2201-2214, doi:10.1016/j.celrep.2017.08.010 (2017).

9 Koleti, A. *et al.* Data Portal for the Library of Integrated Network-based Cellular Signatures (LINCS) program: integrated access to diverse large-scale cellular perturbation response data. *Nucleic acids research* **46**, D558-D566, doi:10.1093/nar/gkx1063 (2018).

CANDIDATE PROFILE	
Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)	
Pre-requisite qualifications of applicants: e.g. BSc or equivalent in specific subject area(s)	BSc or equivalent in a biological science
Intended learning outcomes:	• Patient derived organoid culture • Drug sensitivity analysis • Large scale proteomics and transcriptomics data analysis • Candidate gene/target prioritisation from large scale data analysis • Genetic screens • Phenotyping of drug responses • Team research involving regular interaction with proteomics, transcriptomics and bioinformatics specialsist
ADVERTISING DETAILS	
Project suitable for a student with a background in:	☒ Biological Sciences ☐ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science ☐ Other (provide details)
Keywords:	**1. Drug resistance**

	2. Colorectal cancer
	3. Proteomics
	4. Transcriptomics
	5. Genetic screen
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