Oncogene (2009) 28, 3689–3701

www.nature.com/onc

ORIGINAL ARTICLE

# Upregulation of lactate dehydrogenase A by ErbB2 through heat shock factor 1 promotes breast cancer cell glycolysis and growth

YH Zhao[1,2,6], M Zhou[1,6], H Liu[1], Y Ding[1], HT Khong[1], D Yu[3], O Fodstad[1,4] and M Tan[1,5,7]

[1]*Mitchell Cancer Institute, University of South Alabama, Mobile, AL, USA;* [2]*Department of Biochemistry and Molecular Biology, West China Medical Center, Sichuan University, Chengdu, China;* [3]*Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA;* [4]*Institute for Cancer Research, The Norwegian Radium Hospital, University of Oslo, Oslo, Norway and* [5]*Department of Cell Biology and Neuroscience, University of South Alabama, Mobile, AL, USA*

**ErbB2 has been shown to activate signaling molecules that may regulate glucose metabolism. However, there is no evidence reported to directly link ErbB2 to glycolysis, and the mechanism underlying ErbB2-enhanced glycolysis is poorly understood. In this study, we investigated the role and mechanism of ErbB2 in regulating glycolysis. We found that ErbB2-overexpressing cells possessed a significantly higher level of glycolysis when compared to the ErbB2-low-expressing cells, and the downregulation of ErbB2 markedly decreased glycolysis. Overexpression of ErbB2 increased the expression of glycolysis-regulating molecules lactate dehydrogenase A (LDH-A) and heat shock factor 1 (HSF1). ErbB2 activated HSF1, indicated by the increased HSF1 trimer formation, and promoted HSF1 protein synthesis. HSF1 bound to LDH-A promoter and the downregulation of HSF1 reduced the expression of LDH-A and subsequently decreased cancer cell glycolysis and growth. Moreover, the glycolysis inhibitors, 2-deoxyglucose and oxamate, selectively inhibited the growth of ErbB2-overexpressing cells. Taken together, this study shows that in human breast cancer cells, ErbB2 promotes glycolysis at least partially through the HSF1-mediated upregulation of LDH-A. This pathway may have a major role in regulating glucose metabolism in breast cancer cells. These novel findings have important implications for the design of new approaches to target ErbB2-overexpressing breast cancers.**
*Oncogene* (2009) **28,** 3689–3701; doi:10.1038/onc.2009.229; published online 10 August 2009

**Keywords:** ErbB2; HSF1; LDH-A; glycolysis; Warburg effect; breast cancer

## Introduction

Cancer cells are different from nonneoplastic cells in their metabolic properties. Normal cells mostly rely on the process of mitochondrial oxidative phosphorylation, which consumes oxygen and glucose to produce energy. In contrast, cancer cells depend mostly on glycolysis, the aerobic breakdown of glucose into the energy-storing molecule adenosine triphosphate (ATP). This altered energy dependency is known as the 'Warburg effect' and is a hallmark of cancer cells (Warburg, 1956; Kim and Dang, 2006; Chen *et al*., 2007; Gatenby and Gillies, 2007; DeBerardinis *et al*., 2008; Gillies *et al*., 2008; Hsu and Sabatini, 2008; Kroemer and Pouyssegur, 2008).

*ErbB2* (Her2/neu) is an oncogene that is overexpressed in approximately 30% of breast cancers and is correlated with a poor prognosis (Slamon *et al*., 1989). We and others (Guy *et al*., 1992; Tan *et al*., 1997, 2005, 2006a, 2006b) have previously shown that the overexpression of ErbB2 increases the transformation and/or metastatic potentials of breast cancer cells. In addition, ErbB2 has been shown to activate signaling molecules that regulate bioenergetic metabolism, such as Ras, PI3K/Akt, mTOR and Src (Yarden and Sliwkowski, 2001; Zhou *et al*., 2004; Tan *et al*., 2005, 2006b; Zhang *et al*., 2007). A recent study investigated the role of lactate dehydrogenase A (LDH-A), a critical enzyme in the glycolysis pathway, in the tumor maintenance of neu-transformed mouse mammary epithelial cells, showing that compared to normal cells, cancer cells have deregulated bioenergetic metabolism and increased glycolysis (Fantin *et al*., 2006). However, this study did not systemically investigate the role of ErbB2 in cancer cell glycolysis and this was performed in mouse mammary epithelial cells. So far, there is no reported evidence that directly links the overexpression of ErbB2 to increased glycolysis in human breast cancer cells. Moreover, the impact of ErbB2-mediated changes in energy dependency on human breast cancer progression and the mechanism underlying ErbB2-mediated glycolysis are still not fully understood.

Heat shock factor 1 (HSF1) is a transcriptional factor that has a critical role in the regulation of the heat shock response in eukaryotes (Westerheide and Morimoto, 2005). On exposure to a variety of stresses, HSF1 oligomerizes into active trimers, translocates into the nucleus and binds with heat shock elements located in the promoter region of target genes, subsequently activating the heat shock response (Westerheide and Morimoto, 2005). Although still not well understood,

Correspondence: Dr M Tan, Center for Basic and Translational Cancer Research, USA Mitchell Cancer Institute, 1660 Spring Hill Avenue, Mobile, AL 36604, USA.
E-mail: mtan@usouthal.edu
[6]These authors contributed equally to this work.
[7]M Tan is a Vincent F Kilborn, Jr Cancer Research Scholar.
Received 15 December 2008; revised 1 July 2009; accepted 8 July 2009; published online 10 August 2009

the functions of HSF1 are beyond the classical induction of heat shock responses. Recent reports have shown that HSF1 may have an important role in cancer development (Khaleque *et al*., 2005, 2008; Min *et al*., 2007). A recent study showed that the elimination of HSF1 protects mice from tumors induced by mutations of the *Ras* oncogene or a hot spot mutation in the tumor suppressor *p53* (Dai *et al*., 2007). HSF1 supports malignant transformation by orchestrating a network of core cellular functions including proliferation, survival, protein synthesis and glucose metabolism. Thus, HSF1 functions as a powerful multifaceted modifier of carcinogenesis (Dai *et al*., 2007).

We report here that the overexpression of ErbB2 leads to increased glucose uptake, lactate production and decreased oxygen consumption in multiple human breast cancer cell lines. ErbB2 overexpression increases LDH-A expression and its activity through the upregulation of HSF1, resulting in increased glycolysis. This pathway may have a major role in glucose metabolism in cancer cells. Moreover, we report that ErbB2-overexpressing human breast cancer cells are more sensitive to the glycolysis inhibitors 2-deoxyglucose (2-DG) and oxamate. These novel findings provide insight into the future development of therapeutics for ErbB2-overexpressing cancers.

## Results

### *Overexpression of ErbB2 promotes glycolysis in human breast cancer cells*

To examine whether the overexpression of ErbB2 alters glucose metabolism in human breast cancer cells, we used the isogenic ErbB2-stable transfectants derived from breast cancer cell lines, MCF7, and from MDA-MB-435 cells (Benz *et al*., 1992; Tan *et al*., 2002, 2005). ErbB2 expression levels were confirmed by immunoblot analysis (Figure 1a). ErbB2 overexpression was shown in MCF7/ErbB2 and 435ErbB2 cells, compared to the much lower ErbB2 levels in their corresponding control cells (MCF7 and 435 neo). Glucose uptake, lactate production and oxygen consumption, which are hallmarks of glycolysis, were measured and compared in ErbB2-low-expressing and ErbB2-high-expressing cells. Both MCF7/ErbB2 and 435ErbB2 cells showed a significantly higher glucose uptake (Figure 1b) and lactate production (Figure 1c) but lower oxygen consumption rates (Figure 1d) than the MCF7 and 435 neo cells, respectively. These results strongly suggest an important link between ErbB2 expression and glycolysis, indicating that ErbB2 overexpression may promote glycolysis in human breast cancer cells.

### *Overexpression of ErbB2 transcriptionally activates LDH-A and promotes glycolysis*

LDH-A has a critical role in glycolysis and neu-transformed mouse tumor maintenance (Fantin *et al*., 2006). To examine the mechanism of ErbB2-induced glycolysis in human breast cancer cells, we compared the LDH-A protein levels between ErbB2-low and ErbB2-overexpressing cells by immunoblotting (Figure 2a, left top). Compared with MCF7 and 435 neo cells, MCF7/ErbB2 and 435ErbB2 cells showed higher LDH-A protein levels. To test whether other LDH isoforms would be affected by ErbB2, we detected LDH-B expression in these cells. As shown in Figure 2a (left bottom), LDH-B proteins were not detectable in MCF7 cells, as reported earlier (Balinsky *et al*., 1983), and the levels of LDH-B were similar between 435 neo and 435ErbB2 cells, indicating that ErbB2 has no effect on LDH-B expression. To examine whether ErbB2 upregulates LDH-A at the transcriptional level, the mRNA of LDH-A were measured by quantitative RT–PCR (qRT–PCR; Figure 2a, upper middle and upper right) and semiquantitative RT–PCR (Figure 2a, lower middle and lower right). The LDH-A mRNA expression in ErbB2-overexpressing cells was higher than that in their ErbB2-low-expressing counterparts, indicating that ErbB2 activates LDH-A at the transcriptional level. When the LDH enzyme activity was measured in these cells, we found that the LDH activity in MCF7/ErbB2 cells was much higher than that of MCF7 cells, and the LDH activity of 435ErbB2 was also higher than that of 435 neo cells (Figure 2b). These results indicated that ErbB2 overexpression upregulates LDH-A expression and activity in human breast cancer cells.

To confirm the causal relationship between ErbB2, LDH-A and glycolysis, the naturally ErbB2-overexpressing BT474M1 breast cancer cells were used to test whether LDH-A expression and glycolysis would decrease with the knockdown of ErbB2 expression. Downregulation of ErbB2 by siRNA led to decreased LDH-A expression in BT474M1 cells (Figure 2c, left). Furthermore, the downregulation of ErbB2 decreased both glucose uptake and lactate production but increased oxygen consumption rate (Figure 2c, right). We performed similar experiments in MCF7/ErbB2 cells and the results were similar (Figure 2d). To avoid off-target effects, these results were confirmed using a second ErbB2-targeting siRNA sequence (data not shown). These results confirmed the link between ErbB2, LDH-A expression and activity. To examine whether the ErbB2-upregulated LDH-A is essential in ErbB2-enhanced glycolysis in human breast cancer cells, an LDH-A targeted siRNA was used to treat ErbB2-overexpressing MCF7/ErbB2 or BT474M1 cells. LDH-A siRNA effectively downregulated LDH-A, inhibited the glucose uptake, LDH activity (Supplementary Figure 1) and proliferation of these cells (Supplementary Figure 2), indicating that LDH-A is essential in ErbB2-mediated glycolysis and proliferation in human breast cancer cells.

### *Overexpression of ErbB2 leads to the upregulation of HSF1*

Recently, it has been reported that HSF1 modulates glucose metabolism and has an important role in cancer development (Dai *et al*., 2007). To investigate the role of HSF1 in ErbB2-mediated glycolysis, we examined the

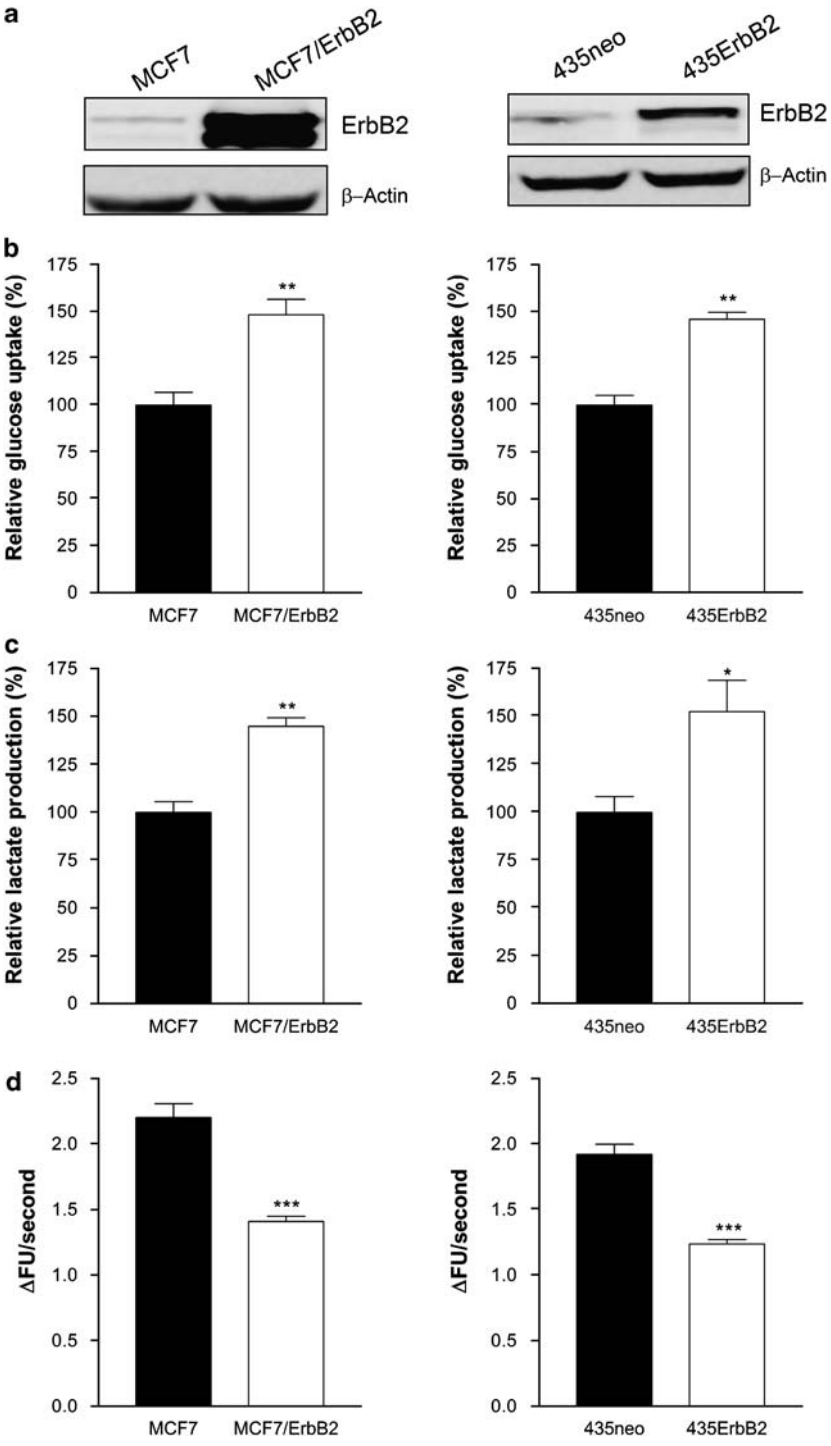


**Figure 1** ErbB2 overexpression enhances glycolysis in breast cancer cells. (**a**) ErbB2 protein expressions in breast cancer cells MCF7, MCF7/ErbB2 (left), MDA-MB-435 neo, MDA-MB-435ErbB2 (right) were detected by using an ErbB2 antibody. β-Actin was used as a loading control. (**b**) ErbB2 increased the glucose uptake. Cells were cultured in medium containing 10% fetal bovine serum (FBS) and glucose uptake was measured. Data are shown in percentage relative to MCF7 (left) or 435 neo (right). (**c**) ErbB2 increased lactate production. Cells were cultured in medium containing 10% FBS and lactate production in the medium was measured as described in Materials and methods. Data are shown in percentage relative to MCF7 (left) or 435 neo (right). (**d**) ErbB2 decreased oxygen consumption. Oxygen consumption rates of MCF7, MCF7/ErbB2 (left), 435 neo and 435ErbB2 cells (right) were measured. The oxygen consumption rate was calculated on the basis of the maximal rate of change in relative fluorescence units (ΔFU/second). Columns, mean of three independent experiments; bars, s.e. $*P<0.05$ $**P<0.01$; $***P<0.001$.

HSF1 expression in our breast cancer cell variants by immunoblotting. The HSF1 protein levels were higher in MCF7/ErbB2 and 435ErbB2 cells than in MCF7 and 435 neo cells, respectively (Figure 3a, left and middle). Silencing of ErbB2 by siRNA in BT474M1 cells led to a marked reduction of HSF1, and this result was verified

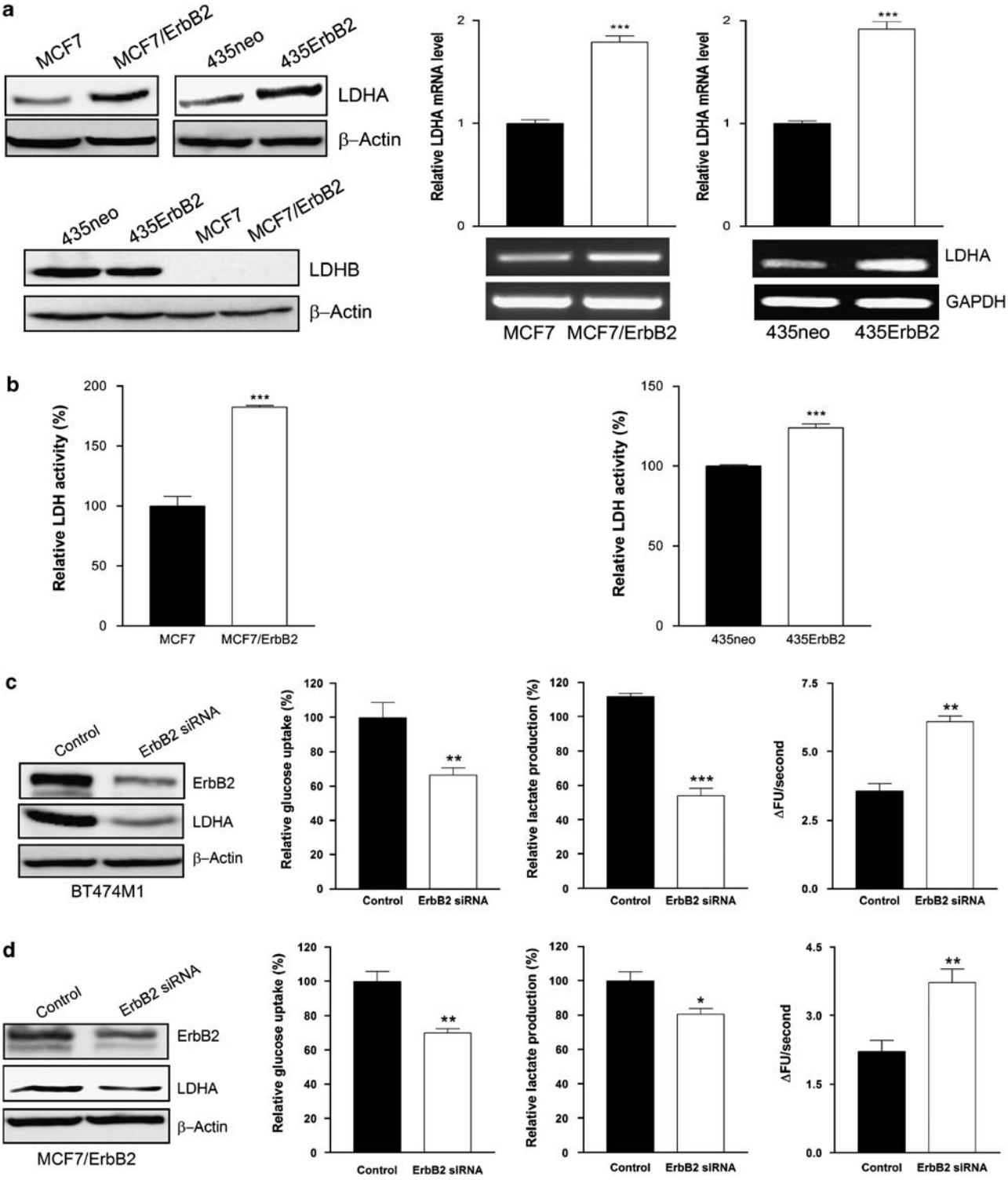


**Figure 2** ErbB2 upregulates lactate dehydrogenase A (LDH-A). (**a**) Western blot analysis with anti-LDH-A (left top) or anti-LDH-B (left bottom) antibody of total cell extract from MCF7, MCF7/ErbB2, 435 neo and 435ErbB2 cells. The β-actin protein served as a loading control. LDH-A mRNA levels were measured by quantitative RT–PCR (qRT–PCR; top) and semiquantitative PCR (bottom) using the total RNA samples isolated from MCF7, MCF7/ErbB2 (middle), 435 neo and 435ErbB2 cells (right). The relative LDH-A mRNA levels were normalized relative to GAPDH; top). GAPDH was used as a loading control (bottom). (**b**) LDH activity in MCF7, MCF7/ErbB2 (left), 435 neo and 435ErbB2 cells (right). Activities are presented as the percentage of the activity measured in MCF7 or 435 neo cells. (**c**) BT474M1 cells were transfected with scramble siRNA (Control) or ErbB2 siRNA. Forty-eight hours after siRNA transfection, cell lysates were prepared and immunoblot analyses were carried out with antibodies against ErbB2, LDH-A and β-actin (left). Forty-eight hours after transfection, cells were transferred to 24-well plates for glucose uptake, lactate production and oxygen consumption rate assay (right). Data are shown in percentage relative to control-transfected cells. (**d**) Similar experiments were performed in MCF7/ErbB2 cells as described under panel c. Columns, mean of three independent experiments; bars, s.e. $*P<0.05$, $**P<0.01$, $***P<0.001$.

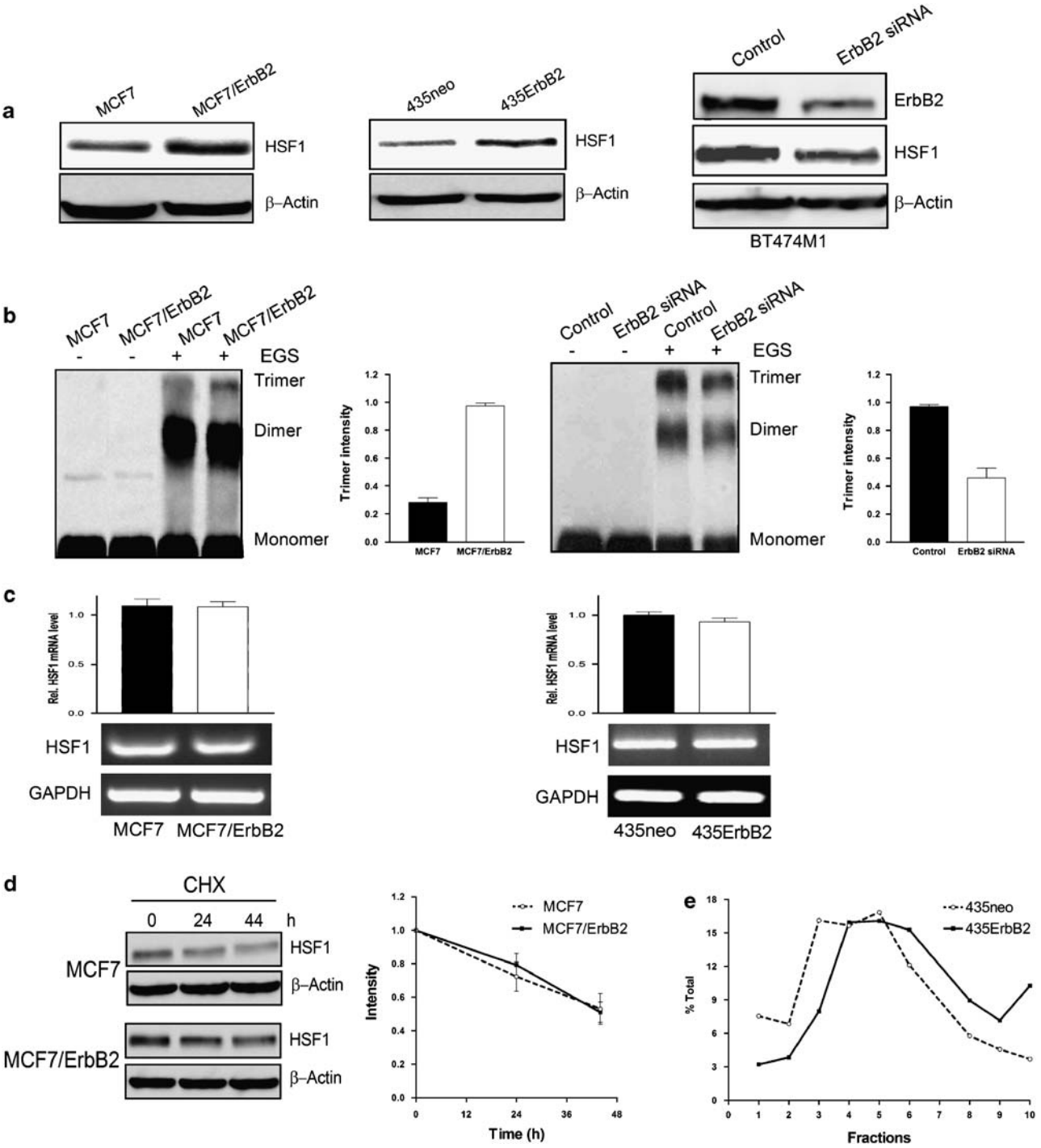


**Figure 3** ErbB2 upregulates heat shock factor 1 (HSF1). (**a**) Western blot analysis with anti-HSF1 antibody of total cell extract from MCF7, MCF7/ErbB2 (left), 435 neo and 435ErbB2cells (middle), BT474M1 cells transfected with siRNA (Control) or ErbB2 siRNA (right). The β-actin protein was used as a loading control. (**b**) Cell extracts from MCF7, MCF7/ErbB2 (left) or BT474M1 cells (right) transfected with scramble siRNA (Control) or ErbB2 siRNA were incubated in the absence or in the presence of cross-linker ethylene glycol bis (succinimidylsuccinate) (EGS) at a final concentration of 1.0 mM. The reaction mixture was run on an SDS–6% polyacrylamide gel electrophoresis (PAGE) and subjected to western blot assay using the HSF1 antibody. The relative intensity of HSF1 trimer was estimated by densitometry. Columns, mean of three independent experiments; bars, s.e. (**c**) HSF1 mRNA levels were measured by quantitative RT–PCR (qRT–PCR; top) and semiquantitaitve PCR (bottom) using the total RNA samples isolated from MCF7, MCF7/ErbB2 (left), 435 neo and 435ErbB2 cells (right). The relative HSF1 mRNA levels were normalized relative to GAPDH; top). GAPDH was used as a loading control (bottom). Columns, mean of three independent experiments; bars, s.e. (**d**) MCF7 and MCF7/ErbB2 cells were treated with 200 μg/ml cycloheximide (CHX) for 24 and 44 h; cell lysates were collected for western blot with antibodies against HSF1 and β-actin (left). The relative intensity of HSF1 band was normalized to its β-actin loading. Degradation curves were drawn on the basis of three independent experiments (right). (**e**) HSF1 mRNA distribution in polysome fractions of 435 neo and 435ErbB2 cells. The results are presented as percent of the HSF1 mRNA in each fraction, where the total amount of the HSF1 mRNA in all fractions was set at 100%.

by using a second ErbB2-directed siRNA sequence (Figure 3a, right and data not shown). Moreover, when cells were treated with Herceptin, an ErbB2-targeting antibody, the ErbB2-mediated induction of HSF1 was inhibited in a dose-dependent manner (Supplementary Figure 3). These results show that overexpression of ErbB2 upregulates HSF1.

In normal cells, HSF1 exists as an inactive monomer that is rapidly converted to trimer after heat shock resulting in binding to DNA. We examined HSF1 trimer formation in MCF7 and MCF7/ErbB2 cells. Cell extracts were treated with the chemical cross-linker, ethylene glycol bis (succinimidylsuccinate) (EGS), and subjected to SDS–polyacrylamide gel electrophoresis (PAGE) and western blot. In the presence of EGS, a portion of HSF1 in MCF7/ErbB2 cells was converted to its trimer form, which was not detectable in MCF7 cells (Figure 3b, left). To confirm the result, we downregulated ErbB2 by siRNA in BT474M1 cells and analysed the oligomeric state of HSF1. Compared to control cells, the downregulation of ErbB2 led to a decrease in HSF1 trimer formation (Figure 3b, right). These results indicate that overexpression of ErbB2 activates HSF1 in breast cancer cells.

To test whether the increased HSF1 protein levels resulted from the upregulation of HSF1 mRNA by ErbB2, qRT–PCR (Figure 3c, upper) and semiquantitative RT–PCR (Figure 3c, lower) were performed. The mRNA levels in ErbB2-overexpressing and -low-expressing cells were similar, indicating that ErbB2 upregulates HSF1 at the posttranscriptional level. We then asked if ErbB2 could stabilize HSF1. After cycloheximide (CHX) treatment, HSF1 protein degraded at a similar rate in MCF7 cells as in MCF7/ErbB2 cells (Figure 3d), indicating that ErbB2 upregulation of HSF1 is not by stabilizing HSF1 protein. In conjunction with the results that ErbB2 does not increase the mRNA level of HSF1, we hypothesize that ErbB2 may upregulate HSF1 by promoting the translational mechanism of HSF1. We performed polysome analysis in 435 neo and 435ErbB2 cells as we did earlier (Lu *et al*., 2009) to examine whether ErbB2 promotes HSF1 protein translation. As shown in Figure 3e, HSF1 mRNA was distributed to the heavier polysome fractions in 435ErbB2 cells than in 435 neo cells, indicating an increased number of ribosomes associated with HSF1 transcripts in 435ErbB2 cells. These results indicate that ErbB2-mediated HSF1 upregulation is largely at the translational level. As HSF1 was shown to be involved in cancer cell glycolysis, these results also suggested that ErbB2-mediated HSF1 upregulation might serve as a mechanism for ErbB2-enhanced glycolysis in breast cancer cells.

*Downregulation of HSF1 leads to decreased LDH-A expression and decreased glycolysis*

To study the role of HSF1 in ErbB2-induced glycolysis, the effect of HSF1 on LDH-A expression, glucose uptake and lactate production were examined in MCF7/ErbB2 cells. When HSF1 was downregulated by siRNA, the protein levels of LDH-A also decreased (Figure 4a, left). Furthermore, HSF1 siRNA dramatically reduced lactate production (Figure 4a, right), indicating that HSF1 is essential in regulating LDH-A expression and activity in breast cancer cells. We performed similar experiments using another specific HSF1 siRNA sequence and the results were similar (data not shown). To further confirm these results, we compared LDH-A protein levels and lactate production between wild-type $Hsf1^{+/+}$ and HSF1 knockout $Hsf1^{-/-}$ mouse embryonic fibroblasts. Compared to $Hsf1^{+/+}$ cells, $Hsf1^{-/-}$ cells had a lower LDH-A protein level and a lower lactate production (Figure 4b), which is consistent with the results obtained after the HSF1 siRNA experiment. To examine how HSF1 regulates LDH-A expression, qRT–PCR was performed in control, HSF1 siRNA-treated MCF7/ErbB2 cells, and in $Hsf1^{+/+}$ and $Hsf1^{-/-}$ cells (Figure 4c). The results show that the downregulation of HSF1 in MCF7/ErbB2 cells and elimination of HSF1 in $HSF1^{-/-}$ cells led to decreased mRNA levels of LDH-A, indicating that HSF1 regulates LDH-A at the transcription level. Next, we investigated whether HSF1 binds to the LDH-A promoter. We searched the motif database on http//motif.genome.jp and found that there is a putative HSF1 binding site (−3819 to −3810) on the LDH-A promoter. A chromatin immunoprecipitation (ChIP) assay was performed in MCF7 cells to test whether HSF1 binds to this putative binding site *in vivo*. As shown in Figure 4d, after ChIP, a 329 bp segment of the LDH-A promoter (lane 3) was amplified by PCR as well as by the positive control, a 185 bp segment of the Hsp70 promoter (lane 2) containing a known HSF1 binding site (Wadekar *et al*., 2004). A negative control PCR designed to amplify a 228 bp region of glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Liu *et al*., 2008) did not yield a PCR product following ChIP using the HSF1 antibody (lane 1) and IP with the control immunoglobulin G (IgG) also did not yield a product (lane 4). These data together with Figure 2a indicate that HSF1 regulates LDH-A at the transcriptional level by binding to the LDH-A promoter. These results proved that HSF1 has a critical role in the upregulation of LDH-A and LDH-A-mediated glycolysis in ErbB2-overexpressing cancer cells.

It was reported earlier that heregulin β1 (HRGβ1), which activates signaling of ErbB family receptors, induces heat shock proteins by inducing HSF1 (Khaleque *et al*., 2005). We asked whether HRGβ1 induces LDH-A expression in an HSF1-dependent manner. We downregulated HSF1 by siRNA in MCF7 cells and then treated the cells with HRGβ1. HRGβ1 induces HSF1 expression and subsequently induces LDH-A expression in control cells (Supplementary Figure 4, left). However, there is no obvious induction of LDH-A by HRGβ1 in HSF1 siRNA-treated cells (Supplementary Figure 4, right). These data suggest that HRGβ1 induces LDH-A expression by upregulating HSF1, and supports that HSF1 has an important role in ErbB2-mediated glycolysis.

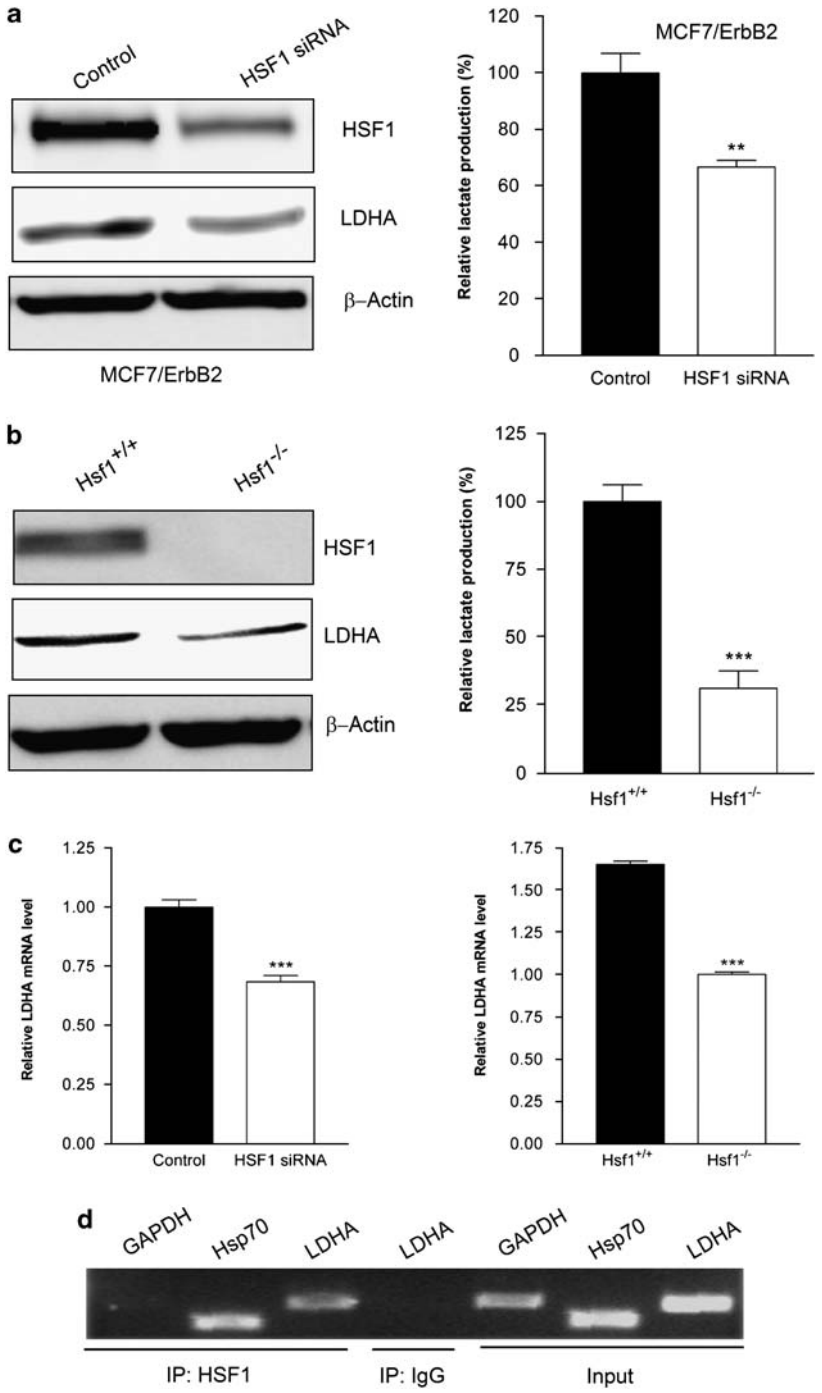


**Figure 4** Downregulation of heat shock factor 1 (HSF1) decreases lactate dehydrogenase A (LDH-A) expression and glycolysis. (**a**) MCF7/ErbB2 cells were transfected with scramble siRNA (Control) or HSF1 siRNA. Forty-eight hours after siRNA transfection, cell lysates were prepared and immunoblot analyses were carried out with antibodies against HSF1, LDH-A and β-actin (left). Forty-eight hours after siRNA transfection, cells were transferred to 24-well plates for lactate production assay (right). Data are shown in percentage relative to control-transfected cells. (**b**) Western blot analysis with antibodies against HSF1 and LDH-A of total cell extract from $Hsf1^{+/+}$ and $Hsf1^{-/-}$ mouse embryonic fibroblasts (MEFs). The β-actin protein was used as a loading control (left). Lactate production of $Hsf1^{+/+}$ and $Hsf1^{-/-}$ MEFs (right) was measured. (**c**) MCF7/ErbB2 cells (left) were transfected with scramble siRNA (Control) or HSF1 siRNA. Twenty-four hours after siRNA transfection, the total RNA was isolated and LDH-A mRNA levels were measured by quantitative RT–PCR (qRT–PCR; left). LDH-A mRNA levels were measured by qRT-PCR using the total RNA samples isolated from $Hsf1^{+/+}$ and $Hsf1^{-/-}$ cells (right). The relative LDH-A mRNA levels were normalized relative to GAPDH). Columns, mean of three independent experiments; bars, s.e. $^{**}P<0.01$, $^{***}P<0.001$. (**d**) Chromatin immunoprecipitation (ChIP) was performed in MCF7 cells. Cells were fixed with formaldehyde, lysed and then sonicated. *In vivo* cross-linked chromatin was then precipitated using an antibody against HSF1. Non-specific rabbit immunoglobulin G (IgG) was used as a negative control. After washing and reversal of cross-linking, PCR was performed using primers against the LDH-A promoter. An unrelated genomic region of GAPDH was used as negative control and a known HSF1 binding region on the Hsp70 promoter was used as positive control.

*Overexpression of ErbB2 increases the sensitivity of breast cancer cells to glycolysis inhibitors*

It is well accepted that glycolysis is an important process that supports cancer cell malignant phenotypes (Zu and Guppy, 2004; Garber, 2006; Gatenby and Gillies, 2007; Gillies *et al*., 2008; Hsu and Sabatini, 2008). As ErbB2-overexpressing cells have enhanced glycolysis, we reasoned that glycolysis inhibitors may selectively inhibit the growth of ErbB2-overexpressing cells. 2-DG, a synthetic glucose analog, is one of the glycolysis inhibitors that has been shown to have remarkable effects in inhibiting the growth of cancer cells and is currently under phase I/II clinical trials (Pelicano *et al*., 2006). When we treated ErbB2-low and -overexpressing cells with 2-DG, it effectively inhibited the growth of both 435 neo and 435ErbB2 cells (Figure 5a). Interestingly, 2-DG showed a stronger inhibitory effect on 435ErbB2 cells compared to 435 neo cells, indicating that ErbB2-overexpressing cells are more sensitive to this glycolysis inhibitor (Figure 5a). Oxamate, a more specific glycolysis inhibitor that uses a different mechanism of action from 2-DG (Ramanathan *et al*., 2005), also showed selectivity in inhibiting ErbB2-overexpressing cells (Figure 5b). As glycolysis and mitochondrial oxidative phosphorylation are linked processes (Fantin *et al*., 2006), we tested if ErbB2-overexpressing cells, which possess a higher level of glycolysis, are less sensitive to inhibitors of oxidative phosphorylation. The MCF7 and MCF7/ErbB2 cells were treated with oligomycin, an ATPase inhibitor that specifically inhibits mitochondrial oxidative phosphorylation (Nguyen *et al*., 2008). In contrast to 2-DG and oxamate, oligomycin showed a stronger inhibitory effect on MCF7 cells compared to MCF7/ErbB2 cells (Figure 5c), suggesting that ErbB2-overexpressing cells may have a lower level of mitochondrial oxidative phosphorylation. To further confirm that the different sensitivity to glycolysis inhibitors between ErbB2-low

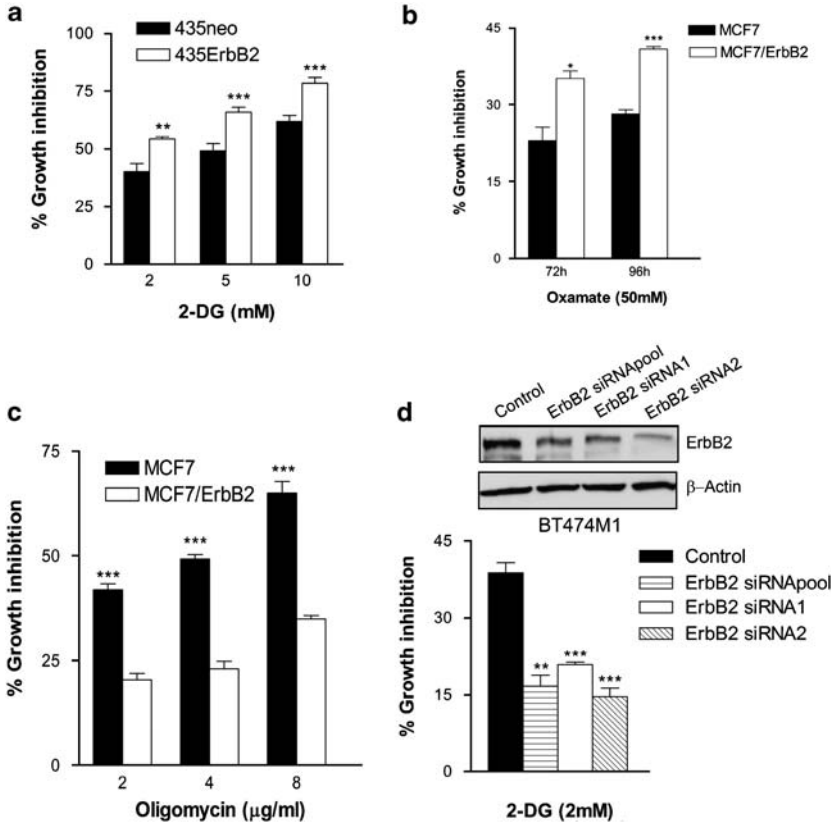


**Figure 5** ErbB2-overexpressing cells are more sensitive to glycolysis inhibitor. (**a**) 435 neo and 435ErbB2 cells were cultured in a 96-well plate. Twenty-four hours later, the medium was replaced with fresh medium with or without 2-deoxyglucose (2-DG) and incubated for 48 h. Then, the cell viability was detected using an MTS reagent. (**b**) MCF7 and MCF7/ErbB2 cells were cultured in a 96-well plate. Twenty-four hours later, the medium was replaced with fresh medium with or without oxamate and incubated for 72 or 96 h. Then, the cell viability was detected using an MTS reagent. (**c**) MCF7 and MCF7/ErbB2 cells were cultured in a 96-well plate. Twenty-four hours later, the medium was replaced with fresh medium with or without oligomycin and incubated for 48 h. Then, the cell viability was detected using an MTS reagent. Data are presented as the percentage of growth inhibition measured in cells treated without oligomycin. (**d**) BT474M1 cells were transfected with scramble siRNA (Control), ErbB2 siRNApool or two specific ErbB2 siRNA (ErbB2 siRNA1 or ErbB2 siRNA2). Forty-eight hours after siRNA transfection, cell lysates were prepared and immunoblot analyses were carried out with antibodies against ErbB2 and β-actin (upper). Forty-eight hours after transfection, cells were transferred to 96-well plates for 2-DG treatment as described above (lower). Data are presented as the percentage of growth inhibition measured in cells treated without 2-DG, oxamate or oligomycin. Columns, mean of three independent experiments; bars, s.e. $*P<0.05$, $**P<0.01$, $***P<0.001$.

and -overexpressing cells was due to the different expression levels of ErbB2, BT474M1 cells were treated with siRNA to downregulate ErbB2 (Figure 5d, upper), and then treated with 2-DG. Although the overall inhibition of cell growth was more potent by the combination of ErbB2 knockdown and 2-DG treatment (data not shown), the percent inhibition of cell growth by 2-DG treatment was much less in the ErbB2 knocked-down cells compared to the percent inhibition in the control ErbB2-overexpressing cells (Figure 5d, lower). These results indicate that the overexpression of ErbB2 confers an increased sensitivity to the cell growth inhibitory effect of glycolysis inhibitors.

*Downregulation of HSF1 decreases ErbB2-mediated glycolysis, cell growth and sensitivity to glycolysis inhibition*

Our data show that HSF1 has a critical role in ErbB2-mediated glycolysis (Figure 4), whereas the overexpression of ErbB2 increases the sensitivity of cancer cells to glycolysis inhibitors (Figure 5). The link between these was studied through the downregulation of HSF1 by siRNA in MCF7 and MCF7/ErbB2 cells (Figure 6a). Glucose uptake and the cell growth rate were measured. HSF1 siRNA significantly inhibited the glucose uptake (Figure 6b) and cell growth (Figure 6c) in both MCF7 and MCF7/ErbB2 cells. It is noted that the downregulation of HSF1 had a stronger inhibitory effect on both the glucose uptake and cell growth of MCF7/ErbB2 cells than on that of MCF7 cells (Figure 6b and c). These results were confirmed by similar experiments using another specific HSF1 siRNA (data not shown).

To further examine whether HSF1 confers the increased sensitivity of ErbB2-overexpressing cells to glycolysis inhibitors, HSF1 was downregulated by siRNA in BT474M1 cells (Figure 6d, inset). Control siRNA or HSF1 siRNA-treated BT474M1 cells were further treated with 2-DG or oxamate to examine whether downregulation of HSF1 results in decreased sensitivity. As expected, downregulation of HSF1 in BT474M1 cells attenuated their sensitivity to 2-DG (Figure 6d, left) and oxamate (Figure 6d, right). Moreover, compared to $Hsf1^{+/+}$ cells, $Hsf1^{-/-}$ mouse embryonic fibroblasts (MEFs) showed less sensitivity to 2-DG (Figure 6e, left) and oxamate (Figure 6e, right). These results showed that HSF1 has an important role in ErbB2-mediated cancer cell glucose metabolism and in ErbB2-mediated breast cancer cell growth.

## Discussion

Thus far, there is no direct evidence reported to support that ErbB2 overexpression leads to increased glycolysis in human breast cancer cells. In this study, we used two pairs of breast cancer cell lines with engineered ErbB2-overexpression and a natural ErbB2-overexpressing cell line to systemically address the role of ErbB2 in glycolysis in human breast cancer cells. The forced overexpression of ErbB2 increased glycolysis in both MDA-MB-435 and MCF7 cells, and the downregulation of ErbB2 by two different ErbB2-targeted siRNAs in BT474 cells decreased glycolysis, indicating that overexpression of ErbB2 indeed promotes glycolysis in these human breast cancer cells.

We found that, compared to control cells, ErbB2-overexpressing human breast cancer cells had higher expression levels of LDH-A. A further study revealed that ErbB2 upregulates LDH-A at the transcriptional level. Moreover, LDH-A-targeted siRNA and LDH-specific inhibitor oxamate selectively inhibits the glucose uptake, LDH activity, lactate production and the growth of ErbB2-overexpressing cells. These results clearly indicate that overexpression of ErbB2 promotes glycolysis at least in part through the transcriptional upregulation of LDH-A and results in enhanced glycolysis in ErbB2-overexpressing breast cancer cells.

Dai *et al*., 2007 showed that the downregulation of HSF1 led to decreased glycolysis in mouse embryonic fibroblasts. Here, for the first time, we show that ErbB2 induces HSF1 trimer formation and promotes HSF1 protein synthesis. We further show for the first time that HSF1 binds to the LDH-A promoter and that the downregulation of HSF1 reduced the expression of LDH-A and subsequently inhibited cell glycolysis and growth in breast cancer cells. Moreover, the downregulation of HSF1 in ErbB2-overexpressing cells led to reduced cell glycolysis and growth, and the inhibition on cell glycolysis and growth by downregulation of HSF1 is stronger in ErbB2-overexpressing cells. These novel findings indicate that the upregulation of LDH-A by ErbB2 through HSF1 has an important role in ErbB2-enhanced breast cancer cell glycolysis and growth.

Overexpression of ErbB2 results in constitutively activated PI3K/Akt, which in turn activates the hypoxia-inducible transcription factor hypoxia-inducible factor-1α (HIF1α) (Laughner *et al*., 2001; Li *et al*., 2005). It is known that HIF1α transcriptionally upregulates genes that encode glycolytic enzymes, including LDH-A (Dang and Semenza, 1999). Here, for the first time we show that in addition to HIF1α, the activation of transcription factor HSF1 also has a critical role in ErbB2-mediated upregulation of LDH-A, a critical enzyme in cell glycolysis. Although our data show that HSF1 regulates LDH-A at the transcriptional level by binding to the LDH-A promoter, it is possible that HSF1 also regulates LDH-A expression at the post-transcriptional level. Currently, we are studying the relationships among ErbB2, PI3K/Akt, HIF1α and HSF1 in ErbB2-overexpressing breast cancer cells.

The mechanisms that alter the bioenergetic metabolism in cancer cells are still not fully understood. However, it is generally accepted that increased glycolysis offers cancer cells an advantage to allow them to better proliferate, survive and invade. We show that targeting glycolysis-regulating molecules, HSF1 and LDH-A, with siRNA and the glycolysis inhibitors inhibited cancer cell glycolysis and growth. In addition, compared to their counterparts, ErbB2-overexpressing cancer cells showed increased sensitivity to 2-DG and oxamate. The downregulation of ErbB2 or HSF1 in

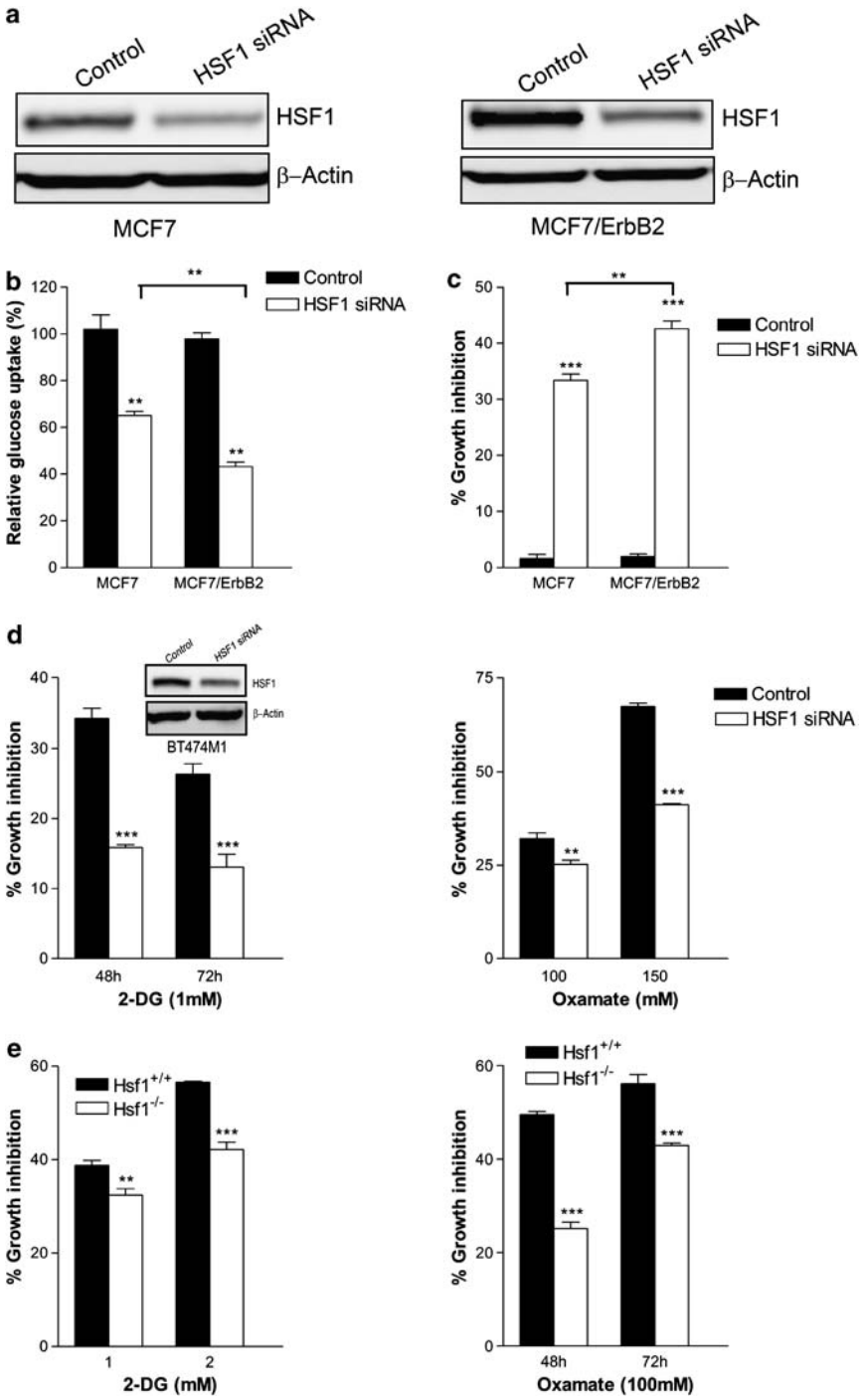


**Figure 6** Downregulation of heat shock factor 1 (HSF1) inhibits ErbB2-enhanced glycolysis, cell growth and decreases the sensitivity to glycolysis inhibitor. (**a**) MCF7 (left) and MCF7/ErbB2 (right) cells were transfected with scramble siRNA (Control) or HSF1 siRNA. Forty-eight hours after siRNA transfection, cell lysates were prepared and immunoblot analyses were carried out with antibodies against HSF1 and β-actin. (**b**) Forty-eight hours after transfection, cells were transferred to 24-well plates for glucose uptake assay. Data are shown in percentage relative to control-transfected cells. (**c**) Forty-eight hours after siRNA transfection, MCF7 and MCF7/ErbB2 cells were cultured in a 96-well plate and viable cells were detected at day 5 with an MTS reagent. Data are presented as the percentage of growth inhibition measured in control-transfected cells. (**d**) BT474M1 cells were transfected with scramble siRNA (Control) or HSF1 siRNA. Forty-eight hours after siRNA transfection, cell lysates were prepared and immunoblot analyses were carried out with antibodies against HSF1 and β-actin (insert). Forty-eight hours after siRNA transfection, cells were plated into a 96-well plate. Twenty-four hours later, the medium was replaced with fresh medium with or without 2-deoxyglucose (2-DG; left) or oxamate (right) and incubated for 48 and 72 h. Then, the cell viability was detected using an MTS reagent. (**e**), $Hsf1^{+/+}$ and $Hsf1^{-/-}$ cells were cultured in a 96-well plate. Twenty-four hours later, the medium was replaced with fresh medium with or without 2-DG (left) or oxamate (right) and incubated for 48 h or the indicated time. Then, the cell viability was detected using an MTS reagent. Data are presented as the percentage of cell growth inhibition measured in cells treated without 2-DG or oxamate. Columns, mean of three independent experiments; bars, s.e. $^{**}P<0.01$, $^{***}P<0.001$.

ErbB2-overexpressing cells reduced the sensitivity. Moreover, although the growth rates of ErbB2-low 435 neo and ErbB2-overexpressing 435ErbB2 cells are similar (Tan *et al*., 1997), the levels of the glycolysis are much higher in 435ErbB2 cells. Thus, our data are consistent with the notion that increased glycolysis contributes to the maintenance of cancer cell malignant phenotypes. However, this study does not exclude the possibilities that ErbB2 overexpression stimulates cell proliferation and that increased glycolysis and its associated parameters are a result of growth stimulation, or that there is a mutual stimulation between ErbB2-mediated cell proliferation and glycolysis.

In summary, we report that the overexpression of ErbB2 leads to increased glycolysis in multiple human breast cancer cell lines. ErbB2 overexpression increased LDH-A through the upregulation of HSF1, and this contributes to increased glycolysis in human breast cancer cells. The study provides direct evidence in support of a causal relationship between ErbB2 and glycolysis in human breast cancer cells. To our knowledge, this is the first report showing that ErbB2 upregulates LDH-A through HSF1 in human breast cancer cells, and also that the glycolysis inhibitors selectively inhibit ErbB2-overexpressing human breast cancer cells. These findings provide insight into how oncogenic ErbB2 regulates bioenergetic metabolism, and the results will help us to elucidate the impact of the changes of energy dependency on cell malignant phenotypes of ErbB2-overexpressing cancer cells. This is important for the future design of ErbB2 targeting therapeutics to treat breast and other ErbB2-overexpressing cancers.

## Materials and methods

*Cell lines and cell cultures*
MCF7, MCF7/ErbB2, MDA-MB-435 neo (435 neo) and MDA-MB-435ErbB2 (435ErbB2) cells were obtained or generated as described earlier (Benz *et al*., 1992; Tan *et al*., 2002, 2005). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/F-12 (Mediatech Inc., Manassas, VA, USA) with 10% fetal bovine serum (FBS). Immortalized $Hsf1^{+/+}$ and $Hsf1^{-/-}$ MEFs were maintained in DMEM (Invitrogen, Carlsbad, CA, USA) with 10% FBS and penicillin/streptomycin.

*Glucose uptake assay*
Cells were seeded in 24-well plates at $5 \sim 10 \times 10^5$ cells/well. Culture media were collected at 4 and 8 h and stored at $-20\,^{\circ}C$ until they were assayed. Glucose uptake was measured using the Amplex Red Glucose/Glucose Oxidase Assay Kit (Molecular Probes, Carlsbad, CA, USA). Absorbance was measured at 563 nm using a SpectraMax M5 plate reader (Molecular Devices, Sunnyvate, CA, USA) and results were normalized on the basis of the total protein amounts of the cells.

*Lactate production assay*
Lactate production in the medium was detected by using the Lactate Assay Kit (BioVision, Mountain View, CA, USA). Results were normalized on the basis of the total protein amounts of the cells.

*$O_2$ consumption assay*
Cells were transferred to 96-well $O_2$ biosensor plates (BD Biosciences, San Jose, CA, USA) with cyclodextran beads, at a density of $1 \times 10^6$ cells/well. Fluorescence was measured at an excitation of 485 nm and an emission of 630 nm. Oxygen consumption rate was expressed as $\Delta$FU per second.

*LDH activity assay*
Cells were extracted for protein, followed by measurement of LDH activity using the LDH-Cytotoxicity Assay Kit II (BioVision). Results were normalized on the basis of the total protein amounts of the cells.

*Western blot*
Western blot was performed as described earlier (Tan *et al*., 2002). The following antibodies were used: ErbB2 (OP15; Calbiochem, Gibbstown, NJ, USA), LDH-A (2012; Cell Signaling Technology, Danvers, MA, USA), LDH-B (ab75167; Abcam, Cambridge, MA, USA), HSF1 (4356; Cell Signaling Technology), β-actin (A2228; Sigma, St Louis, MO, USA). Immunoreactive bands were visualized by horseradish peroxidase-conjugated secondary antibodies (Bio-Rad, Hercules, CA, USA) using ECL western Blotting Substrate (Pierce, Rockford, IL, USA). Cross-linking of HSF1 was performed by adding EGS (Pierce) to whole cell extract and incubated for 30 min at room temperature. Then, the cross-linked proteins were subjected to SDS–PAGE and visualized by probing with the HSF1 antibody (Sarge *et al*., 1993).

*siRNA experiments*
siRNA for ErbB2 were purchased from Dharmacon (Chicago, IL, USA). siRNAs for HSF1 and the control scrambled siRNA were from Sigma. The downregulation of ErbB2 or HSF1 was confirmed with two siRNA sequences to avoid off-target effects. Transfection was performed using the Oligofectamine (Invitrogen). Forty-eight hours after transfection, cell lysates were prepared and assays such as western blot and LDH activity were performed.

*Cell viability assay*
Cells ($5 \times 10^3 \sim 1 \times 10^4$ cells/well) were placed in a 96-well plate. Twenty-four hours later, the medium was replaced with fresh medium with or without 2-DG (Sigma) or sodium oxamate (Sigma) and incubated for the indicated time. Cell viability was determined using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay Kit (Promega, Madison, WI, USA).

*Cell proliferation assay*
Forty-eight hours after siRNA transfection, cells were plated in 96-well plates at 1000 cells/well. The viable cells were detected using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay Kit at different times.

*RT–PCR and qRT–PCR*
Total RNA was isolated from cells using the TRIzol reagent (Invitrogen). cDNAs were synthesized from 1 μg total RNA using Cloned AMV First-Strand cDNA Synthesis Kit (Invitrogen). For semiquantitative PCR, the cDNA was mixed with PCR SuperMix (Invitrogen) and gene-specific primers. The primers used were as follows: LDH-A, 5′ primer, 5′-GGACTT GGCAGATGAACTTG-3′, and 3′ primer, 5′-TCAGAGAGA CACCAGCAACA-3′; HSF1, 5′ primer, 5′-CCTGATGCTGA ACGACAGT-3′, and 3′ primer, 5′-CTACGCTGAGGCACT TTTC-3′; GAPDH, 5′ primer, 5′-ATCCCATCACCATCTTC CAG-3′, and 3′ primer, 5′-ATGAGTCCTTCCACGATACC-3′. The PCR conditions were 25–30 cycles at 95 °C for 30 s,

56 °C for 30 s and 72 °C for 1 min. For qRT–PCR, cDNA was mixed with the 2 × SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and various sets of gene-specific primers and then subjected to reverse transcription PCR (RT–PCR) quantification using the iQ5 Real-Time PCR System (Bio-Rad). The primers used were as follows: LDH-A, 5′ primer, 5′-TGGAGTGGAATGAATGTTGC-3′, and 3′ primer, 5′-ATAGCCCAG;GATGTGTAGCC-3′; HSF1, 5′ primer, 5′-ATGAAGGGGAAGCAGGAGTG-3′, and 3′ primer, 5′-TGTTGACGACTTTCTGTTGC-3′; GAPDH, 5′ primer, 5′-ATCATCCCTGCCTCTACTGG-3′, and 3′ primer, 5′-CTGCTTCACCACCTTCTTGA-3′. All reactions were performed in triplicate. The relative amounts of mRNA were calculated by using the comparative $C_T$ method.

*Polysomal fractionation*

Polysomal fractionation was carried out as described earlier (Lu *et al*., 2009). Briefly, cells were lysed in polysome lysis buffer containing 100 mmol/l KCl, 5 mmol/l $MgCl_2$, 10 mmol/l HEPES, 1% Triton 100, 5 mmol/l dithothreitol, 100 μg/ml CHX and Protease Inhibitor Cocktail, pH 7.4). Cytoplasm fraction was collected and loaded onto a 15–40% (w/v) linear sucrose gradient and centrifuged at 38 000 *g* at 4°C for 90 min. Ten fractions were collected to isolate RNA for RT–PCR.

*Chromatin immunoprecipitation*

The assay was performed using the ChIP kit (Upstate, Billerica, MA, USA). A total of 35 cycles of PCR were carried out with the primers as follows: LDH-A (forward 5′-GATCAAATCTGGGGAACTGGT-3′ and reverse 5′-AA-CATGGATGGAACTGGAGGT-3′) to amplify a 329 bp segment (−4075 to −3747; Rutter *et al*., 2001) of the LDH-A promoter containing the putative HSF1 binding site; Hsp70 (forward 5′-GGAAGGTGCGGGAAGGTTCG-3′ and reverse 5′-TTCTTGTCGGATGCTGGA-3′) to amplify a 185 bp segment of the Hsp70 promoter containing the HSF1 binding site (Wadekar *et al*., 2004); GAPDH (forward 5′-CGACCACTT TGTCAAGCTCA-3′ and reverse 5′-AGGGGTCTACATGG CAACTG-3′) to amplify a 228 bp segment used as negative control (Liu *et al*., 2008).

**Acknowledgements**

We thank Dr Ivor Benjamin for the HSF1 MEFs; Dr Jianrong Lu, Dr Adam Riker and Dr Laurie Owen for reading the article; Ms Amy Brown for editorial assistance; Dr June Ayling, Dr Robert Laush and Ms Judy Miller for assistance with equipments. We are grateful for the support from The Vincent F Kilborn, Jr. Cancer Research Foundation (M Tan) and from the Radiumhospitalets Legater Award Project 334003 (M Tan and O Fodstad).

## References

Balinsky D, Platz CE, Lewis JW. (1983). Isozyme patterns of normal, benign, and malignant human breast tissues. *Cancer Res* **43**: 5895–5901.

Benz CC, Scott GK, Sarup JC, Johnson RM, Tripathy D, Coronado E *et al*. (1992). Estrogen-dependent, tamoxifen-resistant tumorigenic growth of MCF-7 cells transfected with HER2/neu. *Breast Cancer Res Treat* **24**: 85–95.

Chen Z, Lu W, Garcia-Prieto C, Huang P. (2007). The Warburg effect and its cancer therapeutic implications. *J Bioenerg Biomembr* **39**: 267–274.

Dai C, Whitesell L, Rogers AB, Lindquist S. (2007). Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis. *Cell* **130**: 1005–1018.

Dang CV, Semenza GL. (1999). Oncogenic alterations of metabolism. *Trends Biochem Sci* **24**: 68–72.

DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. (2008). The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab* **7**: 11–20.

Fantin VR, St-Pierre J, Leder P. (2006). Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell* **9**: 425–434.

Garber K. (2006). Energy deregulation: licensing tumors to grow. *Science* **312**: 1158–1159.

Gatenby RA, Gillies RJ. (2007). Glycolysis in cancer: a potential target for therapy. *Int J Biochem Cell Biol* **39**: 1358–1366.

Gillies RJ, Robey I, Gatenby RA. (2008). Causes and consequences of increased glucose metabolism of cancers. *J Nucl Med* **49**(Suppl 2): 24S–42S.

Guy CT, Webster MA, Schaller M, Parsons TJ, Cardiff RD, Muller WJ. (1992). Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. *Proc Natl Acad Sci USA* **89**: 10578–10582.

Hsu PP, Sabatini DM. (2008). Cancer cell metabolism: Warburg and beyond. *Cell* **134**: 703–707.

Khaleque MA, Bharti A, Gong J, Gray PJ, Sachdev V, Ciocca DR *et al*. (2008). Heat shock factor 1 represses estrogen-dependent transcription through association with MTA1. *Oncogene* **27**: 1886–1893.

Khaleque MA, Bharti A, Sawyer D, Gong J, Benjamin IJ, Stevenson MA *et al*. (2005). Induction of heat shock proteins by heregulin beta1 leads to protection from apoptosis and anchorage-independent growth. *Oncogene* **24**: 6564–6573.

Kim JW, Dang CV. (2006). Cancer's molecular sweet tooth and the Warburg effect. *Cancer Res* **66**: 8927–8930.

Kroemer G, Pouyssegur J. (2008). Tumor cell metabolism: cancer's Achilles' heel. *Cancer Cell* **13**: 472–482.

Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. (2001). HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. *Mol Cell Biol* **21**: 3995–4004.

Li YM, Zhou BP, Deng J, Pan Y, Hay N, Hung MC. (2005). A hypoxia-independent hypoxia-inducible factor-1 activation pathway induced by phosphatidylinositol-3 kinase/Akt in HER2 overexpressing cells. *Cancer Res* **65**: 3257–3263.

Liu H, Zhou M, Luo X, Zhang L, Niu Z, Peng C *et al*. (2008). Transcriptional regulation of BRD7 expression by Sp1 and c-Myc. *BMC Mol Biol* **9**: 111–124.

Lu J, Tan M, Huang WC, Li P, Guo H, Tseng LM *et al*. (2009). Mitotic deregulation by survivin in ErbB2-overexpressing breast cancer cells contributes to Taxol resistance. *Clin Cancer Res* **15**: 1326–1334.

Min JN, Huang L, Zimonjic DB, Moskophidis D, Mivechi NF. (2007). Selective suppression of lymphomas by functional loss of Hsf1 in a p53-deficient mouse model for spontaneous tumors. *Oncogene* **26**: 5086–5097.

Nguyen T, Ogbi M, Johnson JA. (2008). Delta protein kinase C interacts with the d subunit of the F1F0 ATPase in neonatal cardiac myocytes exposed to hypoxia or phorbol ester. Implications for F1F0 ATPase regulation. *J Biol Chem* **283**: 29831–29840.

Pelicano H, Martin DS, Xu RH, Huang P. (2006). Glycolysis inhibition for anticancer treatment. *Oncogene* **25**: 4633–4646.

Ramanathan A, Wang C, Schreiber SL. (2005). Perturbational profiling of a cell-line model of tumorigenesis by using metabolic measurements. *Proc Natl Acad Sci USA* **102**: 5992–5997.

Rutter J, Reick M, Wu LC, McKnight SL. (2001). Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* **293**: 510–514.

Sarge KD, Murphy SP, Morimoto RI. (1993). Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress. *Mol Cell Biol* **13**: 1392–1407.

Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE *et al*. (1989). Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* **244**: 707–712.

Tan M, Jing T, Lan KH, Neal CL, Li P, Lee S *et al*. (2002). Phosphorylation on tyrosine-15 of p34(Cdc2) by ErbB2 inhibits p34(Cdc2) activation and is involved in resistance to taxol-induced apoptosis. *Mol Cell* **9**: 993–1004.

Tan M, Lan KH, Yao J, Lu CH, Sun M, Neal CL *et al*. (2006a). Selective inhibition of ErbB2-overexpressing breast cancer *in vivo* by a novel TAT-based ErbB2-targeting signal transducers and activators of transcription 3-blocking peptide. *Cancer Res* **66**: 3764–3772.

Tan M, Li P, Klos KS, Lu J, Lan KH, Nagata Y *et al*. (2005). ErbB2 promotes Src synthesis and stability: novel mechanisms of Src activation that confer breast cancer metastasis. *Cancer Res* **65**: 1858–1867.

Tan M, Li P, Sun M, Yin G, Yu D. (2006b). Upregulation and activation of PKC alpha by ErbB2 through Src promotes breast cancer cell invasion that can be blocked by combined treatment with PKC alpha and Src inhibitors. *Oncogene* **25**: 3286–3295.

Tan M, Yao J, Yu D. (1997). Overexpression of the c-erbB-2 gene enhanced intrinsic metastasis potential in human breast cancer cells without increasing their transformation abilities. *Cancer Res* **57**: 1199–1205.

Wadekar SA, Li D, Sanchez ER. (2004). Agonist-activated glucocorticoid receptor inhibits binding of heat shock factor 1 to the heat shock protein 70 promoter *in vivo*. *Mol Endocrinol* **18**: 500–508.

Warburg O. (1956). On respiratory impairment in cancer cells. *Science* **124**: 269–270.

Westerheide SD, Morimoto RI. (2005). Heat shock response modulators as therapeutic tools for diseases of protein conformation. *J Biol Chem* **280**: 33097–33100.

Yarden Y, Sliwkowski MX. (2001). Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* **2**: 127–137.

Zhang H, Berezov A, Wang Q, Zhang G, Drebin J, Murali R *et al*. (2007). ErbB receptors: from oncogenes to targeted cancer therapies. *J Clin Invest* **117**: 2051–2058.

Zhou X, Tan M, Stone Hawthorne V, Klos KS, Lan KH, Yang Y *et al*. (2004). Activation of the Akt/mammalian target of rapamycin/4E-BP1 pathway by ErbB2 overexpression predicts tumor progression in breast cancers. *Clin Cancer Res* **10**: 6779–6788.

Zu XL, Guppy M. (2004). Cancer metabolism: facts, fantasy, and fiction. *Biochem Biophys Res Commun* **313**: 459–465.

Supplementary Information accompanies the paper on the Oncogene website (http://www.nature.com/onc)