Research shows the way to more efficient EPO production

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To many people, EPO rhymes with doping and cycling. But in fact, EPO is an important medical drug. This hormone works naturally in the body by stimulating red blood cell production. Patients suffering from anemia caused by for instance chronic kidney disease, AIDS or hematologic disorders can benefit immensely from EPO therapy. Furthermore, many cancer patients who are anemic from receiving chemotherapy are also in great need of EPO therapy. It is estimated that the market for EPO therapy is around 11 billion dollars annually.

Today, medical EPO is produced in mammalian cell lines using so-called recombinant DNA technology, where human genes are inserted to produce recombinant human EPO (rhEPO).

Recombinant human hormones such as EPO can be challenging to produce effectively, though, because they are quickly degraded by, amongst other things, the cells' own protein- and hormone-eating enzymes.

Using CRISPR technology, researchers from The Novo Nordisk Foundation Center for Biosustainability at Technical University of Denmark (DTU) have looked into how to reduce several cellular 'stressors' in order to improve production and product quality. This work has recently been published in the journal *Metabolic Engineering*.

The researchers found that by knocking out three genes involved in sugar group degradation and two involved in programmed cell death, they

could improve production by 1.4-fold in Chinese Hamster Ovary (CHO) cells. Furthermore, the quality was greatly improved. The knockout cells contained around 40% of the most active EPO form, while the non-knockouts only contained around 2% of the most active EPO form (the highly sialylated EPO). The highly sialylated EPO form is important as it has the longest half-life. Only active EPO can treat conditions of anemia, so improving this feature is imperative in order to keep prices low and quality high.

How to avoid EPO degradation

The optimised cell line was actually designed to work in so-called fed-batch cultures, which is a way of feeding the cells continuously over the course of 10-14 days. Fed-batch is a commonly used method to make human hormones for various conditions and antibodies for cancer treatment.

But it is hard to produce EPO in fed-batch, because it is destroyed over time. Thus, EPO is nowadays produced in a 'one-feeding' manner (batch cultures), even though this method is less efficient. In order to do fed-batch cultures despite these obstacles, others have tried adding supplements to the mixture to avoid degradation. But with this new cell line, there is no need for workarounds, explains first author Tae Kwang Ha, Postdoc at The Novo Nordisk Foundation Center for Biosustainability:

"With our cell line, you don't have to add anything—it just works."

These results have only been shown at small scale, so scale-up is needed to see if cells perform equally well in big production tanks. But this research verifies that knockout of specific genes can definitely improve productivity and product quality in single cells.

More information: Tae Kwang Ha et al, Knockout of sialidase and pro-apoptotic genes in Chinese hamster ovary cells enables the production of recombinant human erythropoietin in fed-batch cultures, *Metabolic Engineering* (2019). DOI: 10.1016/j.ymben.2019.11.008