

Hijacking intracellular storage bodies to produce difficult to express proteins

Proof of Concept funding from BioProNET has enabled Marek Brzozowski and Tim Ganderton from the University of York to investigate a novel protein packaging and secretion system, which might be used to make recombinant proteins that are tricky to produce.

Many potentially useful therapeutic or diagnostic proteins are difficult to express in the mammalian cell lines that are, or could be, used for their production. The proteins may be fragile, misfolded, harmful to the host, or require complicated, time consuming purification strategies.

With the aim of creating a novel mammalian cell-based system for the production of difficult to express proteins, the scientists investigated a naturally occurring system of intracellular storage bodies — known as Weibel Palade bodies. In nature these have a crucial role in the storage and controlled secretion of several haemostatic, and very complex, proteins, most notably von Willebrand factor.

The formation of functional pseudo-Weibel Palade bodies which behave like ‘wild type’ bodies can be induced in certain non-endothelial cell lines by expression of von Willebrand factor. “The ability of intracellular storage bodies to store, protect, and regulate the release of functionally active proteins, has yet to be exploited as part of a novel production strategy for difficult-to-express proteins,” says Tim.

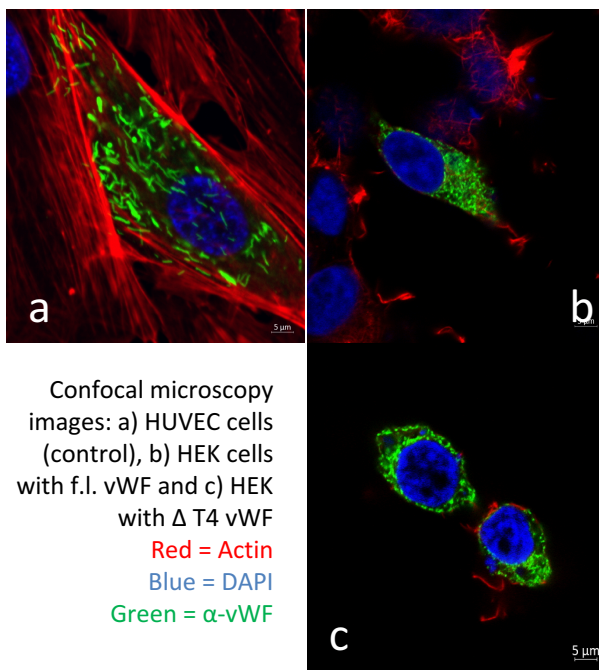
The main part of the work investigated which parts of the von Willebrand factor protein are responsible for promoting accumulation and storage in Weibel-Palade body vesicles “Our aim with is to be able to turn the von Willebrand factor fragment into a Trojan horse for the re-direction and packaging of other proteins into WPB so that it can be hijacked for the production of novel difficult to express proteins,” says Tim.

Using green fluorescent protein as a test

protein they identified a ‘minimal critical region’ of von Willebrand factor that was needed to generate functional pseudo-WPB that packaged and secreted the green protein.

As well as demonstrating the feasibility of using Weibel-Palade bodies as part of a protein expression system, the project also generated several novel observations about the fundamental biology of Weibel-Palade bodies. Tim was an invited speaker at the 2017 ESACT-UK meeting, where he presented preliminary results from this project.

Further work studying protein expression levels — to help differentiate stimulated and constitutive protein release — are currently underway. A priority for future studies is to test the system with other proteins. “It will be really interesting to use more relevant proteins — for example, insulin, insulin-like growth factor 1 and erythropoietin to demonstrate effectiveness as a protein expression system,” highlights Tim.



“The ability of pseudo-Weibel Palade bodies to store and protect functionally active proteins has yet to be exploited as part of a novel protein production strategy.”