Project Details	
Project Code	MRC23IIARCa Bowen
Title	Investigating novel chronic kidney disease therapies that regulate expression of long noncoding RNA hyaluronan synthase 2-antisense 1 (HAS2-AS1)
Research Theme	Infection, Immunity, Antimicrobial Resistance and Repair
Summary	Chronic kidney disease (CKD) affects 15% of the global population but has no cure. This project will investigate a novel therapeutic approach to halting CKD progression, by using a long noncoding (lnc)RNA that is relevant to kidney disease. The student will explore the role of lncRNA HAS2-AS1 in the regulation of kidney fibrosis using cell and in vivo models, and then translate these findings by manipulating HAS2-AS1 expression in models of CKD therapy.
Description	BACKGROUND This project will provide skills and facilitate knowledge transfer to address the global clinical challenge of chronic kidney disease (CKD), which is associated with high morbidity and mortality, by focusing on understanding mechanisms of tissue repair. Kidney fibrosis is a key determinant of CKD progression and correlates strongly with increased synthesis of the extracellular matrix glycosaminoglycan hyaluronan (HA). Expression of the enzyme HA synthase 2 (HAS2), which produces HA, is significantly upregulated in CKD. We have shown that increased HAS2 expression is causally linked to kidney fibrosis in vivo and is a critical mediator of profibrotic cell phenotypes in vitro. However, since HAS2 knockout mice die before birth, indirect methods are required to explore its therapeutic potential. Advanced sequencing techniques have revealed that most human gene expression transcribes RNAs that do not code for proteins. Analysis of these noncoding RNAs, or genomic “dark matter”, is revolutionising our understanding of the mechanisms underpinning human disease. The student will develop novel therapeutic approaches by exploring the role of long noncoding (lnc)RNAs in CKD progression, focusing on HAS2-antisense 1 (HAS2-AS1). HAS2-AS1 is transcribed from the opposite genomic DNA strand to HAS2. HAS2 messenger RNA and HAS2-AS1 lncRNA share a region of sequence complementarity that varies in length, and therefore potential function, due to formation of two HAS2-AS1 variants by alternative splicing. We have detected these HAS2-AS1 splice variants and HAS2-AS1:HAS2 heteroduplexes in kidney cells. We have also demonstrated increased HAS2-AS1 expression in kidney fibrosis in vivo, and that functional HAS2 expression and activity in vitro is dependent on HAS2-AS1. HYPOTHESIS Manipulation of HAS2-AS1 expression can be used to control HAS2 expression, HA production and thereby CKD progression. PLAN OF INVESTIGATION HAS2-driven renal HA synthesis drives kidney fibrosis and CKD progression. Regulation of HAS2-AS1 transcription will be used to manipulate HAS2 expression and identify novel therapies. The student will elucidate the mechanisms by which HAS2-AS1 modulates HA-dependent profibrotic cell development and use nanoparticle technology to manipulate HAS2-AS1 expression in CKD animal models. 1. Characterising regulation of HAS2-AS1 expression and splice variation Our previous data show that HA-degrading enzyme hyaluronidase 2 (HYAL2) and transcription factor GATA3 regulate HAS2-AS1 transcription, and GATA3 directs stromal fibroblast cell heterogeneity. The student will

	characterise transcriptional regulation of HAS2-AS1 in renal stromal fibroblasts, including analysis of GATA3/HYAL2. The regulatory elements governing HAS2-AS1 exon 2 splice variation will be identified by bioinformatic mapping. Expression of selected variants will then be enforced in fibroblast models using splice-switching oligonucleotides, and the effects on cell differentiation analysed. 2. Manipulating HA synthesis in interventional animal models The student will use next-generation biocompatible kidney-targeting nanocarriers in interventional animal CKD models to modulate expression of HAS2-AS1 splice variants in the renal stroma, determining the therapeutic efficacy of each variant by analysing attenuation of CKD progression, renoprotective effects and functional recovery. 3. Analysis of noncoding RNAs in renal fibrosis and regulation of HAS2-driven HA synthesis Using historical RNA sequencing data, the student will employ bioinformatics analysis to i) identify other lncRNA transcripts that are differentially expressed during kidney fibrosis and ii) examine the interactions between microRNAs, HAS2 mRNA and HAS-AS1 lncRNA. Time permitting, selected lncRNA transcripts will be analysed as above. The student will revise the research objectives according to their areas of specific interest and outcomes from data generated.
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