Implementing an *in vitro* human cardiomyocyte platform to characterise and control transcriptional responses evoked by changes in histone acylation driven by disturbed propionyl-CoA in cardiometabolic disease

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Project outline:

Please contact Professor Pawel Swietach to request a copy of manuscript describing discovery of propionylation marks in the heart, currently in revised form with *Nature Cardiovascular Research*

Please watch short video about the project: https://www.youtube.com/watch?v=LnZOrIYYOqE

Background: The heart generates considerable turnover of short-chain acyls, notably acetyl-CoA and propionyl-CoA. Disturbances to these fluxes in cardiometabolic diseases – including inborn errors of metabolism, gut microbiome disorders, diabetes and heart failure – can dynamically alter the concentration of CoA esters, potentially affecting histone acylation and hence gene expression. However, the relationship between epigenetic changes and transcriptional responses is not intuitive and rarely stoichiometric; for example, promoters with a high level of acylated histones may not necessarily produce a transcriptional response when acylation increases further. Whilst measurements on diseased hearts can inform about correlations, *causation* must be inferred from systems that alter acyl-CoAs selectively, robustly and reproducibly. Such systems are also needed to test novel targets for therapy and identify biomarkers related to histone modifications. Recently, signalling by propionyl-CoA – the second-smallest acyl-CoA – has garnered attention due to its propensity to accumulate in the nucleus and reach high levels in disease. Unlike acetyl-CoA, which can be incorporated rapidly into the Krebs cycle, spikes in propionyl-CoA tend to be more persistent and evoke chemical modifications to proteins, including histones. To study the consequences of stably raised propionyl-CoA *in vivo*, we used a hypomorphic mouse model of propionic acidaemia (PA) and found that elevated propionate has the dual effect of raising *histone propionylation* (via propionyl-CoA) and *acetylation* (via HDAC inhibition by propionate). These histone modifications induce a subset of genes, including those implicated in contractile dysfunction. Additionally, we described sex-dependent differences in baseline histone acylation, which leads to differential transcriptional

responses upon comparable metabolic disruptions. Thus, we have defined the scope for propionyl-CoA to influence transcription via histone modifications.

Hypothesis. (1) Nuclear propionyl-CoA levels respond profoundly to disturbances in metabolic fluxes in heart disease, leading to changes in histone acylation and gene expression. (2) The selective actions of propionyl-CoA can be studied in a tractable *in vitro* system based on human iPSC-derived cardiomyocytes with genetically-altered levels of propionyl-CoA carboxylase (PCC), the enzyme catalysing the rate-limiting step in propionyl-CoA breakdown. (3) This approach can identify biomarkers of epigenetic changes and test therapies for controlling or reversing histone acylation in human disease.

Aims. Much of our understanding of the role of epigenetics in heart disease is borne from correlations, rather than controlled interventions. To address this, we will establish an *in vitro* system, based on human iPSC-derived cardiomyocytes, that will phenocopy the metabolic disturbance to propionyl-CoA across the range observed in diseases (gut microbiome disorders, T2D, PA). This provides a platform for linking metabolic disturbances to altered gene expression, and testing drugs that affect this coupling. An unbiased approach will relate propionyl-CoA levels (metabolomics) with histone marks (ChIP-seq) and differential gene expression (RNA-seq). We will raise antibodies against specific propionyl-CoA-evoked histone modifications for site-specific studies. These analyses will identify the genomic loci that respond most robustly to changes in propionyl-CoA. Subsequent analysis of population-based human genetic data (GWAS, eQTLs, pQTLs) will determine the prevalence of these responding genes in cardiac disease. Biomarkers based on novel histone modifications and their transcriptional responses will be tested in samples of diseased myocardium to establish the prominence of propionyl-CoA disturbances. Finally, we will implement pharmacological and genetic approaches that target propionyl-CoA handling to seek ways of influencing epigenetic responses. These will be tested for efficacy in reversing gene responses (ChIP-qPCR, RT-qPCR). Putative targets include specific histone acyl-transferases, enzymes of alternative propionyl-CoA handling pathways (e.g. generation of beta-alanine), or enzymes responsible for compartmentalizing propionyl-CoA in the nucleus.

Work to be Undertaken. A range of propionyl-CoA levels will be established in iPSC-derived cardiomyocytes by genetically altering (overexpression or knockout) *PCCA* or *PCCB* genes coding for PCC subunits. Metabolomics will measure steady-state acyl-CoA levels in cell compartments. Initial chromatin immunoprecipitation (ChIP) experiments will use H3K27ac and pan-Kpr antibodies, which we have used successfully in cardiac samples. Specific histone modifications will be identified by mass spectrometry of histone extracts and used to raise site-specific antibodies for further ChIP experiments. This work will identify changes in histone acylation across the genome for correlations with transcriptional responses (RNA-seq) over a wide range of propionyl-CoA levels. Specific loci of cardiac interest will be verified by ChIP-qPCR, and common signatures to those observed *in vivo* (PA mouse) will be sought. Readouts of these genes-of-interest and/or specific histone modifications will be tested in samples of failing hearts, with and without diabetes, to determine the contribution of disturbed propionyl-CoA signalling over a spectrum of metabolic disorders. The *in vitro* system will be used as a screening platform to identify targets for attenuating or reversing the actions of propionyl-CoA. Processes targeted by pharmacological or genetic methods will include: *(i)* acyltransferases such as GCN5, KAT6 and PCAF (proposed to propionylate histones), *(ii)* enzymes of the beta-alanine pathway (postulated as a 'sink' for excess propionate), and *(iii)* enzymes involved partitioning propionyl-CoA into the nucleus). Our outcomes will *(a)* link cardiac metabolism, histone acylation and gene expression, and *(b)* identify strategies for reversing this new and emerging component of cardiometabolic disease.

Supervisor's recent relevant publications:

PAWEL SWIETACH

1. Alkaline nucleoplasm facilitates contractile gene expression in the mammalian heart. Hulikova A, Park KC, Loonat AA, Gunadasa-Rohling M, Curtis MK, Chung YJ, Wilson A, Carr CA, Trafford AW, Fournier M, Moshnikova A, Andreev OA, Reshetnyak YK, Riley PR, Smart N, Milne TA, Crump NT, **Swietach P**. *Basic Res Cardiol.* 2022 Mar 31;117(1):17.
2. Acidic environments trigger intracellular H^+-sensing FAK proteins to re-balance sarcolemmal acid-base transporters and auto-regulate cardiomyocyte pH. Wilson AD, Richards MA, Curtis MK, Rohling M, Monterisi S, Loonat AA, Miller J, Ball V, Lewis A, Tyler DJ, Moshnikova A, Andreev OA, Reshetnayh YK, Carr, C, **Swietach P**. *Cardiovasc Res.* 2022 Nov 10;118(14):2946.
3. Oxidation of Protein Kinase A Regulatory Subunit PKARIα Protects Against Myocardial Ischemia-Reperfusion Injury by Inhibiting Lysosomal-Triggered Calcium Release. Simon JN, Vrellaku B, Monterisi S, Chu SM, Rawlings N, Lomas O, Marchal GA, Waithe D, Syeda F, Gajendragadkar PR, Jayaram R, Sayeed R, Channon KM, Fabritz L, **Swietach P**, Zaccolo M, Eaton P, Casadei B. *Circulation.* 2021 Feb 2;143(5):449.
4. Nitric oxide modulates cardiomyocyte pH control through a biphasic effect on sodium/hydrogen exchanger-1. Richards MA, Simon JN, Ma R, Loonat AA, Crabtree MJ, Paterson DJ, Fahlman RP, Casadei B, Fliegel L, **Swietach P**. *Cardiovasc Res.* 2020 Oct 1;116(12):1958-1971.
5. Iron-deficiency anemia reduces cardiac contraction by downregulating RyR2 channels and suppressing SERCA pump activity. Chung YJ, Luo A, Park KC, Loonat AA, Lakhal-Littleton S, Robbins PA, **Swietach P**. *JCI Insight.* 2019 Apr 4;4(7):e125618.

THOMAS MILNE

1. A human fetal liver-derived infant MLL-AF4 Acute Lymphoblastic Leukemia model reveals a distinct fetal gene expression program. Rice, S., Jackson, T., Crump, N.T., Fordham, N., Elliott, N., O'Byrne, S., Inglott, S., Ladon, D., Wright, G., Bartram, J., Ancliff, P., Mead, A.J., Halsey, C., Roberts, I., **Milne, T.A**.*, and Roy, A.* *Nat Comm,* 2021. PMID: 34824279. *co-corresponding
2. A Phenotypic Screen Identifies a Compound Series That Induces Differentiation of Acute Myeloid Leukemia Cells In Vitro and Shows Antitumor Effects In Vivo. Josa-Cullere, L., Madden, K.S., Cogswell, T.J., Jackson, T.R., Carter, T.S., Zhang, D., Trevitt, G., Davies, S.G., Vyas, P., Wynne, G.M., **Milne, T.A.***, and Russell, A.J.* (2021). *J Med Chem*. 64: 15608-15628. *co-corresponding
3. H3K79me2/3 controls enhancer-promoter interactions and activation of the pan-cancer stem cell marker PROM1/CD133 in MLL-AF4 leukemia cells. Godfrey, L., Crump, N.T., O'Byrne, S., Lau, I.J., Rice, S., Harman, J.R., Jackson, T., Elliott, N., Buck, G., Connor, C., Thorne, R., Knapp, D., Heidenreich, O., Vyas, P., Menendez, P., Inglott, S., Ancliff, P., Geng, H., Roberts, I., Roy, A.*, and **Milne, T.A.*** (2021). *Leukemia*. 35: 90-106. *co-corresponding
4. BET inhibition disrupts transcription but retains enhancer-promoter contact. Crump, N.T., Ballabio, E., Godfrey, L., Thorne, R., Repapi, E., Kerry, J., Tapia, M., Hua, P., Lagerholm, C., Filippakopoulos, P., Davies, J.O.J., and **Milne, T.A.** (2021). *Nat Commun*. 12: 223.
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