ICR The Institute of Cancer Research

PhD Project Proposal

Funder details

Studentship funded by:	ICR

Project details

Project title:	*In situ* analysis of the TCR repertoire in colorectal cancer

Supervisory team

Primary Supervisor:	Trevor Graham
Associate Supervisor(s):	Annie Baker
Secondary Supervisor:	Alan Melcher

Divisional affiliation

Primary Division:	Centre for Evolution and Cancer / Division of Molecular Pathology
Primary Team:	Genomics and Evolutionary Dynamics
Site:	Sutton

Project background

As cancer cells divide their DNA accumulates new mutations, some of which will lead to the generation of neoantigens – mutant peptides that can be recognised by immune cells as non-self. Specific (neo)antigen recognition is mediated by T cell receptors (TCRs), which are encoded by a hypervariable sequence that is unique to a TCR clone. Binding of a TCR with its cognate antigen can lead to expansion of that T cell clone and immune destruction of cancer cells bearing that antigen. For neoantigen-bearing cells to survive, they need to escape T cell recognition and/or destruction – this immune evasion has been recognised as a hallmark of cancer (1).

The dynamics of tumour-immune evolution remain undetermined. As the TCR is incredibly diverse, it has traditionally been very difficult to sequence, and even more challenging to spatially map T cell clones using in situ methods. Similarly, there are only limited efforts to spatially map neoantigens across tumours. To address this, our lab have recently optimised a method for next generation sequencing of the TCR, and we have successfully applied this even to poor quality tissue, such as archival formalin-fixed paraffin-embedded (FFPE) samples.

Project aims

- Characterise the T cell repertoire in colorectal cancers containing TP53 mutant subclones
- Describe the neoantigen burden in colorectal cancers containing TP53 mutant subclones
- Define genetic mechanisms of immune escape in colorectal cancers containing TP53 mutant subclones
- Develop and apply a new method for in situ analysis of T cell clones

Research proposal

Hypothesis: Immune escape co-evolves with TP53 mutation in colorectal cancer.

We will look for evidence of this in the tumour genome and in the T cell repertoire, firstly by using established methods (bulk sequencing and multiplex staining) and secondly by developing and applying a novel in situ TCR sequencing methodology.

Our team has broad experience in the analysis of tumour-immune evolution in colorectal cancer, using multiplex staining, next generation sequencing and mathematical modelling. However, sequencing of the TCR has thus far been limited to bulk approaches, with loss of spatial and morphological information.

In this PhD project, we would like to take our analysis of the TCR repertoire a step further and visualise the distribution of TCR clones (and neoantigens) in situ, using thin sections of patient tissue. This has the advantage of providing single cell resolution, whilst preserving spatial context. These approaches could be probe based (analogous to our recent development of the Basescope method (2)) or may leverage the rapidly advancing technologies of in situ transcriptomics (3). Developing this new technology will be an exciting central challenge of the PhD.

The student will test their new methods of in situ TCR analysis using a cohort of colorectal cancers (CRCs) that harbour TP53 mutant subclones. The tumour suppressor TP53 is the most commonly mutated gene in cancer, and recent studies have shown it can mediate tumour-immune crosstalk (4). As a key regulator of DNA damage repair, the mutation of TP53 could lead to a sharp increase in genetic variants (and neoantigens) in the tumour. Thus our hypothesis is that immune evasion must precede or co-evolve with TP53 mutation, and we will look for evidence of this in the immune microenvironment and in the tumour DNA.

This project will use immunohistochemistry and targeted sequencing to identify CRCs containing both TP53 wild-type and TP53 mutant tumour cells. Whole exome sequencing will be used to derive neoantigen burdens for each subclone. Our recently established TCR sequencing methodology will then be used to characterise the overall TCR repertoire in the wild-type and mutant subclones, then novel in situ approaches will be applied to map the spatial distribution of the identified TCR clones and neoantigens. This in situ mapping will be combined with multiplex staining for T cell phenotype, to characterise the nature of the immune response (or immune evasion) to TP53 mutation.

We note that the application of novel in situ TCR analyses has far-reaching potential. Our lab has a long-standing interest in the evolution of colorectal cancer over time and therapy (including metastases) (5-8), as well as looking at pre-cancer evolution in inflammatory bowel disease (9) and familial cancer syndromes. We will actively encourage the student to explore the potential applications of their new methodology to align their PhD project with their own research interests.

The studentship will be based in the Genomics and Evolutionary Dynamics group, within the Centre for Evolution and Cancer. We are a highly diverse and interdisciplinary team of about 15 people, consisting of clinicians, biologists, mathematicians and computational scientists. Our lab has around 10 years of experience in the application of next generation sequencing and in situ methodology, and full training will be provided to the successful candidate. In addition, the candidate will receive “dry-lab” training in bioinformatics analysis of TCR sequencing data.

Literature references

[1] Hallmarks of cancer: the next generation, Hanahan D, Weinberg RA, Cell, 2011 Mar 4;144(5):646-74

[2] Robust RNA-based in situ mutation detection delineates colorectal cancer subclonal evolution, Baker AM, Huang W, Wang XM, Jansen M, Ma XJ, Kim J, Anderson CM, Wu X, Pan L, Su N, Luo Y, Domingo E, Heide T, Sottoriva A, Lewis A, Beggs AD, Wright NA, Rodriguez-Justo M, Park E, Tomlinson I, Graham TA., Nature Comms, 8(1), 2017

[3] Method of the Year: spatially resolved transcriptomics, Marx V, Nature Methods, 2021 Jan;18(1):9-14

[4] p53, cancer and the immune response, Blagih J, Buck MD, Vousden KH, J Cell Sci, 2020 Mar 6;133(5)

[5] Evolutionary dynamics of neoantigens in growing tumours, Lakatos E, Williams MJ, Schenck RO, Cross WCH, Househam J, Werner B, Gatenbee C, Barnes CP, Anderson AR, Sottoriva A*, Graham TA*, Nature Genetics, 52, 1057-1066 (2020)

[6] Immunosuppressive niche engineering at the onset of human colorectal cancer., Gatenbee CD, Baker AM, Schenck RO, Strobl M, West J, Neves MP, Hasan SY, Lakatos E, Martinez P, Cross WCH, Jansen M, Rodriguez-Justo M, Whelan CJ, Sottoriva A, Leedham S, Robertson-Tessi M, Graham TA, Anderson ARA., Nat Comms, 2022 Apr 4;13(1)

[7] The co-evolution of the genome and epigenome in colorectal cancer, Heide T, Househam J, Cresswell GD, Spiteri I, Lynn C, Mossner M, Kimberley C, Fernandez-Mateos J, Chen B, Zapata L, James C, Barozzi I,

Chkhaidze K, Nichol D, Berner A, Schmidt M, Lakatos E, Baker AMC, Costa H, Mitchinson M, Jansen M, Caravagna G, Ramazzotti D, Shibata D, Bridgewater J, Rodriguez-Justo M, Magnani L, Graham TA*, Sottoriva A* https://www.biorxiv.org/content/10.1101/2021.07.12.451121v1, Nature, accepted

[8] Phenotypic plasticity and genetic control in colorectal cancer evolution, Househam J, Heide T, Cresswell GD, Lynn C, Spiteri I, Mossner M, Kimberley C, Gabbutt C, Lakatos E, Fernandez-Mateos J, Chen B, Zapata L, James C, Berner A, Schmidt M, Baker AMC, Nichol D, Costa H, Mitchinson M, Jansen M, Caravagna G, Shibata D, Bridgewater J, Rodriguez- Justo M, Magnani L, Sottoriva A*, Graham TA* https://www.biorxiv.org/content/10.1101/2021.07.18.451272v1, Nature, accepted

[9] The evolutionary history of human colitis-associated colorectal cancer, Baker AM, Cross W, Curtius K, Al Bakir I, Choi CR, Davis HL, Temko D, Biswas S, Martinez P, Williams MJ, Lindsay JO, Feakins R, Vega R, Hayes SJ, Tomlinson IPM, McDonald SAC, Moorghen M, Silver A, East JE, Wright NA, Wang LM, Rodriguez-Justo M, Jansen M, Hart AL, Leedham SJ, Graham TA., Gut 68(6), 2019

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 biological science or related subject (First or 2:1) or a Master's degree in biological science or related subject

Intended learning outcomes:

- Wet lab training in in situ methodology, such as immunohistochemistry and multiplex staining approaches
- Wet lab training in genomics, such as whole exome sequencing and TCR sequencing
- Learn how to develop and implement a new wet lab method (in situ TCR sequencing)
- Gain skills in bioinformatic analysis of next generation sequencing data
- Become an independent scientist, confident in hypothesis generation, experimental design and implementation
- Attain thorough knowledge of the subject area and associated literature, including critical review of research papers
- Training in scientific writing and presentation to large and diverse audiences

Advertising details

Project suitable for a student with a background in:

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