CellPress

Engineering Skin with Skinny Genes

Anika Böttcher,[1] Heiko Lickert,[1,2,*] and Matthias Tschöp[3,4,*]
[1]Institute of Diabetes and Regeneration Research, Helmholtz Diabetes Center, Helmholtz Center Munich, Am Parkring 11, 85748 Garching, Germany
[2]Technische Universität München, Ismaninger Strasse 22, 81675 München, Germany
[3]Institute of Diabetes and Obesity, Helmholtz Diabetes Center, Helmholtz Center Munich, Am Parkring 13, 85748 Garching, Germany
[4]Division of Metabolic Diseases, Technische Universität München, Ismaninger Strasse 22, 81675 München, Germany
*Correspondence: heiko.lickert@helmholtz-muenchen.de (H.L.), matthias.tschoep@helmholtz-muenchen.de (M.T.)
http://dx.doi.org/10.1016/j.stem.2017.07.013

The increasing prevalence of obesity and diabetes is an urgent worldwide health challenge. Now in *Cell Stem Cell*, Yue et al. (2017) report a proof-of-concept study using genetically engineered skin transplants that produce the incretin GLP-1 to prevent diet-induced obesity, suggesting a powerful approach for treating metabolic disorders.

Obesity has more than doubled over the last 30 years according to the World Health Organization (WHO) with approximately 600 million obese people worldwide today. One of the direct consequences is the steady increase in the number of diabetic patients, currently reaching more than 415 million around the globe (http://www.diabetesatlas.org). Thus, therapeutic intervention is urgently needed, but at present no pharmacological treatment is available with the potential to prevent, stop, or reverse the obesity and diabetes epidemics along with their deleterious complications (Scheen and Van Gaal, 2014). Only bariatric surgeries produce significant and durable weight loss with the potential to cure these diseases (Clemmensen et al., 2017; Evers et al., 2017). However, surgeries are expensive, highly invasive and irreversible; hence, there is a major interest in developing new treatment options. Recently, there has been progress into dissecting the molecular underpinnings through which surgical interventions lead to metabolic benefits (Evers et al., 2017). Leveraging these insights, research has shown that combinations of neuroendocrine factors that signal from the gastrointestinal system to the brain induce rapid, impressive, and separate improvements in metabolism and body weight. Unimolecular polyagonists that mimic bariatric interventions have shown promising preclinical results and are currently being tested in numerous human studies in many countries (Tschöp et al., 2016). However, like the approved and mildly anti-obesogenic glucagon-like peptide-1 (GLP-1) agonist liraglutide, all of these drug candidates are peptides and therefore will have to be injected life-long without endogenous or demand-related control. In this respect, somatic stem or progenitor cell-based CRISPR/Cas9-mediated gene therapy could open new avenues to deliver temporally and spatially regulated hormone therapy with the potential to stop the obesity and diabetes epidemics.

The skin, and in particular epidermal cells, are well suited for somatic gene therapy because of their easy accessibility and low immunogenicity. Not only are isolation, cultivation, and propagation protocols well established, but cutaneous gene therapy has been successfully applied to treat devastating skin adhesion disorders such as junctional epidermolysis bullosa (Mavilio et al., 2006).

With this in mind, Yue and colleagues employed CRISPR/Cas9 technology to genetically engineer primary mouse and human epidermal keratinocytes ex vivo to create skin transplants, with inducible expression of the body-weight-lowering gut hormone GLP-1, for the treatment of obesity and diabetes (Yue et al., 2017). The incretin GLP-1 targets its G protein-coupled receptor (GLP-1R) in multiple organs and has anorectic and cardioprotective effects, but most importantly it is known to improve glucose metabolism by stimulating insulin secretion from pancreatic beta cells (Campbell and Drucker, 2013). GLP-1R agonists and dipeptidyl-peptidase 4 inhibitors (which prolong the half-life of endogenous incretins) are among the more recent and most successful additions to the diabetes and obesity treatment portfolio. Thus, GLP-1 is an ideal candidate to test the applicability of inducible cutaneous delivery for the treatment of obesity and diabetes.

For this purpose the authors developed an organotypic culture model that enables stratification of epidermal progenitor cells and formation of skin-like organoids, which can then be grafted onto an immunocompetent host (Figure 1). Therapeutic genome editing by CRISPR/Cas9 might alter the fitness of edited cells, which in turn can affect the efficacy and duration of gene therapy (Dai et al., 2016). However, the targeted and transplanted skin-like organoids were tolerated well in vivo and exhibited normal epidermal stratification and proliferation without premature cell death or signs of immunogenicity. Importantly, the newly formed skin patch stably and dose-dependently expressed and released GLP-1 through the basement membrane into the blood circulation upon oral or intra-peritoneal administration of doxycycline for more than 3 months, offering an impressive proof-of-principle. To further investigate the potential therapeutic effect of GLP-1 induction in vivo, Yue et al. transplanted GLP-1-expressing and control organoids onto the skin of mice and then induced obesity by high-fat diet (HFD) feeding. Strikingly, induction of GLP-1 expression not only prevented diet-induced weight gain and insulin resistance but maintained glucose homeostasis. Together, these results demonstrate a systemic beneficial effect of cutaneous gene therapy.

While food consumption was not determined in the reported study, it is well

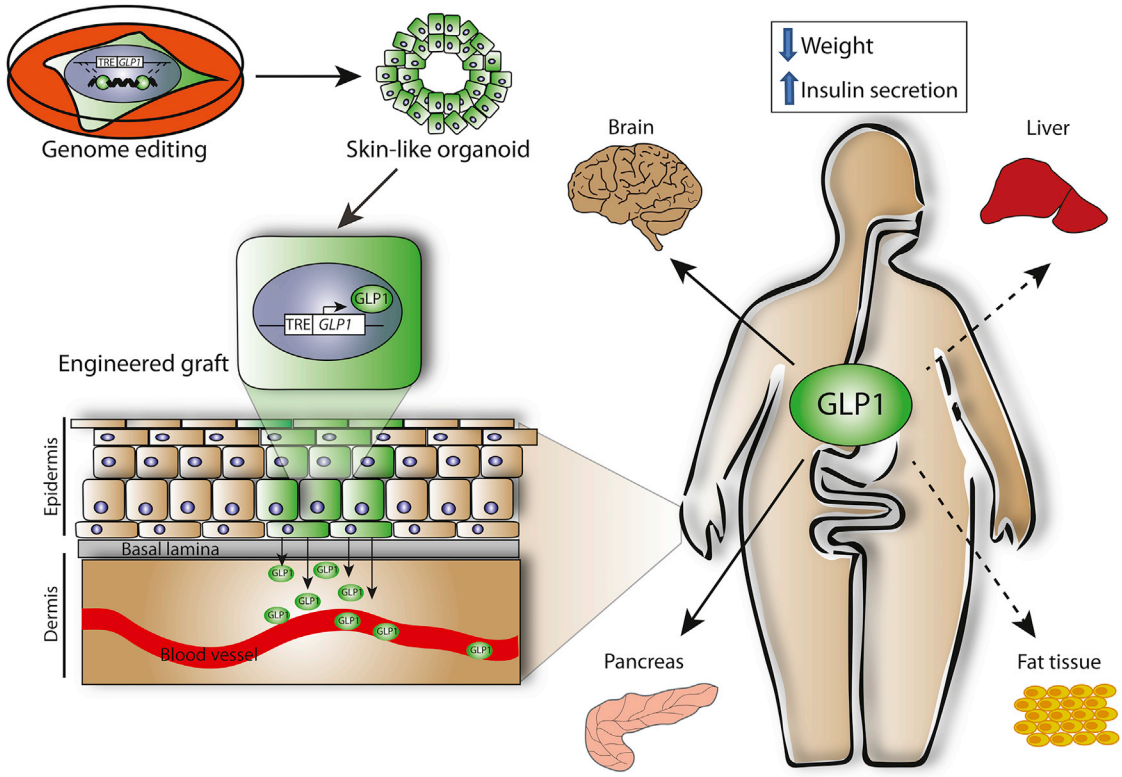

Figure 1. Engineered Skin Cells for Gene Therapy to Fight Metabolic Diseases
The schematic shows the potential therapeutic effects of cutaneous gene therapy. Epidermal stem cells/progenitor cells are genetically modified in vitro using CRISPR/Cas9 technology to inducibly express the incretin hormone GLP-1. An organotypic culture model enables stratification of epidermal progenitor cells and formation of skin-like organoids, which can then be transplanted to a patient for treatment. Cutaneous delivered GLP-1 may prevent diet-induced weight gain and insulin resistance, directly affecting pancreas and brain with indirect influence on adipose tissue and liver.

known that GLP-1 prevents weight gain by suppressing caloric intake. It is very likely that the effects of genetically enhanced GLP-1 release on reduced weight gain in the context of HFD reported here resulted from diminished appetite and food intake. It is also known that GLP-1 exerts pleiotropic effects on beta cells. Beyond its well-known function to amplify glucose-stimulated insulin release, GLP-1 stimulates beta cell proliferation and also triggers activation of pro-survival and anti-apoptotic pathways in rodent beta cells, at least in some experimental set-ups (Migliorini et al., 2014). Yue et al. do show convincingly that in their model, basal insulin level is increased 2-fold in homeostatic conditions upon GLP-1 release. Surprisingly, however, increased GLP-1 did not seem to induce beta cell proliferation either in homeostasis or during HFD exposure. One explanation could of course be an insufficiently high increase in plasma GLP-1. Obesity causes insulin resistance of peripheral tissues, which triggers beta cell compensation followed by exhaustion, dedifferentiation, and death (Migliorini et al., 2014). In this respect, it would be interesting to test whether (1) cutaneous delivered GLP-1 not only prevents but also reverses HFD-induced obesity and insulin resistance, and (2) GLP-1 induces proliferation of unhealthy (dedifferentiated) beta cells. To test this, GLP-1 expression could be induced in mice that are already obese and insulin resistant.

The findings by Yue et al. show that cutaneously delivered GLP-1 prevents weight gain. However, it remains to be shown if this cutaneous gene therapy approach offers sufficient efficacy for treating metabolic diseases in humans. The system reported here could also be used to interrogate the interplay between different factors (hormones) at the organismal level, which is important for developing combinatorial therapy approaches and to assess side effects (e.g., cancer risk) associated with long-term treatments. Thus, one striking feature of this system is that it is a step toward personalized medicine.

Despite the excitement of these findings, there are concerns regarding their translation to patients. In particular, and as mentioned above, glucose metabolism needs to be tightly controlled to prevent hypoglycemic episodes. It will be technically challenging, yet critically important, to develop an advanced system with the capability to provide fine-tuned, glucose-dependent release of GLP-1. If patients have to undergo multiple, carefully timed doxycycline doses per day, they might want to resort to the already existing, well established, and more convenient once-per-week injections of long-acting GLP-1 analogs (Marso et al., 2016).

Still, the findings reported here by Yue and colleagues suggest applicability of cutaneous gene therapy beyond skin diseases. If replicable, most desirably in larger mammalian models, this elegant model offers exciting promise for the

development of long-lasting, safe, and minimally invasive precision therapy for human obesity and type 2 diabetes (Figure 1).

REFERENCES

Campbell, J.E., and Drucker, D.J. (2013). Cell Metab. *17*, 819–837.

Clemmensen, C., Müller, T.D., Woods, S.C., Berthoud, H.R., Seeley, R.J., and Tschöp, M.H. (2017). Cell *168*, 758–774.

Dai, W.-J., Zhu, L.-Y., Yan, Z.-Y., Xu, Y., Wang, Q.-L., and Lu, X.-J. (2016). Mol. Ther. Nucleic Acids *5*, e349.

Evers, S.S., Sandoval, D.A., and Seeley, R.J. (2017). Annu. Rev. Physiol. *79*, 313–334.

Marso, S.P., Bain, S.C., Consoli, A., Eliaschewitz, F.G., Jódar, E., Leiter, L.A., Lingvay, I., Rosenstock, J., Seufert, J., Warren, M.L., et al. (2016). N. Engl. J. Med. *375*, 1834–1844.

Mavilio, F., Pellegrini, G., Ferrari, S., Di Nunzio, F., Di Iorio, E., Recchia, A., Maruggi, G., Ferrari, G., Provasi, E., Bonini, C., et al. (2006). Nat. Med. *12*, 1397–1402.

Migliorini, A., Bader, E., and Lickert, H. (2014). Mol. Metab. *3*, 268–274.

Scheen, A.J., and Van Gaal, L.F. (2014). Lancet Diabetes Endocrinol. *2*, 911–922.

Tschöp, M.H., Finan, B., Clemmensen, C., Gelfanov, V., Perez-Tilve, D., Müller, T.D., and DiMarchi, R.D. (2016). Cell Metab. *24*, 51–62.

Yue, J., Gou, X., Li, Y., Wicksteed, B., and Wu, X. (2017). Cell Stem Cell *21*, this issue, 256–263.

Do Not Adjust Your Mind: The Fault Is in Your Glia

Robin J.M. Franklin[1,*] and Edward T. Bullmore[2,3]
[1]Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute, Clifford Allbutt Building, Cambridge Biomedical Campus, University of Cambridge, Cambridge, UK
[2]Department of Psychiatry, University of Cambridge, Cambridge, UK
[3]GlaxoSmithKline R&D, Stevenage, UK
*Correspondence: rjf1000@cam.ac.uk
http://dx.doi.org/10.1016/j.stem.2017.07.005

Glia have been implicated in schizophrenia, although whether they play a primary role is uncertain. In this issue of *Cell Stem Cell*, Windrem et al. (2017) transplant human glial progenitors from schizophrenia patients into mouse brains, which develop abnormalities and behaviors characteristic of schizophrenia, thereby suggesting a primary role for glia in the complex disease pathogenesis.

Our understanding of the causes of schizophrenia, one of the most severe and widely occurring psychiatric disorders, has come a very long way from its characterization during most of the 20th century as a functional psychosis without an organic cause in terms of brain disorder. The biological basis of the disease is now beyond dispute, based on an ever-increasing body of evidence from imaging, pathology, and response to medication. However, at a cellular level, the mechanistic basis of the disease remains uncertain. In the study in this issue of *Cell Stem Cell* by Windrem et al. (2017), the authors employ a highly imaginative stem cell-based approach to provide evidence for a primary role of glia, the non-neuronal cells of the CNS, in the etiology of schizophrenia and in so doing, not only make a significant step forward in understanding the mechanistic basis of the disease, but also resolve a current "chicken-and-egg" debate within the field.

The CNS comprises several types of cell. In addition to neurons, which, through their extensive network of processes and connections, establish the functional circuitry of the brain, there are two other major cell types—astrocytes and oligodendrocytes, the latter being the myelin-forming cells of the CNS. Collectively the astrocytes and oligodendrocytes are called glia—a term coined by Rudolf Virchow in the 19th century to describe the non-neuronal cells of the CNS. The term glia, derived from the Greek for glue, reflects the mystery that originally surrounded these cells—in the absence of any more informed understanding they were regarded as the cells that "glued" the apparently more important neurons together. The historical uncertainty about what glia did led to a neuron-centric view of the CNS and its diseases. It is not surprising therefore that one of the earlier and now best established biological models of the underlying mechanisms of schizophrenia was based on a primary dysfunction in dopamine neurons (the dopamine hypothesis) (Howes and Kapur, 2009). However, in recent years, a primary pathogenic role for glia has emerged in several diseases that ultimately manifest themselves as neuronal disorders including some of the classic neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS, or motor neuron disease) and Huntington's disease (Benraiss et al., 2016; Kang et al., 2013). There is even growing evidence for a role for glia in Alzheimer's disease. These discoveries are entirely consistent with the emergent recognition of a central trophic role for both astrocytes and myelinating oligodendrocytes in maintaining neuronal (and axonal) health and integrity (Nave, 2010). If glia play key roles in these diseases, then why not in a highly complex psychiatric disorder such as schizophrenia? Several lines of evidence have emerged over the last few years to indicate that astrocytes and oligodendrocytes are perhaps not