Hypo- and hyperactivated Notch signaling induce a glycolytic switch through distinct mechanisms

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A switch from oxidative phosphorylation to glycolysis is frequently observed in cancer cells and is linked to tumor growth and invasion, but the underpinning molecular mechanisms controlling the switch are poorly understood. In this report we show that Notch signaling is a key regulator of cellular metabolism. Both hyper- and hypoactivated Notch induce a glycolytic phenotype in breast tumor cells, although by distinct mechanisms: hyperactivated Notch signaling leads to increased glycolysis through activation of the phosphatidylinositol 3-kinase/AKT serine/threonine kinase pathway, whereas hypoactivated Notch signaling attenuates mitochondrial activity and induces glycolysis in a p53-dependent manner. Despite the fact that cells with both hyper- and hypoactivated Notch signaling showed enhanced glycolysis, only cells with hyperactivated Notch promoted aggressive tumor growth in a xenograft mouse model. This phenomenon may be explained by that only Notch-hyperactivated, but not -hypoactivated, cells retained the capacity to switch back to oxidative phosphorylation. In conclusion, our data reveal a role for Notch in cellular energy homeostasis, and show that Notch signaling is required for metabolic flexibility.

Cancer cells frequently rely on glycolysis rather than oxidative phosphorylation (OXPHOS) for energy generation. This phenomenon was first observed by Otto Warburg, who more than 80 y ago found that cancer cells, despite ample access to oxygen, prefer to metabolize glucose by aerobic glycolysis (1). The reason to opt for aerobic glycolysis is not fully understood, but it has been proposed that intermediates of the glycolytic pathway are important for biosynthesis required for rapid growth or to prime cancer cells for survival in hypoxic areas. Down-regulation of OXPHOS may also induce resistance to apoptosis by compromising intrinsic apoptotic programs. Recent data indicate that metabolic reprogramming promotes unrestricted growth and constitutes an essential component of the invasive phenotype (2–5).

The molecular mechanisms underlying the metabolic reprogramming are complex and only partially understood. Activation of oncogenic signals and the loss of tumor suppressors are critical modulators of tumor cell metabolism. Notably, activation of the phosphatidylinositol 3-kinase (PI3K)/AKT serine/threonine kinase pathway (6), Ras (7, 8), Myc (9), loss of the tumor suppressor p53 (10, 11), and activation of the cellular hypoxic response (12, 13) are linked to enhanced glycolysis. There is an emerging view that the glycolytic phenotype is not caused by permanent mitochondrial damage but that mitochondrial activity in many instances is retained (14), and that metabolic flexibility rather than a permanent switch to glycolysis is important for tumor progression. Cancer cells appear to have a substantial reserve capacity for OXPHOS (15). Recent data in fact suggest an important role for functional mitochondria in oncogenic transformation and tumor growth (16, 17).

In this report we have explored the role of Notch in metabolic control of tumor cells. The Notch pathway is important for differentiation in most cell types (18) and frequently deregulated in cancer (19, 20). Activating mutations in the Notch1 receptor are found in the majority of patients with acute lymphoblastic T-cell leukemia (T-ALL) (21), and deregulated Notch signaling is observed in solid tumors such as breast cancer (22–26). Notch signaling also cross-talks with the cellular hypoxic response, which is an important glycolysis driver (27, 28). We show that both activation and inhibition of Notch enhance glycolysis, although by different mechanisms. Activation of Notch resulted in activation of PI3K/AKT signaling, whereas inhibition of Notch reduced the activity of the mitochondrial respiratory chain and decreased p53 protein levels, accompanied by enhanced glycolysis. Notch inhibition rendered cells dependent on glucose and blocked growth under restricted conditions, whereas hyperactivated Notch signaling showed uncontrolled invasive tumor growth. The data indicate that Notch is important for maintenance of metabolic flexibility and that the glycolytic phenotype does not automatically enhance the tumorigenic potential.

Results

Hyperactive, but Not Hypoactive, Notch Signaling Promotes Tumor Growth and Invasiveness in Vivo. Notch signaling is activated by ligands on juxtaposed cells, liberating the Notch intracellular domain (NICD), which translocates to the nucleus where it interacts with the DNA-binding protein CSL (for CBF-1/Suppressor of Hairless/Lag-1) to regulate expression of downstream genes (18, 29). To explore the role of Notch signaling in breast tumor growth and cellular metabolism, we engineered MCF7 luminal-type breast cancer cells to express high, normal, and reduced Notch activity by stable expression of constructs NICD1-GFP, GFP, and dominant-negative CSL–GFP, respectively (Fig. 1 *A*–*C*). The cells are hereafter referred to as N^{high}, N^{medium}, and N^{low} cells, respectively.

Upon orthotopical xenotransplantation, tumors developed from all three cell lines, and the N^{high} cell-derived tumors showed dramatically enhanced tumor size (Fig. 1*D*) coupled with increased proliferation compared with N^{medium} cells (Fig. 1*E*). Furthermore, the N^{high} tumors exhibited an aggressive basal-like phenotype with a spindle-shaped cellular morphology, invasive growth pattern (including local invasion into blood vessels), and a blurred tumor-stroma boundary (Fig. 1 *F* and *G*).

In contrast, despite initial growth, N^{low} tumors later regressed, and at 8 wk only scar tissue was visible (Fig. 1 *E* and *F*). In sum, these data show that elevated Notch signaling leads to enhanced tumor growth and a more aggressive tumor phenotype in vivo, whereas cancer progression was retarded in hypoactive Notch tumors.

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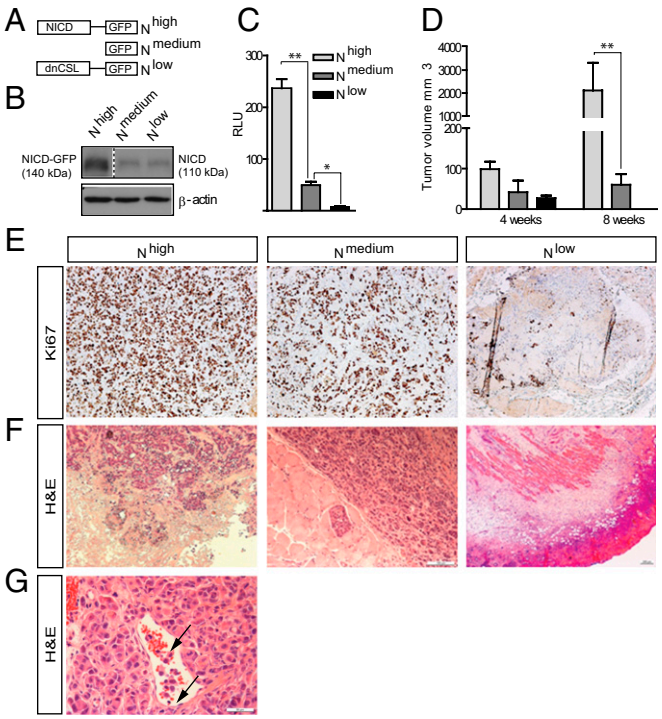

Fig. 1. Notch induces enhanced tumor growth and invasiveness. (*A*) Schematic depiction of the N^{high}, N^{medium}, and N^{low} cells. (*B*) NICD levels in N^{high}, N^{medium}, and N^{low} cells. NICD in N^{high} cells is expressed as a GFP fusion form. (*C*) Notch signaling activity from the 12xCSL-luc reporter in N^{high}, N^{medium}, and N^{low} cells. (*D*) Tumor volume measured at 4 and 8 wk after xenografting. (*E*) Expression of the proliferation marker Ki67 in N^{high}, N^{medium}, and N^{low} tumors at 8 wk. N^{low} shows mainly connective tissue. (*F*) Histological analyses of N^{high}, N^{medium}, and N^{low} tumors showing invasive growth pattern of N^{high} tumors and the remaining scar tissue of N^{low} tumors. (*G*) Infiltration of N^{high} tumor cells (arrows) into surrounding vessels. (Scale bar: *E*–*G*, 100 μm.) Values are significant at $^{**}P < 0.01$ and $^{*}P < 0.05$ as indicated in *C* and *D*. Values indicate the average of at least three independent experiments.

Both Hyper- and Hypoactive Notch Signaling Induce a Glycolytic Switch in Vivo and in Vitro. Analysis by ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET revealed that tumors from N^{high} cells showed increased glucose uptake, compared with N^{medium} tumors (Fig. 2 *A* and *B*). Unexpectedly, we also observed higher uptake in the N^{low} tumors (Fig. 2 *A* and *B*).

We next analyzed the metabolic state in the different cell lines in vitro. In the N^{high} cell line, glucose consumption and lactate production were increased (Fig. 2 *C* and *D*), indicating that the glycolytic phenotype occurred independently of the in vivo context. Transient activation of Notch in naïve MCF7 by immobilized Notch ligands Jagged1 (Jagged-FC) yielded a similar increase in glucose consumption (Fig. 2*E*). Further, blocking Notch in breast cancer MDA-MB-231 cells with high endogenous Notch activity (Fig. S1) affected metabolism. MDA-MB-231 cells showed high glucose uptake and lactate production (Fig. S1), which was reduced by inhibition of Notch signaling (Fig. 2 *F* and *G*). In keeping with these data, tumor samples from patients with aggressive basal carcinoma showed increased expression of Notch1 ICD and glucose transporter 1 (GLUT1), a marker of glycolytic cancers, compared with a less aggressive luminal type of cancer. This finding further links Notch to glycolytic aggressive cancers and GLUT1 expression (Fig. 2*H*), and extends a recent report on Notch and glycolysis (30).

The N^{low} cells also showed elevated glucose consumption and increased lactate production (Fig. 2 *C* and *D*), indicating that sustained Notch inhibition forces the cells into a glycolytic phenotype. Both N^{high} and N^{low} cells were shown to use more glucose and produce more lactate to equivalent ATP content compared with N^{medium} cells (Fig. 2*I*). Together, these data show that both hyper- and hypoactive Notch signaling boost glycolysis in a cell-intrinsic manner.

Hyperactive Notch Signaling Induces the Glycolytic Phenotype Through PI3K/AKT Signaling. We next assessed whether Notch affected cellular metabolism via regulation of two signaling mechanisms known to induce glycolysis: the cellular hypoxic response and PI3K/AKT signaling. Notch signaling has been shown to cross-talk with the cellular hypoxic response in both stem cell differentiation and epithelial-to-mesenchymal transition (27, 28, 31), but up-regulation of the cellular hypoxic response in N^{high} cells was observed only during hypoxia, and was not significantly higher than in N^{medium} cells (Fig. S2).

In contrast, N^{high} cells showed increased phosphorylation of AKT on serine 473 (Fig. 3*A*), accompanied by increased levels of PI3K class III and p110β (Fig. S3). Conversely, blocking Notch by *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine *t*-butyl ester (DAPT) reduced the amount of phosphorylated AKT in naïve MCF7 cells (Fig. 3*B*). An increase in S473-phosphorylated AKT levels was observed also after transient transfection of naïve MCF7 cells with Notch1 ICD or Notch1ΔE, a membrane-tethered form of Notch that generates Notch1 ICD after γ-secretase cleavage (Fig. 3*C*). PI3K/AKT signaling affects expression of several glycolytic genes, as well as the activity and localization of several rate-limiting glycolytic enzymes. Hexokinase 2 (HK2) was up-regulated on both protein and mRNA levels (Fig. 3*D* and Fig. S4), whereas GLUT1, aldolase A (ALDOA), and pyruvate dehydrogenase kinase 2 (PDK2) were up-regulated at the mRNA level (Fig. 3*D*). Furthermore, GLUT1 showed predominant membrane localization in N^{high} and N^{low} cells, compared with N^{medium} cells (Fig. S4). In contrast, in the N^{low} cells, only PDK2 was up-regulated (Fig. 3*D*).

We next tested whether PI3K/AKT signaling was required for the Notch-induced increase in AKT phosphorylation and glucose consumption. Phosphorylation of AKT by Notch required PI3K activity, as the PI3K inhibitor LY294002 significantly reduced the Notch-mediated increase in S473-phosphorylated AKT (Fig. 3*C*). Although the expression of GLUT1, HK2, and ALDOA was insensitive to PI3K/AKT inhibition, blocking of PI3K/AKT by LY294002 abrogated the Notch-induced increase in glucose consumption (Fig. 3*E*). Furthermore, AKT inhibition reduced proliferation and 3D growth, but did not affect the invasive capacity of N^{high} cells (Fig. S3). In sum, these data suggest that hyperactivated Notch signaling operates via the PI3K/AKT signaling pathway to induce glycolysis and growth.

Hypoactive Notch Signaling Induces Glycolysis by Altering Mitochondrial Function. In N^{low} cells, the PI3K/AKT signaling pathway was unaffected. In contrast, we observed that oxygen consumption was reduced in N^{low} cells (Fig. 4*A*), which prompted us to assess mitochondrial function, as deregulated mitochondrial activity may lead to a glycolytic switch (14). OXPHOS is carried out by the respiratory complexes I–V in the inner mitochondrial membrane, where complexes I, III, and IV generate the proton gradient across the inner membrane, which is used to drive the ATP synthase activity of complex V for the generation of ATP (32, 33). Treatment of the cells with oligomycin, which inhibits the ATP synthase, resulted in a substantial decrease in oxygen consumption for N^{high} and N^{medium} cells, but a considerably smaller decrease in N^{low} cells (Fig. 4*A*). ATP levels were considerably reduced in N^{low} cells and in naïve MCF7 cells treated with DAPT for 3 d (Fig. 4*B*). To corroborate the data, we analyzed an MCF7 cell line stably expressing dominant-negative Mastermind-like (dnMAML) and demonstrated that oxygen consumption and ATP production were reduced in these cells (Fig. S5). Interestingly, there was no significant reduction in oxygen consumption in N^{high} cells, indicating that OXPHOS was operative in these cells. When we measured glycolytic dependency, i.e., the relative contribution from glycolysis to the mitochondrial ATP budget, the N^{low} cells showed the highest dependency (Fig. 4*C*). In sum, these data indicate that N^{high} cells are not dependent on glycolysis, and have the capacity

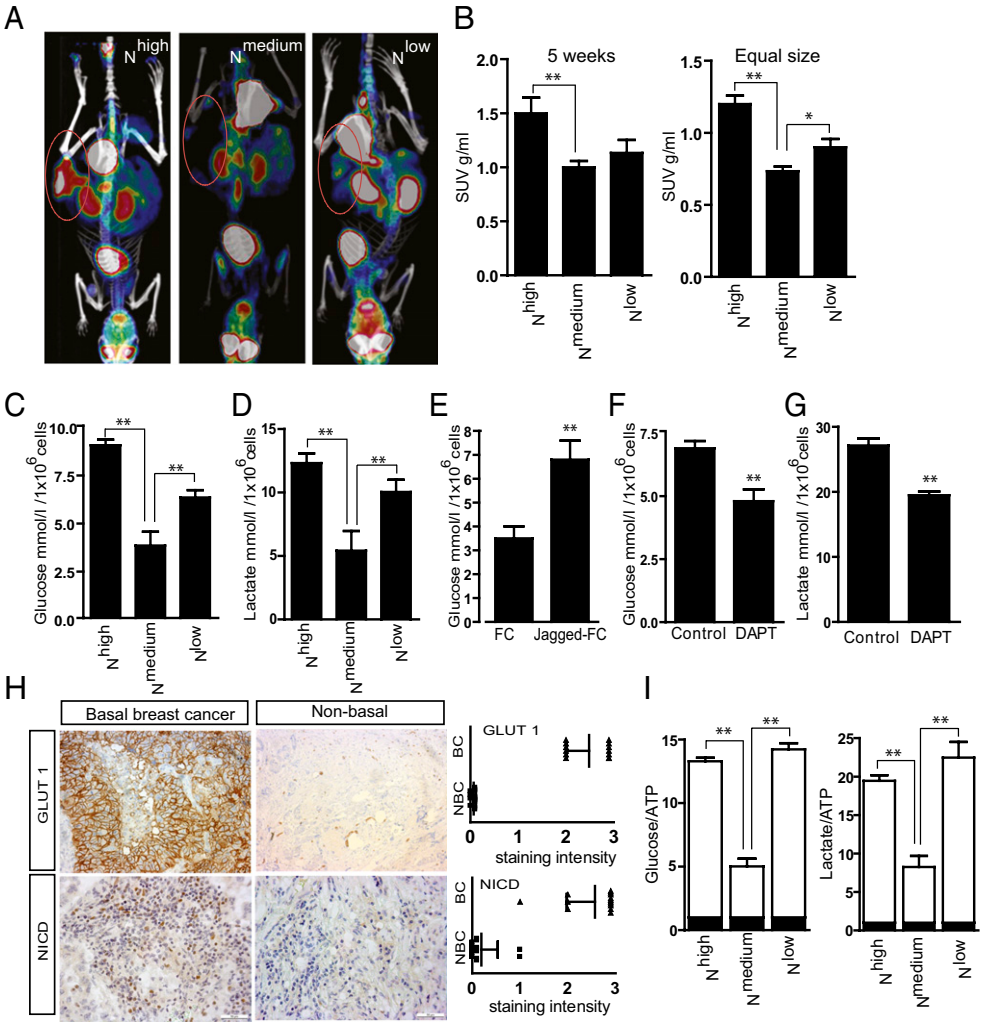

Fig. 2. Tumors with hyper- and hypoactive Notch signaling show enhanced glucose uptake and lactate production. (*A*) Representative ^{18}F-FDG PET images of mice 5 wk after grafting (tumor areas circled). (*B*) Standardized glucose uptake value (SUV) in N^{high}, N^{medium}, and N^{low} tumors 5 wk after transplantation (*Left*) or in tumors of equal size (*Right*). The graph summarizes three different experiments with four animals in each group. (*C* and *D*) Glucose consumption (*C*) and lactate production (*D*) in N^{high}, N^{medium}, and N^{low} cells. (*E*) Glucose consumption in naïve MFC7 cells with endogenous levels of Notch signaling (FC) and after Jagged ligand-induced activation (Jagged-FC). *C–E* represent the average of a minimum of three experiments with three technical replications within each experiment. (*F* and *G*) Glucose uptake (*F*) and lactate production (*G*) in glycolytically active MDA-MB-231 breast cancer cells treated with DAPT. (*H Left*) Representative images of GLUT1 and NICD expression in breast cancer tissue from patients with basal and nonbasal breast cancer. (*Right*) Scoring of staining intensities (n = 14). BC, basal cancer; NBC, nonbasal cancer. (*I*) Relative uptake of glucose (*Left*) or lactate (*Right*) production per unit of ATP content. Values are significant at $^{**}P < 0.01$ and $^{*}P < 0.05$ as indicated in *B–I*. Values indicate the average of at least three independent experiments.

to revert back to OXPHOS when needed, whereas N^{low} cells have an impaired mitochondrial function and to a larger extent depend on glycolysis for energy production.

Hypoactive Notch Signaling Attenuates the Activity of Respiratory Complexes I, IV, and V. We next analyzed the activity of isolated mitochondrial complexes. The activity of complex I was reduced in N^{low} cells (Fig. 5*A*), and the cells showed decreased sensitivity (i.e., reduced cell death) in response to the complex 1 inhibitor rotenone (Fig. 5*B*). The activity (Fig. 5*C*) and amount (Fig. 5*D*) of complex IV was also reduced in N^{low} cells. Furthermore, we observed a decrease in the protein level of COXII (Fig. 5*E*), a subunit of complex IV previously shown to be linked to diminished activity of electron transfer chain complex IV in p53-deficient cancer cells (34).

To assess complex V function, we analyzed whether Notch signaling status affected the mitochondrial membrane potential ($\Delta\psi m$), which is built up if complex V functions suboptimally. The $\Delta\psi m$ was significantly increased in N^{low} cells (Fig. 5*F*), and a similar increase in $\Delta\psi m$ was observed after DAPT treatment (Fig. 5*G*). Treatment with oligomycin resulted in increased cell death in N^{low} cells and in DAPT-treated naïve MCF7 cells (Fig. 5*H*), further supporting a link between Notch and the activity of complex V. In contrast, we observed that the ATPase hydrolyzing activity in isolated mitochondria was not affected (Fig. 5*I*). Taken together, these data indicate that mitochondrial function is deregulated at several steps in the respiratory chain when Notch signaling is blocked.

Hypoactive Notch Signaling Induces Glucose Addiction in Tumor Growth. We next subjected the cells to glucose deprivation, to test whether the switch to glycolysis was irreversible or if OXPHOS could be resumed. N^{medium} cells were not sensitive to glucose deprivation, in keeping with the nonglycolytic phenotype of these cells. In contrast, the N^{low} cells demonstrated significantly increased cell death upon glucose deprivation (Fig. 6*A*). Similarly, blocking of Notch signaling by DAPT increased sensitivity to glucose withdrawal in ligand-induced naïve MCF7 cells and in control cells (Fig. 6*B*). Cell death in N^{high} cells or naïve MCF7 cells

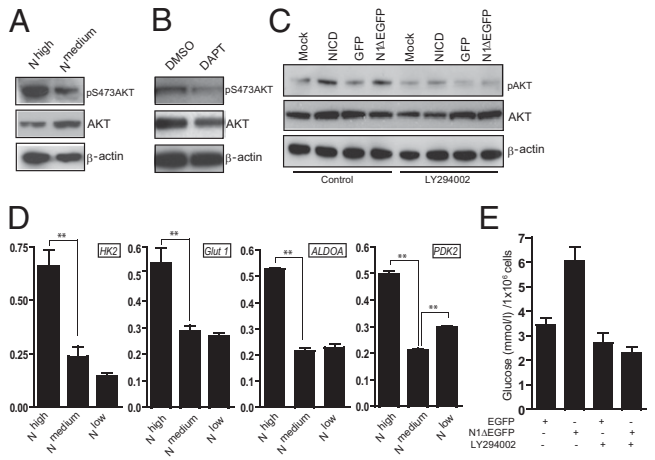

Fig. 3. Notch induces glycolysis via PI3K/AKT signaling. (*A*) Immunoblotting of N^{high} and N^{medium} cells with antibodies for activated (pS473), total AKT, and β-actin. (*B*) Immunoblotting of MCF7 cells with pS473, total AKT, and β-actin antibodies in the presence or absence of DAPT. (*C*) Immunoblotting of MCF7 cells transfected with Notch1 ICD or N1ΔEGFP with pS473 and total AKT antibodies in the presence or absence of the PI3K inhibitor LY294002. (*D*) Analysis of mRNA expression levels of HK2, GLUT1, ALDOA, and PDK2. Average of three different experiments. Values are significant at $^{**}P < 0.01$ and $^{*}P < 0.05$. Values indicate the average of at least three independent experiments. (*E*) Glucose consumption in MCF7 cells expressing EGFP or N1ΔEGFP in the presence or absence of LY294002. Average of two separate experiments, including three technical replications within each experiment.

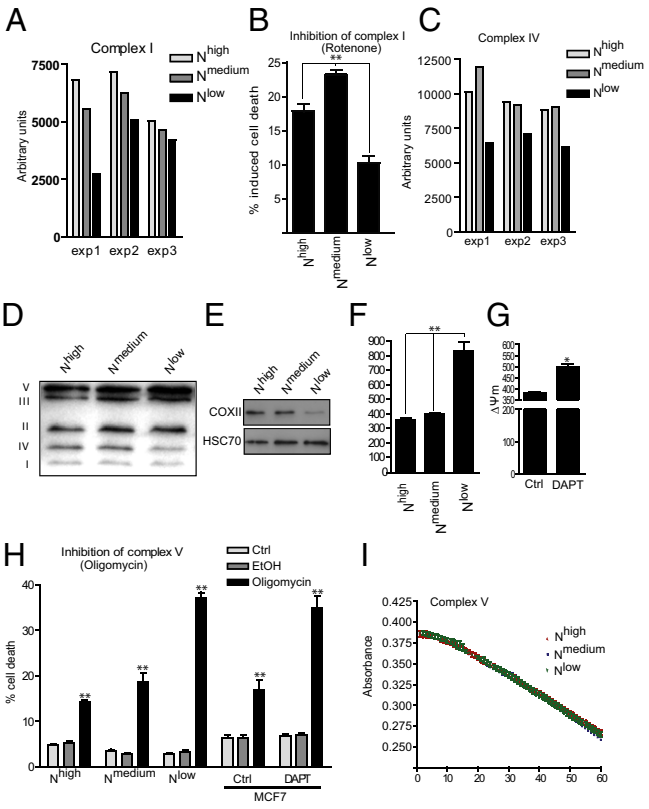

Fig. 5. Notch signaling is important for mitochondrial integrity and function. (*A* and *B*) The activity of the respiratory complex I (*A*) and the sensitivity to inhibition of complex I activity by rotenone (*B*) was determined on mitochondria isolated from N^{high}, N^{medium}, and N^{low} cells. (*C*) The activity of complex IV in isolated mitochondria. (*D*) Immunoblotting of mitochondrial preparations using antibodies against mitochondrial proteins for detection of different complexes. (*E*) Immunoblotting of whole-cell lysates of N^{high}, N^{medium}, and N^{low} cells with an antibody against subunit COXII. (*F* and *G*) The mitochondrial membrane potential in N^{high}, N^{medium}, and N^{low} cells (*F*) and in control or DAPT-treated naïve MCF7 cells (*G*). The cells were analyzed after 3 d of DAPT treatment. (*H*) Oligomycin-induced cell death (oligomycin 50 μM for 48 h) in N^{high}, N^{medium}, and N^{low} cells and in naïve MCF7 cells where Notch is inhibited with DAPT. (*I*) The hydrolyzing activity of complex V did not differ among N^{high}, N^{medium}, and N^{low} cells. Values are significant at $^{**}P < 0.01$ and $^{*}P < 0.05$. Values indicate the average of at least three independent experiments as indicated in *C*, *F*, *G*, and *I*.

cultured on Jagged1 ligand was not significantly elevated (Fig. 6 *A* and *B*). When cultured in a 3D growth assay, the growth and survival of both N^{medium} and N^{low} cells was not significantly hampered by low glucose levels (Fig. 6*C*), but the N^{low} cells developed considerably fewer colonies. Together, these data indicate that the mitochondrial defects in N^{low} cells are irreversible, whereas glycolytic N^{high} cells have a respiratory backup function under conditions when glucose is scarce but oxygen is available.

Hypoactive Notch Signaling Reduces p53 Levels. Loss of p53 has been associated with increased glycolysis, mitochondrial dysfunction, and deregulation of COXII (35, 36), which was also observed in N^{low} cells (Fig. 5*E*). We therefore analyzed whether reduced Notch signaling affected p53. N^{low} cells exhibited reduced p53 levels, whereas N^{high} cells showed elevated p53 protein levels, compared with N^{medium} cells (Fig. 7*A*). Transient transfection of Notch1 ICD into naïve MCF7 cells increased the p53 protein level (Fig. 7*B*), whereas introduction of dominant-negative CSL (dnCSL) or treatment with DAPT for 5 d led to a reduction in the amount of p53 protein (Fig. 7 *B* and *C*). The p53 mRNA levels were not significantly altered by altering the level of Notch signaling (Fig. 7*D*). Reintroducing p53 into N^{low} cells reversed the increase in glucose consumption (Fig. 7*E*). Conversely, knockdown of p53 by siRNA in N^{medium} cells increased the mitochondrial membrane potential (Fig. S6). Furthermore, transient transfection of dnCSL into naïve MCF7 cells led to an increase in glucose uptake, which could be abrogated by simultaneously introducing p53 (Fig. 7*E*). In sum, the data show that reduced Notch signaling lowers p53 protein levels, and that the elevated glucose consumption resulting from low Notch signaling can be abrogated by restoring high p53 levels in cells.

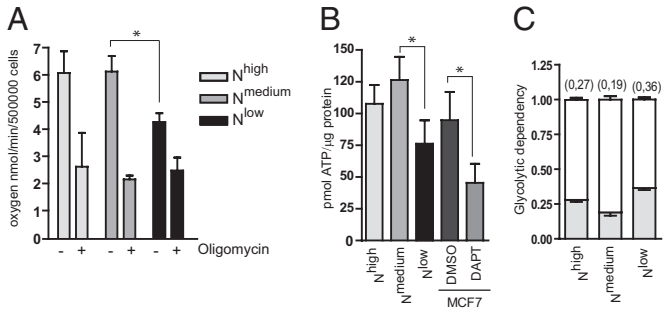

Fig. 4. Oxygen consumption and ATP production are reduced in cells with blocked Notch signaling. (*A*) Oxygen consumption in untreated or oligomycin-treated N^{high}, N^{medium}, and N^{low} cells (*B*) ATP levels in N^{high}, N^{medium}, and N^{low} cells and in naïve MCF7 cells in the absence or presence of the Notch inhibitor DAPT. Values are significant at $^{**}P < 0.01$ and $^{*}P < 0.05$. Values indicate the average of at least three independent experiments. (*C*) Glycolytic dependency, i.e., the relative contribution of glycolysis to mitochondrial ATP production as calculated: lactate accumulation rate/lactate accumulation rate + 4.5× oxygen consumption rate.

Discussion

In this report, we show that deregulation of Notch signaling resets cellular metabolism, and that both hyper- and hypoactivation of Notch induces a glycolytic phenotype, although by distinct

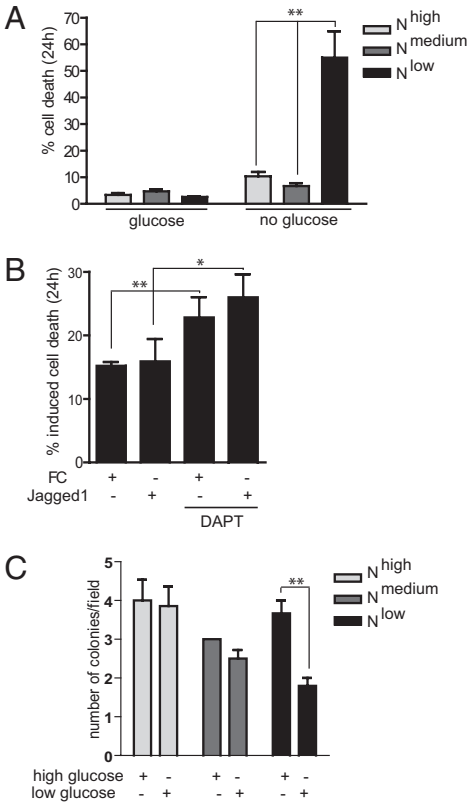

Fig. 6. Block of Notch signaling induces glucose addiction. (*A* and *B*) Cell death upon glucose deprivation in N^{high}, N^{medium}, and N^{low} cells (*A*), or in naïve control (FC) or ligand (FC-Jagged)-activated MCF7cells grown in the absence or presence of the Notch inhibitor DAPT (*B*). Average of three different experiments is shown. (*C*) Tumor growth of N^{high}, N^{medium}, and N^{low} cells in high- or low-glucose conditions as assessed by a 3D growth assay. Values are significant at $^{**}P < 0.01$ and $^{*}P < 0.05$. Values indicate the average of at least three independent experiments.

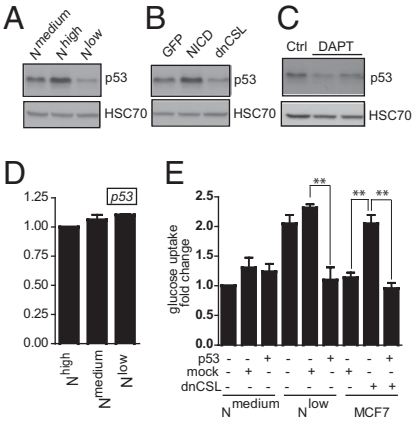

Fig. 7. Notch regulates p53 protein levels. (*A* and *B*) Immunoblotting of N^{high}, N^{medium}, and N^{low} cells (*A*) or naïve MCF7 cells transiently transfected with GFP, NICD, or dnCSL (*B*) using an antibody against p53. (*C*) Western blot of naïve MCF7 cells cultured in the presence or absence of DAPT. HCS70 was used as loading control. (*D*) Quantitative PCR of p53 mRNA levels in response to activation of blocking of Notch. (*E*) Fold change in glucose uptake in N^{medium} and N^{low} cells and in naïve MCF7 cells transfected with dnCSL in the presence and absence of reintroduced p53 as related to glucose uptake in nontransfected N^{medium} cells (which was set at 1). Values are significant at $^{**}P < 0.01$ and $^{*}P < 0.05$. Values indicate the average of at least three independent experiments.

mechanisms. Hyperactive Notch enhanced glycolysis in a PI3K/AKT-dependent manner. These data extend previous observations in normal and leukemic T cells, where AKT was activated by Notch, accompanied by increased glucose uptake (37) and a prosurvival function of Notch1-PI3K/AKT under metabolic challenge in malignant mesothelioma (38), suggesting that Notch and PI3K/AKT signaling synergize in different tumor cell types. High Notch activity boosted glycolysis without significantly lowering mitochondrial activity. This combination of glycolysis and retained mitochondrial activity may ensure a balance between the need for building blocks and the need for energy production through ATP synthesis to promote a highly proliferative state.

Notch signaling can occur in a canonical form that requires CSL, and in a noncanonical form that bypasses CSL or is independent of the ligand (18). A recent report indicates that canonical Notch signaling is dispensable for mammary tumorigenesis in mice (39). Notch1 ICD lacking the CSL binding domain did not revert the metabolic phenotype of N^{low} cells (Fig. S6), suggesting that canonical Notch signaling is required for metabolic regulation in cancer. Whether this difference reflects species difference between human and mouse mammary tumors, or activation of Notch in endogenous mammary cells vs. the use of established cell lines, remains to be analyzed.

Canonical Notch signaling was required for mitochondrial integrity, and mitochondrial function was compromised at several steps in the respiratory chain in cells expressing dnCSL. The cells were dependent on glycolysis for energy production and more sensitive to glucose deprivation, because they could not revert to OXPHOS when glucose was scarce. Our study links Notch activity to mitochondrial respiration, and may shed light on the observation that patients with cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy harboring *Notch3* mutations exhibit mitochondrial dysfunction (40, 41).

Our data implicate p53 in the metabolic effects in N^{low} cells and show that blocking canonical Notch signaling diminishes p53 levels, adding a new link to the multifaceted cross-talk between Notch and p53 (42). In keeping with this finding, Notch was recently shown to restore p53 in glioblastoma (43) and to enhance p53 protein levels in hepatocellular carcinoma (44). Activated Notch1 has been reported to directly bind p53 and inhibit its phosphorylation and transactivation (45), and a direct interaction between NICD4, p53, and murine double minute 2 (Mdm2) was recently shown to be important for Mdm2-mediated degradation of Notch (46). Conversely, modulation of p53 levels directly altered Notch levels in hepatocellular carcinoma, and p53 was required for Notch-induced growth and invasiveness (47). Notch may also be part of p53's antiapoptotic activity (48). Collectively, our findings suggest the existence of an intricate cross-talk between Notch and p53, and, although the precise details remain to be worked out, this may be of interest for possible future combination therapies (48). There is also an emerging view that p53 regulates mitochondrial functions. In p53-deficient mice, mitochondrial biogenesis is impaired, accompanied by reduced oxygen consumption and increased glycolysis (11, 35). Moreover, parkin, a novel p53 target gene, mediates the effect of p53 on metabolism, and parkin deficiency leads to mitochondrial dysfunction and reduced mitochondrial respiration (49). p53 has also been shown to regulate the level of COXII (34), which is in keeping with the observed reduction of COXII after blocking Notch signaling.

Though N^{high} and N^{low} cells both enhanced glycolysis, N^{high} cells generated rapidly growing, metastasizing tumors, whereas N^{low} cells grew only transiently, and then regressed. This finding shows that a glycolytic phenotype alone is not sufficient for promotion of tumor growth. Growth of N^{low} in vitro was hampered under glucose-restricted conditions, and the cells showed increased sensitivity to glucose deprivation, which suggests that metabolic flexibility is more important than glycolysis for tumor progression. Though it was initially thought that mitochondrial dysfunction accompanied aerobic glycolysis in tumor cells (1), there is accumu-

lating evidence that tumor cells have intact mitochondria (14, 15, 50, 51) and can revert to OXPHOS in oxygenated regions to more efficiently use the limited amount of glucose available (17, 52).

In summary, our data show that Notch signaling plays an important role in cellular energy homeostasis, which is crucial for better understanding the physiological effects of deregulated Notch signaling in tumors and inspiring new strategies for cancer therapy.

Materials and Methods

Details for plasmid constructs; cell culture; metabolic and mitochondrial assays; quantitative RT-PCR; immunohistochemistry and cytochemistry; Western blot analysis; and tumor xenotransplantation are given in *SI Materials and Methods*.

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