Published in final edited form as:
Clin Cancer Res. 2013 July 1; 19(13): 3703–3713. doi:10.1158/1078-0432.CCR-13-0623.

Lactate Dehydrogenase B: A Metabolic Marker of Response to Neoadjuvant Chemotherapy in Breast Cancer

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Abstract

Purpose—Although breast cancers are known to be molecularly heterogeneous, their metabolic phenotype is less well understood and may predict response to chemotherapy. This study aimed to evaluate metabolic genes as individual predictive biomarkers in breast cancer.

Methods—mRNA microarray data from breast cancer cell lines were used to identify bimodal genes – those with highest potential for robust high/low classification in clinical assays. Metabolic function was evaluated in vitro for the highest scoring metabolic gene, lactate dehydrogenase B (*LDHB*). Its expression was associated with neoadjuvant chemotherapy response and relapse within clinical and PAM50-derived subtypes.

Results—LDHB was highly expressed in cell lines with glycolytic, basal-like phenotypes. Stable knockdown of LDHB in cell lines reduced glycolytic dependence, linking LDHB expression directly to metabolic function. Using patient datasets, *LDHB* was highly expressed in basal-like cancers and could predict basal-like subtype within clinical groups (odds ratio = 21 for hormone-receptor (HR)-positive/HER2-negative; odds ratio = 10 for triple-negative). Furthermore, high *LDHB* predicted pathological complete response (pCR) to neoadjuvant chemotherapy for both HR-positive/HER2-negative (odds ratio = 4.1, $P < .001$) and triple-negative (odds ratio = 3.0, $P = .$

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Conflicts of interest: none

003) cancers. For triple-negative tumors without pCR, high LDHB post-treatment also identified proliferative tumors with increased risk of recurrence (hazard ratio = 2.2, $P = .006$).

Conclusions—Expression of *LDHB* predicted response to neoadjuvant chemotherapy within clinical subtypes independently of standard prognostic markers and PAM50-subtyping. These observations support prospective clinical evaluation of LDHB as a predictive marker of response for breast cancer patients receiving neoadjuvant chemotherapy.

Keywords

breast cancer; LDHB; lactate; glycolysis; Warburg

INTRODUCTION

Molecular subtyping of breast cancers has identified multiple gene clusters that can predict outcomes independently of clinical characteristics and the standard biomarkers: estrogen-receptor (ER), progesterone-receptor (PR), and human epidermal growth factor receptor 2 (HER2) (1,2). Subtype gene signatures are often composed of different mRNAs but are highly correlated and can equally predict outcomes, reflecting fundamental biological differences between breast cancer lineages (3,4).

While able to provide clinically useful information for a subset of ER-positive, node negative tumors (5,6), mRNA profiling approaches like Oncotype DX are limited by type and purity of the specimen. For instance, Oncotype DX and immunohistochemistry (IHC)/ fluorescent in-situ hybridization (FISH) scoring of HER2 may not agree if the stromal component of the tumor contaminates the mRNA pool (7,8). Even with a relatively pure specimen, clinically useful results with Oncotype DX testing are not guaranteed; 37% of scored tumors are “intermediate” without a treatment recommendation (9). In clinical research, mRNA profiling approaches can be problematic if tumors are necrotic or have a low concentration of malignant cells as often found in cancers pretreated with chemotherapy (10).

Additional predictive biomarkers, particularly for intermediate-grade or chemotherapy-resistant tumors, may complement existing mRNA profiling efforts for ER, HER2, and proliferation-related genes. We hypothesized that new types of biomarkers could be discovered by exploring functional differences in metabolic pathways as these pathways may predict response to therapy.

While many cancers preferentially generate ATP via anaerobic glycolysis in the presence of oxygen, a process termed the “Warburg effect” (11), the metabolic phenotypes of breast cancers are heterogeneous; more than a 20-fold range in glucose uptake has been reported as quantified by ^{18}FDG-PET maximum standard uptake values (12). To study metabolic differences between breast cancers and avoid interference from the microenvironment, mRNA microarray data from breast cancer cell lines were used to identify bimodal metabolism-related genes, those with high/low expression. This type of analysis was chosen because clinically useful biomarkers, including ER and HER2, are often bimodal enabling a more robust threshold determination during assay development (13). The biomarker performance of the highest scoring metabolic bimodal gene, lactate dehydrogenase B (*LDHB*), was evaluated and compared to standard clinical characteristics and intrinsic subtyping by PAM50 (14). Lactate dehydrogenase tetrameric enzymes, composed of lactate dehydrogenase A (LDHA) and/or LDHB subunits, are utilized by cancer cells to bypass oxidative phosphorylation and produce lactate from pyruvate (15). We propose that LDHB expression marks fundamental metabolic differences between breast cancers and may predict response to neoadjuvant chemotherapy.

MATERIALS AND METHODS

Samples and clinical data

Two publically available mRNA microarray datasets of breast cancers were used to evaluate pCR and the predictive ability of LDHB for PAM50 subtyping: Microarray Quality Control (MAQC) II- study (16) [GSE20194] and MD Anderson Cancer Center Super Series (MDACCSS) (17) [GSE25066]. Any overlapping patient samples with the MAQC were removed from the MDACCSS dataset. Because HER2-positive cases were not part of the MDACCSS cohort, only HER2-negative breast cancers were included in the evaluation of *LDHB* levels to predict pCR. Two additional publically available datasets were used to evaluate the predictive ability of LDHB on breast cancer intrinsic subtyping: The Cancer Genome Atlas (TCGA, Supplementary Table S1) and Xeloda in NeoAdjuvant Trial (XeNA) (18) [GSE22358]. *LDHB* mRNA expression was quantified by platform-dependent probe sets [201030_x_at], [A_23_P53476], or those as defined by TCGA. To compare thresholds, *LDHB* levels were median-centered to the HR-positive/HER2-negative group within each cohort.

For the tissue microarray (TMA), archival formalin-fixed, paraffin-embedded (FFPE) blocks were from patients with clinically-diagnosed triple-negative breast cancer (2008–2009) who received at least 3 cycles of anthracycline-based neoadjuvant chemotherapy (with or without taxanes) and had residual disease in the breast or lymph nodes at surgery. All patients were treated at the Instituto Nacional de Enfermedades Neoplásicas in Lima, Perú. Blocks were from post-neoadjuvant chemotherapy mastectomy specimens with residual disease. Recurrence-free survival was defined as the time between the date of surgery and the date of diagnosis of recurrence. Samples and associated clinical data were collected under an institutionally approved protocol (INEN #10-018, Supplementary Table S2). PAM50 intrinsic subtyping and scoring of LDHB and standard markers including Ki67, androgen receptor (AR), and HER2 are described in the supplementary methods. The IHC protocol for LDHB was validated using FFPE blocks of MDAMB231 cell lines with shRNA knockdown of LDHA or LDHB (Supplementary Fig. S1).

Cell Culture and Glycolytic Phenotyping

Breast cancer cell lines (SKBR3, BT474, MDAMB231, HCC38, BT20, MDAMB468, DU4475, HCC70, HCC1937, HCC1187, HCC1806, CAMA1, T47D, HCC1428, ZR751, MDAMB175, MCF7, MCF10A, and MCF12A, and MDAMB453) were cultured in DMEM supplemented with 5% fetal bovine serum (FBS) at 37°C in 5% carbon dioxide atmosphere. MCF10A and MCF12A were supplemented with additional cholera toxin (100 ng/mL), hydrocortisone (0.5 mg/mL), insulin (10 μg/mL), and epidermal growth factor (20 ng/mL). Cell lines were routinely tested for *Mycoplasma* infection using a MycoTect Kit (Invitrogen). Stable isogenic cell lines of MDAMB231 and HCC1937 were generated using LDHA, LDHB, or the non-silencing control Expression Arrest GIPZ lentiviral shRNA particles from Open Biosystems (ThermoFisher Scientific Inc, Fremont, CA). Infected cells were selected and routinely cultured with 1 μg/mL puromycin (Sigma-Aldrich, St. Louis, MO). The identities of all cell lines were verified using AmpF/STR Identifier kit (Applied Biosystems). Protein quantification of whole cell lysates and Western blotting using primary antibodies for LDHA (Cell Signaling; 3582S; 1:500) and LDHB (Abcam; ab85319; 1:2,000) and secondary antibodies, antirabbit or antimouse immunoglobulin G (IgG) horseradish peroxidase–linked secondary antibody (Cell Signaling Technology, Danvers, MA; 1:2,000), were as described previously (19).

The oxygen consumption rates (OCR) and the extracellular acidification rates (ECAR) of cell lines were quantified using the Seahorse Extracellular Flux Analyzer (XF96, Seahorse

Biosciences, North Billerica, MA). For adherent lines, at least 5 wells for each cell line were seeded on XF 96-well microplates (Seahorse Biosciences), 0.6–1.6x10^4 cells/well in 5% FBS DMEM, and left overnight to attach. Approximately 1 h prior to the Seahorse readings, the medium was replaced with exchange medium: serum-free, bicarbonate-free DMEM with phenol red (5 mM glucose, 0.5 glutamine, 1 mM sodium lactate). For suspension cell lines, wells were pretreated with CellTak (BD Biosciences, San Jose, California) per the manufacturer instructions, and 1.6x10^4 cells were added to the wells in the exchange medium on the day of the readings. OCR and ECAR readings were determined for 6 cycles (2 min mixing, 5 min measuring), and the baseline measurements were the average of the last 3 readings prior to oligomycin addition (1 μg/mL final concentration). The absolute OCR reduction after oligomycin addition was defined at the ATP-dependent OCR (OCR_{ATP}).

Analytical and Statistical Methods

Publically available mRNA microarray data from a panel of 54 breast cancer cell lines (20) were used to identify bimodal genes based on KEGG function from central-carbon metabolic pathways. For probe sets with at least a 10-fold range, R code (web site http://bioinformatics.mdanderson.org/Software/OOMPA) was used to compute the bimodality index as previously described (13). A probe set was considered bimodal if the bimodality index was >1.1, and the proportion of samples in one group was >10%. Bimodal genes were selected based on their KEGG function including genes from central-carbon metabolic pathways (21).

mRNA and protein expression differences between groups of breast cancers and cell lines were assessed using t-tests and one-way ANOVA. Survival curves were estimated using the Kaplan-Meier method, and differences were evaluated using the log-rank test or univariate Cox regression. Fisher's exact test for single variables and binary logistical regression for multiple variables were used to determine the impact of potential markers on pCR. Statistical analyses were performed using Prism 5.0c (GraphPad Software, La Jolla, CA) or SPSS Statistics (Version 19.0, SPSS, Chicago, IL). $P \leq .05$ (two-sided) was considered significant.

RESULTS

Identification of LDHB as a Bimodal Metabolism Gene

Using mRNA microarray data from a panel of breast cancer cell lines, metabolism-related genes were ranked by their bimodality index values (Supplementary Table S3). As compared to clinical markers *ERBB2* and *ESR1*, 20 metabolism-related genes with similar bimodality were identified including the highest-ranked gene, *LDHB* (Fig. 1A, B). LDHB expression was the highest in basal-like or triple-negative cell lines (Fig. 1C, D). Because LDHA and LDHB form active tetrameric enzymes with each other, the relative expression levels of mRNA and protein were evaluated for both subunits (Fig. 1B, D). Consistent with the mRNA data (Fig. 1B) and a recent study (22), differential protein expression of LDHB but not LDHA was confirmed by Western blot (Fig. 1D).

While LDHA was generally high, LDHB when present contributed substantially to the total lactate dehydrogenase activity in the cell lines (>50% for HCC1187, HCC1937, and MDAMB175; Supplementary Fig. S2). Certain basal A cell lines like BT20 and MDAMB468 did not express LDHB, but LDHB was highly expressed in basal B lines which include the stem cell-like, claudin-low subset of breast cancers (23,24) (Supplementary Fig. S2). Within the ER-positive/HER2-negative lines, *LDHB* was

expressed in a minority of cell lines, typically those with lower *ESR1* mRNA levels (*LDHB* vs *ESR1*: Pearson $r = 0.58$, $P = .04$).

Glycolytic Phenotyping of Breast Cancer Cell Lines and Role of LDHB

To determine how LDHB expression related to metabolic state, the rates of oxygen consumption (OCR) and extracellular acidification (ECAR) were quantified using a panel of cell lines (N = 19) with representatives from each of the known subtypes (20). The ratios of the ATP-synthase dependent OCR in the mitochondria (OCR_{ATP}) and the ECAR (a measure of lactate production rates) were used to rank order the cell lines (Fig. 2A, B). Consistent with previous reports of high ^{18}FDG-PET uptake in basal-like tumors (12), the metabolic phenotypes as determined by OCR_{ATP}/ECAR ratios of breast cancer cell lines were highly variable (20-fold range) and most glycolytic in basal-like as compared to luminal-like cells (Fig. 2B, $P = .01$). Importantly, *LDHB* was equally predictive of metabolic phenotype with the highest expression in the most glycolytic lines (Fig. 2B, $P = .005$).

To evaluate the functional metabolic role of LDHB, we created two sets of isogenic cell lines with stable knockdowns of LDHA or LDHB using breast cancer cells with high LDHB in the parental lines (Supplementary Fig. S3). Knockdown of LDHA or LDHB promoted a more oxidative metabolic state as shown by increased OCR_{ATP}/ECAR ratios for both cell lines (Fig. 2C). The changes were primarily attributable to increased OCR_{ATP} (Supplementary Fig. S3) and consistent with previous reports of mitochondrial compensation after stable knockdown of LDHA (25).

Expression of LDHB is Sufficient to Predict Basal Phenotype

To determine whether the cell line data translated to primary tumors, the mRNA expression of *LDHB* was evaluated using multiple cohorts of patients' cancers. As expected based on the cell line data and a recent report (22), basal-like breast cancers as defined by PAM50 expressed higher levels of *LDHB* in multiple datasets (Fig. 3A, Supplementary Fig. S4). Using standard clinical markers, *LDHB* was also higher in triple-negative breast cancers and the lowest in HR-positive/HER2-negative cancers (Fig. 3B, Supplementary Fig. S5). In contrast, *LDHA* was not differentially expressed in the breast cancer subtypes (data not shown) in agreement with a previous report (26).

Clinical classification of breast cancers by ER/PR/HER2 status did not exclusively define PAM50 intrinsic subtype in our cohorts; 17 to 28% of triple-negative tumors were not basal-like, and 31 to 38% of HR-positive/HER2-negative tumors were not luminal-like (Table 1). Thus, we evaluated the ability of *LDHB* to predict intrinsic subtype within HR-positive/HER2-negative and triple-negative cancers. *LDHB* was highly associated with basal-like phenotype independently of HR status (Supplementary Fig. S6, S7). The threshold for high/low expression of *LDHB* was determined by optimizing the odds ratio of the MAQC cohort based on the separation of basal-like subtype within the HR-positive/HER2-negative group (0.60, Supplementary Fig. S6). Using this threshold for all remaining cohorts, *LDHB* was able to predict basal-like subtype within the HR-positive/HER2-negative and triple-negative breast cancer groups with a high degree of power (Fig. 3C, D). The odds ratio for prediction of basal phenotype was overall lower for triple-negative as compared to the HR-positive/HER-negative cancers. However, this trend was not observed for the TCGA cohorts; *LDHB* levels were as predictive of basal phenotype for the triple-negative group as compared to the HR-positive/HER2-negative group (Fig. 3C and D), perhaps reflecting the higher purity of the TCGA specimens and consequently a reduced number of normal-like breast cancers (27). In fact, exclusion of the normal-like breast cancers in the other cohorts increased the odds ratios for basal prediction within the triple-negative groups (data not shown).

LDHB Predicts Response to Neoadjuvant Chemotherapy

Given that pCR after neoadjuvant chemotherapy predicts reduced risk of relapse independently of clinical subtype in breast cancer (28), pCR was used to evaluate LDHB as a biomarker of response. The ability of *LDHB* to predict pCR after neoadjuvant chemotherapy was evaluated in two independent cohorts (MAQC and MDACCSS).

First, the HR-positive/HER2-negative groups were evaluated for differences in pCR using the *LDHB* threshold determined from the basal-like prediction (0.60, Fig. 3C). High nuclear grade, basal-like subtyping, and high *LDHB* predicted pCR to neoadjuvant chemotherapy by univariate analyses for two independent cohorts (Table 1) and in the combined cohort (odds ratio = 4.1; 95% CI, 2.0 to 8.3; $P < .001$ for high *LDHB*). In a logistic regression model for the combined cohorts with these characteristics as predictors, only *LDHB* and nuclear grade remained significant (Table 2).

For triple-negative cancers, adjustment of *LDHB* threshold from 0.60 to 0.94 optimized the ability of *LDHB* to predict pCR within the MDACCSS cohort (approaching statistical significance, $P = .087$, Table 1). Using this threshold, *LDHB* predicted pCR in the MAQC cohort ($P = .020$) and in the combined cohort (odds ratio = 3.0; 95% CI, 1.4 to 6.2; $P = .003$). Although the basal-like subtype predicted pCR in the MAQC cohort by univariate analysis, only *LDHB* and tumor size were significant in a multivariate, logistic regression model of the combined cohort (Table 2). Interestingly, *LDHB* was highly expressed in the most aggressive triple-negative cancers within the basal-like subtype as shown by its association with the proliferation marker *CCNB1* (Supplementary Fig. S8). Therefore, an adjustment of the *LDHB* threshold may allow stratification within basal-like cancers.

LDHB Expression in Triple-Negative Breast Cancers with Residual Disease

To further evaluate the potential predictive role of LDHB, an independent sample set of relatively advanced-stage, triple-negative disease (primarily stage III, Supplementary Table S2) biopsied after neoadjuvant chemotherapy was quantified for LDHB protein expression by IHC. As expected based on the cell line data (Fig. 1), intertumoral expression of LDHB was heterogeneous in the breast cancer cells (Fig. 4A). Interestingly, LDHB expression was generally ubiquitous in the tumor microenvironment, which may in part explain why differential expression of LDH isoforms was not previously detected in breast cancer (29). Consistent with mRNA microarray data of patient tumors, high LDHB was also able to predict basal-like phenotype (threshold = 180 for lowest *P*; sensitivity = 96% (47/49); specificity = 60% (18/30); Fig. 3D and Fig. 4C). Using the same LDHB intensity threshold as the basal-like prediction analysis, LDHB was the only predictive marker for relapse in this cohort (Fig. 4B and Supplementary Table S2). High LDHB marked the most aggressive disease that was more common in young women as shown by association of LDHB with Ki67 and age (Fig. 4D). Cancers with low LDHB were also more likely to have high AR and HER2 (Fig. 4D).

DISCUSSION

Molecular profiling methods in breast cancer used for prognosis and to predict lack of benefit from chemotherapy such as Oncotype DX and PAM50 primarily focus on HER2, ER, and proliferation-related genes (9,14). Here, we evaluate another functional hallmark of cancer, deregulation of cellular energetics (30). By starting with mRNA microarray data of cell lines, bimodal genes within metabolic pathways were detected in the cancer cells without influence or dilution by the tumor microenvironment. This approach led to the evaluation of LDHB as a putative biomarker, which proved to be highly expressed in aggressive, glycolytic breast cancers primarily of the basal subtype.

Bimodal expression of LDHB may have clinical relevance because cancers with high *LDHB* were most responsive to neoadjuvant chemotherapy independently of established prognostic factors (grade, tumor size) and molecular markers (HR-status and PAM50 subtyping). Although it might be expected that higher response rates to neoadjuvant chemotherapy would lead to reduced rates of relapse, high LDHB tumors without pCR post neoadjuvant chemotherapy were also more likely to relapse in our triple-negative cohort. One possible explanation is that LDHB marks cancers with higher metastatic potential leading to more residual micrometastases. Also, LDHB is reported to facilitate tumor growth in basal-like breast cancers (22), so faster rates of relapse are consistent with higher rates of proliferation as shown by association with Ki67 and proliferation markers in high LDHB cancers. However, LDHB not Ki67 predicted relapse in our triple-negative cohort supporting the hypothesis that LDHB is more than a surrogate proliferation marker.

We demonstrated that LDHB expression levels in breast cancers were bimodal, an important characteristic for a robust biomarker. The most striking observation was the near total depletion of LDHB in many samples. For breast cancer cell lines with low LDHB levels, the contribution of LDHA to the total LDH activity was almost 100%. Likewise, in most luminal tumors, LDHB was highly expressed in the tumor microenvironment but not detected in cancer cells (Fig. 4). In contrast, LDHB levels were high in many basal-like cancer cells, in some cell lines exceeding that of LDHA. To determine whether overexpression was regulated at the gene level, we evaluated copy number gain for *LDHB* at chromosome 12p12, the same amplicon as *KRAS*. While we did see gain in approximately 40% of TCGA basal-like tumors that correlated with mRNA expression, the differences in mRNA expression of *LDHB* between luminal and basal cancers were independent of gene copy number (Supplementary Fig. S9). This observation for breast cancer is in contrast to that of lung cancer; LDHB was recently shown to be highly expressed in lung adenocarcinomas with *KRAS* amplification or mutations (31). Recently reported for breast cancer (32) and similar to regulation of *LDHB* in prostate cancer (33), DNA hypermethylation of *LDHB* may contribute to low expression of *LDHB* mRNA in luminal breast cancer (Supplementary Fig. S9).

While LDHB expression levels were associated with breast cancer subtype as defined by PAM50, high LDHB identified aggressive cancers were predominantly but not exclusively basal-like (Fig. 4). Importantly, as compared to basal-like phenotype, LDHB was a more robust predictor of response to neoadjuvant chemotherapy (Table 2). Our TMA of triple-negative breast cancers with matched PAM50 subtyping also demonstrated that LDHB was highly expressed not only in basal but in a subset of HER2-enriched and luminal B cancers with increased rates of relapse (Fig. 4). Consistent with these findings, another study reported that only basal-like, HER2-enriched, and luminal B cancers with the worst clinical outcomes were able to form stable grafts in mice (34); importantly, we determined that 11 out of the 12 stable grafts (out of 49 tumors transplanted) expressed high levels of *LDHB* (GSE32532, data not shown). Based on these results, we anticipate that LDHB expression may provide additional information beyond the standard proliferation and hormone markers used in molecular profiling tests like Oncotype DX and PAM50. However, additional studies will be required to understand the prognostic or predictive value of LDHB expression as compared to other molecular classifiers and signatures for breast cancer.

LDHB expression is likely to also provide information on the metabolic phenotype of breast cancers that could contribute to selection of new treatment modalities. High LDHB marked the most glycolytic breast cancer cells, often basal-like, within the HR-positive/HER2-negative and triple-negative groups. Our cell line studies demonstrated a direct effect and strong positive association with LDHB and glycolytic phenotype (Fig. 2). Consistent with ^{18}FDG-PET reports (12,35), cell lines with a more glycolytic phenotype in our study

were primarily basal-like: those with genetic instability or increased proliferation often caused by loss of *TP53, MYC* amplification, or *BRCA1* mutations. High glycolytic flux may provide these types of cancers a survival advantage because the Warburg effect allows more rapid consumption of glucose and consequently can support increased rates of proliferation, regardless of tumor oxygenation (15). This adaptation may be less important for luminal-like cancers whose survival may be more dependent on antiapoptotic mechanisms such as Bcl-2 expression (36).

Although the general enzymatic function of LDHB is known, the functional role of LDHB is cell type and context dependent. In tissues like the brain that utilize lactate as energy, LDHB promotes lactate uptake during exercise; other cells like erythrocytes use LDHB for glycolysis to synthesize lactate (37). While LDHB is thought to convert lactate to pyruvate in certain tissues, our breast cancer cell line results demonstrate that LDHB is functionally similar to LDHA and contributes to the conversion of pyruvate to lactate (Fig. 2). We propose that LDHB is constitutively expressed in most basal-like breast cancers and significantly contributes to the overall LDH activity. Consequently, inhibition of LDHA, a proposed therapeutic target in breast cancer (25,38), would have less effect on the total lactate dehydrogenase activity if LDHB were coexpressed. Indeed, high LDHB expression may identify tumors less likely to respond to LDHA inhibitors.

While the function of LDHB is consistent with an increased glycolytic phenotype, LDHB expression was insufficient to completely explain the metabolic variability between breast cancer cell lines (Fig. 1B). LDHB is likely part of a network of metabolic proteins that together create a specific glycolytic phenotype. Low expression of fructose-1,6-bisphosphatase 1 (*FBP1*) and high expression of monocarboxylic acid transporter 1 (*SLC16A1*) and glutaminase (G*LS*), previously reported for basal-like phenotype (39,40), were associated with high *LDHB* (data not shown). We propose that LDHB expression may mark a particular lineage or state of differentiation with altered metabolic demands. Consistent with this hypothesis, LDH isoform distribution is well known to be a fundamental property of cell type and developmental stage (41). Also, low LDHB in HER2-positive and luminal-like cancers is consistent with a model of breast cancer lineage (42), from least to most differentiated (LDHB levels: claudin-low/basal > HER2 > luminal). Given that *LDHB* mRNA levels were unchanged by chemotherapy in breast cancer (GSE28844, N = 28 pairs, data not shown), LDHB expression appears to be a property of lineage and independent of the tumor microenvironment.

In conclusion, LDHB expression in breast cancer cells was bimodal, a desirable property for a clinical biomarker. As expected based on its enzymatic function, high LDHB was associated with glycolytic, basal-like phenotype. While additional prospective studies are required, LDHB in our cohorts was an independent predictive marker of pCR for HER2-negative cancers (HR-positive or HR-negative) and relapse in triple-negative disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Pamela K. Johnston for help with the IHC assay for LDHB, Ju-Seog Lee and Fan Zhang for assistance with patient datasets, and Xiudong Lei for suggestions on the biostatistical analyses.

Grant Support

This work was supported by the Susan G. Komen Foundation (KG081694); GlaxoSmithKline; Vanderbilt Breast Cancer Specialized Program of Research Excellence (SPORE) (P50CA98131); American Cancer Society Clinical

Research Professorship Grant (CRP-07-234); Entertainment Industry Foundation; and Susan G. Komen for the Cure Scientific Advisory Council Grant (SAC100013). S.M. is supported by a Susan G. Komen Postdoctoral Fellowship. J.B.D. is supported by a GlaxoSmithKline TRIUMPH post-doctoral fellowship and the American Cancer Society, Joe and Jessie Crump Medical Research Fund Postdoctoral Fellowship.

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Statement of Translational Relevance

Even within the most proliferative, aggressive subtypes of breast cancer, only a fraction of breast cancers respond to neoadjuvant chemotherapy. While mRNA profiling methods and standard clinical markers (ER/PR/HER2) help identify those tumors more likely to respond to chemotherapy, other robust markers could benefit patient care. We present evidence that a metabolic enzyme, LDHB, is highly expressed in the microenvironment but essentially absent in a subset of breast cancers. Independent of existing biomarkers, LDHB is a novel metabolic marker for breast cancer, identifying cancer cells with a more glycolytic phenotype. Importantly, high *LDHB* expression predicted pCR independently of ER expression and PAM50-subtyping. In an independent triple-negative cohort, high LDHB predicted relapse. Thus, measurement of LDHB may help identify breast cancers most likely to respond to neoadjuvant chemotherapy as well as those with the highest risk of relapse that may benefit from additional adjuvant therapy.

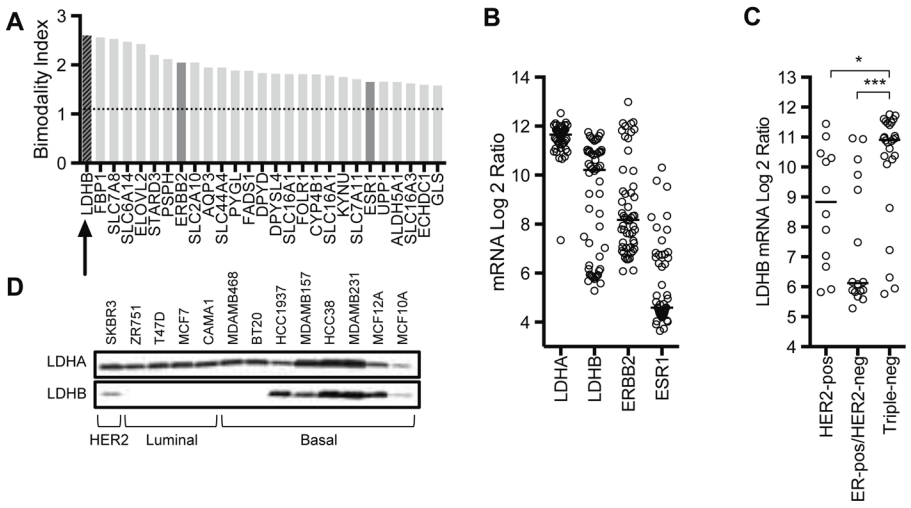

Figure 1.

LDHB is highly expressed in basal-like breast cancer cells and contributes to glycolytic phenotype. **A**, *LDHB* was the highest scoring of metabolic genes in a bimodal analysis, higher than *ERBB2* and *ESR1*. >1.1 bimodality index was considered bimodal. The top scoring 25 genes for bimodality index are shown; see Supplementary Table S3 for the entire list. **B**, mRNA expression by microarray of *LDHB, ERBB2,* and *ESR1* were bimodal but not that of *LDHA*. **C**, *LDHB* expression was the highest in triple-negative lines. mRNA microarray expression of *LDHB* separated by HER2 and ER-status of cell lines. Lines represent the median values. **D**, LDHB protein levels were differentially expressed in cell lines as shown by Western blot of LDHA and LDHB in a panel of cell lines including HER2-amplified, luminal, and basal-like subtypes as defined previously (20). 20 μg protein was loaded per lane.

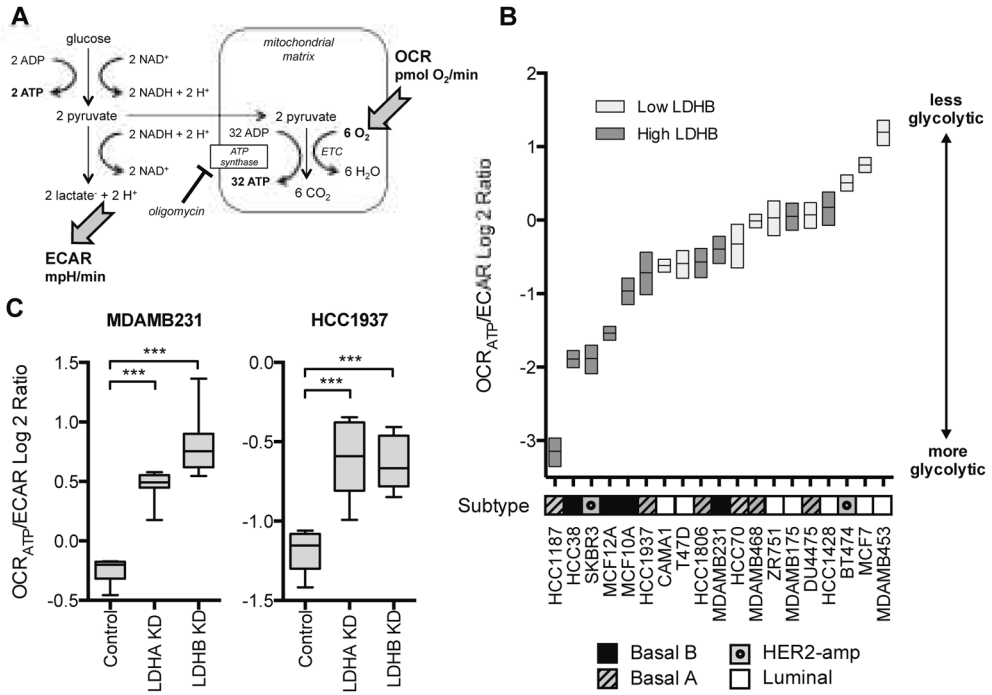

Figure 2.

A, schematic of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) as they relate to ATP production in the cell. OCR_{ATP} is defined as the OCR by ATP synthase experimentally determined by inhibition with oligomycin. High OCR_{ATP}/ECAR ratios occur with higher mitochondrial dependence for ATP production. **B**, ratio of OCR_{ATP} normalized to ECAR was determined for a panel of breast cancer cell lines. Intrinsic subtype as determined previously (20) is included in the legend. Floating bars represent the average ± one standard deviation. **C,** stable shRNA knockdown of LDHA or LDHB increased mitochondrial dependence for ATP production in MDAMB231 and HCC1937 cell lines. The box and whiskers plots show the minimum and maximum values. Knockdown was confirmed by non-denatured electrophoresis of LDH (Supplementary Fig. S3). *, $P < .05$; ***, $P < .001$

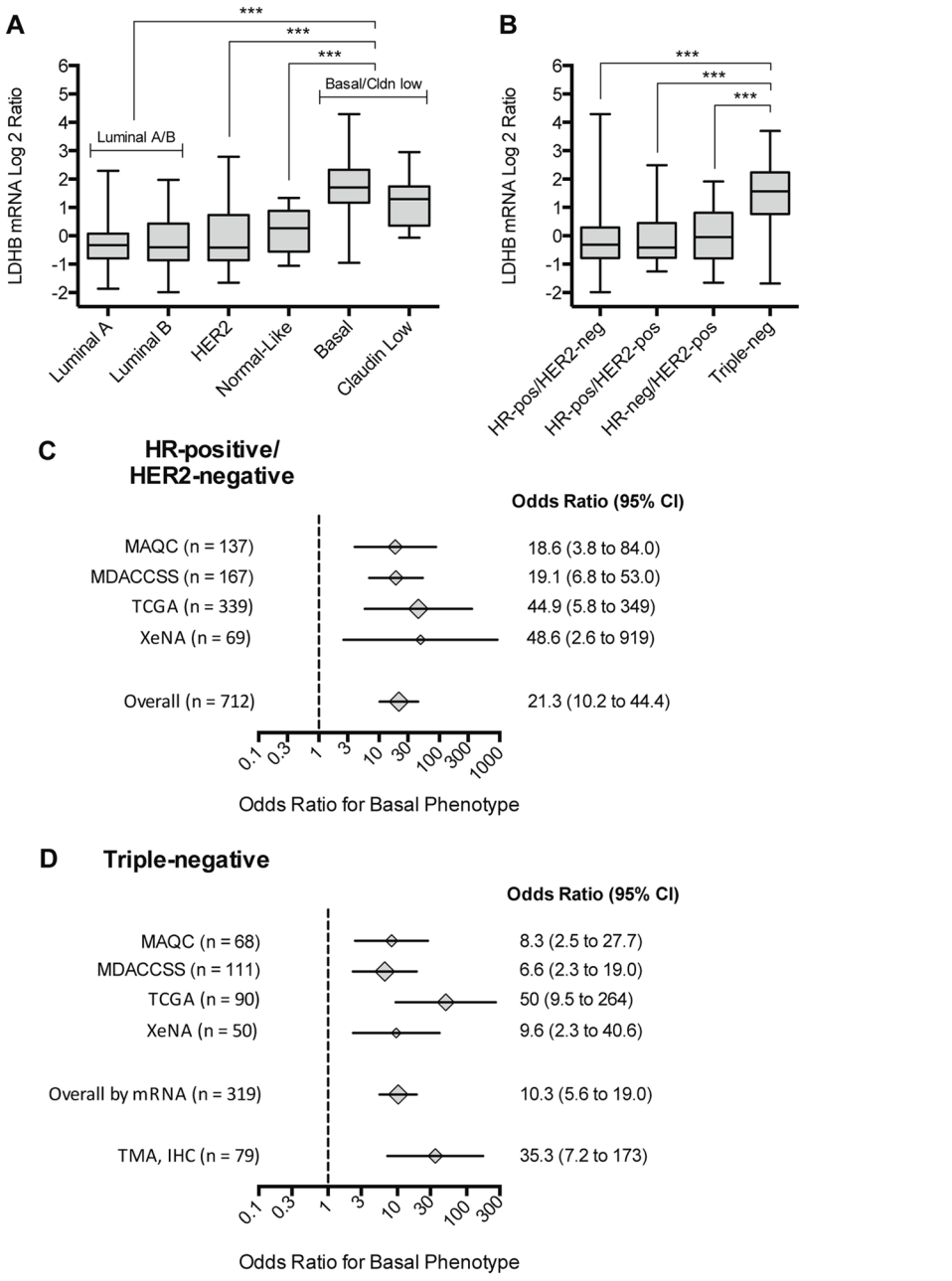

Figure 3.

LDHB mRNA was highly expressed in basal-like and triple-negative disease and predicted basal-like subtyping of breast cancers independently of HR-status. *LDHB* mRNA expression separated by **A**, PAM50 intrinsic subtype and **B**, clinical ER/HER2 status (TCGA, Supplementary Table S1). The box and whiskers plots **A**–**B** show the minimum and maximum values. Forest plots for *LDHB* prediction of basal-like phenotype by cohort for **C**, HR-positive/HER2-negative, and **D**, triple-negative breast cancers. Marker size represents the size of the cohort, and the bars cover the 95% CI. Results from a triple-negative TMA stained for LDHB (Fig. 4) are also plotted (**D)** but are not part of the average. HR = hormone receptor (ER and/or PR), TN = triple-negative, ***, $P < .001$

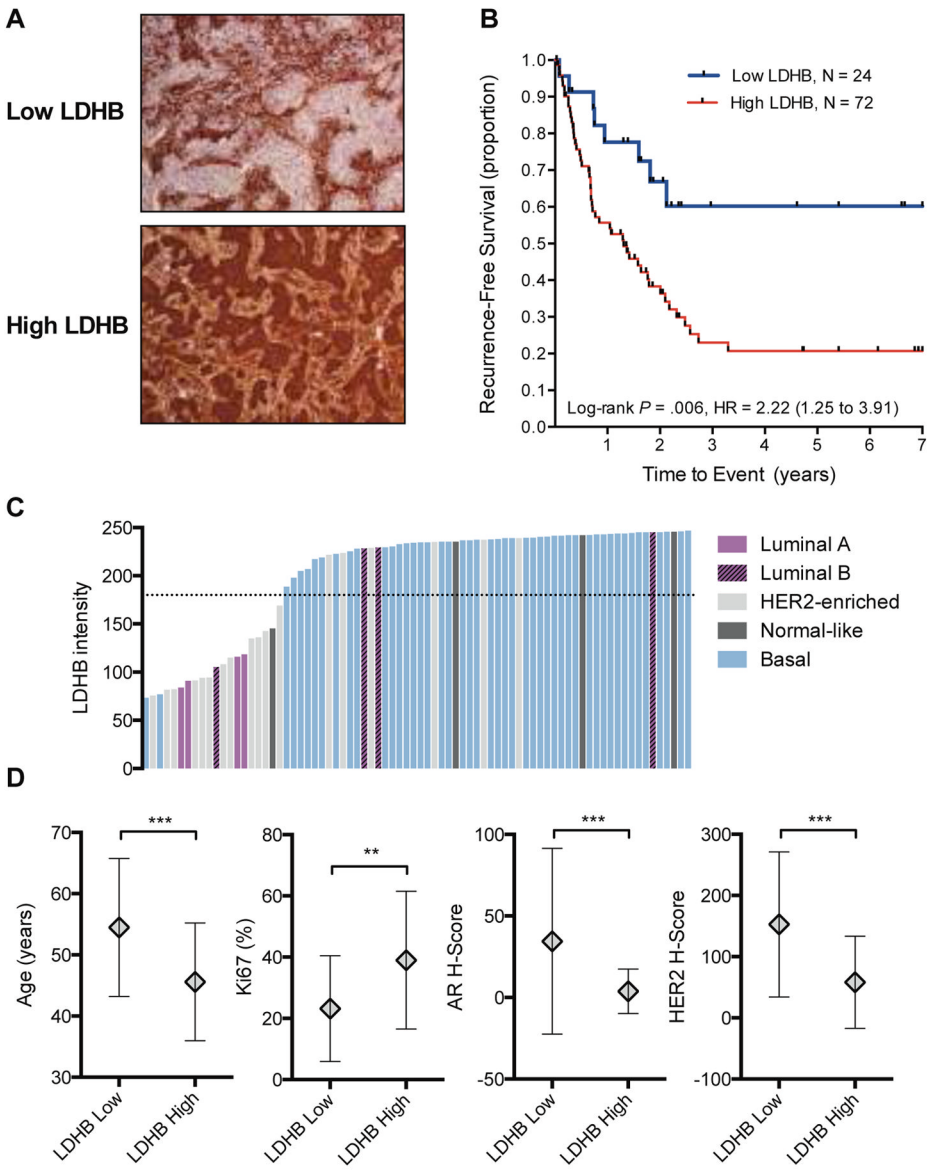

Figure 4.

High LDHB expression by IHC was associated with relapse, PAM50 basal-like phenotype, and other standard breast cancer markers in triple-negative breast cancers with residual disease post-neoadjuvant chemotherapy. **A**, representative immunostaining of LDHB in low and high-expressing triple-negative breast cancers. **B**, Kaplan-Meier curve of recurrence-free survival of patients separated by LDHB expression. **C**, LDHB intensity by IHC identified by PAM50 subtype. The dotted line represents the high/low separation for LDHB in all analyses. **D**, association of LDHB with various patient characteristics and markers including age, Ki67, AR, and HER2. Symbols and error bars represent the average ± one standard deviation. **, $P < .01$; ***, $P < .001$

Table 1

Association of clinical characteristics to neoadjuvant pCR by cohort*

	MAQC						MDACCSS					
	HR+/HER2-neg			Triple-negative			HR+/HER2-neg			Triple-negative		
Characteristic	**No.**	**% pCR**	***P***	**No.**	**% pCR**	***P***	**No.**	**% pCR**	***P***	**No.**	**% pCR**	***P***
No. of Patients	137	6.6	–	68	35.3	–	167	16.2	–	111	30.6	–
Age			1.000			.309			.676			.148
< 50 years	62	6.5		30	43.3		88	14.8		63	36.5	
≥ 50 years	75	6.7		37	29.7		79	17.7		48	22.9	
Tumor size at diagnosis (TNM)			.139			.608			1.000			.064
T0/T1/T2	94	4.3		38	39.5		86	16.3		50	40.0	
T3/T4	43	11.6		29	31.0		81	16.0		61	23.0	
Lymph Node			1.000			.304			.828			.154
Negative	48	6.3		11	18.2		62	14.5		28	42.9	
Positive	89	6.7		56	39.3		105	17.1		83	26.5	
Modified Black's Nuclear Grade			.006			.182			.003			.542
1–2	90	2.2		11	18.2		84	7.1		14	21.4	
3	45	15.6		50	42.0		71	25.4		82	31.7	
unknown or indeterminate	2	0.0		7	14.3		12	25.0		15	33.3	
PAM50 Intrinsic Subtype			.050			.048			.016			1.000
Luminal A	52	5.8		0	–		73	4.1		2	0.0	
Luminal B	33	9.1		4	25.0		42	23.8		0	–	
HER2-enriched	1	0.0		0	–		11	18.2		8	25.0	
Normal	37	0.0		15	13.3		15	20.0		9	44.4	
Basal	14	21.4		49	42.9		26	34.6		92	30.4	
LDHB (0.60 threshold)			.027			.083			.001			.359
Low	94	3.2		18	16.7		126	10.3		31	22.6	
High	43	14.0		50	42.0		41	34.1		80	33.8	

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	MAQC						MDACCSS					
	HR+/HER2-neg			Triple-negative			HR+/HER2-neg			Triple-negative		
Characteristic	**No.**	**% pCR**	***P***	**No.**	**% pCR**	***P***	**No.**	**% pCR**	***P***	**No.**	**% pCR**	***P***
LDHB (0.94 threshold)			.117			.020			.006			.087
Low	106	4.7		24	16.7		134	11.9		40	20.0	
High	31	12.9		44	45.5		33	33.3		71	36.6	

*P was calculated by Fisher's exact test. For PAM50 intrinsic subtyping, basal-like subtype was compared to other subtypes pooled (luminal A, luminal B, HER2-enriched, and normal-like).

Table 2

Binary logistical regression for odds of pCR after neoadjuvant chemotherapy by HR-status, combined MAQC and MDACCSS cohorts*

	HR-positive/HER2-negative				Triple-negative			
		95% CI for OR				95% CI for OR		
Characteristic	**Odds Ratio**	**Low**	**High**	***P***	**Odds Ratio**	**Low**	**High**	***P***
Tumor size at diagnosis (TNM)								**.017**
T0/T1/T2					**1**			
T3/T4					**0.44**	**0.23**	**0.87**	
Modified Black's Nuclear Grade				**.001**				
1–2	**1**							
3	**4.30**	**1.78**	**10.42**					
PAM50 Intrinsic Subtype				.580				.898
Luminal A	1				1			
Luminal B	1				1			
HER2-enriched	1				1			
Normal	1				1			
Basal	1.32	0.49	3.58		1.06	0.42	2.61	
LDHB (0.60 threshold)				**.018**				
Low	**1**							
High	**2.86**	**1.19**	**6.84**					
LDHB (0.94 threshold)								**.004**
Low					**1**			
High					**3.18**	**1.45**	**6.99**	

*Characteristics were included in the models if $P \leq .05$ by univariate analysis in at least one cohort (Table 1) or the combined cohort. Cases were excluded if all characteristics were not reported. The odds ratios were not statistically significant if basal-like and luminal B cases were considered as one group.