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Regulation of glucose homeostasis using radiogenetics and magnetogenetics in mice

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Expression of tailored nanoparticles in cells and application of low frequency radio-waves or a magnetic field can be used to non-invasively modulate gene expression. This approach successfully increases insulin transgene expression in diabetic mice.

A major goal in biomedical research is to establish mechanisms to control gene expression and cellular activity in a living organism in a non-invasive manner. One way to tightly control gene expression and availability of proteins in a time- and location-dependent way is to establish a precise and efficient endogenous switch mechanism. While photo-activation of caged compounds or genetically encoded light-sensors, as in optogenetics, allows for rapid turning on or off of signals, truly non-invasive application of this approach is restricted to accessible tissue such as the skin or the eye. To access deeper tissue, probes are required to allow light to reach the tissue. Other non-invasive strategies include chemical regulation of gene expression, by insertion of tetracycline-dependent activators or repressors¹ and mutant estrogen receptor ligand binding domains that can be switched on or off by administration of synthetic estrogens². However, compared to light-activation systems, drug-regulated gene expression systems are slow and imprecise. While switching on gene expression in such a system can be titrated by dose, time and mode of drug administration, switching off is dependent on the turnover of the drug and thus difficult to control. In this issue of *Nature Medicine*, Stanley *et al.*³ present an elegant solution to the problem of non-invasively controlling gene expression in a living animal.

Previous work by the authors led to the design of a nanoparticle based switch, which can be applied exogenously to regulate gene expression *in vivo*, or endogenously produced to regulate gene expression *in vitro*⁴. The present study validates the use of a genetically engineered nanoparticle system *in vivo* and uses it to remotely control expression of an insulin transgene in a diabetic mouse, and thereby restore glucose homeostasis. The switch in their system (Figure 1) is a genetically encoded ferritin-iron-nanoparticle co-expressed with an epitope-tagged TRPV1 Ca²⁺ channel that allows regulated Ca²⁺ influx triggered by either low frequency radio-waves or magnetic fields. More specifically, the genetically encoded iron oxide containing nanoparticles are synthesized intracellularly as a GFP-tagged fusion of

ferritin heavy and light chains, and the TRPV1 Ca^{2+} channel is tagged with an epitope that binds GFP. Upon stimulation with radio-waves or magnetic fields, the GFP-tagged iron-nanoparticles that are tethered to the anti-GFP-tagged TRPV1 Ca^{2+} channel, activate the Ca^{2+} channel which leads to an increase in cytoplasmic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$). The authors first tested the efficiency of three different constructs encoding the ferritin-nanoparticles on TRPV1 activation *in vitro*; the constructs differed in their expression at different intracellular locations: in the cytoplasm, at the membrane, and tethered to TRPV1 at the membrane. Upon radio-wave stimulation, the latter combination of GFP-tagged ferritin with $\alpha\text{GFP-TRPV1}$ was found to most robustly regulate Ca^{2+} influx *in vitro* using HEK cells.

The authors then combined the expression of the switch (GFP-ferritin-iron-nanoparticle/ $\alpha\text{GFP-TRPV1}$ Ca^{2+} channel) with that of an insulin transgene under control of a Ca^{2+} -dependent promoter in mice. In order to demonstrate the applicability of this ‘remote control’ system *in vivo*, they first genetically engineered murine mesenchymal stem cells (MSC) to stably express the switch and insulin-transgene combination and then implanted these MSC cells into streptozotocin (STZ) treated C57Bl6 mice, a model of type I diabetes. Treatment of STZ-mice bearing the engineered MSC implants by whole-body exposure to low-frequency radio-waves led to an increase in MSC insulin transgene expression and a decrease in blood glucose levels.

Next the authors generated replication-deficient adenoviruses encoding the switch/insulin-transgene combination. Adenoviral delivery of the switch/insulin-transgene cassette by intravenous injection into STZ-mice, followed by one hour of low-frequency radio-wave stimulation, induced expression of the insulin transgene in the liver, among other tissues, and increased circulating levels of insulin protein. This approach was also successful in alleviating the temporary phenomenon of anesthesia-induced hyperglycemia in WT mice, which is caused by reduced insulin release. Repeated treatment with radio-waves, once a week from week 2 up to week 6 post viral injection, resulted in an increase in plasma insulin levels and lowering of blood glucose levels in diabetic mice. This suggests that viral mediated gene transfer followed by radio-wave treatment could be a useful strategy for long term control of gene expression and physiological responses *in vivo*.

Because iron-ferritin-nanoparticles are paramagnetic⁵ and TRPV1 channels have been implicated in mechanosensing⁶, the authors speculated that the switch (GFP-ferritin-iron-nanoparticle tethered to $\alpha\text{GFP-TRPV1}$ Ca^{2+} channel) could not only be activated by radio-waves but also by a static magnetic field. This indeed was the case, as application of a magnetic field resulted in increased $[\text{Ca}^{2+}]_i$ *in vitro* as well as increased insulin transgene expression *in vitro* and *in vivo*. Having two independent but efficient ways to specifically activate *in vivo* gene expression adds to the versatility of the system described by Stanley *et al.*

In this remote control system the central component of the switch is the genetically encoded ferritin-iron-nanoparticle, which could potentially allow target activation by either heat (in

response to radio-wave treatment) or by mechanical sensing/change in 3D-conformation (in response to a magnetic field). The endogenous expression of the ferritin-iron-nanoparticle is advantageous over exogenous delivery of nanoparticles since the latter allows regulation of gene expression by radio-waves only in local cell populations (those accessed by injected nanoparticles) and requires repeated nanoparticle administration to elicit serial or chronic responses. The secondary component of the switch is the thermo/mechanical sensitive Ca^{2+} channel TRPV1, which allows a regulated increase in $[\text{Ca}^{2+}]_i$ by both radio-waves and a magnetic field. However, theoretically the secondary component of the switch could be composed of thermally or mechanically sensitive proteins other than the TRPV1 Ca^{2+} channel including other ion channels or membrane receptors. This would allow the conversion of the radio-wave or magnetic field signal into intracellular signals other than Ca^{2+} . A further inherent advantage of a genetically encoded switch over a non-genetic system such as delivery of exogenously derived nanoparticles is the possibility of tissue or cell type-specific expression, by employing appropriate promoter elements to drive transcription. This will restrict the expression and action of the switch to the tissue or cell type of interest and thereby reduce unwanted side-effects as a result of expression in unwanted tissues or cells.

Interestingly, the fact that Stanley *et al.*³ can also activate the switch by a magnetic field suggests that an applied oscillatory magnetic field could be converted into an oscillatory $[\text{Ca}^{2+}]_i$ pattern. If this is to be the case, modulating the frequency of $[\text{Ca}^{2+}]_i$ oscillations would allow the regulation of discrete downstream targets that are distinctly regulated by different $[\text{Ca}^{2+}]_i$ frequencies, e.g. insulin secretion, insulin gene expression and pancreatic β -cell survival⁷⁻⁹.

It is feasible to combine the non-invasive modulation of gene expression now described by Stanley *et al.*³ with non-invasive monitoring systems to assess efficiency of expression, or study the effects of the expressed molecule within its target tissue. For example engraftment of a sample of deep tissue, such as the pancreatic islets, in the anterior chamber of the eye allows for non-invasive, longitudinal imaging of pancreatic islet/ β -cell function by fluorescence microscopy^{10,11}. Hence, the immediate effects of biomolecules expressed by the remote-control system described by Stanley *et al.*³, could be efficiently evaluated with regard to for example *in situ* pancreatic islet cell function and survival by reporter islets in the eye. This may be a valuable tool for investigating the efficacy of a novel personalized medicine approach.

A genetically encoded switch to control biological systems in the living organism by either low frequency radio-waves or by a magnetic field is an exciting non-invasive approach with many potential applications. Time will tell if this is the beginning of a new era of radiogenetics and magnetogenetics, which could potentially prove even more versatile as tools than optogenetics.

Legend to Figure 1

(a) Stanley *et al* have generated a system in which viral mediated transfer of a construct encoding a switch (GFP-ferritin-iron-nanoparticle/ α GFP-TRPV1 Ca^{2+} channel) and an insulin-transgene under control of a Ca^{2+} -dependent promoter. Upon stimulation with either radio-waves or a static magnetic field, the iron-nanoparticles activate the tethered TRPV1 Ca^{2+} channel which leads to an increase in $[\text{Ca}^{2+}]_i$ in these cells. This leads to subsequent expression of a Ca^{2+} -dependent insulin transgene, and increased circulating insulin levels.

(b) Activation of insulin expression in the liver by radio-waves/magnetic field combined with non-invasive *in vivo* imaging of reporter islets in the anterior chamber of the eye by fluorescence microscopy. Insulin-dependent effects on pancreatic islets cells (α -cells in red, β -cells in green, δ -cells in purple) imaged *in vivo* by two-photon laser scanning microscopy (TP-LSM) or confocal laser scanning microscopy (CLSM).

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Figure 1