Identification of Itgbl1: a Novel Regulator of Adipogenesis

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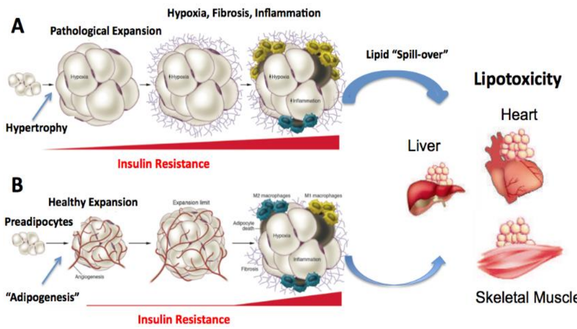
Introduction

It is widely appreciated that obesity confers significant risk for developing metabolic disorders; however, upwards of one-third of obese individuals are resistant to developing metabolic syndrome, at least for a period of time. This suggests that additional factors, outside of increased adiposity per se, determine metabolic health in obesity. The mechanism by which **white adipose tissue (WAT)** expands in obesity is widely viewed as a critical determinant of metabolic health. The expansion of WAT by **adipocyte hypertrophy** (increased cell size) is a defining feature of pathologic obesity. WAT expansion through **adipocyte hyperplasia** (de novo adipocyte formation, or "adipogenesis") results in smaller, healthier fat cells within WAT depots, and is commonly observed in metabolically healthy obese individuals. Increasing the number of adipocytes, and/or maintaining the function of existing adipocytes, may lead to metabolically healthy WAT expansion in the setting of caloric excess. However, ***factors that control the balance between adipocyte hyperplasia and adipocyte hypertrophy remain unknown***. Since adipocytes are post-mitotic, adipocyte hyperplasia must occur through de novo adipocyte formation, or **"adipogenesis".** Thus, **identifying viable strategies to improve healthy energy storage will depend on a deeper understanding of what factors induce or repress adipogenesis in adult animals.**

A significant barrier to advancing our understanding of adipogenesis in adults has been a lack of knowledge regarding which genetic factors are most important *in vivo* to promote or repress adipogenesis. Our lab previously established that the transcription factor **Zfp423** is a regulator of preadipocyte commitment, serving as an activator of **Ppary**, the "master regulator" of adipocyte differentiation. Based on this finding, the lab has generated several genetic tools suitable for the identification, isolation, and manipulation of distinct adipose precursor cell populations in mice.

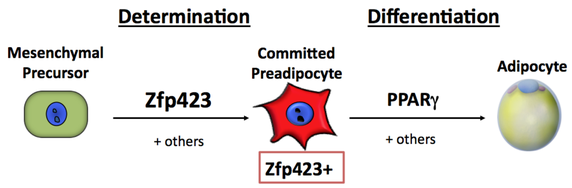
The goal of the study presented here is the identification of novel genetic factors that regulate adipogenesis. Using our animal models, we have previously shown that Zfp423-expressing cells are a specialized pericyte-like cell population that is primed for adipogenesis. We now investigate what genes are expressed in these primed preadipocytes *in vivo*, and seek to determine the impact of those genes on the regulation of adipogenesis. **The identification of a novel repressor of adipogenesis *in vitro* offers a new opportunity to investigate the role of this factor in regulating adipogenesis—and ultimately, metabolic health—in the setting of obesity.**

1 Adipocyte differentiation is important for healthy adipose tissue expansion in obesity



Healthy vs. Unhealthy Obesity. A) Pathological adipose tissue expansion is characterized by enlarged adipocytes, poor adipogenesis and angiogenesis, hypoxia, fibrosis, and pro-inflammatory M1 macrophage recruitment. This adipocyte dysfunction leads to ectopic lipid accumulation in peripheral tissues. **B**) Healthy adipose tissue remodeling involves adipocyte hyperplasia and appropriate angiogenesis. This limits the burden on individual fat cells and delays the acquisition of insulin resistance and lipitoxicity. **(Figure adapted from Sun and Scherer JCI 2011)**

2 Transcriptional control of preadipocyte determination and differentiation

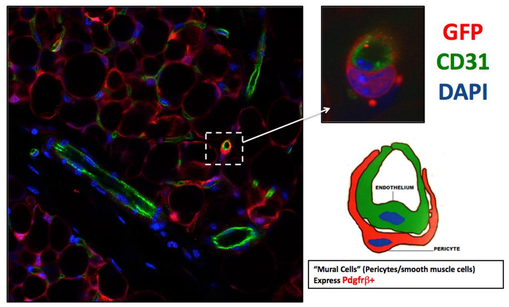


Zfp423 expression identifies Committed Preadipocytes

Gupta et al *Nature* 2010

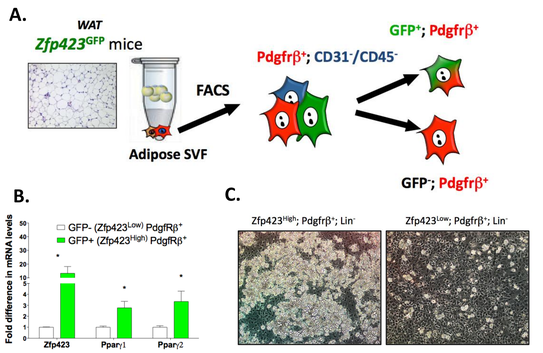
The expression of the transcriptional regulator Zfp423 identifies committed preadipose fibroblasts. Zfp423 functions to control preadipocyte levels of PPARγ, the "master regulator" of adipocyte differentiation.

3 Zfp423 is expressed in a subset of adipose tissue mural cells



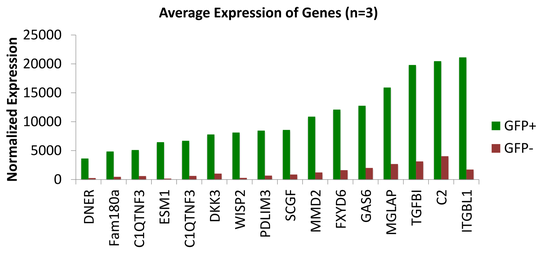
Immunostaining of Zfp423/GFP expression in adipose tissue of *Zfp423*^{GFP} transgenic reporter mice. Zfp423/GFP expression **(Red)** is found in differentiated fat cells and a subset of peri-endothelial mesenchymal cells that express the mural cells marker Pdgfrβ. CD31 **(green)** expression marks endothelial cells. DAPI **(Blue)** labels cell nuclei.

4 Zfp423^{High};Pdgfrβ^{+} cells express adipose lineage-selective transcripts and differentiate into adipocytes



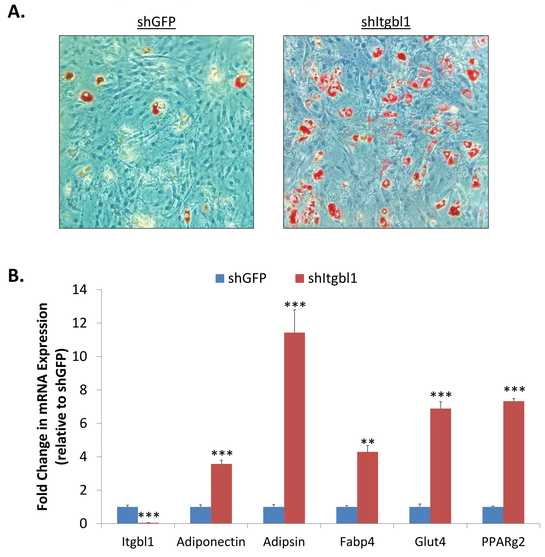
Molecular and Functional analysis of adipose Zfp423^{+}; Pdgfrβ^{+} mural cells: A) GFP^{+};Pdgfrβ^{+} and GFP^{-};Pdgfrβ^{+} cells from adult white adipose tissue were isolated by FACS. **B)** qPCR analysis of gene expression reveals the enrichment of lineage specific transcripts in the Zfp423-expressing population of cells. **C)** Zfp423^{High};Pdgfrβ^{+} and Zfp423^{Low};Pdgfrβ^{+} cells shown in a) were tested *in vitro* for their propensity for adipocyte differentiation. Upon reaching confluence, cells within the Zfp423^{High};Pdgfrβ^{+} population underwent spontaneous differentiation, as indicated by lipid accumulation and the expression of adipocyte-specific transcripts (data not shown).

5 Microarray analysis reveals Itgbl1 as the top regulated gene in Zfp423^{High};Pdgfrβ^{+} cells



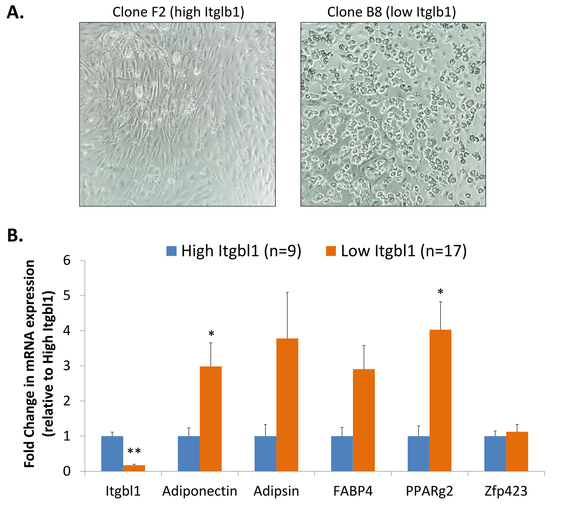
***Zfp423*^{GFP} microarray data.** Analysis of gene expression in *Zfp423*^{Low} mural cells vs. *Zfp423*^{High} mural cells identifies Itgbl1 as the gene with the largest difference in expression. This differential expression—highly expressed in the *Zfp423*^{High} mural cells, and almost absent in the *Zfp423*^{Low} mural cells—suggests that Itgbl1 has a significant role in the differentiation of a committed preadipocyte.

6 shRNA knockdown of Itgbl1 expression promotes adipogenesis in pluripotent C3H/10T1/2 mesenchymal stem cells



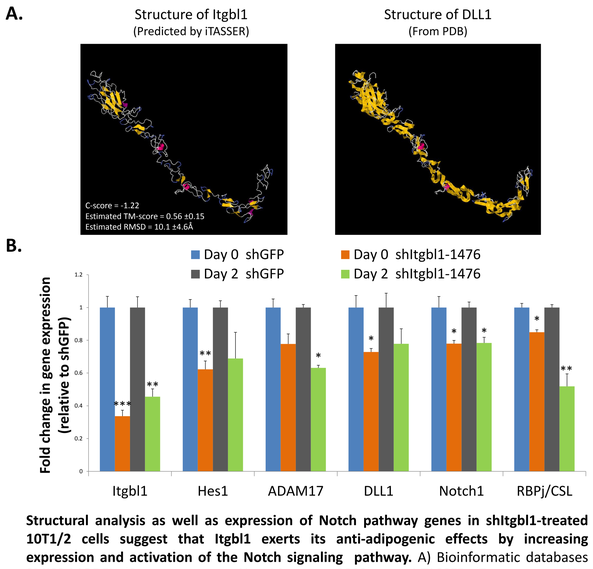
10T1/2 cells treated with shItgbl1 are more likely to undergo adipogenesis. 10T1/2 mesenchymal stem cells were infected with a retrovirus in order to express either shGFP (control) or shItgbl1. Two out of three constructs yielded consistent results, and the results from shItgbl1-1476, which yielded the best knockdown (95%), are shown. A) Oil Red O lipid staining shows increased number of adipocytes six days after induction of differentiation with DMI (dexamethasone, insulin, and IBMX) and rosiglitazone. B) Adipocyte-specific transcripts are upregulated 3-11 fold on day six of differentiation in 10T1/2 cells expressing shItgbl1.

7 CRISPR-cas9 knockout of the ITGBL1 gene promotes adipogenesis in clonal cell lines



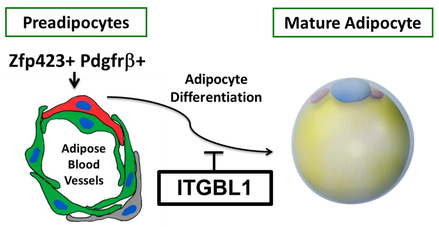
Isolation of clonal lines of 10T1/2 cells modified with CRISPR-cas9 knockout of the ITGBL1 gene shows increased adipogenesis in ITGBL1 KO clones. 10T1/2 mesenchymal stem cells were treated with lentivirus in order to stably express both the cas9 nuclease and a gRNA targeting the second exon of ITGBL1. Three gRNAs were tested, and the most effective guide RNA, targeting Exon 2, was used to generate clonal lines of 10T1/2 cells with varied levels of Itgbl1 expression. A) Bright-field photographs of representative high- and low-Itglb1 colonies, on day six post-DMI +rosiglitazone. B) Average fold change in expression of adipocyte genes in 26 clonal lines on day six post DMI +rosiglitazone.

8 Structural analysis predicts that Itgbl1 exerts its effects through the Notch signaling pathway



Structural analysis as well as expression of Notch pathway genes in shItgbl1-treated 10T1/2 cells suggest that Itgbl1 exerts its anti-adipogenic effects by increasing expression and activation of the Notch signaling pathway. A) Bioinformatic databases SMISS-MODEL (Schwede et al, *Nat Methods*, 2009) and I-TASSER (Zang et al., *Nat Methods*, 2015) were used to generate models of ITGBL1's tertiary protein structure. These models predicted high homology between ITGBL1 and DLL1, a known Notch ligand. B) Expression of Notch pathway and target genes were significantly decreased in shItgbl1-1476-treated 10T1/2 cells on Day 0 and Day 2 of differentiation.

9 Working hypothesis: Itgbl1 acts to repress adipogenesis of mesenchymal stem cells



Conclusions

- Adipose tissue contains functionally distinct mural cell populations; high levels of the preadipocyte commitment factor Zfp423 in these perivascular cells (Pdgfrβ^{+}) distinguish adipogenic mesenchymal stem cells in adipose tissue
- Itgbl1 is highly expressed in Pdgfrβ^{+}, *Zfp423*^{High} cells, and acts to maintain the precursor cell population by preventing progression of adipogenesis
- Itgbl1 likely exerts these repressive effects by increasing activation of the Notch signaling pathway

The identification of Itgbl1 as a novel regulator of adipogenesis provides new opportunities to investigate the mechanisms by which adipose tissue expands in the setting of obesity, and the impact that expansion has on metabolic health.

References:

Vishvanath, L, MacPherson KA, Hepler C, Wang QA, Shao M, **Spurgin SB**, Wang MY, Kusminski CM, Morley TS, Gupta RK. (2015) "Pdgfrβ+ Mural Preadipocytes Contribute to Adipocyte Hyperplasia Induced by High-Fat-Diet Feeding and Prolonged Cold Exposure in Adult Mice." Cell Metabolism. 2015 Nov 13. PMID: 26626462