

Notch signaling as a novel regulator of metabolism

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Evolutionarily unprepared for modern high-calorie diets and sedentary lifestyles, humans are now unprecedentedly susceptible to metabolic disorders such as obesity, type 2 diabetes (T2D), nonalcoholic fatty liver, and cardiovascular disease. These metabolic conditions are intertwined, together known as metabolic syndrome, and compromise human life quality as well as lives. Notch signaling, a fundamental signal transduction pathway critical for cell–cell communication and development, has recently been recognized as a key player in metabolism. This review summarizes the emerging roles of Notch signaling in regulating the metabolism of various cell and tissue types, with emphasis on the underlying molecular mechanisms and the potential of targeting this signal axis to treat metabolic diseases.

An overview of Notch signaling

The Notch signaling pathway is an evolutionarily conserved pathway important for cell–cell communication and cell-fate determination during development and is required for adult tissue homeostasis. It comprises Notch receptors (see [Glossary](#)) and Notch ligands as well as intracellular proteins that function to transmit the Notch signal to the cell's nucleus. Notch receptors (Notch1–4) are single-pass transmembrane proteins comprising an extracellular domain (NECD), a transmembrane (TM) domain, and an intracellular domain (NICD). Notch ligands are also transmembrane proteins and cells expressing Notch ligands must be in close proximity to Notch-expressing cells for signaling to occur. Ligands bind to the Notch NECD to induce proteolytic cleavage and release of the NICD, which enters the cell nucleus to modify gene expression. Notch ligands are members of the Delta/Serrate/LAG-2 (DSL) family of proteins that includes Delta-like (Dll1, Dll3, Dll4) and Jagged (Jag1, Jag2) in mammals [1,2].

Notch signal transduction is initiated on binding of a Notch receptor to a ligand located on a neighbor cell. Endocytosis of Notch-bound ligand generates a mechanical pulling force that drives conformational changes of the Notch receptor and facilitates its sequential proteolytic cleavage [3]. The first cleavage, mediated by a disintegrin

and metalloproteinase (ADAM) family peptidase, releases the NECD, whereas the second cleavage, mediated by γ -secretase, releases the NICD [1]. The NICD then translocates to the nucleus where it binds with recombination signal binding protein for immunoglobulin kappa j region (Rbpj) and recruits a transcriptional complex to activate the transcription of downstream targets including Hairy/enhancer of split (Hes) and Hes-related with YRPW motif protein family genes. Simple in design, activation of Notch is tightly orchestrated at multiple levels [1] and the

Glossary

Atherosclerosis: a type of vascular disease characterized by plaque accumulation in arteries resulting from increased cytokines due to metabolic dysfunction, which leads to activation of the innate immune system and chronic inflammation.

Beige adipocytes: a newly defined type of adipocyte within the WAT. They are similar to brown adipocytes in that they express UCP1 and have the capacity for thermogenesis. Their gene expression signature is distinct from those of both brown adipocytes and white adipocytes.

Brown adipocytes: a type of adipocyte that is abundant in rodents and newborn humans but less abundant in adult humans and has a high capacity for adaptive thermogenesis. Brown adipocytes contain numerous mitochondria expressing UCP1, which uncouples the proton gradient from ATP production to generate heat. Due to their ability to burn lipids (through β -oxidation) to generate heat, brown adipocytes increase energy expenditure and are negatively associated with obesity.

Delta/Serrate/Lag-2 (DSL) family protein: single-pass transmembrane proteins whose extracellular domain acts as a ligand for Notch receptors on a neighboring cell. In mammals, the family members include Delta-like (Dll1, Dll3, Dll4) and Jagged (Jag1, Jag2).

Gluconeogenesis: a biochemical process that generates glucose from non-carbohydrate carbon substrates like pyruvate.

Glycogenolysis: a biochemical process whereby glycogen is broken down to glucose 1-phosphate.

Glycolysis: a biochemical process that converts glucose to pyruvate, releasing free energy in the form of ATP.

Hairy/enhancer of split (Hes): a transcription repressor that belongs to the bHLH protein family with important roles in the Notch signaling pathway.

Hes-related with YRPW motif protein (Hey): a nuclear protein that belongs to the Hes-related (HESR) family of basic helix–loop–helix (bHLH)-type transcriptional repressors. Hey expression is induced by Notch signaling.

Lipogenesis: a metabolic pathway that has two separate processes: fatty acid synthesis and triglyceride synthesis.

M1 and M2 macrophages: also known as classically and alternatively activated macrophages, respectively. M1 macrophages are activated in response to bacterial infections or lipopolysaccharide and IFN- γ and are highly inflammatory. By contrast, M2 macrophages are activated in response to parasitic infections or IL-4 and -13 and are anti-inflammatory.

Notch receptors (Notch1–4): a family of single-pass transmembrane receptors comprising an NECD, a TM domain, and an NICD. Activation of Notch receptors leads to release of the NICD, which then acts as a transcription factor to regulate gene expression.

Recombination signal binding protein for immunoglobulin kappa j region (Rbpj): also known as CBF1 in humans; a highly conserved DNA-binding protein that mediates canonical Notch signaling.

White adipocytes: a major type of adipocyte in animals and humans that store energy in the form of triglycerides.

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biological output is highly cellular-context dependent. One unique and important feature of Notch signaling is the lack of secondary amplification: NICD is part of the Notch receptor as well as the direct activator of Notch targets. Therefore, every event of Notch activation engages and consumes one Notch receptor. A similar turnover scenario also applies to Notch ligands. Notch ligand and receptor turnover together establish an oscillating pattern of Notch activation based on the availability of replenished Notch receptors and ligands. Nuclear NICD is eventually targeted for proteasomal degradation mediated by the E3 ubiquitin ligase F box- and WD repeat domain-containing 7 (FBW7) [4,5]. A recent study showed that FBW7 transcription is repressed by the Notch target gene *Hes5*, thus creating a positive feedback loop that prolongs Notch signaling [6].

Notch signaling is a highly conserved intercellular communication mechanism critical for many cellular processes including survival, proliferation, and differentiation, as well as maintaining stem cell quiescence and identity [7]. Thus, Notch signaling is widely employed to orchestrate proper development and perturbation of the Notch pathway is linked to various devastating genetic disorders and cancers [8]. In addition, recent studies employing transgenic mouse models of tissue-specific manipulation of Notch signaling have begun to reveal the roles of the Notch pathway in regulating metabolism in several key metabolic organs.

Notch signaling in diabetic and fatty liver

Notch signaling is involved in embryonic development, postnatal regeneration, and carcinogenesis of the liver [9], the central hub for glucose and lipid metabolism. On feeding, an increase in blood glucose stimulates the secretion of insulin from the pancreas. Circulating insulin inhibits liver glucose production, including glycogenolysis and gluconeogenesis, and stimulates glucose utilization, including glycolysis and lipogenesis. Recent studies have revealed a key role of Notch signaling in regulating both processes, with abnormal activation of Notch signaling in hepatocytes leading to hyperglycemia and fatty liver disease (Figure 1) [10,11].

The effect of Notch signaling on hepatic glucose production is mainly mediated through synergy of NICD with the transcription factor forkhead box protein O1 (FoxO1) (Figure 1). FoxO1 directly activates the transcription of the catalytic subunit of glucose-6-phosphatase (G6pc), a rate-limiting enzyme involved in hepatic glycogenolysis and gluconeogenesis [11]. Compound haploinsufficiency of FoxO1 and Notch1 (Foxo1^{+/-}:Notch1^{+/-}) markedly ameliorates insulin resistance in diet-induced obese (DIO) mice [11]. Liver-specific knockout of *Rbpj* using albumin-Cre phenocopies FoxO1:Notch1 haploinsufficiency, indicating that Notch signaling is the key driver of hepatic insulin resistance. Consistently, adenovirus-mediated activation of Notch1 in liver induces G6pc expression and exacerbates insulin resistance in a FoxO1-dependent manner [11].

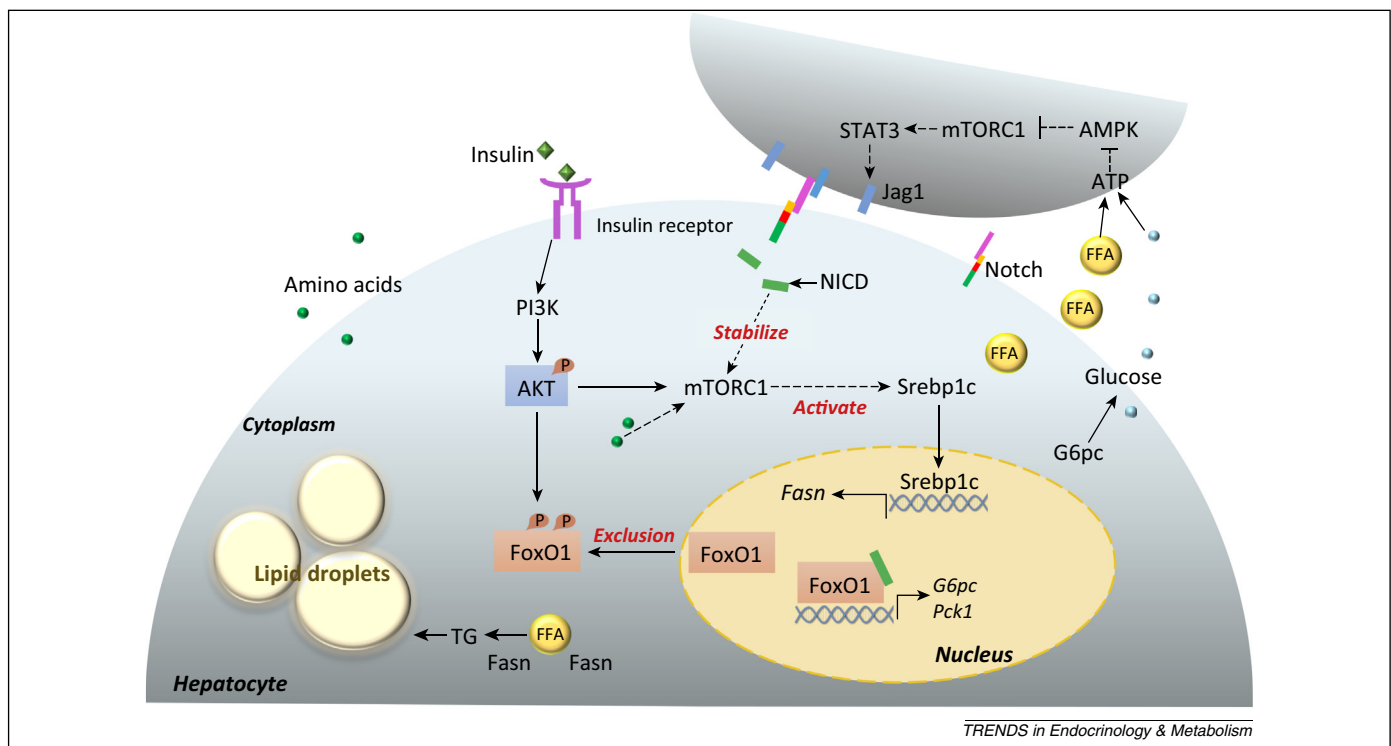


Figure 1. Notch regulates gluconeogenesis and lipogenesis of hepatocytes. Notch signaling regulates hepatic glucose production through synergy with forkhead box protein O1 (FoxO1), which directly activates the transcription of glucose 6-phosphatase, catalytic subunit (G6pc) and phosphoenolpyruvate carboxykinase 1 (Pck1), the rate-limiting enzymes in hepatic glycogenolysis and gluconeogenesis, respectively. Transcriptionally active FoxO1 is phosphorylated by AKT and excluded from the nucleus. In addition, Notch signaling promotes hepatic lipogenesis through an unknown factor that stabilizes mammalian target of rapamycin complex 1 (mTORC1), which is normally activated by amino acids, as well as the insulin-phosphatidylinositol-3-kinase (PI3K)-AKT pathway. mTORC1 in turn activates sterol regulatory element-binding protein 1c (Srebp1c), a key factor that turns on the transcription of fatty acid synthase (Fasn), which encodes a rate-limiting enzyme in lipogenesis. In obesity, high levels of glucose and free fatty acids (FFAs) activate the AMP-activated protein kinase (AMPK)-mTORC1-signal transducer and activator of transcription 3 (STAT3) pathway, which eventually upregulates Jagged 1 (Jag1) and activates Notch signaling in the neighboring hepatocyte. Broken line indicates indirect effect. Abbreviation: TG, triglyceride.

Importantly, pharmacological inhibition of Notch signaling by blocking γ -secretase-mediated cleavage of NICD improves glucose tolerance and insulin sensitivity in DIO mice [11].

Another arm of insulin action in the liver is its stimulatory effect on lipogenesis. In mouse and human diabetes mellitus, hepatic insulin resistance is selective, whereby insulin fails to suppress gluconeogenesis but continues to stimulate lipogenesis, resulting in hyperglycemia and hypertriglyceridemia. By contrast, mice with total hepatic insulin resistance elicited by liver-specific deletion of the insulin receptor develop hyperglycemia but not hypertriglyceridemia [12–14]. These results suggest the existence of divergent pathways controlling hepatic gluconeogenesis and lipogenesis. Intriguingly, activation of hepatic Notch signaling leads to a selective insulin resistance phenotype with hyperglycemia and hepatosteatosis (fatty liver) [10]. This indicates that Notch signaling is a key point in the web of the hepatic insulin paradox, where the two branches of insulin action converge. Mechanistically, Notch stimulates lipogenesis through an unknown factor that stabilizes mammalian target of rapamycin complex 1 (mTORC1) [10], a central player in lipid metabolism (Figure 1) [15]. Importantly, in both mouse and human, the hepatic Notch signaling is positively correlated with insulin resistance and fatty liver disease [10,16].

One unanswered yet important question concerns the upstream regulator of Notch signaling in hepatocytes. A recent study indicates that the energy sensor AMP-activated protein kinase (AMPK) regulates Notch signaling through mTORC1 under the influence of nutrient status [17]. Specifically, excessive amino acids cause insulin resistance in cultured hepatocytes, accompanied by attenuation of AMPK activity and activation of mTORC1–signal transducer and activator of transcription 3 (STAT3)–Notch1 signaling (Figure 1) [17,88]. The phenotypes are ameliorated by chronic administration of either the AMPK activator metformin or the mTORC1 inhibitor rapamycin [17]. Hence, the synergy between Notch and FoxO1 and the positive feedback loop between Notch and mTORC1 in hepatocytes may be targeted to improve liver insulin sensitivity and ameliorate the hyperglycemia and hypertriglyceridemia caused by diabetic fatty liver.

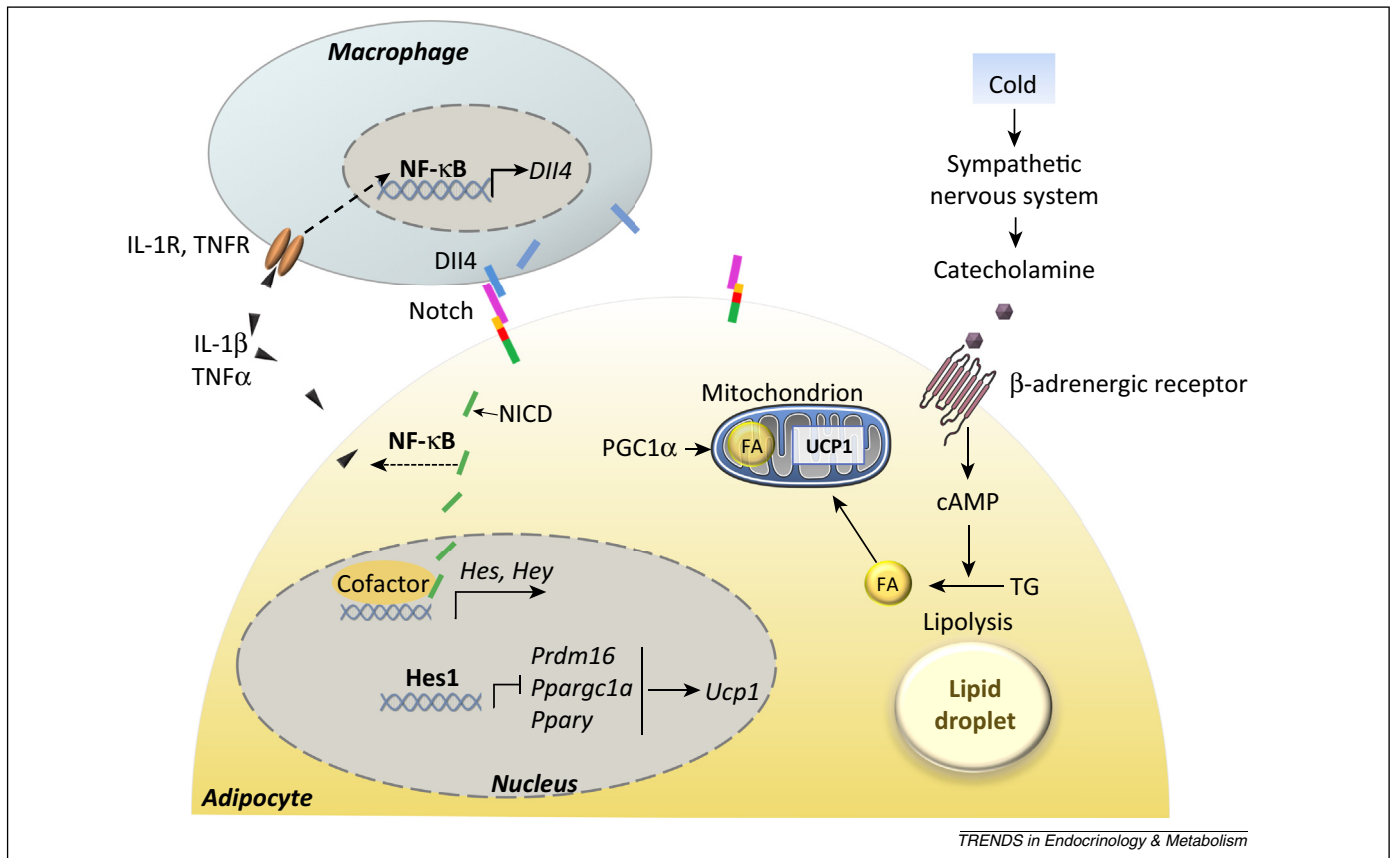
Notch signaling regulates adipocyte homeostasis

White adipose tissue (WAT) is the primary site of long-term energy storage. In response to excess calorie intake, the size of the WAT expands through hyperplasia and hypertrophy of adipocytes. Understanding the pathways that regulate adipocyte homeostasis is thus fundamental to the treatment of obesity. Characterization of the role of Notch signaling in adipocyte differentiation by various groups has generated inconsistent results. In 3T3-L1 preadipocytes, the Notch target *Hes1* is shown to inhibit adipogenic differentiation by repressing expression of CCAAT/enhancer binding protein alpha (C/EBP α) and peroxisome proliferator-activated receptor gamma (Ppar γ) [18]. Paradoxically, knockdown of *Hes1* also inhibits adipogenic differentiation of 3T3-L1 cells, accompanied by increased expression of Delta-like 1 homolog (Dlk1), an inhibitor of adipogenic differentiation [18]. In human primary cell cultures, inhibition of Notch

promotes, whereas activation of Notch inhibits, adipogenic differentiation of mesenchymal stem cells and adipose-derived precursor cells [19–21]. However, genetic ablation of several key components of the Notch pathway fails to elicit any obvious deficiencies in adipogenic differentiation of embryonic fibroblasts [22]. The contradictory findings of these cell-culture experiments could be attributed to the timing and dose of Notch intervention and the distinct cell types used. For instance, initiation of adipogenic differentiation of immortalized 3T3-L1 cells requires sequential control of the cell cycle, which is directly affected by Notch signaling [23,24]. Another confounding factor is the heterogeneity of primary preadipocyte cultures compared with 3T3-L1 cells [25]. It is possible that adipocytes of different origins (or differentiation stages) employ diverse combinations of Notch ligands and receptors to achieve context-dependent versatility of Notch signaling.

Adipocytes can be classified into white, beige (brite), and brown [26]. White adipocytes are the predominant cell type in various depots of subcutaneous and visceral WAT. Brown adipocytes are mainly found in brown adipose tissue (BAT), which is scarcely dispersed along the neck and shoulders of humans [26]. Beige adipocytes are a newly defined type of adipocyte that coexist with white adipocytes in subcutaneous WAT and with brown adipocytes in BAT [26]. While white adipocytes are primarily involved in energy storage, brown and beige adipocytes are highly specialized for energy expenditure due to their higher mitochondrion content and abundant expression of uncoupling protein 1 (UCP1), which uncouples the electron transport chain from ATP production to generate heat [27]. Notably, UCP1 is activated by fatty acids that are produced by the lipolysis of lipid droplets on adrenergic stimulation in brown and beige adipocytes (Figure 2) [28]. Functionally, brown adipocytes contain more mitochondria, express higher levels of UCP1, and have stronger thermogenic activity than beige adipocytes [26]. Beige adipocytes can be generated through *de novo* differentiation of preadipocytes [29,30] or from direct conversion of mature white adipocytes [31–33]. Several recent lineage-tracing studies have shown that brown, beige, and white adipocytes have distinct developmental origins. Specifically, brown adipocytes arise from Myf5⁺ lineages while white and beige adipocytes arise from Myf5⁻ lineages [34]. In addition, in WAT, beige and white adipocytes are enriched in Pax3⁻ and Pax3⁺ cell populations, respectively [35].

It was recently reported that Notch signaling plays a role in regulating the plasticity (conversion) of white and beige adipocytes *in vivo*, consequently affecting body energy metabolism [36]. Adipocyte-specific ablation of *Notch1* or *Rbpj* driven by aP2-Cre decreases the size of various adipose depots and increases the abundance of beige adipocytes in WAT, accompanied by increased metabolic rate, improved glucose tolerance, and insulin sensitivity [36]. These phenotypes are associated with elevated expression of beige adipocyte-specific genes in WAT but not BAT. In addition, mice depleted of *Notch1* or *Rbpj* exhibit accelerated browning (appearance of beige adipocytes within WAT) in response to cold environments. The adipose-specific Notch1 mutant mice are also resistant to high-fat diet (HFD)-induced obesity. Importantly, intraperitoneal administration



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Figure 2. Notch signaling regulates adipocyte thermogenesis. In response to cold ambient temperatures, the sympathetic nervous system releases catecholamine, which binds β -adrenoceptors and activates lipolysis through the cAMP pathway. Fatty acids (FAs) can directly activate uncoupling protein 1 (UCP1) for heat production. Notch target gene Hairy/enhancer-of-split 1 (Hes1) directly binds to the promoter region of PR domain-containing 16 (*Prdm16*), peroxisome proliferator-activated receptor gamma (*Ppar γ*) coactivator 1 alpha (*Ppargc1a*), and *Ppar γ* and inhibits their transcription. This leads to reduced mitochondrion numbers and expression of UCP1. Notch signaling promotes the activation and production of proinflammatory cytokines mediated by nuclear factor kappa light chain enhancer of activated B cells (NF- κ B), which attracts macrophages and together causes low-grade systemic inflammation and exacerbates insulin resistance. In obesity, infiltrated macrophages activate transcription of Notch ligand Delta-like 4 (DII4) through NF- κ B. Broken line indicates indirect effect. Abbreviations: IL-1 β , interleukin-1 β ; TNF α , tumor necrosis factor alpha; IL-1R, interleukin-1 receptor; TNFR, tumor necrosis factor receptor.

of a γ -secretase inhibitor reduces the adiposity and body weight of obese mice [36]. Currently it is unclear whether the reduced size of adipose depots is due to increased energy expenditure (and thus less deposition of fat) or a requirement of Notch signaling in normal adipogenesis. Because aP2-Cre is activated in adipose progenitors [37], using a mature adipocyte-specific Cre line to delete *Notch1* or *Rbpj* will distinguish these two possibilities. In addition, as the aP2-Cre mouse has been reported to drive weak Cre expression in the brain and macrophages [38–41], future studies using more stringent adipocyte-restricted Cre driver mice are necessary to confirm these observations. As an initial attempt to confirm the role of Notch signaling in adipose tissues, activation of Notch signaling using a highly adipocyte-specific adiponectin-Cre mouse is shown to inhibit browning of WAT and induce whitening of BAT, manifested by lipid deposition and the emergence of white adipocytes in the classic interscapular BAT [36]. Adiponectin-Cre-induced Notch activation also renders the mice glucose intolerant and insulin resistant [36]. These phenotypes are in sharp contrast to those observed in Notch-deficient mice. Mechanistically, the Notch target gene *Hes1* directly binds to the promoter regions of PR domain-containing 16 (*Prdm16*) and *Ppar γ* coactivator 1 alpha (*Ppargc1a*) to inhibit the transcription of these two master regulators of

mitochondrial biogenesis [42] and beige adipogenesis (Figure 2) [43–45]. Taken together, these results indicate that Notch signaling is a negative regulator of beige adipocyte biogenesis.

Several key questions remain to be answered. Considering the distinct developmental origins of beige and white adipocytes, it is important to examine whether Notch signaling differentially stimulates the specification, fate determination, or differentiation of white versus beige preadipocytes. Alternatively, Notch ligands and receptors may be differentially expressed by beige and white adipocytes to achieve distinct activation patterns or engage different target genes in these two types of adipocyte. Addressing these questions requires the ability to prospectively isolate white preadipocytes and beige progenitors [46] or to definitively identify mature white and beige adipocytes [47].

Notch signaling in skeletal muscle homeostasis

Skeletal muscle utilizes both glucose and free fatty acids as fuel for ATP production during contraction. In addition, skeletal muscle is a major site of glucose storage, hence coordinately maintaining blood glucose levels within the normal range. In particular, skeletal muscle accounts for 80–90% of postprandial insulin-stimulated glucose uptake

[48,49]. Importantly, skeletal muscle insulin resistance is identified as the primary defect in T2D [50]. Furthermore, muscle exercise elicits strong benefits against metabolic disorders. These benefits are not limited to the direct energy expenditure during muscle contraction but are also attributed to the muscle secretome – cytokines and peptides produced by and released from skeletal muscle cells (myofibers) to regulate body metabolism [51]. Therefore, maintaining muscle insulin sensitivity and its proper motor and secretory functions are important prerequisites for the treatment of metabolic diseases.

Within the skeletal muscle, a pool of well-defined stem cells called satellite cells are indispensable for the postnatal growth, maintenance, and regeneration of myofibers [52]. Notch signaling plays dose-dependent roles in satellite cells [53]. High, intermediate, and low Notch activities are essential for the quiescence (self-renewal), activation (cell cycle entry), and differentiation of satellite cells, respectively [54–57]. Specifically, deletion of either *Rbpj* or *Dll1* leads to premature differentiation and depletion of satellite cells, resulting in loss of postnatal muscle growth and severe muscle hypotrophy [53,56,58,59]. Conversely, constitutive activation of Notch1 promotes the self-renewal but inhibits the differentiation of satellite cells, resulting in poor muscle regeneration [57].

In addition to its well-established role in myogenesis, recent studies have indicated a potential role of Notch signaling in regulating muscle metabolism. In this regard, the interaction between Notch and FoxO1 again appears to be essential. Muscle-specific knockout of FoxO1 promotes conversion of oxidative slow-twitch to glycolytic fast-twitch myofibers in the soleus muscle [60]. Using the gold-standard euglycemic hyperinsulinemic clamp technique, Pajvani *et al.* found that the muscles of Foxo1^{+/-}:Notch1^{+/-} mice have considerably higher rates of glucose uptake than those of wild type and Foxo1^{+/-} mice [11], although the fiber type composition in Foxo1^{+/-}:Notch1^{+/-} mice was not characterized [60]. This phenotype can be explained by either muscle-specific action of Notch1 and FoxO1 or as a secondary effect of Notch1:Foxo1 haploinsufficiency in non-muscle organs. In the future, it would be interesting to directly examine whether myofiber-specific perturbations in Notch signaling affect muscle glucose metabolism and insulin sensitivity.

Notch in the central nervous system (CNS)

The CNS plays a key role in orchestrating proper central neuroendocrine function and regulating systemic glucose and energy metabolism [61]. Notably, obesity is associated with structural and functional impairment of the CNS [62], whose maintenance relies on neurogenesis mediated by adult neural stem cells (NSCs) [63]. Using tamoxifen-inducible conditional *Rbpj* knockout mice, Imayoshi *et al.* recently found that deletion of *Rbpj* in the adult brain caused transient differentiation of NSCs into neurons, leading to a total loss of NSCs and blockage of subsequent neurogenesis [64]. This indicates an indispensable role of Notch signaling in maintaining the quiescence of NSCs in addition to its widely accepted role in inhibiting neuronal differentiation. NSCs in the hypothalamus of DIO mice show impaired survival and neurogenic functions

[65]. Mechanistically, HFD feeding activates the I kappa B kinase beta (IKK β)/nuclear factor kappa light chain enhancer of activated B cells (NF- κ B)–Notch signaling axis, which promotes apoptosis and impairs neurogenic differentiation of NSCs [65]. Hypothalamus-specific activation of the proinflammatory IKK β /NF- κ B pathway phenocopies the effect of HFD on NSCs and ultimately leads to the development of obesity and diabetes [65]. Intriguingly, the proinflammatory factor NF- κ B directly binds to the promoters and activates the expression of *Dll4*, *Notch1*, and *Notch4* genes in NSCs [65]. Consistently, inhibition of either IKK β or Notch signaling reverses the differentiation defect of hypothalamic NSCs in DIO mice [65]. Collectively, NF- κ B not only mediates the systemic low-grade inflammation that is critical for the initiation, development, and exacerbation of metabolic syndrome [66] but also impairs neurogenesis through transcriptional upregulation of Notch ligands and receptors during metabolic stress.

Notch signaling also functions as an important NSC niche factor in the CNS. Endothelial cells of cerebral vessels enforce the quiescence of adult NSCs by presenting Jag1, which activates Notch signaling in the neighboring NSCs [67]. Endothelial cell lineage-specific ablation of Jag1 results in aberrant activation and depletion of quiescent NSCs [67]. In addition, both HFD and a high-cholesterol diet increase the expression of *Dll4* in the blood vessels of an atherosclerotic mouse model [68]. In summary, Notch signaling must be temporally regulated in NSCs for proper neurogenesis. Low Notch activity facilitates the differentiation of NSCs to immediately supply neurons to repair nerve damage and high Notch activity facilitates quiescence and self-renewal of NSCs. Dysregulation of Notch signaling in adult NSCs by obesity-associated systemic low-grade inflammation or proatherosclerotic vessels represents a novel neurodegenerative mechanism in obese patients.

Notch in metabolic angiogenesis

It has been well established that Notch signaling regulates the development of the embryonic vasculature and that perturbation of Notch pathway genes results in severe vascular defects in mutant mice [69]. During development, Notch signaling specifies the arterial fate of endothelial cells [70]. Notch signaling also plays a critical role in controlling the differentiation of vascular smooth muscle cells and senescence of endothelial cells in the postnatal vasculature [71]. Moreover, the Notch pathway actively participates in vascular remodeling by inhibiting the formation and function of endothelial tip cells via the regulation of vascular endothelial growth factor (VEGF) signaling [71]. As vascular sprouting is fundamentally important for the growth and expansion of WAT [72], understanding how Notch regulates angiogenesis has important implications for the prevention and treatment of obesity. Compared with WAT, BAT is hypervascularized to meet the nutrient and oxygen demands of its active thermogenic metabolism. Additionally, brown adipocytes rely on the blood flow to diffuse heat throughout the body to defend against hypothermia. Investigating whether and how Notch regulates angiogenesis in cold-activated BAT will extend the therapeutic scope of current preclinical

trials of Notch inhibition-based intervention in tumor angiogenesis [72].

Notably, vascular dysfunction is not only involved in the development of obesity [72], but is also a consequence of metabolic disorders manifested as atherosclerosis and other types of cardiovascular disease. Strikingly, blockade of Dll4–Notch signaling using neutralizing anti-Dll4 antibody elicited a broad range of benefits, dramatically attenuating the development of atherosclerosis, reducing inflammation, improving insulin resistance, and ameliorating obesity [68].

The vasculature also serves as the main niche factor for both brown and white adipocyte precursors; therefore, it may regulate adipose mass and body insulin sensitivity [72]. Currently, it is unclear how vasculature-derived adipocyte stem cells contribute to the heterogeneity of adipocytes in terms of their distinct differentiation potential toward white versus beige/brown adipocytes. Future work in characterizing how Notch signaling regulates the fate choice of adipocyte stem cells in response to physiological (VEGF) and metabolic (insulin, energy status) cues is warranted.

Notch regulates metabolic immunity

Notch signaling is emerging as an important regulator of both innate and adaptive immune system development and function [73], which have crucial implications in metabolic syndrome [74]. Among various cell types in innate immunity, macrophages are the major players in terms of abundance and functional significance in metabolic disorders. Macrophages can be divided into M1 and M2 subtypes. M1 macrophages are proinflammatory and secrete chemokines [e.g., monocyte chemoattractant protein 1 (MCP-1)] and proinflammatory cytokines [e.g., tumor necrosis factor alpha (TNF α)] that directly increase inflammation in peripheral tissues and impair local insulin sensitivity (Figure 3) [75]. By contrast, M2 macrophages ameliorate obesity-induced inflammation and secrete anti-inflammatory cytokines [e.g., interleukin (IL)-10] that promote insulin sensitivity (Figure 3) [75]. Importantly, Notch signaling regulates M1-versus-M2 macrophage specification, through various mechanisms (Figure 3) [76]. First, Rbpj promotes expression of the transcription factor interferon regulatory factor 8 (IRF8) while suppressing expression of the histone H3 Lys27 (H3K27) demethylase Jumonji domain-containing 3 (JMJD3) [77], which are inducers of M1 and M2 polarization, respectively [76]. Second, Notch signaling boosts the inflammatory property of M1 macrophages by interacting with NF- κ B [78,79]. Compared with lean animals, DIO mice showed a shift from a M2- to a M1-polarized state [75]. Consistently, Notch signaling in adipose tissue is activated by HFD feeding [36], which in part can be explained by the infiltration of Notch-primed M1 macrophages. It will be interesting to determine in the future whether and how inhibition of Notch signaling promotes the transition from M1 to M2 macrophages in obese adipose tissues and its therapeutic potential against obesity and other metabolic disorders.

Notch-mediated macrophage polarization contributes to the development of metabolic disorders not only through the abovementioned inflammatory actions but also

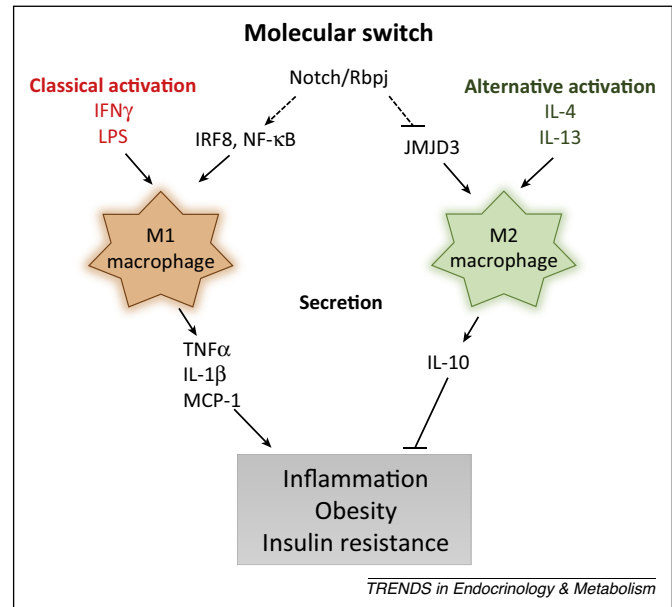


Figure 3. Notch signaling and macrophage polarization. M1 (classically activated) and M2 (alternatively activated) macrophages are activated by distinct and mutually exclusive activation programs. Notch signaling promotes M1 macrophage polarization through synergy with nuclear factor kappa light chain enhancer of activated B cells (NF- κ B) and by upregulating expression of the M1 macrophage regulator interferon regulatory factor 8 (IRF8). Notch signaling inhibits M2 macrophage polarization through repressing Jumonji domain-containing 3 (JMJD3), an M2 macrophage regulator. M1 and M2 macrophages have distinct secretory profiles that oppositely control inflammation and impact systemic insulin sensitivity. Abbreviations: IFN- γ , interferon gamma; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein 1.

through direct interaction with adipocytes. Notch ligands present on the surface of macrophages can potentially activate Notch signaling in neighboring adipocytes (Figure 2), hepatocytes, and myofibers and consequently exacerbate the insulin resistance of these key metabolic tissues. Interferon gamma (IFN- γ), which activates M1 macrophages, induces Jag1 expression rapidly, thereby amplifying Notch signaling in neighboring cells [80].

The macrophage–adipocyte interaction is of significance in obesity and T2D, where systemic infiltration of macrophages and other immune cells is prevalent. In this scenario, dynamic Notch ligand presentation on these circulating cell types may produce functional diversity in Notch activation, a direction deserving future investigation. The complexity is encoded by the diverse signaling capacity of Notch ligands and receptors [81] and the preferential binding of ligands to different Notch receptors [82]. For instance, during angiogenesis, Dll4 has strong while Jag1 has weak signaling potential and the relative abundance of these ligands creates distinct outcomes in angiogenesis [83]. Similarly, the vasculature may represent a crucial metabolic niche factor by presenting Notch ligands to fine-tune Notch signaling in peripheral tissues and metabolic disorders, either through regulating stem cells to maintain homeostasis of neurons, myocytes, and adipocytes or through directly regulating enzyme expression in hepatocytes. These observations and speculations warrant future investigations into the metabolic phenotypes of mice with macrophage-specific deletion of Notch ligand genes. Answers to this question will shed light on the distinct functions of different Notch ligands in

Box 1. Outstanding questions

- What are the ligand-presenting cell types that activate Notch signaling in metabolic organs under normal and disease conditions?
- Do different Notch receptors have diverse, or redundant, roles in regulating energy metabolism?
- How does Notch signaling regulate browning? Is it through determining the fate of adipocyte precursors or regulating the interconversion of mature white and beige adipocytes?
- What is the metabolic function of Notch signaling in mature muscle cells (myofibers)?
- How do energy-sensing kinases, such as AMPK and mTOR, modulate Notch signaling transduction?

peripheral tissues and potentially enable tissue-specific Notch-based therapies for metabolic disorders.

Concluding remarks and future perspectives

Notch signaling is a key regulator of cell fate and cellular homeostasis in virtually every metabolic organ. In the liver, Notch signaling boosts the gluconeogenesis and lipogenesis programs, which leads to hyperglycemia and fatty liver disease. In adipose tissue, genetic activation of Notch signaling induces whitening of BAT and insulin resistance, whereas genetic or pharmacological inhibition of Notch signaling promotes browning of white adipocytes and improves insulin sensitivity. In skeletal muscle and brain, Notch activation actively enforces quiescence of local adult stem cells, thereby limiting their tissue-repair potential and subsequently influencing body metabolism. In the immune system, activation of Notch signaling promotes M1 macrophage polarization, producing a systemic low-grade inflammatory state that exacerbates insulin resistance in peripheral tissues. Inhibition of Notch signaling in several of these tissues consistently improves glucose tolerance and insulin sensitivity and ameliorates obesity and atherosclerosis. Several outstanding questions, summarized in **Box 1**, merit future investigations. With the availability of pharmaceutical-grade γ -secretase inhibitors used in various clinical trials to treat Alzheimer's disease and cancers [84], it would be feasible to examine the effect of these inhibitors in the treatment of diabetes and obesity in humans. Due to the gastrointestinal toxicity and other off-target effects of γ -secretase inhibitors [85–87], however, alternative Notch inhibitors should also be developed. In this regard, antibodies targeting Notch ligands and receptors have recently been used in clinical trials [84]. The antiobesity and antidiabetic effect of these promising therapeutic agents should be investigated in the future.

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