Identification of downstream target genes and analysis of obesity-related variants of the bHLH/PAS transcription factor Single-minded 1

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ABSTRACT

Single-minded 1 (SIM1) is a basic Helix-Loop-Helix/PER-ARNT-SIM (bHLH/PAS) transcription factor essential for survival in mice. The early post-natal lethality exhibited by *Sim1*$^{-/-}$ mice is believed to be the consequence of severely compromised hypothalamus development, although the contribution of reduced SIM1 signalling in the numerous other tissues in which it is expressed has never been formally investigated. The presence of a single *Sim1* allele is sufficient to avoid this perinatal lethality, and instead confers an early onset, hyperphagic obesity phenotype, potentially via disruption of critical intracellular signalling pathways that are activated in *Sim1*-expressing hypothalamic neurons in response to food intake. Similar correlations between reduced *SIM1* gene dosage and severe obesity have also been documented in humans. Alterations in *SIM1* expression and/or function therefore have important implications in health and disease, and warrant a detailed investigation into the downstream target genes and regulatory behaviours of this critical transcription factor, which are thus far almost entirely lacking in the literature.

The studies presented in this thesis describe a twofold approach to dissecting the gene regulatory properties of the SIM1 protein. Firstly, we optimised and performed a range of functional assays, including a cell-based luciferase reporter gene assay, a subcellular localisation assay, a co-immunoprecipitation assay, and an electrophoretic mobility shift assay, which were designed to assess the contribution of nineteen unique point mutations within the SIM1 protein sequence to altered SIM1 expression and behaviour. These nineteen mutations were identified in multiple cohorts of severely obese humans, and therefore represent potentially pathogenic alterations in the SIM1 sequence. Indeed, we observed a significant loss of function for many of these variants in luciferase reporter gene assays relative to wild type SIM1. The severe loss of function observed for one of these variants, SIM1 T292A, could be further attributed to altered subcellular localisation, thus impacting on its ability to form a stable heterodimer with ARNT2 in co-immunoprecipitation experiments. Secondly, we performed microarray studies on cultured kidney-derived cells inducibly overexpressing Myc-tagged SIM1 and its obligate partner factor ARNT2, and subsequently identified several genes that selectively responded to SIM/ARNT2 overexpression in this context. Further validation in hypothalamus-derived cultured cells highlighted *Myomesin 2* (*Myom2*) as a potentially

genuine downstream SIM1 target gene in both kidney and hypothalamus. We also present data that are the first to indicate *Somatostatin* (*Ss*) as a hypothalamic target gene regulated by SIM1 in a cell-autonomous manner.

These data are among the first to dissect the downstream target genes and regulatory properties of the SIM1 protein, and therefore make an important contribution to our understanding of the molecular basis to the hyperphagic obesity exhibited by *Sim1*$^{+/-}$ mice. They are also the first to link reduced activities of mis-sense mutations in the SIM1 coding sequence to increased weight gain in humans, and give further credence to the possibility that *SIM1* represents a novel genetic contributor to obesity disorders in the wider population. This knowledge may inform future attempts to develop therapies for obese phenotypes in humans, and broaden our understanding of the molecular events that underpin *Sim1*-mediated survival and maintenance of homeostasis.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Anne Raimondo, and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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