Investigation of the Role of the X-Linked Opitz Syndrome Gene, *MID1*, in Craniofacial Development

By

Saidi Jaafar
BSc.Ed (Hons) (Malaya), MSc (Malaya)

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Dental School
Faculty of Health Sciences

&

School of Molecular & Biomedical Science
Faculty of Sciences
The University of Adelaide
Adelaide 5005
Australia

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THESIS SUMMARY

Normal formation of the vertebrate face requires appropriate growth, contact and fusion of craniofacial primordia in the ventral midline. Perturbations in these steps in facial development can result in an array of facial defects, the most common of which are cleft lip with or without cleft palate (CLP). Mutations in the \textit{Mid1} gene result in the X-linked form of Optiz GBBB syndrome (OS) in which CLP is a prominent feature. In fact, \textit{Mid1} represents one of only a few genes causally linked to CLP. The human \textit{Mid1} gene encodes a \textit{c}67 amino acid microtubule-associated RING finger (2BCC) protein that functions as part of a large multi-protein complex. \textit{In situ} hybridisation studies carried out in chick, mouse and human have indicated the highly conserved \textit{MID1/Mid1} is expressed widely throughout embryogenesis although at varying levels depending on the tissue and cell type. However, the specific role of the \textit{Mid1} protein in the development of these structures/tissues and how mutations of this gene gives rise to the various features seen among OS patients remains to be elucidated. As the various affected systems in OS patients appear to arise as a result of defective tissue fusion or remodelling during embryogenesis, elucidation of the molecular and cellular mechanisms by which the primordia of the face grow and then fuse to form the lip and primary palate will therefore shed light not only on our understanding of the developmental basis of CLP but also the processes leading to other common malformations (such as hypospadias and cardiac septal defects that also characterise OS patients).

A standard knockout of the mutate \textit{Mid1} gene was developed as part of a larger project to delineate the functional roles of \textit{Mid1} in a mouse model. The generation involved replacement of the first coding exon of \textit{Mid1} in ES cells with a \textit{LacZ} reporter gene such that the reporter would be under the control of the endogenous \textit{Mid1} cis-regulatory elements. Although the \textit{Mid1} null mice do not display any gross external malformation in the current 129SvJMF1 genetic background, use of a \textit{LacZ} reporter gene in the targeted DNA constructs enabled study of the expression pattern of \textit{Mid1} by staining for \textit{β}-galactosidase activity during early embryogenesis. Data presented in this thesis have revealed that \textit{Mid1} is expressed in specific cell types within the craniofacial complex, the urogenital organ and surprisingly in the developing heart consistent with the defects seen in OS patients.
Detection of Mid1 expression in the specific tissues during outgrowth and fusion of facial primordia suggests an important function of this gene in regulating these complex morphogenetic events. However, overlapping expression with the highly homologous Mid2 gene suggests perhaps a level of functional redundancy between Mid1 and its protein homologue, Mid2. This would be consistent with: (1) the marked clinical variability in the presentation of CS, even among male patients from the same family and thus share identical Mid1 mutations, (2) the failure of the Mid1 targeted knockout lines to display any gross facial malformation, at least in the current genetic background. To assist in addressing the functional redundancy between Mid1 and Mid2, specific antibodies recognised each protein were developed. Taken with recent evidence from early chick studies and the results presented in this thesis are consistent with this notion of redundancy also during later embryological stages.

In order to understand the cellular and developmental functions of Mid1, inducible Madin-Darby canine kidney (MDCK) (epithelial) and Cos-1 (mesenchymal) cell lines that stably express either wild-type GFP-Mid1 or one of a number of different mutant GFP-Mid1 fusion proteins were then developed. These cells were used in preliminary investigations to address the role of Mid1 in cellular processes such as cell migration, proliferation, cell death and the ability of cells to undergo epithelial-mesenchymal transitions (EMT), a key event in the fusion of epithelial-lined tissue such as in the facial prominences. These preliminary results showed that both epithelial and mesenchymal cell lines stably overexpressing wild-type or mutant Mid1 did not effect either proliferation or apoptosis levels. However, in wound healing assays, MDCK cells stably overexpressing wild-type Mid1 displayed delayed closure of the wounding area, in contrast to that seen with both Mid1ΔCTD expressing cell lines where the rate of wound closure was notably more rapid than control cells. These early observations provide evidence that Mid1 regulates the activation of epithelia, an early step in both EMT and cell migration.

This study has demonstrated that Mid1 expression is expressed in all tissues normally affected in CS patients and, in particular, characterised in detail the expression of Mid1 during the fusion of the facial prominences. This, together with an increasing knowledge about the cellular role of Mid1, will greatly facilitate our understanding of the developmental processes controlled by the MID proteins and how their disruption
contributes to the clinical presentation of OS, and more specifically at least one of the pathways that lead to the susceptibility to CLP.
1.6.5 How disruption of MIDI function can lead to the development of the malformations seen in OS.................................................. 31
1.7 Aims of the thesis and approaches........................................... 33

Chapter Two: Materials and Methods.......................................... 35
2.1 Abbreviations........................................................................... 35
2.2 Materials.................................................................................. 37
   2.2.1 Drugs, chemicals and reagents............................................. 37
   2.2.2 Enzymes........................................................................... 37
   2.2.3 Stains and dyes.................................................................. 38
   2.2.4 Antibiotics and indicators.................................................. 38
   2.2.5 Kits and assays.................................................................. 39
   2.2.6 Nucleic acid and protein molecular weight marker............. 39
   2.2.7 Solutions and buffers....................................................... 40
   2.2.8 Radiolabeled nucleotides.................................................... 42
   2.2.9 Cloning and expression vectors......................................... 42
   2.2.10 Synthetic oligonucleotides................................................. 42
   2.2.11 MIDI domain deletions..................................................... 43
   2.2.12 Bacterial strains.............................................................. 43
   2.2.13 Bacterial growth media.................................................... 43
   2.2.14 Tissue culture cell lines and media.................................... 44
      2.2.14.1 Cell lines................................................................. 44
      2.2.14.2 Media.................................................................... 44
   2.2.15 Antibodies...................................................................... 44
   2.2.16 Miscellaneous materials.................................................. 45
2.3 Recombinant DNA methods...................................................... 45
   2.3.1 General molecular biology methods................................... 45
   2.3.2 Restriction endonuclease digestions of DNA..................... 45
   2.3.3 Agarose gel electrophoresis of DNA................................... 46
   2.3.4 Phenol/chloroform extraction of DNA................................ 46
   2.3.5 Ethanol precipitation of DNA............................................. 47
   2.3.6 Preparation of cloning vectors.......................................... 47
   2.3.7 Preparation of DNA restriction fragments.......................... 47
   2.3.8 End-filling restriction endonuclease digested DNA............. 48
2.3.9 Ligation of DNA ......................................................... 48
2.3.10 Transformation of E. coli with recombinant plasmids ............... 48
   2.3.10.1 Preparation of competent E. coli .......................... 48
   2.3.10.2 Transformation of competent bacteria ....................... 49
2.3.11 Isolation of plasmid DNA ........................................ 49
2.3.12 Determination of DNA concentration ............................ 49
2.3.13 Automated sequencing of PCR products ........................... 50
2.3.14 Polymerase chain reaction (PCR) amplification of DNA .......... 50
2.3.15 Preparation of PCR products for cloning ........................ 50

2.4 Methods for RNA analysis and β-galactosidase activity .................. 51
   2.4.1 Embryo collection and fixation .................................. 51
   2.4.2 Preparation of hybridisation probes ......................... 52
      2.4.2.1 Synthesis of DIG-labelled RNA probes .................. 52
      2.4.2.2 Synthesis of 32P-labelled RNA probes .................. 53
   2.4.3 Whole-mount in situ hybridisation to mouse embryos .......... 53
   2.4.4 In situ hybridisation to tissue sections ..................... 55
   2.4.5 Reverse-transcriptase-PCR (RT-PCR) .......................... 56
   2.4.6 LacZ staining .................................................... 57

2.5 Methods for generation of polyclonal and monoclonal antibodies .... 57
   2.5.1 Generation of polyclonal antibodies ......................... 57
   2.5.2 Generation of monoclonal antibodies ....................... 58
   2.5.3 ELISA procedures ............................................. 59

2.6 Methods for mammalian cell culture .................................. 59
   2.6.1 Maintaining cultured cell lines .............................. 59
   2.6.2 Transfection of cultured cells ................................ 60
   2.6.3 Generation of stable cell lines ............................... 60
   2.6.4 Assay for Luciferase activity .................................. 61
   2.6.5 Immunofluorescent analysis of cultured cells ................ 61
   2.6.6 Non-denaturing protein extraction from cultured cells ..... 61
   2.6.7 Protein concentration determination (Bradford assay) ....... 62
   2.6.8 Protein gel electrophoresis and Western blotting ............ 62
   2.6.9 Isolation of genomic DNA from tissue culture cells .......... 63
   2.6.10 Proliferation and apoptosis assays ........................... 63
Chapter Three: Characterisation of Mid1 expression during early mouse embryogenesis

3.1 Introduction .................................................................................. 65
3.2 Results ......................................................................................... 67
  3.2.1 Mid1 developmental expression analysis by *in situ* hybridisation studies... 67
  3.2.2 Generation and characterisation of Mid1 knockout mice ..................... 68
  3.2.3 Mid1 developmental expression analysis by LacZ staining .................. 69
  3.2.3.1 Mid1 expression during early mouse embryogenesis ......................... 69
  3.2.3.2 Expression of Mid1 during fusion of facial primordia to form lip and primary palate ......................................................... 70
  3.2.4 Regulatory relationships between Mid1 and other molecules involved in craniofacial morphogenesis .................................................. 71
3.3 Discussion ..................................................................................... 72

Chapter Four: Investigation into the functional relationship between MID1 and MID2

4.1 Introduction .................................................................................. 78
4.2 Results ......................................................................................... 79
  4.2.1 Preparation and production of MID1- and MID2-specific antibodies ... 79
    4.2.1.1 Selection of peptides for raising anti-peptide sera ........................ 79
    4.2.1.2 Production of anti-MID1 and MID2 antibodies ............................. 80
  4.2.2 Characterisation of MID1- and MID2-specific antibodies ................... 82
  4.2.3 Investigating the functional relationship between MID1 and MID2 ...... 83
4.3 Discussion ..................................................................................... 84

Chapter Five: Investigation into the cellular and developmental functions of MID1 protein

5.1 Introduction .................................................................................. 89
5.2 Results ......................................................................................... 92
  5.2.1 Construction of inducible gene-specific expression plasmids ............. 92
  5.2.2 Establishment and characterisation of tetracycline-inducible cell lines ... 92
5.2.3 Investigating the cellular function(s) of MID1 .................................................. 95
  5.2.3.1 Cell proliferation and apoptosis assays of cells stably expressing wild-type and mutant MID1 proteins ....................................................... 95
  5.2.3.2 The effects of ectopic wild-type and mutant MID1 on wound closure .............................................................................................................. 95
5.3 Discussion .............................................................................................................. 96

Chapter Six: Final discussion and future directions ................................................. 102
6.1 Final discussion ...................................................................................................... 102
6.2 Future directions .................................................................................................. 110

References ................................................................................................................. 113