**University of Leicester**

**BBSRC MIBTP Studentship Project 2025-6 entry.**

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**Section 2 – *Project Information***

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| **Project Title** | Evaluating the impact of chemically reactive metabolites in premature ageing  |
| **Project Summary**  |
| While most biological molecules are not that chemically reactive, some are very reactive and can significantly affect our health. Some reactive metabolites (RMs) are known toxins and carcinogens that act by damaging DNA and proteins. However, the vast majority of biological functions involving RMs are unknown. We believe that RMs are key regulators of human biology. Given their chemical reactivity, it is likely that RMs can affect multiple biochemical pathways by reacting with DNA and proteins. However, it is currently unclear what reactions occur in human cells and how these reactions affect human biology at the molecular and systems levels. Recent pioneering work has revealed a potential function for aldehyde RMs in human ageing. These studies using mouse models suggest a link between genotoxic aldehyde stress (as caused by aldehydes derived from alcohol and cigarette smoke) and the p53 response, leading to early cell senescence. This is a hallmark of premature ageing (Wang et al., Mol. Cell, 2023). While these findings are extremely exciting, there is currently little known about the underpinning molecular mechanism. Identifying this mechanism is essential as it would redefine our understanding of human ageing and could lead to the development of anti-ageing therapies. Defining how RMs induce cell senescence requires the development of a simple and robust cell model where (i) cellular RM levels can be easily modulated and quantified, and (ii) where the molecular mechanisms underpinning senescence, e.g. p53-dependent pathways, can be analysed in a sensitive and controlled manner. This project aims to establish such a cell model and to use it to discover how RMs affect ageing-related biology. **Objective 1**: To establish human cell lines where endogenous RM levels can be manipulated.  The project will generate cell models deficient in RM metabolism. In particular, we will focus on the metabolism of aldehyde RMs, with which we have extensive experience. Our cell models will be derived from immortalised but ‘normal’ cells (unlike normally used cancer-derived cells), which will make them ideal for studying senescence-related biological pathways in healthy models without inducing senescence. Derivatives of these cells will also be generated with single and double knock-outs of ADH5 and ALDH2, which are key aldehyde-metabolising enzymes. This will enable us to test how aldehyde metabolism affects ageing. **Objective 2**: To develop methods to modulate and quantify cellular aldehyde levels. The project will develop methods to both deliver aldehydes to cells and to quantify cellular aldehyde levels in response to aldehyde exposure and to knock-out of aldehyde metabolism enzymes. This work will build on existing research in the Hopkinson group with aldehyde-releasing small molecules and aldehyde detection methods. The releasers and detection methods will ultimately be used in cell assays (see below).  **Objective 3**: To study the effects of aldehydes on ageing-related cell functions  The project will determine the aldehyde sensitivity and aldehyde metabolism efficiency of the cell models using reported cytotoxicity assays and NMR studies on cell lysates. The project will also study the effects of aldehydes and aldehyde releasers (see above) on cell cycle progression and p53-dependent biological pathways. These studies will include analysis of p53 levels and downstream markers of p53 induction. Any changes will be correlated with aldehyde levels using the aldehyde quantification methods. The status of DNA damage will also be assessed, while later work will test whether combinatorial exposure to aldehyde releasers and p53 modulators induces synergistic effects. Overall, these studies will give unprecedented insight into the role of aldehyde in p53 biology and ageing.  Techniques that will be undertaken during the projectCRISPR-Cas9-mediated gene editing Cell culture Organic synthesis Spectroscopic techniques, most notably NMR spectroscopy and mass spectrometry Western blotting Cell cycle analysis, including flow cytometry Indirect fluorescence microscopy  |
| **References** |
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