Using NanoBiT™ to measure the binding interactions between ANGPTL3, ANGPTL8, and lipoprotein lipase

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Background

- Dietary fats are absorbed by the intestine and packaged into triglyceride-rich particles called chylomicrons.
- Chylomicrons travel throughout the body via blood vessels.
- The enzyme lipoprotein lipase (LPL) breaks down chylomicrons to release fatty acids for cellular use.
- Three angiopoietin-like proteins (ANGPTLs) are involved in regulating lipid metabolism by selectively inhibiting LPL activity.
- ANGPTL3 is a liver-secreted inhibitor of LPL.
- ANGPTL4 is secreted by white adipose tissue during fasting and inhibits fat storage.
- ANGPTL8 is released after feeding and indirectly inhibits triglyceride uptake by cardiac and skeletal muscle.
- It is believed that ANGPTL8 interacts with and promotes the function of ANGPTL3 to inhibit LPL.
- The exact mechanism behind the ANGPTL3-8 pathway remains unclear.

Hypothesis

When co-expressed, ANGPTL8 binds to ANGPTL3 and increases ANGPTL3 binding and inhibition of LPL.

NanoLuc® Binary Technology

- NanoLuc® Binary Technology (NanoBiT™) is a luminescent reporter optimized for investigating protein-to-protein interactions.
- NanoBiT™ is a form of luciferase (the enzyme that makes fireflies glow) that is split into two parts: a Large BiT and a Small BiT.

Methods

- Cloning
- Transfection
- Verification
- Binding Assay 1
- Binding Assay 2

Results

- Verification of ANGPTL8-Lg
- NanoBiT™ Binding Assay

Conclusions

- The NanoBiT™ reporter system successfully demonstrated ANGPTL3 binding to LPL.
- ANGPTL8 enhances ANGPTL3 binding to LPL, but only when co-expressed in the cell.
- Preliminary results suggest that ANGPTL8 tagged with the Large BiT is potentially viable.
- The limited ANGPTL3 secretion with ANGPTL8-Lg could prevent its use in binding assays.

Future Directions

- Confirm ANGPTL8-Lg viability with activity assay.
- Conduct binding assay 2 (See Figure 4).
- NanoBiT™ binding assays with ANGPTL3 and ANGPTL8 with LPL bound to its rino anchoring protein glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 to observe differences in binding affinity.

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Selected References