The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title	Mapping the interaction landscape and cellular dynamics of the SWI/SNF chromatin remodelling complex
Short Project Title	**SWI/SNF interaction landscape and cellular dynamics**

SUPERVISORY TEAM	
Primary Supervisor	Jessica Downs
Secondary Supervisor	Jyoti Choudhary

DIVISIONAL AFFILIATION	
Primary Division	Cancer Biology
Primary Team	Epigenetics and Genome Stability
Site	Chelsea

PROJECT PROPOSAL

Mammalian cells possess multiple closely related SWI/SNF chromatin remodelling complexes. These complexes have been implicated in multiple cellular pathways, including transcription, replication, and DNA repair. Notably, genes encoding subunits of the SWI/SNF complexes, such as PBRM1, are frequently altered in cancer and evidence supports a role for these genes as tumour suppressors. SWI/SNF complexes are important for responding to replication stress and maintaining genome stability, which are important functions in preventing tumourigenesis. However, very little is known about how the complexes are normally regulated in space and time, or how this is altered in response to replication stress. Nor is it understood how the regulation of the complexes is impaired when key subunits such as PBRM1 are absent. In this project, we will address these outstanding questions by investigating the SWI/SNF interaction landscape and its cellular dynamics. The insights generated in these studies will yield important insights into the mechanisms by which SWI/SNF complexes prevent tumourigenesis.

PROJECT AIMS

- **To elucidate the dynamic chromatin binding pattern, transcriptional profile, and interactome of SWI/SNF complexes in normal cells across the cell cycle to understand how SWI/SNF is regulated in space and time**
- **To determine how these patterns are altered when cells are under replication stress and understand the mechanism by which SWI/SNF helps to buffer cells from the negative effects of replication stress**
- **To determine how these patterns are altered in cells lacking the PBRM1 subunit of SWI/SNF, which is mutated in approximately 40% of clear cell renal cell carcinoma**

RESEARCH PROPOSAL

Mammalian cells possess multiple closely related SWI/SNF chromatin remodelling complexes that are defined by their subunit composition. These complexes have been implicated in multiple cellular pathways, including transcription, replication, and DNA repair. Genes encoding SWI/SNF subunits are frequently mutated in human cancers, and evidence indicates that they act as tumour suppressors. We previously found that the PBRM1 (or BAF180) subunit of SWI/SNF complexes is important for the cellular response to replication stress and helps to prevent genome instability (Brownlee

et al., 2104; Kakarougkas et al., 2014; Niimi et al., 2012). However, it is not entirely clear how this occurs and whether this is a major driver of tumourigenesis.

The multiple cellular activities combined with the variation in complex composition make the SWI/SNF complexes challenging to study, and relatively little is known about how the complexes are regulated normally in space and time, or how this regulation changes in response to replication stress. Moreover, it is not clear how SWI/SNF regulation is impaired in cells lacking key subunits or how this might influence tumourigenesis. In this project, we will address these outstanding questions by investigating the SWI/SNF interaction landscape and its cellular dynamics.

The key aims of this studentship are:

1. **To elucidate the dynamic chromatin binding pattern, transcriptional profile, and interactome of SWI/SNF complexes in normal cells across the cell cycle to understand how SWI/SNF is regulated in space and time**
2. **To determine how these patterns are altered when cells are under replication stress and understand the mechanism by which SWI/SNF helps to buffer cells from the negative effects of replication stress**
3. **To determine how these patterns are altered in cells lacking the PBRM1 subunit of SWI/SNF, which is mutated in approximately 40% of clear cell renal cell carcinoma**

To address these aims, we will use a combination of cutting-edge technologies to map the interaction landscape and cellular dynamics of the SWI/SNF complexes. First, we will use synchronised cell cultures to perform genome-wide mapping of SWI/SNF chromatin binding through the cell cycle. This will be done using the newly developed Cut&Run methodology that overcomes many of the limitations of traditional ChIP-seq approaches (Skene et al., 2018). In collaboration with the Choudhary lab in the ICR (see Chen et al., Cell 2020), we will map the SWI/SNF interactome through the cell cycle using quantitative mass spectrometry to identify determinants of SWI/SNF regulation.

The Downs lab has generated a library of CRISPR-Cas9 engineered cell lines with loss of function mutations in key SWI/SNF subunits, including the PBRM1 subunit. RNA-seq data generated in these cell lines will be used to identify the SWI/SNF regulated transcriptome, and in combination with the chromatin binding data, map the genes that are directly and indirectly regulated by SWI/SNF.

While there is considerable evidence that SWI/SNF promotes the cellular response to replication stress, which is a central factor in cancer cell biology, it is not yet clear how SWI/SNF achieves this. To understand this, we will use the approaches described above (Cut&Run and mass spectrometry) to map the changes in genome binding patterns and the interactome of SWI/SNF that take place in response to replication stress. This will be done in several cellular model systems, including both immortalised non-cancer cells and cancer cells, in order to provide insights into the mechanisms by which SWI/SNF contributes to replication stress responses.

Finally, we will focus on a key subunit of SWI/SNF – PBRM1. This subunit is inactivated in approximately 40% of clear cell renal cancers (Brownlee et al., 2015), but its specific role in preventing tumourigenesis is not yet clear. We will utilise the library of isogenic cell lines with PBRM1 alterations generated in the Downs lab to explore the changes in SWI/SNF dynamics arising as a result of PBRM1 loss by making further use of Cut&Run, mass spectrometry and RNA-seq. These data will provide insights into the PBRM1-dependent alterations in the transcriptome, interactome and genome binding profile of SWI/SNF that contribute to tumourigenesis.

The outcomes of the studentship include high resolution mapping of SWI/SNF complex dynamics in normal and cancer cells to elucidate the mechanisms of regulation and replication stress responses. In addition, insights into the mechanism by which PBRM1 loss leads to tumourigenesis will be generated and explored. The student will develop proficiency in cell biology, next generation sequencing-based techniques (Cut&Run and RNA-seq), proteomics (in collaboration with the Choudhary lab), and analysis of large datasets.

LITERATURE REFERENCES

BROWNLEE, P. M., CHAMBERS, A. L., CLONEY, R., BIANCHI, A. & DOWNS, J. A. 2014. BAF180 promotes cohesion and prevents genome instability and aneuploidy. *Cell Rep,* 6**,** 973-981.

BROWNLEE, P. M., MEISENBERG, C. & DOWNS, J. A. 2015. The SWI/SNF chromatin remodelling complex: Its role in maintaining genome stability and preventing tumourigenesis. *DNA Repair (Amst),* 32**,** 127-133.

CHEN, Y. J., ROUMELIOTIS, T. I., CHANG, Y. H., CHEN, C. T., HAN, C. L., LIN, M. H., CHEN, H. W., CHANG, G. C., CHANG, Y. L., WU, C. T., LIN, M. W., HSIEH, M. S., WANG, Y. T., CHEN, Y. R., JONASSEN, I., GHAVIDEL, F. Z., LIN, Z. S., LIN, K. T., CHEN, C. W., SHEU, P. Y., HUNG, C. T., HUANG, K. C., YANG, H. C., LIN, P. Y.,

YEN, T. C., LIN, Y. W., WANG, J. H., RAGHAV, L., LIN, C. Y., CHEN, Y. S., WU, P. S., LAI, C. T., WENG, S. H., SU, K. Y., CHANG, W. H., TSAI, P. Y., ROBLES, A. I., RODRIGUEZ, H., HSIAO, Y. J., CHANG, W. H., SUNG, T. Y., CHEN, J. S., YU, S. L., CHOUDHARY, J. S., CHEN, H. Y., YANG, P. C. & CHEN, Y. J. 2020. Proteogenomics of Non-smoking Lung Cancer in East Asia Delineates Molecular Signatures of Pathogenesis and Progression. *Cell,* 182**,** 226-244 e17.

KAKAROUGKAS, A., ISMAIL, A., CHAMBERS, A. L., RIBALLO, E., HERBERT, A. D., KUNZEL, J., LOBRICH, M., JEGGO, P. A. & DOWNS, J. A. 2014. Requirement for PBAF in transcriptional repression and repair at DNA breaks in actively transcribed regions of chromatin. *Mol Cell,* 55**,** 723-32.

NIIMI, A., CHAMBERS, A. L., DOWNS, J. A. & LEHMANN, A. R. 2012. A role for chromatin remodellers in replication of damaged DNA. *Nucleic Acids Res,* 40**,** 7393-403.

SKENE, P. J., HENIKOFF, J. G. & HENIKOFF, S. 2018. Targeted in situ genome-wide profiling with high efficiency for low cell numbers. *Nat Protoc,* 13**,** 1006-1019.

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 or MSc or equivalent in biochemistry or molecular biology
Intended learning outcomes	• Knowledge of epigenetics, chromatin, genome stability, and cancer biology • Ability to design, execute and interpret experiments in this field • Excellent technical skills in cell biology, microscopy, molecular biology, next generation sequencing, and proteomics • Project management and ability to work as part of a fast-paced research team • Communication skills, including scientific writing and presentation
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. chromatin remodelling and epigenetics**
	2. cancer biology
	3. proteomics and next generation sequencing
	4. replication stress
	5. genome instability
	6. DNA damage responses