Role of Zfp367 in ATF6-Induced Beta Cell Proliferation

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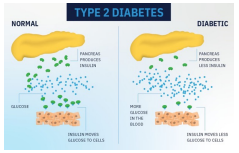
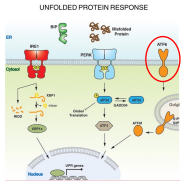
Introduction

Type 2 Diabetes (T2D) is caused by a deficiency in mass or function of beta cells in pancreatic islets, leading to failure to meet insulin demand.



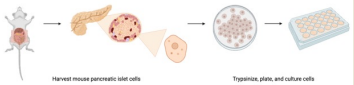
A previous study aimed to increase beta cell mass and proliferation through activation of the ATF6 pathway of the Unfolded Protein Response, which in high glucose conditions significantly increases beta cell proliferation[1]. Proliferation-related genes upregulated by high glucose and further increased by ATF6 activation were identified by RNA-seq. ChIP-X Enrichment Analysis suggested a role for the transcription factor Zfp367 in the regulation of these genes. Zfp367 is known to promote proliferation and tumorigenesis in various cancers[2,3]. In contrast, there is also evidence to support the opposite biological function of Zfp367 in blocking proliferation in cancer cell lines and during neurogenesis[4,5,6]. Preliminary CUT&RUN sequencing data showed potential direct binding of ATF6 to the Zfp367 promoter; however, the role of Zfp367 on beta cell proliferation induced by ATF6 is unknown.



We hypothesize that Zfp367 promotes ATF6-induced beta cell proliferation.

Materials and Methods

Islet Cell Culture:



Mouse pancreatic islets were dispersed into single cells and transduced with:

- shRNA to knockdown Zfp367 (sh-Zfp367)
- Adenovirus to overexpress Znf367 (Ad-Znf367)
- Adenovirus to activate ATF6 (DHFR-ATF6)

Beta cell proliferation was quantified by BrdU uptake in Insulin+ cells via immunofluorescence microscopy. mRNA levels were estimated by qPCR.

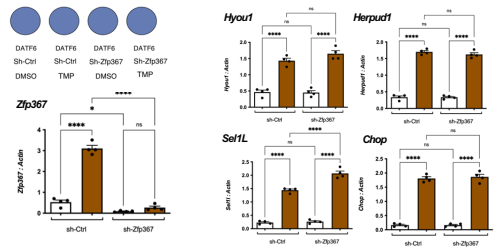
DAPI – Nuclei
Insulin – Beta Cells
BrdU – Proliferation (S phase)

Results

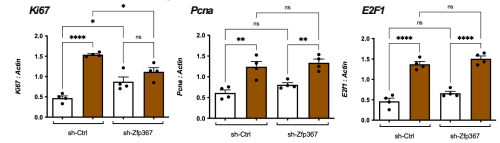
Zfp367 Knockdown Upregulates High Glucose-Induced Proliferation

DMSO: Control | TMP: ATF6 Activation

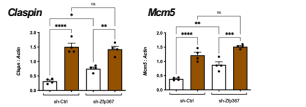
Confirmation of Zfp367 Knockdown and ATF6 Activation



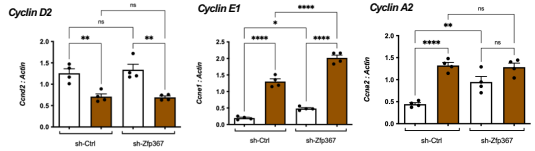
Transcriptional Markers of Proliferation



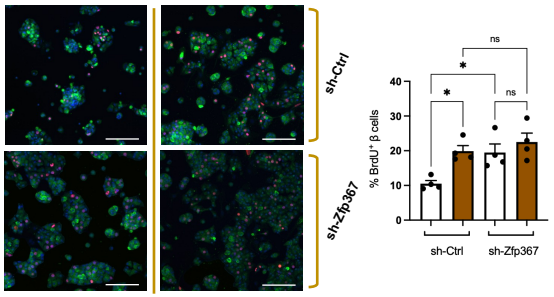
E2F1 Transcriptional Targets



Cell Cycle Progression Markers



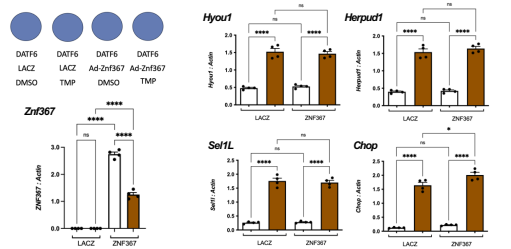
BrdU Quantification Confirms Increase in Proliferation



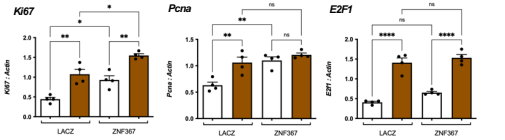
Zfp367 Overexpression Yields Mixed Results

DMSO: Control | TMP: ATF6 Activation

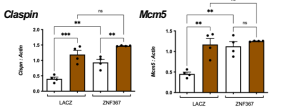
Confirmation of Znf367 Overexpression and ATF6 Activation



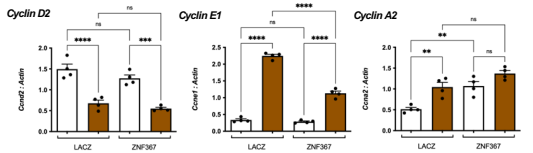
Transcriptional Markers of Proliferation



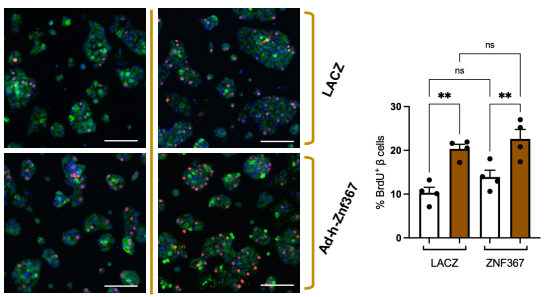
E2F1 Transcriptional Targets



Cell Cycle Progression Markers

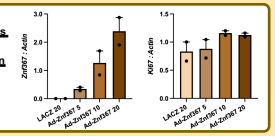


BrdU Quantification Reveals Failure to Increase Proliferation in General



Results (cont.)

MOI Test Shows Failure of Overexpression to Impact Proliferation

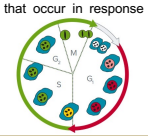


Conclusion

- Zfp367 knockdown increased beta cell proliferation in high glucose and reduced the capacity of ATF6 activation to further upregulate it.
- There was differing expression of Ki67 and Cyclin E1 in response to ATF6 activation in comparison to other factors.
- Zfp367 overexpression in islets cells increased the expression of transcriptional markers of proliferation; however, it did not change beta cell proliferation as measured by BrdU uptake.
- Further study is needed to fully understand the role of Zfp367 overexpression in ATF6-induced beta cell proliferation.

Future Directions

- Test the Zfp367 knockdown in normal glucose (7.5 mM glucose).
- Test transcriptional targets of Zfp367 from literature supporting both arguments.
- Conduct RNAseq analysis to explore the transcriptional changes that occur in response to Zfp367 knockdown and overexpression.
- Conduct the FUCCI assay to analyze cell cycle progression in greater detail than the BrdU assay.



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Diagrams were made using BioRender.

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