

The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS

Project Title:	Exploring tankyrase regulation and cellular roles by CRISPR-based phenotypic screens and functional studies
Short Project Title:	A genetic approach to studying tankyrase regulation and function

SUPERVISORY TEAM

Primary Supervisor(s):	Dr. Sebastian Guettler and Professor Chris Lord
Other supervisory team members:	Dr. Dragomir Krastev Professor Ian Collins

DIVISIONAL AFFILIATION

Primary Division:	Breast Cancer Now Research Centre
Primary Team:	Gene Function (Lord)
Other Division:	Structural and Cancer Biology
Other Team:	Structural Biology of Cell Signalling (Guettler)

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Tankyrase is a poly(ADP-ribose)polymerase (PARP) with complex cellular roles pertinent to the function of cancer cells (Riffell et al., 2012). For example, tankyrase supports oncogenic Wnt/ β -catenin signalling (reviewed by Mariotti et al., 2017), is required for telomere extension in human stem and cancer cells (Smith and de Lange, 2000), and counteracts the tumour-suppressive Hippo signalling pathway (Wang et al., 2015). Tankyrase has therefore attracted considerable interest as a potential anti-cancer target (Riffell et al., 2012). There are two human tankyrase paralogues, TNKS and TNKS2 (here collectively referred to as “tankyrase” where the discussed aspects apply to both paralogues). A multi-domain protein, tankyrase consists of five N-terminal ankyrin repeat clusters (ARCs) that mediate substrate recruitment, followed by a polymerising sterile alpha motif (SAM) domain and a catalytic PARP domain (Figure 1). With the exception of polymerisation-induced auto-PARylation (Mariotti et al., 2016) and an NAD⁺ concentration-dependent activity of tankyrase (Zhong et al., 2015), we currently do not understand how tankyrase activity is regulated in cells.

In Wnt/ β -catenin signalling, tankyrase poly(ADP-ribosyl)ates (PARylates) AXIN1/2, an essential scaffolding component of the β -catenin destruction complex (reviewed by Mariotti et al., 2017). AXIN1/2 PARylation either targets AXIN1/2 for ubiquitination and proteasomal degradation or, under Wnt-stimulated conditions, assists in the conversion of the β -catenin destruction complex to a receptor-associated ‘Wnt signalosome’, which is incapable of initiating β -catenin degradation (reviewed by Mariotti et al., 2017). Moreover, tankyrase can also promote Wnt/ β -catenin signalling through PARP-activity-independent scaffolding (Mariotti et al., 2016), but the underlying mechanisms remain poorly understood. Early evidence points to different requirements for tankyrase PARP activity under basal and Wnt-stimulated conditions (reviewed by Mariotti et al., 2017).

Efforts to develop catalytic tankyrase inhibitors, including those at ICR (CL and colleagues, unpublished work), have resulted in highly specific compounds (reviewed by Mariotti et al., 2017). While some cellular and *in vivo* models of colorectal cancer have shown promise of tankyrase inhibitors (reviewed by Mariotti et al., 2017), sensitivity determinants of cells, and therefore potential biomarkers for tankyrase inhibitors in cancer, remain largely unknown.

The aims of this project are:

- (1) to establish and perform a CRISPR-X genetic screen to identify novel regulatory mechanisms that govern tankyrase catalytic and non-catalytic functions in Wnt/ β -catenin signalling.
- (2) to use the information gained from the CRISPR-X screen to further explore tankyrase regulatory mechanisms.
- (3) to identify the homeostatic mechanisms through which cells respond to tankyrase inhibition, as a means to extending our understanding of tankyrase's roles in maintaining cell fitness.

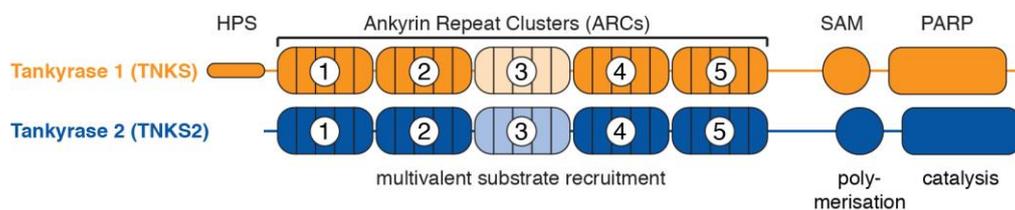


Figure 1: Domain organisation of the two human tankyrase paralogs. Known domain functions are indicated.

PROJECT AIMS

- Establish a phenotypic screening system for tankyrase by CRISPR-X
- Perform a CRISPR-X mutagenesis screen for tankyrase to identify novel regulatory principles in the context of Wnt/ β -catenin signalling
- Explore identified regulatory mechanisms in cell-based assays and biochemical studies using purified proteins
- Perform a genome-wide CRISPR-Cas9 screen to identify homeostatic mechanisms that allow cells to tolerate the loss of tankyrase function

RESEARCH PROPOSAL

The functional redundancy of the two tankyrase paralogues (TNKS, TNKS2) has limited genetic screening efforts aimed at elucidating regulatory mechanisms of tankyrase. To overcome this obstacle, Guettler (co-supervisor) obtained cells lacking either TNKS or TNKS2. We have established that the parental cells maintain a low basal level of Wnt/ β -catenin signalling which can be induced by Wnt3a. Using the TOPFlash reporter system, the student will first (in Guettler's laboratory) establish which of the two tankyrases makes the predominant contribution to Wnt/ β -catenin signalling in the cell model. The student will next introduce an mCherry fluorescent tag at the C-terminus of the identified tankyrase paralogue, using CRISPaint technology (Schmid-Burgk et al., 2016 – optimised in the Lord Laboratory), which allows tagging of the endogenous allele, and hence preserve its normal regulation and expression levels. This will enable the separation of missense from nonsense mutations in tankyrase at the screening stage. A β -catenin-responsive reporter based on destabilised GFP (TOP:dsGFP) (Reya et al., 2003) will next be stably introduced into the resulting tankyrase-mCherry cell line. Upon validation of the reporter line with tankyrase inhibitors and Wnt3a, a high-throughput screening setup will be established.

The student will exploit these models to establish structure-function relationships within tankyrase that govern Wnt/ β -catenin signalling. To do this, the student will design, in the Lord Laboratory with Dr. Krastev, a high-density CRISPR-Cas9 guide (g)RNA library encompassing a “tiled” library of gRNA covering the coding sequence of *TNKS* or *TNKS2* (depending on the choice of paralogue for screening) (Figure 3). Using lentiviral delivery, this gRNA library will be introduced into TOP:dsGFP+ve cells together with either Cas9 or catalytically dead Cas9 (dCas9) fused to a mutagenic enzymes including the cytidine deaminases, rat APOBEC1 (Komor et al., 2016), or AID (Ma et al., 2016) to generate libraries of cells with a variety of different TNKS or TNKS2 mutations, an approach we have recently used to establish the relationship between PARP1 mutations and function (manuscript under review). To account for potentially different functions of tankyrase depending on the status of the Wnt/ β -catenin pathway, screening will be performed both under basal conditions and upon Wnt3a stimulation. Subsequent isolation by FACS sorting of GFP-positive and -negative cells from these mutagenised cell libraries (i.e., cells with high and low expression of TOP:dsGFP) will allow us to identify TNKS and TNKS2 nonsense and missense mutations associated with a change in Wnt/ β -catenin signalling (Figure 3). We anticipate the identification of both loss-of-function (lost PARP activity or scaffolding functions) or gain-of-function phenotypes (increased PARP activity or augmented scaffolding functions). Nonsense and missense mutations will be distinguished from each other by the absence and presence, respectively, of the mCherry signal. Mutations in TNKS and TNKS2 associated with changes in the TOP:dsGFP signal will be identified from FACS sorted cell populations by sequencing of tankyrase-mCherry cDNA generated from the clones by RT-PCR (Figure 3).

Clones will next be isolated and expanded for further functional studies, to be carried out in the Guettler Laboratory.

These will include:

- focussed TOP:dsGFP reporter assays and an assessment of endogenous β -catenin target gene expression (e.g, *AXIN2*)
- localisation studies of tankyrase-mCherry mutants by fluorescence microscopy. Punctate staining characterises intact tankyrase scaffolds.
- co-immunoprecipitation-based interaction studies to assess binding of AXIN1/2 and potentially other β -catenin destruction complex components
- *in-vitro* PARylation studies to assess catalytic PARP activity of tankyrase-mCherry mutants isolated by immunoprecipitation.

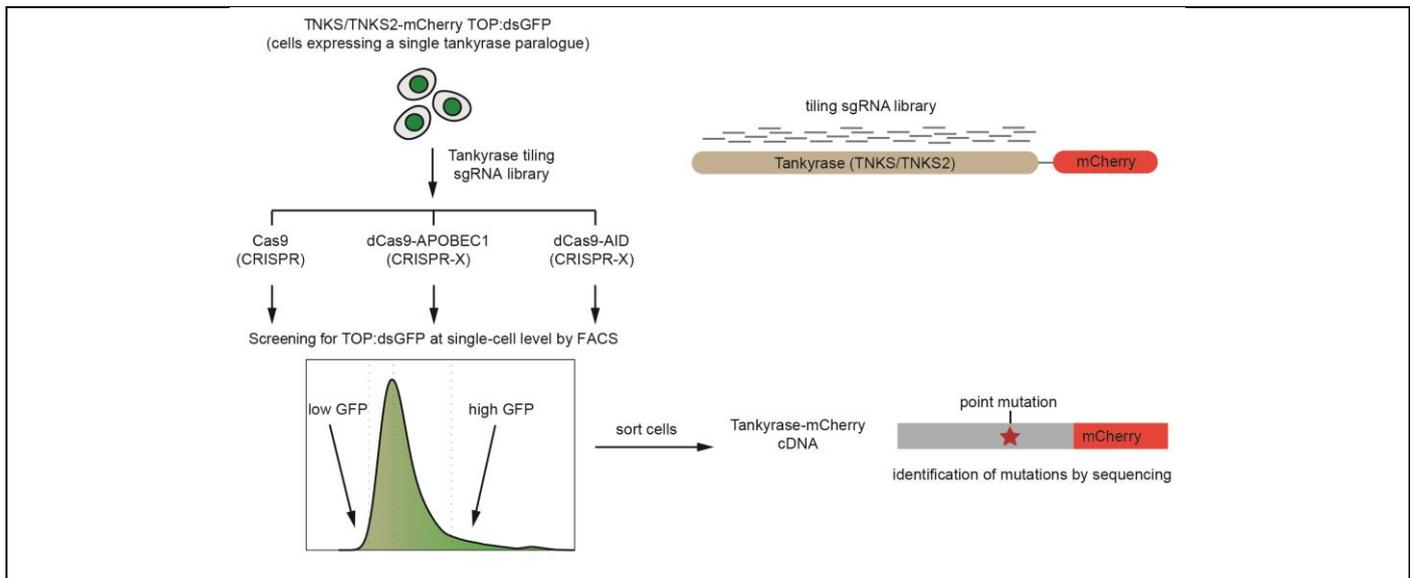


Figure 3: CRISPR-X screening strategy for elucidating regulatory mechanisms of tankyrase in Wnt/ β -catenin signalling.

In collaboration with members of Dr Sebastian Guettler’s team, selected mutant variants of tankyrase will be produced recombinantly and purified for mechanistic studies, including kinetic PARylation assays (both auto-PARylation and AXIN1/2 PARylation), an analysis of PAR chain length by PAGE to assess potential effects of the mutation on PARP activity and PARylation processivity, the ability to bind substrates or form filamentous polymers. The impact of the mutations on protein folding/stability will be assessed by differential scanning fluorimetry (thermofluor) assays. Identified mutations will form the basis for hypotheses on the regulation of tankyrase function. With available structural information, and informed by identified mutation ‘hotspots’, a series of tankyrase mutant variants for transient expression in mammalian cells (e.g., HEK293T cells or SW480 colorectal cancer cells) will be generated and evaluated by TOPFlash reporter assays, catalytic PARP activity and subcellular localisation (Mariotti et al., 2016). The findings will guide follow-up structural studies of tankyrase in the Guettler laboratory, for example exploring potential cis-regulatory interaction analogous to those occurring in PARP1 (Langelier et al., 2012).

In addition, using the CRISPR-X technology, the student will introduce specific knock-in mutations in identified regulatory ‘hotspots’ in tankyrase, informed by experiments outlined above. This will provide valuable models that express tankyrase variants under endogenous regulation, circumventing potential caveats of overexpression studies.

In parallel, the student will also use similar CRISPR-screening technology to understand how cells homeostatically respond to inhibition of tankyrase enzymes, thus informing the identification of feedback signalling loops associated with these PARP family members. To do this, the student will learn how to carry out negative selection genome-wide CRISPR-Cas9 genetic screens in Lord’s laboratory and carry out a screen to identify gRNAs that cause sensitivity to small molecule tankyrase inhibitors, including those developed by Lord. In this particular case, the student will use already established protocols including those used in the Lord Laboratory to identify novel determinants of resistance to PARP1,2,3 inhibitors (Manuscript in review).

In summary, the project will establish novel regulatory mechanisms of tankyrase and identify co-dependencies of cells that could potentially be exploited in cancer therapy.

LITERATURE REFERENCES

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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 MSc in Molecular Biology or Biochemistry

Intended learning outcomes:

- Expert in the theory and practice of cell-based genetic (CRISPR) screens

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| | <ul style="list-style-type: none">• Experience in protein expression, purification and biochemical assays• Knowledge of protein structure• Knowledge of cancer signalling networks |
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