Investigating Function and Evolution of Genes and Proteins Involved in Metabolic Control in Mammals

by

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Abstract

The duck-billed platypus and the short-beaked echidna represent the most basal lineage of living mammals and therefore provide important information about mammalian evolution. Monotremes have also undergone remarkable anatomical, physiological and genetic changes. One of the most radical changes involves the monotreme digestive system and genes associated with metabolic control. I have investigated several genes that are vital for metabolic control (specifically genes in the ghrelin and incretin pathways) as well as the histology of the monotreme pancreas.

Given the glandless gut in monotremes, I first sought to investigate genes in the appetite regulating ghrelin pathway. Surprisingly, I discovered that genes encoding ghrelin and ghrelin *O*-acyl transferase (GOAT) are missing in the platypus and echidna genome, whilst, its receptor, growth hormone secretagogue receptor 1a (GHS-R 1a) is present. This is the first report suggesting the loss of ghrelin in a mammal. The conservation of the ghrelin receptor gene despite the lack of the ghrelin and GOAT genes in platypus suggests that another ligand maybe acting via this receptor in monotremes (Chapter 2).

Ghrelin is expressed in human pancreatic ε-cells. Hence the lack of ghrelin in platypus led us to investigate in more detail the structure of the monotreme pancreas, another key organ in both metabolic control and digestion (Chapter 3). Generally, the monotreme pancreata share the basic characteristics of other mammalian pancreata, including both endocrine islets and exocrine acini. I performed immunohistochemical analysis to reveal the detailed architecture of the platypus and echidna endocrine islets of Langerhans. The unique phenotypes of the PP-lobe, smaller size of islets, and the

abundance of α-cells indicate the monotreme pancreata have more resemblance to that of birds and marsupials than eutherian species.

One of the key functions of the pancreas is to release insulin upon food intake. Glucagon-like peptide 1 (GLP-1), a hormone released from the small intestine upon food intake, triggers insulin release via the GLP-1 receptor (GLP-1R) in β-cells of the pancreas. In human, GLP-1 is rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4) and thereby has a very short serum half-life (<2 min). Searching for long-acting GLP-1 analogs to improve insulin sensitivity has been a key strategy in Type 2 diabetes (T2D) treatment. We identified and characterised *Glp-1*, *Glp-1r* and *Dpp-4* and found both *Glp-1* and *Dpp-4* are expressed in gut and pancreas as expected, and interestingly also in venom. Importantly, evolutionary changes in monotreme GLP-1 sequences led us to predict that it would be resistant to enzymatic degradation. Extensive biochemical analysis of monotreme GLP-1 revealed that this variant is in fact resistant to DDP-4 degradation, however can be degraded by other enzymes (trypsin-like) in their own sera. Moreover, we demonstrated that monotreme GLP-1s can bind and activate both pGLP-1R and hGLP-1R with similar potency and stimulate insulin release in isolated mouse islets (Chapter 4).

Together this work provides fundamental new insights into the molecular and anatomical characteristics of the monotreme digestive system, the evolution of metabolic control and potential novel avenues for diabetes treatment based on monotreme GLP-1.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Chuan He Date

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List of Publications

Four publications will arise from this thesis. One has been published, one has been submitted and two are in preparation and will be submitted soon.

Published:

1. He C, Tsend-Ayush E, Myers MA, Forbes BE, Grützner F (2013) Changes in the ghrelin hormone pathway maybe part of an unusual gastric system in monotremes. Gen Comp Endocrinol 191:74-82

Accepted for publication:

1. He C, Myers MA, Forbes BE, Grützner F. Immunohistochemical analysis of pancreatic islets of platypus (*Ornithorhynchus anatinus*) and echidna (*Tachyglossus aculeatus ssp*)
Journal of Anatomy

Manuscripts in preparation:

1. Tsend-Ayush E, He C (co-first author), Myers MA, Andrikopoulos S, Wong N, Sexton PM, Wootten D, Forbes BE, Grützner F. In monotremes glucagon-like peptide 1 (GLP-1) is dipeptidyl peptidase 4 (DPP-4) resistant and expressed in venom
Prepared for submission to *Nature*

2. He C, Forbes BE, Grützner F Monotremes provide unique insights into the evolution of metabolic control in mammals
Prepared for submission to *BioEssays*