Hepatic SirT1-Dependent Gain-of-Function of Stearoyl-CoA Desaturase-1 Conveys Dysmetabolic and Tumor Progression Functions

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SUMMARY

Obesity is associated with higher incidence of cancer but the predisposing mechanisms remain poorly understood. The NAD+–dependent deacetylase SirT1 orchestrates metabolism, cellular survival, and growth. To date, there is no unifying mechanism to explain the metabolic and tumor-related effects of SirT1. In this work, we demonstrate that genetic ablation of the endogenous inhibitor of SirT1, Deleted-in-Breast-Cancer-1 (Dbc1), unexpectedly results in obesity and insulin-resistance. Dbc1 deficiency promoted SirT1-dependent gain-of-function of stearoyl-coenzyme A desaturase 1 (Scd1), increasing plasma and tissue levels of unsaturated fatty acids. The metabolic abnormalities in Dbc1−/− mice were reversed by ablation of hepatic SirT1 or by inhibition of Scd1 activity. Furthermore, loss of Dbc1 impaired master tumor suppressor p53 activation and treatment of Scd1 inhibitor extended survival of tumorigenic TP53−−/− mice by decreasing tumor-related death. Together, our findings illustrate a shared mechanism of obesity and tumor progression through hepatic SirT1 gain-of-function in a metabolic control step, with potential therapeutic implications.
INTRODUCTION

The rising prevalence of obesity and overweight is expected to result in ~500,000 excess deaths from cancer by 2030 (Wang et al., 2011). Although a well-documented association exists between obesity and cancer, especially among patients at the highest extremes of body mass index distribution, it remains unclear whether the association of obesity with cancer portends shared cellular and biochemical mechanisms, or simply reflects prevalent environmental, behavioral, and genotoxic susceptibilities (Wang et al., 2011). At the cellular level, different signaling pathways, such as insulin/IGF, AKT and mTORC, share metabolic and oncogenic functions (Gallagher and LeRoith, 2013).

The NAD+-dependent, class III histone deacetylase (HDAC) SirT1 is another important control node of metabolism and oncogenesis (Chang and Guarente, 2014). SirT1 deacetylates proteins with complex roles in metabolism, inflammation, aging, cancer cell proliferation, and apoptosis. For example, it regulates hepatic gluconeogenesis through FOXO1 (Frescas et al., 2005; Qiang et al., 2010), white fat remodeling through peroxisome proliferator-activated receptor gamma (PPARγ) (Qiang et al., 2012), mitochondrial biogenesis through peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) (Rodgers et al., 2005), cholesterol metabolism through liver X receptor (LXR) (Li et al., 2007b), and other metabolic processes. The overall metabolic effects of moderate SirT1 gain-of-function are to lower energy efficiency and protect against obesity-induced diabetes (Banks et al., 2008; Pfluger et al., 2008). With regard to cancer biology, SirT1 deacetylates p53 to dampen its tumor-suppressor function (Luo et al., 2001), and may also regulate retinoblastoma protein (Rb) (Wong and Weber, 2007), E2F1 (Wang et al., 2006), c-Myc (Mao et al., 2011) and Poly (ADP-ribose) polymerase 1 (PARP-1) (Rajamohan et al., 2009). Nevertheless, to date, there is no unifying mechanism to explain the metabolic and tumor-related effects of SirT1.

Deleted-in-Breast-Cancer-1 (Dbc1) is an endogenous inhibitor of SirT1 (Kim et al., 2008; Zhao et al., 2008) that is expected to regulate the latter’s ability to acetylate p53, thus promoting cell cycle arrest, apoptosis and senescence (Li et al., 2012). In support of this notion, it is found that regulation of SirT1 by Dbc1 is altered in various types of cancers (Hiraike et al., 2011; Kim et al., 2009; Sung et al., 2010), implicating a tumor suppressor-like function of Dbc1. However, there is no direct evidence that Dbc1 is a tumor suppressor. Similarly, there is evidence that Dbc1 deletion prevents diet-induced hepatosteatosis in mice, but the mechanism of this effect is not known (Escande et al., 2010). Besides its function to inhibit SirT1, Dbc1 also regulates other chromatin remodeling enzymes, such as HDAC3 and SUV39H1, as well as transcription factors, including androgen receptor, estrogen receptor α and β, RARα, Rev-erβα, MYC, and BRCA1, either as an activator or repressor (Joshi et al., 2013). Interestingly, the interaction between Dbc1 and SirT1 is regulated during diet-induced obesity (Escande et al., 2010; Escande et al., 2015), raising the question of whether Dbc1 participates in the etiology of the metabolic syndrome.

In this work, we set out to investigate the metabolic functions of Dbc1 in association with its growth regulatory functions, and asked whether these functions are mediated by SirT1. In studies of Dbc1-deficient mice, we found insulin resistance with increased body fat content.
and impaired activation of tumor suppressor p53. These changes were associated with increased SirT1-dependent expression of Scd1, and altered tissue and plasma levels of unsaturated fatty acids. When the increase of Scd1 was reversed by pharmacological inhibition, metabolic and tumor-progression phenotypes were reversed, consistent with the possibility that Scd1 mediates both metabolic and oncogenic branches of the SirT1 pathway, and providing a potential mechanism for the baffling increase of cancer in metabolic diseases.

RESULTS

\textit{Dbc1}^{−/−} Mice Develop Insulin Resistance and Increased Body Fat

Given the pivotal functions of SirT1 in the pathophysiology of metabolic disorders, we investigated whether its native inhibitor Dbc1 also plays a role in metabolism. To this end, we generated \textit{Dbc1} knockout mice (Figures S1A–D). The knockouts were born in Mendelian ratios and showed normal features, growth rates, and reproductive behavior. Since ablation of \textit{Dbc1} increases SirT1 activity (Escande et al., 2010; Escande et al., 2015), we expected that \textit{Dbc1}^{−/−} mice would be more insulin-sensitive and protected against obesity-induced diabetes (Banks et al., 2008; Pfluger et al., 2008). Surprisingly, \textit{Dbc1}^{−/−} mice developed impaired tolerance to an intraperitoneal glucose load as early as 16 weeks after birth and remained glucose intolerant throughout life (Figure 1A and Figures S2A–S2E). Circulating insulin levels also rose, consistent with systemic insulin resistance (Figure 1B). These changes were not accompanied by changes in body weight (Figure 1C), but rather by an increase in body fat content beginning at puberty (Figure 1D and Figure S2F). To explain these changes, we studied energy balance using indirect calorimetry. \textit{Dbc1}^{−/−} mice had similar respiratory exchange ratios (RER) as littermate controls (Figure S3A), but decreased locomotor activity (Figure 1E) and O\textsubscript{2} consumption (Figure 1F), as well as energy expenditure (Figure S3B). \textit{SirT1} transgenics have similar decreases (Banks et al., 2008), supporting a SirT1 gain-of-function in \textit{Dbc1} knockouts. But unlike \textit{SirT1} transgenics, \textit{Dbc1}^{−/−} had similar food intake to control littermates (Figure S3C).

Metabolic Abnormalities in \textit{Dbc1}^{−/−} Are Exacerbated by High Fat Diet

Overexpression of SirT1 protects mice from obesity-induced insulin resistance, but not obesity (Banks et al., 2008). In contrast, when fed an obesogenic high-fat diet, \textit{Dbc1}^{−/−} mice displayed increased body weight (Figure 2A) and body fat content (Figure 2B), further impairment of glucose tolerance (Figure 2C), and higher insulin levels than wild-type controls (Figure 2D), without changes to glucose levels (not shown). These findings, consistent with impaired insulin sensitivity, were borne out by insulin tolerance tests (Figure 2E). Given the role of SirT1 in regulating hepatic glucose production (Banks et al., 2008), we tested whether glucose intolerance in \textit{Dbc1}^{−/−} was due to hepatic insulin resistance. During hyperinsulinemic-euglycemic clamps, \textit{Dbc1}^{−/−} mice displayed similar rates of glucose infusion (GIR) and disposal (Rd) (not shown), but increased basal hepatic glucose production (Figure 2F). In agreement with these findings, we found that glucose production by primary hepatocytes isolated from \textit{Dbc1}^{−/−} mice was also increased by ~ 50\%, consistent with a cell-autonomous effect (Figure 2G). In summary, \textit{Dbc1}^{−/−} mice are prone to insulin resistance and diet-induced obesity.
Increased Scd1 in Dbc1−/− Mice

Dbc1−/− mice have been reported to have increased browning of white adipose tissue (WAT), a process against the development of obesity (Qiang et al., 2012). Thus, the normal body weight in Dbc1−/− (Figure 1C) could represent a compensatory mechanism from enhanced thermogenesis at ambient temperature. As expected, knockouts gained more body weight than controls at thermo neutrality (32–33°C) (data not shown), further demonstrating a predisposition to obesity in Dbc1−/−. We then hypothesized that their metabolic alterations affect lipid homeostasis. Therefore, we surveyed expression of genes required for lipid synthesis and turnover in livers of Dbc1−/− mice. Strikingly, whereas most regulators of lipid transport and synthesis—including Srebf1, Fasn, Acc2, Dgat2, Pparγ, and Cd36—were expressed at or near wild-type levels, expression of Scd1, the rate-limiting enzyme in fatty acid (FA) desaturation, increased threefold above controls (Figure 3A). Elevation of Scd1 also occurred in livers of HFD-fed Dbc1−/− mice.

In this instance, it was accompanied by smaller increases in other lipogenic genes (Figure 3B). Scd1 protein levels mirrored the mRNA findings (Figures 3C and 3D). To determine whether the increase in Scd1 was secondary to obesity and/or hepatocyte cell-autonomous, we assessed Scd1 activity in primary hepatocytes isolated from young Dbc1−/− mice prior to the onset of obesity and insulin resistance. To this end, we measured the ratio of the Scd1 product, stearoyl-CoA (C18:1), to its substrate oleoyl-CoA (C18:0). We found that the C18:1/C18:0 ratio increased threefold in Dbc1−/− vs. wild-type hepatocytes (Figure 3E), parallel to the levels of Scd1 (Figure 3F). These data indicate that Scd1 activation is a cell-autonomous effect of Dbc1 loss that precedes the onset of obesity in Dbc1 knockouts.

Paradoxical Protection from Atherosclerosis by Deletion of Dbc1

Scd1 deficiency has a paradoxical effect to increase atherosclerosis even though these mice are protected from obesity and insulin resistance (Brown et al., 2008; MacDonald et al., 2009). We then hypothesized that, if the metabolic phenotypes of Dbc1−/− are mediated by Scd1, Dbc1 knockouts should also be protected from atherosclerosis. In Dbc1−/−:Ldlr−/− double knockouts fed a western-type diet (WTD), we confirmed the increase of Scd1 (Figures S4A and S4B) as well as a constellation of atherogenic findings, including increased body weight, hepatosteatosis, dyslipidemia, and insulin resistance (Table S1). However, atherosclerotic lesion size was decreased in Dbc1−/−:Ldlr−/− mice, consistent with a protective function of Scd1 against plaque formation (Erbay et al., 2009), and in line with the anti-atherogenic function of Dbc1 deficiency in ApoE−/− mice (Escande et al., 2015). These data buttress the conclusion that Scd1 activation is mechanistically responsible for the phenotype of Dbc1−/− mice. Indeed, the metabolic sub-phenotypes of Dbc1−/− mice are opposite to those found in Scd1-deficient mice (Table S2).

Dbc1 Deletion Further Impairs Metabolic Control in ob/ob Mice

Scd1 ablation reduces body weight in leptin-deficient ob/ob mice (Cohen et al., 2002). Thus, we asked whether deletion of Dbc1 affects obesity in ob/ob mice. As expected, we saw increased body weight, fat content, decreased lean mass, and impaired glucose tolerance in Dbc1−/−:ob/ob mice (Figures S4C–S4E). The additive effects of the Dbc1 and ob mutations

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indirectly rule out impaired leptin signaling as a primary cause of obesity and insulin resistance in *Dbc1*<sup>−/−</sup> mice.

**Inhibition of Scd1 Restores Metabolic Homeostasis in Dbc1<sup>−/−</sup> Mice**

To test whether the metabolic abnormalities of *Dbc1*<sup>−/−</sup> mice were mediated by Scd1, we treated HFD-fed mice with the Scd1 inhibitor, A939572 (Flowers et al., 2011; Paton and Ntambi, 2010). Treatment with low doses of inhibitor affected weight gain in control mice as expected, but more importantly, normalized body weight gain (Figure 4A) and abnormal body composition in the knockouts (Figure 4B and 4C). Consistent with our findings in primary hepatocytes (Figure 3F), monounsaturated FA content (MUFA, C18:1) increased in livers of *Dbc1*<sup>−/−</sup> mice and were normalized by treatment with the inhibitor (Figure 4D). There was no effect on hepatic lipid content (Figures S5A–S5C). Furthermore, glucose intolerance in *Dbc1*<sup>−/−</sup> mice was also improved by low-dose Scd1 inhibitor treatment (5 vs. 10–100 mg/kg/day) (Figure 4E). These data support the conclusion that the metabolic effects of *Dbc1* ablation require Scd1 gain-of-function.

**Metabolic Abnormalities in Dbc1<sup>−/−</sup> Mice Are SirT1-Dependent**

Next we asked whether the metabolic functions of Dbc1 are mediated through SirT1. To this end, and given the lethality associated with complete SirT1 deletion, we generated 1 (Figures S6A and S6B), *L-SirT1:Dbc1*<sup>−/−</sup> mice showed normal body weight (Figure S6C), fat content (Figure 5B), and glucose tolerance (Figure 5C). In fact, obesity and glucose intolerance in young *Dbc1*<sup>−/−</sup> mice were completely reversed by hepatic *SirT1* deletion (Figures S6D and S6E). When fed HFD, *L-SirT1:Dbc1*<sup>−/−</sup> mice gained less weight than controls (Figure 5E), and showed no increase in body fat (Figure 5F). More importantly, *SirT1* ablation prevented the increase of Scd1 in livers of *L-SirT1:Dbc1*<sup>−/−</sup> mice (Figure 5D). Taken together, the metabolic abnormalities in *Dbc1*<sup>−/−</sup> mice were offset by blocking SirT1-dependent up-regulation of Scd1 in the liver.

**Scd1 Is Regulated by Dbc1 and SirT1**

To understand the biochemical basis of the interaction among SirT1, Dbc1, and Scd1, we examined whether SirT1 and Dbc1 regulate activity of a minimal Scd1 promoter encompassing −1537/+155 (Chu et al., 2006). Wild-type SirT1, but not its catalytically inactive mutant (H363Y), stimulated Scd1 promoter activity, while Dbc1 repressed it (Figure 6A). Chemical inhibition of SirT1 repressed Scd1 promoter activity, without affecting the related *Fasn* promoter (Figure 6B). Deletion mapping localized the Dbc1/SirT1 interaction site between bp −981 and −589 (Figure 6C). Chromatin immunoprecipitation assays further confirmed binding of Dbc1 to Scd1 promoter between bp-807 to −428 (Figure 6D). Moreover, transient knockdown of *DBC1* increased SCD1 levels in human non-small cell lung cancer cells (Figure 6E), indicating that Dbc1 is a conserved negative regulator of Scd1 in normal and carcinoma cells. Collectively, these data suggest that the deacetylase activity of SirT1 increases Scd1 expression, and that Dbc1 inhibits Scd1 by blocking SirT1.
**Impaired p53 Activation in Dbc1−/− Mice**

Next, we investigated the tumor-promoting properties of Dbc1. As predicted, Dbc1 ablation reduced p53 acetylation and activation of its target genes p21 and puma in embryonic fibroblasts (MEFs), following doxorubicin-induced DNA damage (Figures S7A–S7B). We then asked whether Dbc1 is a de facto tumor suppressor. Ionizing radiation induces p53, resulting in apoptosis, as indicated by the presence of cleaved Caspase-3 in thymus and spleen of irradiated wild-type animals (Figures 7A and 7B). Consistent with the findings in MEFs, Dbc1−/− mice showed a blunted p53 response and reduced Caspase-3 cleavage (Figure 7B). Based on the reduced p53 activation by DNA damage, we expected increased tumorigenesis in Dbc1−/− mice. But to our surprise, spontaneous tumors arose in aging Dbc1−/− mice at the same rate as in wild-type controls (not shown). Thus, Dbc1 deletion is not oncogenic per se, but is expected to accelerate cancer progression owing to impaired p53 activation.

**Scd1 Inhibition Prevents Cancer Progression in TP53−/− Mice**

Similar metabolic abnormalities to those observed in Dbc1 knockouts are associated with increased risk of cancer (Gallagher and LeRoith, 2013). Mice deficient in p53 (TP53−/−) develop spontaneous tumors and die by 6 months of age (Jacks et al., 1994). Interestingly, lack of p53 in primary hepatocytes increased levels of Scd1 and MUFAs (Figures 7C and 7D). Therefore, we investigated whether the metabolic alterations in Dbc1−/− mice affected cancer susceptibility in an Scd1-dependent manner. Although SCD1 inhibitor has been shown to induce apoptosis in cultured cancer cells (Hess et al., 2010), and decrease tumor growth in a human gastric xenograft model at high doses (100mg/kg/BID) (Roongta et al., 2011), its anti-tumor function has never been tested in vivo. As a critical test of our hypothesis, we fed TP53−/− mice HFD and treated them with the Scd1 inhibitor at physiological dose (5mg/kg/day). Inhibitor-treated mice mimicked aspects of Scd1-deficient mice, including fur loss, eyelid closure, and body weight reduction (Miyazaki et al., 2001; Ntambi et al., 2002). Importantly, we observed a substantial reduction of tumor-related death and a 28% increase of median lifespan among inhibitor-treated mice, compared to untreated controls (Figure 7E).

**DISCUSSION**

Here we report SirT1-dependent elevation of Scd1 accounts for obesity and insulin resistance in Dbc1−/− mice under multiple genetic and dietary manipulations. Loss of Dbc1 enhanced SirT1 activity, consistent with previous studies in Dbc1 knockout mice (Escande et al., 2010; Escande et al., 2015).

It is surprising that the gain-of–function of SirT1 associated with Dbc1 ablation caused obesity and insulin resistance, unlike the gain-of–function caused by SirT1 overexpression in transgenic mice (Banks et al., 2008; Pfluger et al., 2008). In the latter models, SirT1 is overexpressed at levels about twofold higher than endogenous, and is still under control by Dbc1, whereas in Dbc1−/− mice, SirT1 is constitutively active. This provides a potential explanation for the fact that Scd1 is increased in Dbc1−/−, but not in SirT1 transgenic mice (Qiang et al., 2011). In support of this notion, increased Scd1 expression was observed in β
cells with 12- to 18-fold overexpression of Sirt1 (Moynihan et al., 2005; Ramsey et al., 2008). The increase of Scd1 by unchecked SirT1 activation is likely responsible for the unexpected obesity and insulin resistance of Dbc1−/− mice.

Several transcriptional factors, including SREBP-1c, LXRα and PPARs, activate Scd1 (Chu et al., 2006; Paton and Ntambi, 2009). However, to our knowledge no Scd1 repressors were known thus far. Our mapping data have identified a region in the promoter through which Dbc1 represses Scd1. The consensus binding sites for C/EBP, NF-Y/NF-1, SRE, Sp1 in the mouse Scd1 promoter are conserved in the human SCD1 promoter (Bene et al., 2001). Notably, we also saw regulation of SCD1 by DBC1 knockdown in human cancer cells (Figure 6E and data not shown). We don’t know whether the repression is direct or indirect, but it’s unlikely to be mediated by Srebp-1c, as it’s not associated with increases of other Srebp-1c targets, such as Fasn. Moreover, expression patterns of Scd1 and Srebp-1c are distinct in Dbc1−/− mice. Finally, Scd1 repression by Dbc1 requires SirT1 deacetylase activity, suggesting that it involves deacetylation of transcriptional regulators.

Ablation of Dbc1 is not oncogenic per se, but likely favors tumor progression by impairing p53 activation through unchecked SirT1 activity. The function of SirT1 in cancer is context-dependent (Deng, 2009). SirT1 haploinsufficiency promotes spontaneous tumor development in TP53+/− mice, while overexpression of SirT1 in lymphocyte progenitors reduces the incidence of thymic lymphomas in TP53+/− mice post γ-irradiation, consistent with a tumor-suppressor function of SirT1. On the other hand, SirT1 is elevated in human leukemia, as well as prostate, colon, skin, and some hepatocellular cancers. Moreover, SirT1 levels correlate with malignancy of hepatocellular carcinoma. As SirT1 also impairs p53 acetylation and regulation of cell cycle, apoptosis and senescence (Li et al., 2012), a possible explanation of this apparent discrepancy is that SirT1 inhibits tumor initiation in normal cells, but facilitates progression in established tumors (Chang and Guarente, 2014; Chen et al., 2012). This view is consistent with the present observation that the tumor-promoting function of Dbc1 ablation is associated with SirT1 activation, as well as with evidence of impaired regulation of SirT1 by Dbc1 in certain cancers (Hiraike et al., 2011; Kim et al., 2009; Sung et al., 2010).

Our findings suggest a mechanism whereby tumor progression genes can leverage a metabolic control step to promote cancer growth. Scd1 is the rate-limiting enzyme in generating mono-unsaturated FAs (MUFA). In addition to its critical role in lipid storage and obesity, unsaturated FA synthesis is required for membrane biogenesis, fluidity, and permeability, and hence by rapidly growing tumor cells. MUFAbs are also involved in signal transduction to support cancer cell growth (Atilla-Gokcumen et al., 2014; Igal, 2011). Elevated Scd1 levels have been found in various human cancers with increased lipid desaturation (Fritz et al., 2010; Ide et al., 2013; Roongta et al., 2011; von Roemeling et al., 2013). These types of cancer are also commonly associated with obesity (Calle et al., 2003; Wang et al., 2011). Treatment with Scd1 inhibitor decreases proliferation and induces apoptosis in cancer cells (Hess et al., 2010; Roongta et al., 2011). Of particular interest is that theses anti-proliferative and pro-apoptotic effects of Scd1 inhibition are selective to tumor cells, but not to normal cells (von Roemeling et al., 2013). Therefore, the coordinated
regulation of Scd1 by Dbc1 and SirT1 provides a heretofore-unknown mechanism for the predisposition to cancer associated with obesity.

The metabolic abnormalities in our Dbc1−/− mice are largely consistent with those in knockouts generated by genetrap methodology (Escande et al., 2010; Escande et al., 2015; Nin et al., 2014). In the latter model, female mice developed a “fit obese” phenotype, i.e., obesity with insulin sensitivity, and reduced adipocyte lipolysis (Escande et al., 2015). Neither in two previous reports (Escande et al., 2015; Nin et al., 2014), nor in our study are males protected from diet-induced insulin resistance, raising the possibility of sex-dimorphic actions of Dbc1. We did not observe protection from diet-induced steatosis on chow/HFD feeding (not shown) or through Scd1 inhibition (Figure S5), possibly due to different knockout strategies or experimental conditions. However, both Dbc1 knockout models showed protection from atherosclerosis (Escande et al., 2015), in line with the anti-atherogenic functions of Scd1 (Erbay et al., 2009). The implication of the atherosclerosis studies is that targeting Dbc1/Scd1/MUFA may lessen the burden of atherosclerosis in obesity and type 2 diabetes.

In sum, we have uncovered a shared obesogenic and tumor-promoting mechanism resulting from gain-of-function of the fatty acid desaturase, Scd1, through derepression of SirT1. Our study, along with our previous report of pro-atherogenic effects of SirT1 (Qiang et al., 2011), adds to a body of work that invites caution in exploring the therapeutic applications of SirT1 activators (Alcain and Villalba, 2009; Chang and Guarente, 2014; Chen et al., 2008; Li et al., 2007b; Ng and Tang, 2013). In addition, our study contributes to mapping pathways of disease progression connecting obesity, insulin resistance, and cancer thus may pave the way to treating metabolic syndrome-related cancers.

**EXPERIMENTAL PROCEDURES**

**Generation of Dbc1 Knockout Mice**

To construct Dbc1 gene targeting vector, a fragment of Dbc1 genomic DNA, containing 5.5 kb DNA 5’ of exon 1 and 1.8 kb 3’ of exon 1, was cloned by recombineering. Next, a promoter-less EGFP was inserted in exon 1 in frame next to the translation start codon (ATG) along with a neomycin resistance gene cassette allowing selection by G418. The targeted allele permits expression of EGFP controlled by endogenous Dbc1 promoter (Figure S1A). The clones containing EGFP-neo cassette insertion through homologous recombination were screened by Southern blotting using EcoRV digested ES cell genomic DNA and an external 3’ probe, amplified using forward primer 5’-GTT GAT GAT ACA TTT TGA TCT CA-3’ and reverse primer 5’-AAG GTA GGA GCA GCA GAA ACC TG-3’ (Figure S1B). Targeted ES cell clones were injected into C57BL/6j blastocysts to derive chimeras, from which the targeted allele was transmitted successfully.

**Animal Studies**

Dbc1−/− mice were on 129/J x C57BL/6J background and TP53−/− mice on C57BL/6J background. Lepob/+ mice were from Jackson Laboratories (Bar Harbor, ME). Ldlr−/−, α1-antitrypsin-cre transgenic (AT-Cre), and Sirt1fllox/fllox mice (Li et al., 2007a) have been
described. Mice were housed at ambient temperature in a 12-hr light/dark cycle and fed ad libitum normal chow (PicoLab rodent diet 20) (LabDiet 5053), 60 kcal% high fat diet (Research Diet, D12492), or Western-type diet (TD88137, Harlan Teklad). Metabolic characterization was performed as described (Banks et al., 2008; Qiang et al., 2011; Qiang et al., 2012). All experiments were performed in male mice unless indicated. The Columbia University Animal Care and Utilization Committee approved all procedures.

**Scd1 Inhibitor Treatment**

For acute Scd1 inhibitor treatment, we injected A939572 (Biofine, Vancouver, Canada) intraperitoneally (i.p.) daily at a dose of 5 mg/kg starting on the same day as HFD. For chronic treatment in TP53−/− mice, the inhibitor was mixed with 60 kcal% HFD at a dose of 100mg/kg. Scd1 activities were determined by measuring saturated and unsaturated CoA concentrations at the Biomarkers Core Lab of the Irving Institute for Clinical and Translational at Columbia University.

**Cell Culture and Scd1 Luciferase Reporter Assay**

We transiently transfected HEK-293T cells (12-well) with 0.2µg Luciferase reporter plasmids and 0.02µg Renilla control plasmid, together with 0.2µg indicated SirT1 or Dbc1 plasmids using TransIT-LT1 reagent (Mirus Bio). We then performed Dual-luciferase reporter assay by following the manufacturer’s instructions (Promega, Madison, WI). Mouse Scd1 promoter luciferase reporters (−1537/+155, −981/+155, −589/+89) are generously provided by Dr. James Ntambi (Chu et al., 2006). The Fasn luciferase reporter was used as a control. Glucose production in primary hepatocytes was performed as described (Pajvani et al., 2013).

**Protein Analysis**

Proteins were extracted into protein lysis buffer (50mM Tris, pH 7.4, 150mM NaCl, 10% glycerol, 2% NP-40, 1mM EDTA pH 8.0, 0.1% SDS, 0.5% NaDOC) supplemented with protease and phosphatase inhibitors. Antibodies used were: Dbc1 (Bethyl Laboratories, note: nonspecific signal at 135kD for freshly made working solution), p53 (CM5, Leica Microsystems), Puma (Sigma-Aldrich), SirT1 (Millipore), p21 and tubulin (Santa Cruz). Scd1, Fas, cleaved Caspase 3, Ac-p53, p-Akt (S473), p-Akt (T308), Akt and actin antibodies were purchased from Cell Signaling. HRP-conjugated secondary antibodies (GE Healthcare) were used for detecting ECL western blot signals.

**Gene Expression Analysis**

Total RNA was isolated with RNeasy Lipid Tissue kit (Qiagen) and 1µg RNA was subjected to cDNA synthesis by using High-capacity cDNA Reverse Transcription kit (Applied Biosystems). We performed Q-PCR with goTaq qPCR Master Mix (Promega) on CFX96 Real-Time PCR system (Bio-Rad). The relative gene expression levels were calculated by ΔΔCt method with cyclophilin A as the reference gene. Q-PCR primers were as listed (Qiang et al., 2011; Qiang et al., 2012).
ChIP Assay

We performed ChIP analysis of Dbc1 on Scd1 promoter by using ChIP assay kit following manufacturer’s instructions (EMD Millipore). Anti-Dbc1 (Bethyl Laboratories) was used.

Histology

We used paraffin-embedded sections for Dbc1, p53 and Caspase-3 immunohistochemistry (all at 1:100 dilution) and frozen liver sections for Oil red-O staining.

Statistical Analysis

We performed unpaired 2-tail student’s t-tests for single measurements and two-way ANOVA with Tukey post-hoc analysis where appropriate by using JMP software (SAS, NC) to evaluate statistical significance. \( P < 0.05 \) was considered as a significant change. All values are presented as means ± standard deviations (SD).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Dbc1 ablation causes obesity and insulin resistance
- Dbc1 ablation increases Scd1 levels
- Metabolic abnormalities of \( Dbc1^{-/-} \) are mediated by Scd1 and hepatic SirT1
- Inhibition of Scd1 decreases tumor-related death
Figure 1. Dbc1−/− mice develop insulin resistance and obesity
(A) Areas under the curve (AUC) of glucose levels following intraperitoneal glucose tolerance tests (IPGTT) in chow-fed male mice of different ages (n=6 each).
(B) Plasma insulin levels of ad libitum-fed male mice of the indicated ages (n=9, 12).
(C) Body weight in both males and females (n=12, 9, 10, 10 each).
(D) Body composition of 8-week-old, chow-fed male mice (n=11, 8).
(E–F) Total activity counts (E) and oxygen consumption (F) in chow-fed 6-month-old male mice (n=6 each). Data represent means ± SD, * P<0.05, ** P<0.01. See also Figures S1–S3.
Figure 2. Metabolic abnormalities of Dbc1<sup>−/−</sup> are exacerbated by DIO
(A) Body weight of mice of both genders during high-fat diet (HFD). *P<0.05 between male wild-type and knockout mice (n=9, 9, 6, 9 respectively).
(B) Body composition following HFD for 8 weeks (n=9 each).
(C) IPGTT following HFD for 13 weeks (n=9 each).
(D) Plasma insulin levels in mice fed HFD for 24 weeks (n=7 each).
(E) ITT following 9 weeks of HFD (n=8, 10). The metabolic characterization in B–E was conducted in male mice.
(F) Basal endogenous glucose production (EGP) during hyperinsulinemic-euglycemic clamps in chow-fed male mice (n=6, 4).
(G) Glucose production in primary hepatocytes (n=3 each). Data represent means ± SD, * P<0.05, ** P<0.01.
Figure 3. Increased Hepatic Scd1 in Dbc1\(^{-/-}\) mice
(A) Q-PCR analysis of hepatic gene expression from chow-fed male mice after 4-hr re-feeding following 16-hr fasting (n=6 each).
(B) Q-PCR analysis of hepatic gene expression from male mice fed HFD for 9 weeks and fasted overnight prior to sacrifice (n=6, 7).
(C) Western blot analysis of liver protein extracts from chow-fed mice in (A).
(D) Western blot analysis of liver protein extracts from HFD-fed mice in (B).
(E–F) Western blots analysis of protein extracts (E) and Scd1 activity (F) measured by CoA levels (n=4 each) in primary hepatocytes isolated from 6-week-old mice. Data represent means ± SD, * P <0.05, ** P<0.01. See also Figure S4, Tables S1 and S2.
Figure 4. Inhibition of Scd1 reverses metabolic abnormalities in Dbc1−/− mice

(A) 12- to 16-week-old male mice were injected daily with Scd1 inhibitor after starting HFD-feeding. Body weight change after 20 days of treatment. P<0.0001 by two-way ANOVA.

(B) Lean mass after 18 days of treatment. P=0.001 by two-way ANOVA.

(C) Fat mass after 18 days of treatment. P<0.0001 by two-way ANOVA.

(D) Hepatic Scd1 activity plotted as MUFA to saturated FA ratios at four weeks of Scd1 inhibitor treatment. P=0.017 for C16:1/C16 and P=0.004 for C18:1/C18 by two-way ANOVA.

(E–F) IPGTT (E) and areas under curve (AUC) (F) after 3 weeks of inhibitor treatment. P=0.001 for AUC by two-way ANOVA. Statistically significant differences between two groups were calculated by post-hoc Tukey HSD test. Levels not connected by the same letter
(a, b, c) are significantly different. Data represent means ± SD, n=6 each group. See also Figure S5.
Figure 5. Metabolic abnormalities of Dbc1−/− are mediated by hepatic SirT1

(A) Validation of liver-specific SirT1 knockout (L-SirT1) by western blotting of liver protein extracts.

(B–C) Body composition (B) and IPGTT (C) in 19-week-old L-SirT1 mice and double knockouts on chow diet (n=6 each).

(D) QPCR analysis of hepatic gene expression in chow-fed, overnight-fasted 21-week-old mice (n=6 each).

(E) Body weight of L-SirT1 mice on HFD for 5 weeks or 8 weeks.

(F) Body composition after 8-week HFD (n=7, 6). All experiments were performed in male mice. Data represent means ± SD. * P<0.05. See also Figure S6.
Figure 6. Dbc1 and SirT1 regulate Scd1

(A) Scd1 luciferase reporter assay in 293 cells transfected with control plasmid (RFP), plasmids encoding SirT1, deacetylase-defective SirT1 mutant H363Y (HY) or Dbc1 (n=4 each).

(B) Full-length Scd1 luciferase reporter assay in 293 cells treated with the SirT1 inhibitor 10mM nicotinamide (Nam) or vehicle (Veh) for overnight. Fasn reporter was used as control (n=4 each).

(C) Truncated Scd1 promoter luciferase assay in 293 cells transfected with control plasmid (RFP), or plasmids encoding SirT1 or Dbc1. Fasn reporter was used as a control (n=4 each).

(D) Chromatin immunoprecipitation of Dbc1 on Scd1 promoter in MEFs.
(E) Western blots following transient DBC1 knockdown in human lung cancer H1299 cells. Data represent means ± SD, *p<0.05, **p<0.01. Luciferase reporter assays were repeated at least three times.
Figure 7. *Dbc1* ablation facilitates tumor progression through increased Scd1

(A) Prostate immunohistochemistry (IHC) in *Dbc1*−/− mice subjected to the indicated doses of γ-radiation.

(B) Western blots of spleen protein extracts from mice in (A).

(C) Western blot analysis of proteins in primary hepatocytes isolated from *TP53*−/− mice. Cells were treated overnight with 1.67µM insulin.

(D) MUFA content in primary hepatocytes isolated from *TP53*−/− mice. Data represent means ± SD, ** P<0.01 (n=4).
(E) Kaplan-Meier survival plots of $TP53^{-/-}$ male and female mice following treatment with Scd1 inhibitor. Statistical significance was estimated by Log-rank (Mantel-Cox) test (n=23 each). See also Figure S7.