PhD Project Proposal

Funder details

Studentship funded by: MRC iCASE (with Merck)

Project details

Project title: Innate-immune drivers of cell fate during genotoxic stress

Supervisory team

Primary Supervisor: Christian Zierhut

Associate Supervisor(s): Frank Zenke (Merck Healthcare KGaA/EMD Serono)

Secondary Supervisor: Jyoti Choudhary

Divisional affiliation

Primary Division: Cancer Biology

Primary Team: Genome Stability and Innate Immunity

Site: Chelsea

Project background

Most cancer cells undergo periods of DNA damage and chromosomal instability known as genotoxic stress – either due to their inherent genetic defects, or due to cancer therapy. Although genotoxic stress can drive many different cell fates (Fig. 1, top and middle), a predominant fate is apoptotic cell death. Apoptosis is also the most common fate that cells undergo in drug screens and genetic co-lethality screens that are based on loss of proliferation or the prevalence of death. However, apoptosis is generally immunosuppressive, and can promote proliferation (Ichim & Tait 2016). In contrast, other cell death mechanisms are more immunogenic, and beneficial to the patient (Legrand et al. 2019). One of these is pyroptosis, which is driven by inflammasomes, large megadalton signalling complexes (Lamkanfi & Dixit 2014). Similarly, secretion changes in cells that manage to survive but undergo senescence, may be oncogenic or tumour suppressive, with type I interferons being thought to be particularly beneficial. However, we currently are unable to exploit these more immunogenic fates due to a lack of understanding of the underlying mechanisms.

Many ongoing clinical trials seek to improve immune responses by interfering with immune checkpoints in addition to dysregulating genotoxic stress responses through drugs such as ATR inhibitors, but the underlying mechanisms are poorly understood. Due to the similarity of effects between genotoxic stress and the stress elicited by

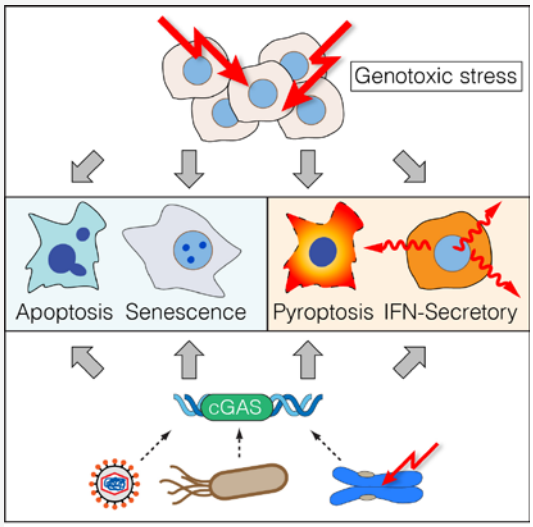

Figure 1. Top and middle, Genotoxic stress can drive different cell fates. Apoptosis and senescence are considered to be immune-cold wheras pyroptosis and interferon (IFN) secretion are considered immune-warm. **Bottom and middle**, The innate immune DNA sensor cGAS becomes activated by intracellular pathogen DNA, but also in response to genotoxic stress, subsequently regulating cell fate and immunogenicity.

pathogen infection, innate immune signalling sits at the crossroads of all these pathways. A key innate immune response is the cGAS pathway, which detects pathogen DNA, but can also respond to self-DNA during genotoxic stress (Fig. 1, bottom), and which can drive inflammation and interferon production, apoptosis, pyroptosis and, senescence, but also promote metastasis and survival (Fig. 1, middle) (Zierhut & Funabiki 2020). However, the determinants of the exact fate this pathway promotes, are currently unknown.

Project aims

- Develop reporters for apoptosis, pyroptosis, type I interferon production and senescence
- Identify regulators of these fates using CRISPRi genetic screens
- Functionally characterise screen hits with cell biology, microscopy and mass-spectrometry
- Establish rationales for driving immunogenic cell fates by targeted manipulation of the identified pathways

Research proposal

Research plan

An overview of the project can be found in Fig. 2. First, we will adapt cGAS wild-type and cGAS knockout MDA-MB-231 triple-negative breast cancer (TNBC) cells, previously established in the Zierhut lab, for screening with an improved CRISPR interference (CRISPRi) system (Yeo et al. 2018). TNBC was chosen as a model due to its high unmet need for effective therapy.

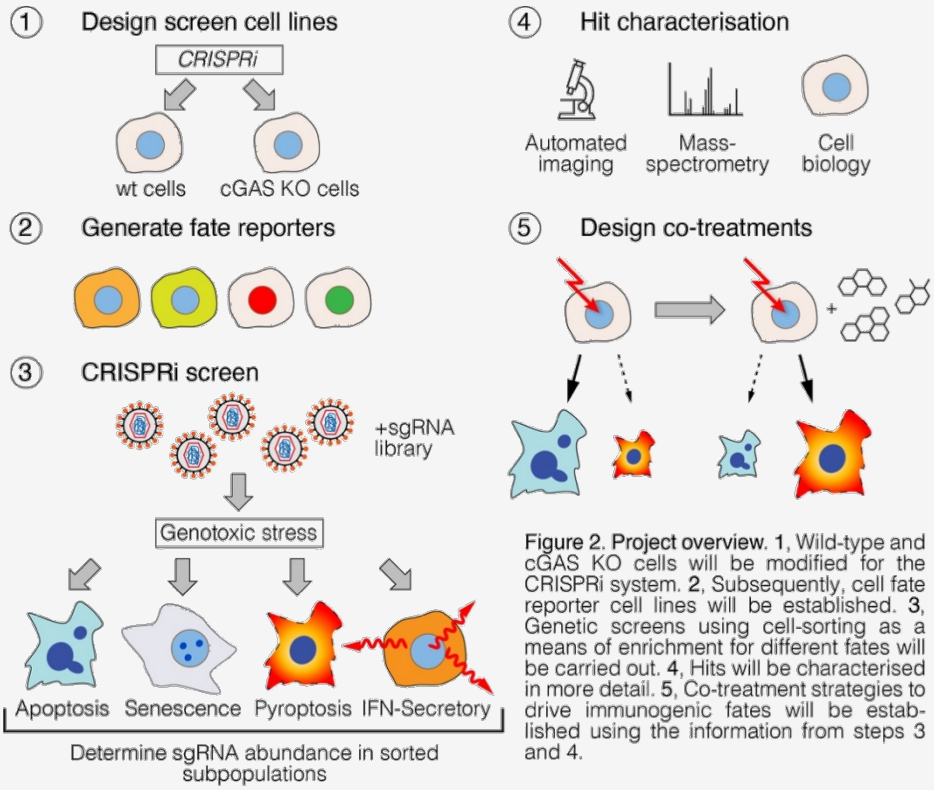

Figure 2. Project overview. 1, Wild-type and cGAS KO cells will be modified for the CRISPRi system. 2, Subsequently, cell fate reporter cell lines will be established. 3, Genetic screens using cell-sorting as a means of enrichment for different fates will be carried out. 4, Hits will be characterised in more detail. 5, Co-treatment strategies to drive immunogenic fates will be established using the information from steps 3 and 4.

Second, we will insert genetic reporters of apoptosis, pyroptosis, interferon production and senescence into these cells. Most of these have previously been established in our lab or others, allowing us to minimise the setup period.

Third, we will carry out phenotypic screens with these cell lines. Cells will be transduced with a library of barcoded sgRNA-encoding lentiviruses, and subsequently subjected to control treatment or treatments inducing DNA damage or chromosome missegregation. Cells that have entered each of the different fates will be identified, and enriched/depleted sgRNAs will be determined by next-generation sequencing. This CRISPRi screen will be supported by the extensive experience present within the ICR, as well as within the industry partners at Merck. We will initially focus on a library of DNA-damage response genes (Knijnenburg et al. 2018), with other libraries that we are using for independent research projects potentially serving as a backup. Following the initial screens, we will carry out further screens with modified DNA damage responses, achieved by inhibitor treatment, with which the

industry partner has extensive experience. Should any difficulty be encountered with the screen setup and readout we can switch to an arrayed format as a backup plan.

In the fourth step, screen hits either promoting or interfering with the specific fates will be ranked based on reproducibility, and by the information available for each hit. Top ranked genes will be used in subsequent analysis, in which we will gather more detailed information about how and when these genes regulate cell fate entry. We will follow the timing and circumstances of each cell fate using time-lapse microscopy and automated analysis methods that are routinely used in the Zierhut lab. In addition, potential novel regulatory networks will be identified by mass-spectrometry analysis of pull downs of these proteins. This latter part will be carried out with help by the secondary supervisor of this studentship, Jyoti Choudhary, a world expert in mass-spectrometry. In addition, standard molecular biology and cell biology (western blot analysis, proliferation assays, fixed-cell microscopy etc.) will be used to characterise the hits.

In the fifth step, we will use the information from the screen and subsequent analysis to help drive cells into immunogenic cell death and interferon-secreting fates by manipulating the identified genes and pathways concomitantly with genotoxic stress treatment. Special emphasis will be put onto available drugs to genes identified in the screen, after consultation with the industry partners at Merck.

Overall, this work will not only identify novel regulators of cell death, survival, inflammation and senescence, but also suggest new targets for co-treatment during cancer therapy. Subsequent, collaborative, experiments will be used to test predictions generated from this work in mouse models of cancer, and to analyse cancer patient data for correlation with treatment responses.

Industry collaboration benefits to this project

The partner researchers at Merck are experts in the analysis of drug sensitivity, cancer immune responses, targeting DNA repair, and CRISPR screening. The main industry partner, Dr Frank Zenke, has extensive experience in modulating DNA damage and immune checkpoint responses. Regular interaction with the Merck team will guide the project at all stages. In addition, part of this project will be carried out by the student within the facilities of Merck Healthcare KGaA, Darmstadt, Germany. Here, we may focus on the characterisation of synthetic interactions with DNA damage response inhibitors, with which the Merck team has extensive experience. The industry insights into the potential application of identified hits – either as drug targets based on druggability, or as predictive biomarkers will be instrumental in shortlisting candidates for subsequent, extensive characterisation. Finally, an established collaboration with Merck will allow this project to quickly be taken to more translational avenues and allow us to create a benefit for patients more quickly.

Literature references

[1] Ichim, G. & Tait, S.W.G., 2016. A fate worse than death: apoptosis as an oncogenic process. Nature reviews Cancer, 16(8), pp.539–548.

[2] Knijnenburg, T.A. et al., 2018. Genomic and Molecular Landscape of DNA Damage Repair Deficiency across The Cancer Genome Atlas. Cell Reports, 23(1), pp.239–254.e6.

[3] Lamkanfi, M. & Dixit, V.M., 2014. Mechanisms and functions of inflammasomes. Cell, 157(5), pp.1013–1022.

[4] Legrand, A.J. et al., 2019. The Diversification of Cell Death and Immunity: Memento Mori. Molecular Cell, 76(2), pp.232–242.

[5] Yeo, N.C. et al., 2018. An enhanced CRISPR repressor for targeted mammalian gene regulation. Nature methods, 15(8), pp.611–616.

[6] Zierhut, C. & Funabiki, H., 2020. Regulation and Consequences of cGAS Activation by Self-DNA. Trends in cell biology, 30(8), pp.594–605.

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:	BSc in Biology or similar, ideally an MSc or MRes degree.
Intended learning outcomes:	• The ability to design, and interpret experiments • The ability to evaluate and interpret published literature

- The ability to present and discuss research data and ideas
- Understand and be able to use state-of-the-art molecular biology techniques
- Become an expert in innate immunity and genome stability
- Become an expert in cell fate decisions following genotoxic stress
- Become an expert in cell biology of genome stability

Advertising details

Project suitable for a student with a background in:

- [x] Biological Sciences
- [ ] Physics or Engineering
- [ ] Chemistry
- [ ] Maths, Statistics or Epidemiology
- [ ] Computer Science