Generation of follistatin overexpressing db/db mice by AAV delivery ameliorates hyperglycemia and preserves pancreatic β-cell function

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The advent of transgenic mice revolutionised basic research into many disease types as well as revealing fundamental biological mechanisms. Despite the progress gained through this approach there remain several major disadvantages to using mouse models such as: the time and cost associated with deriving a genetic knock-in or knock out, confounding development effects when studying post-developmental phenotypes, and effects of changes by the gene of interest on mouse fertility. A recent advance in the genetic manipulation of mice is the application of adeno-associated virus (AAV) to deliver genetic material to a target tissue or organ. AAVs can be produced that overexpress or knockdown genes of interest in specific cell types. This allows the rapid generation of new mouse models at a fraction of the expense and time required for the development of traditional transgenic models. Furthermore, delivery can be made in combination with other AAV, across multiple strains, and into mouse models that are difficult to cross breed. One such strain is the db/db mouse, a gold standard model in obesity and diabetes research, here we used AAV introduce skeletal muscle growth stimulants to assess the impact of increase muscle mass on obesity and diabetes.

Follistatin is a potent antagonist of the muscle growth inhibitor myostatin and its overexpression in a transgenic model or from AAV delivery results in profound muscle hypertrophy. We sought to investigate whether muscle growth caused by follistatin treatment can improve metabolic control in the db/db mouse and attenuate the type II diabetic pathology of this model. Injection of an AAV that carries the gene encoding follistatin (AAV:Fst-288) results in the sustained expression of follistatin in striated muscle of the db/db mice. AAV:Fst-288 administration into the obese and diabetic db/db mouse model resulted in muscle growth with no effect on fat mass when compared with control db/db mice. Follistatin treatment in db/db mice prevented hyperglycaemic progression and lowered fasting blood glucose to levels. The percentage of glycated-haemoglobin (GHb) in control db/db mice was 10% while follistatin treatment reduced this to 6.3%, returning this value to levels observed in lean mice. Follistatin treatment did not reduce insulin resistance and measurements of glucose and insulin tolerance showed no difference between treated and control mice. The db/db mouse experiences transient hyperinsulineamia in response to insulin resistance, but β-cell function eventually fails undergoing de-differentiation/apoptosis. Follistatin treated db/db mice were able to maintain hyperinsulineamia while the insulin levels of untreated mice fell. Immunohistochemical analysis of islets in pancreatic sections revealed a complete preservation of β-cells and insulin content in follistatin treated mice. This suggests that the mechanism for controlled blood glucose levels in treated mice is through a preservation of pancreatic function.

Our study shows that AAV delivery designed to express follistatin in the db/db mouse corrects the key markers of diabetic pathology and prognosis, despite maintained insulin resistance and obesity, via mechanisms that enhance pancreatic function. This highlights the utility of AAV mediated gene delivery in generating otherwise highly refractory transgenic models.

All experiments were approved by the Alfred Medical Research and Education Precinct Animal Ethics Committee in accordance with the current code of practice for the use and care of animals for scientific purposes (National Health and Medical Research Council).