Deregulation of GLUT1 and glycolytic gene expression

by c-Myc

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SUMMARY

Unlike normal mammalian cells, which use oxygen to generate energy, cancer cells rely on glycolysis for energy and are therefore, less dependent on oxygen. The molecular basis for enhanced glycolysis associated with cancers is beginning to emerge with the appreciation that loss of tumor suppressors or activation of oncogenes may directly alter gene expression that affects metabolism.

We previously observed that the c-Myc oncogenic transcription factor regulates lactate dehydrogenase A (LDH-A) and induces lactate overproduction. We, therefore, sought to determine whether c-Myc controls other genes regulating glucose metabolism. In Rat1a fibroblasts transformed by the c-myc gene and murine livers overexpressing c-Myc via in vivo adenovirus-mediated gene transfer, the mRNA levels of the glucose transporter GLUT1, phosphoglucose isomerase, phosphofructokinase (PFK), glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase and enolase were elevated above those in non-transformed Rat1a cells or control LacZ expressing livers. c-Myc directly transactivates genes encoding GLUT1, PFK, and enolase and increases glucose uptake in Rat1 fibroblasts expressing the ligand-inducible Myc-estrogen receptor chimeric protein. These findings were further corroborated by the diminished expression of these genes in c-myc deficient fibroblasts. Nuclear run-on studies confirmed that GLUT-1 transcriptional rate is elevated by c-Myc, and GLUT1 expression is increased in Burkitt’s lymphoma cell lines, which are characterized by chromosomal translocations of c-myc. Hence, our findings suggest that in addition to the physiological induction of glycolysis by hypoxia, overexpression of the c-Myc oncoprotein deregulates glycolysis through the activation of several components of the glucose metabolic pathway.
INTRODUCTION

To form a three dimensional multicellular spheroid mass, neoplastic cells alter their metabolism such that they are able to survive and grow in the hostile microenvironments created by the decreased blood flow found in tumor vasculature [1]. Gradients of oxygen, growth factors, glucose and other nutrients in tumor masses cause a hypoxic and acidic interior due to decreased levels of oxygen and increased levels of lactate [2]. Tumor cells, as compared to normal cells, have simplified metabolic activities and an increased synthesis of material necessary for cell division. The most striking feature of tumor cells is the production of large amounts of lactic acid which is due to the glycolytic conversion of glucose to lactic acid even in the presence of oxygen [3]. This is often accompanied by an increased rate of glucose transport [4-6].

Glucose is a major regulator of gene transcription. In particular, it stimulates transcription of genes encoding glycolytic and lipogenic enzymes in adipocytes and hepatocytes through the carbohydrate response element (ChoRE), a 5'-CACGTG-3' motif [7-11]. The ChoRE is similar to the core binding site for the transcription factors USF2 [12], which is implicated in glucose metabolism, TFE3, and the hypoxia inducible transcription factor (HIF). Hence, the ChoRE serves to integrate physiological signals through transcription factors to regulate glucose metabolism.

During tumor formation, adaptation to hypoxia may be mediated by the HIF-1 family of transcription factors, which induce angiogenesis and other metabolic changes. However, several genetic alterations may also occur, which result in the aberrant constitutive activation of transcription through the ChoREs. HIF-1 has been shown to be induced by v-src and mutant VHL, and several tumors have been noted to overexpress HIF-1 [2, 13, 14]. It is notable that glucose transport and transporter mRNA are induced in cells transformed by ras or src oncogenes (5). The c-myc oncogene is activated in a variety of pathways that are important in controlling cell growth and tumorigenesis. These include the Wnt-APC-β-catenin, c-Src and BCR-ABL pathways [15-18]. Intriguingly, the
ChoRE sequence matches the core E-box (5’-CACGTG-3’) binding site for c-Myc, which binds E-boxes of target genes to stimulate transcription [2, 18, 19]. Previous work showed that c-Myc directly up-regulates the expression of the lactate dehydrogenase gene (LDH-A) through E-boxes [20]. LDH-A is the enzyme that converts pyruvate to lactate and is frequently over-expressed in human cancers. It was further shown that the upregulation of LDH-A is important in the transformed phenotype (anchorage-independent growth) of cells which overexpress c-Myc [20, 21].

The Myc E-boxes found in the LDH-A promoter overlaps with the hypoxia inducible transcription factor HIF-1 response elements, suggesting that hypoxia inducible glycolytic genes might also be regulated by c-Myc. Since ChoREs are found in the promoters of other genes that control glucose metabolism, we sought to determine whether c-Myc controls other genes regulating glucose metabolism by performing Northern blot analysis on cells fibroblasts transformed with c-Myc, c-myc nullizygous fibroblasts, Burkitt’s lymphoma cell lines with endogenous c-Myc overexpression, and murine hepatocytes overexpressing c-Myc via in vivo adenovirus-mediated gene transfer. While many of the genes involved in glycolysis are upregulated in c-Myc transformed cells, we found that only enolase A, the glucose transporter 1 (GLUT1), and phosphofructokinase behave as direct targets of c-Myc. A functional assay measuring glucose uptake in c-Myc transformed cells showed an elevated level of glucose uptake in c-Myc activated cells, which further demonstrates that c-Myc up-regulates glucose transport.
EXPERIMENTAL PROCEDURES

Cell Culture and Transfection. Rat fibroblasts were cultured in 5% CO\textsubscript{2} at 37\textdegree\text C in DMEM supplemented with 10% fetal bovine serum (FBS; GIBCO-BRL Gaithersburg, MD) and antibiotics. Rat1a and Myc-transformed Rat1a-Myc fibroblasts were as previously described [22]. Human lymphoid cells were cultured in Iscove’s modified Dulbecco’s medium with 10% FBS and antibiotics. Cells lacking c-\textit{myc} (HO15), heterozygous HET15 and parental lines (TGR) (a gift of John Sedivy, Brown University) were cultured in DMEM supplemented with 10% FBS and antibiotics [23]. HO15 cells were transfected with either MLVmyc or MLV empty vectors [20] using Lipofectin (GIBCOBRl) according to the manufacturer’s protocol. The human GLUT1 cDNA [24] was kindly provided by Drs. M. Mueckler (Washington University, St. Louis MO) and C. Heilig (Johns Hopkins University, Baltimore MD).

A Rat1 cell line expressing a fusion protein of c-Myc and the human estrogen receptor (MycER) (a gift of J.M. Bishop, University of California, San Francisco) was grown in DMEM with 10% FBS, penicillin and streptomycin [25]. Myc activity was induced when confluent MycER cells were treated with 0.25 \mu M 4-hydroxytamoxifen (4-HOTM; Research Biochemicals, Natrick, MA) for the times indicated as described [20, 26]. To block protein synthesis, 10 \mu M cycloheximide (CHX) was added to the cells 30 minutes prior to 4-HOTM treatment.

Adenoviral in vivo gene transfer. Animal studies were approved by the Johns Hopkins University School of Medicine Animal Use and Care Committee. Adenoviral constructs (a gift from W. El-Deiry, Univ. of Penn., Philadelphia, PA) containing either LacZ (Ad/LacZ) or c-\textit{myc} (Ad/c-\textit{myc}) coding sequence were as described [27]. Two month old male Balb/c mice, weighing 20-25 grams, were intravenously injected with 4x10\textsuperscript{9} plaque forming unit (pfu) of either Ad/LacZ and Ad/c-\textit{myc}. Mice were then sacrificed by exposure to CO2 on day 3, day 4 and day 5 after viral injection.
Their livers were quickly removed and processed for RNAs or frozen in liquid nitrogen.

RNA analysis. Total RNA was isolated by guanidium thiocyanate lysis followed by cesium chloride centrifugation. 15 µg aliquots of RNA were used in Northern blot analysis [22]. IMAGE Consortium cDNA clones [28] were obtained (Research Genetics, Inc.), and the inserts were isolated and $^{32}$P-labeled by Random-Primer synthesis using a Random-PrimeII kit (Stratagene). Hybridization was done at 45°C for 20 hours and blots were washed in 2XSSC/0.1% SDS at room temperature for 30 min and then at 50°C for 2 hours, and exposed for autoradiography.

Nuclear Run-On Assays. Nuclear run-on assays were performed as described [20, 29]. Nuclei were isolated from non-adherent cells (2 x 10⁷) and incubated with buffered $^{32}$P UTP (500 µCi) and labeled RNAs were isolated after treatment with DNase, proteinase K incubation and phenol-chloroform extraction. Labeled RNAs (1.2 x 10⁷ cpm) from either Rat1a-Myc or Rat1a cells were hybridized at 42EC for 40 hours with membrane slot-blotted with denatured glucose transporter 1 and vimentin cDNA fragments. Membranes were washed five times in 0.1x standard saline citrate (SSC)/0.1% SDS at 50°C every 20 min.

$^{3}H$ 2-Deoxyglucose (2-DG) uptake. Uptake experiments were conducted on confluent Rat1a and MycER cells. Cells were treated with 0.25 µM 4-hydroxytamoxifen (4-HOTM; Research Biochemicals, Natrick, MA) 1 hour before 0.1 mM 2-DG with 10 nM $^{3}H$ 2-DG (final concentration) were added. At the end of 10 minute incubation, cells were washed with ice-cold phosphate-buffered saline. Cells were then lysed by the addition of 2 ml of 10 mM NaOH containing 0.1% Triton X-100, and a 100 µl aliquot was assayed for $^{3}H$ by liquid scintillation counting [30]. The protein content was measured by the method of Bradford [31].
RESULTS

Myc induces Glut1 and glycolytic gene expression in Myc-transformed fibroblasts and in hepatocytes in vivo. To identify genes whose expression is deregulated in the presence of increased ectopic c-Myc, c-Myc transformed Rat1a cells were analyzed by Northern blot analysis (Fig. 1A). Using this system of isogenic cell lines, GLUT1, phosphofructokinase (PFK), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), phosphoglucone isomerase (GPI), phosphoglycerate mutase (PGM), and enolase showed elevated mRNA levels in c-Myc transformed cells as compared to the non-transformed Rat1a cells. The expression levels of aldolase A, aldolase C and triose phosphate isomerase (TPI) were decreased and the expression levels of hexokinase I, hexokinase II, and phosphoenolpyruvate carboxykinase (PEPCK) were unchanged. Rat vimentin mRNA levels are independent of Myc expression as determined by Northern blot analysis [20, 32]; hence, vimentin was used as a control in Rat1a fibroblasts.

To determine whether c-Myc could induce GLUT1 and glycolytic gene expression in vivo, we injected adenoviruses expressing either LacZ or c-Myc into mice. Tail-vein injection of adenoviruses has the advantage of very high hepatic clearance, which yields highly efficient gene delivery to the liver [33]. Four days after injection, 40% of hepatocytes were positive for β-galactosidase in LacZ adenovirus treated animals (data not shown; Kim et al., unpublished). c-Myc protein levels were 10-fold elevated in Myc adenovirus treated animals (Fig. 1B). GLUT1, PFK, GAPDH, PGM and enolase mRNAs were significantly induced in the Myc expressing livers as compared to LacZ expressing livers at 4 and 5 days after injection of viruses (Fig. 1C). These observations suggest that transient expression of Myc in vivo induces hepatic expression of the same glycolytic genes that are induced in the Rat1a system.

Glycolytic Genes that are Direct Targets of c-Myc. To determine whether the genes displaying increased or decreased levels of mRNA expression are transcriptionally activated by Myc,
we used a previously described Rat1 fibroblast line expressing a protein that fuses Myc to the estrogen receptor ligand binding domain (MycER). With exposure of cells expressing the MycER protein to estrogenic compounds such as 4-hydroxy tamoxifen (4-HOTM), the ligand-bound MycER protein translocates to the nucleus. The MycER protein then activates target genes without requiring new intervening protein synthesis [26, 34]. Hence, prior exposure of MycER cells to the protein synthesis inhibitor cycloheximide (CHX) would not block activation or repression of direct target genes by 4-HOTM. Activation of MycER by 4-HOTM caused induction of GLUT1, PFK, and enolase mRNAs in the presence of CHX at 2 and 4 hours (Fig. 2A). Note that mRNA levels are diminished in the CHX + TM samples at the 6 hour time point; this is likely due to decreased MycER protein (10% of 0 hour level) with CHX treatment (data not shown). GAPDH, GPI, and PGM expression were not increased in the MycER system. Thus, GAPDH, GPI, and PGM do not meet this criterion for direct targets of c-Myc. Among the potentially down-regulated genes identified in Rat1a-myc cells, only TPI mRNA moderately decreased in MycER cells exposed to 4-HOTM. However, a decrease in TPI mRNA was also seen with CHX treatment alone. The ribosomal phosphoprotein (36B4) mRNA levels were shown to be unresponsive to estrogen and independent of cell cycle progression [35, 36] and was therefore was used as a control. The 18S rRNA is also shown for loading control. To account for experimental variation, a separate tamoxifen induction experiment was performed and GLUT1 expression in MycER cells was determined (Fig. 2B). Following c-Myc induction, GLUT1 expression behaves in a manner that is consistent with a direct c-Myc target.

To determine whether 4-HOTM itself may directly increase the expression of GLUT1, PFK, and enolase independent of the MycER protein, we treated Rat1a cells lacking MycER with 4-HOTM, with or without CHX, and performed Northern analysis. The mRNA levels of these genes were unchanged upon addition of 4-HOTM and cycloheximide in the absence of the MycER protein (Fig. 2C). Hence, the induction of enolase, GLUT1, and PFK expression in the Rat1a-MycER cells
by 4-HOTM is dependent on MycER activity.

Expression of GLUT1 and glycolytic genes in cells lacking c-Myc. To further authenticate GLUT1, PFK and enolase as c-Myc targets, we sought to determine their expression in Rat1 fibroblasts lacking c-myc (Fig. 3). All three transcripts showed decreased levels in c-myc null cells as compared to wild type parental cells. The expression of PFK and enolase genes are elevated in c-myc null cells rescued by a constitutively overexpressed c-Myc (HO15-Myc) when compared to empty vector control transfected myc null cells (HO15-MLV). GLUT1 expression, however, was only slightly elevated in the c-Myc overexpressing HO15 cells as compared to control MLV transfected cells, which display elevated GLUT1 as compared to the parental HO15 cells. Replicate experiments yielded similar results, in which the HO15-MLV cells have elevated expression of all three genes as compared to the HO15 parental cell line. The cause for this elevation in our control HO15-MLV cells is not known. Thus, in this genetically defined system Myc levels parallel the expression of these three target genes.

Among the three up-regulated genes, GLUT1 is most frequently implicated in tumorigenesis [35-38]. We therefore chose to characterize GLUT1 further. Nuclear run-on experiments demonstrated an enhanced transcriptional rate of GLUT1 in Rat1a-Myc cells as compared with Rat1a fibroblasts (Fig. 4A). The nuclear run-on signals for enolase and PFK were low and hence insufficient for interpretation (not shown). These results, nevertheless, underscore the activation of GLUT1 by c-Myc at the transcriptional level.

We further sought to determine whether GLUT1 expression parallels that of c-myc in Burkitt’s lymphoma cells that are characterized by c-myc gene activation by chromosomal translocation. Both c-Myc-transformed lymphoblastoid (CB33-Myc) and Burkitt’s lymphoma cell lines, Ramos and ST486, have elevated c-Myc protein levels [20] that are associated with elevations of GLUT1 mRNA levels compared with the non-transformed lymphoblastoid CB33 cells (Fig. 4B).
We thus observed a correlation between c-Myc expression and the endogenous levels of GLUT1 mRNA in these Myc-transformed human lymphoid cells.

*Increased glucose uptake in Myc transformed cells.* To determine whether increased Myc activity influences glucose transport, $[^3]H$ 2-DG uptake was studied in Myc-ER Rat1 cells as well as Rat1a cells lacking the Myc-ER system. 4-HOTM increased $[^3]H$ 2-DG uptake in Myc-ER cells, while it had no influence in Rat1a cells (Fig. 5).
DISCUSSION

Normal mammalian cells use oxygen to generate energy from glucose and other metabolites through oxidative phosphorylation. In conditions of oxygen deprivation, normal cells rely on glycolysis to generate energy by converting glucose to lactic acid. Neoplastic transformation, however, alters glucose metabolism with enhanced conversion of glucose to lactic acid, thereby making tumor cells less dependent on oxygen. The molecular basis for physiologic and pathologic regulation of glucose metabolism is beginning to emerge with the identification of transcription factors that regulate glycolytic genes [2].

The ability of c-Myc, USF1 and HIF-1 transcription factors to regulate the LDH-A promoter through a Myc consensus binding site 5'-CACGTG-3' suggests that these related bHLH-Zip transcription factors converge onto common cis elements (5'-RCGTG-3') that are found in regulatory sequences of glycolytic genes [20]. Since hypoxia physiologically induces glycolytic gene expression through HIF-1 binding sites, it stands to reason that glycolysis might also be activated by USF or c-Myc. Hence, we sought to determine whether c-Myc regulates genes that are also regulated by HIF-1.

In this study, we observe that the glucose transporter GLUT1, PFK, GAPDH, GPI, PGM and enolase are upregulated by c-Myc in fibroblasts. In addition, we used adenovirus-mediated gene transfer to the liver as a means of studying in vivo gene expression and determined that these genes are also up-regulated by c-Myc in vivo. Our findings agree with a previous study of transgenic mice that also suggests the in vivo regulation of hepatic glycolysis by c-myc [41], although GLUT2, but not GLUT1, was induced in the transgenic livers. While our observation supports the hypothesis that c-Myc induces GLUT1 expression in the liver in vivo, the physiological significance of this induction is unclear since GLUT2 is the dominant form of hepatic glucose transporter. The use of transgenic mice, however, cannot establish whether c-Myc regulates certain genes directly. In our study, only
GLUT1, PFK, and enolase behave as direct target genes in the MycER system. Since HIF-1 and USF lack a corresponding chimeric estrogen receptor system, the directness of activation of glycolytic genes by these factors cannot be assessed. Nevertheless, it is clear that c-Myc and HIF-1 responsive genes can differ remarkably. For example, the mRNA levels for HKI and adolase are unchanged or decreased by c-Myc, but are hypoxia inducible [2, 13, 14]. GPI is increased by c-Myc but is unaffected by hypoxia. Furthermore, we have reported that c-Myc overexpression downregulates VEGF, a HIF-1-mediated hypoxia inducible gene [42]. Thus, while some HIF-1 and c-Myc target genes overlap, the two sets of targets remain distinct.

We observed that c-Myc directly induces GLUT1 and increases glucose uptake in the Rat1 MycER cells. In addition to LDH-A, whose level is elevated in human cancers, GLUT1 is an intriguing c-Myc target when its role in oncogenesis is considered [37]. Elevation of GLUT1 and c-Myc RNA are among the earliest changes in gene expression after H-ras T24 transformation of Rat1a fibroblasts [39]. Reduction of GLUT1 expression through antisense GLUT1 RNA suppresses NIH 3T3 cell transformation by N-ras [40]. Elevated expression of GLUT1 occurs as a late event during in colorectal carcinogenesis and in the neoplastic progression of Barrett’s metaplasia [38]. Hence, the induction of GLUT1 expression by c-Myc may play an important role in tumor glucose metabolism.

The specific roles of PFK and enolase in tumorigenesis are less clear. Glycolytic flux is controlled at multiple steps of glycolysis, such that interruption of a non-rate-limiting step may affect overall flux [43]. PFK is a rate-limiting glycolytic enzyme due to its intricate allosteric responses to ATP/ADP ratios and therefore may be critically important for the control of glycolytic flux. The attachment of glycolytic enzymes to the cytoskeleton appears essential for normal cellular metabolism [44]. This attachment may be disrupted by the microtubule drug paclitaxel, which specifically displaces PFK from the cytoskeleton thereby inhibits glycolysis [45]. Enolase has not been directly implicated in human cancers; however, it is intriguing to note that a negative
transactivating factor of the c-myc promoter (MBP-1) may be identical to enolase A [46, 47]. A role for metabolic enzymes in gene regulation has already been well-illustrated by the iron-containing TCA cycle enzyme aconitase or IRP-1, which regulates mRNAs of transferrin and ferritin in response to changes in iron levels [48]. While the role for enolase in regulating c-myc expression remains to be confirmed and further studied, this finding suggests that the induction of enolase by c-Myc may increase glycolysis and also serves as a negative feedback onto c-myc gene expression.

In summary, we observe that while both c-Myc and HIF-1 up-regulates GLUT1 and glycolytic gene expression, the sets of target genes for these transcription factors are distinct. HIF-1 physiologically induces glycolytic gene expression in hypoxic cells that also undergo growth arrest. By contrast, c-Myc stimulates glucose uptake, glycolysis and overall metabolism as well as activates the cell cycle machinery, which are all necessary for cell proliferation [49]. The widespread deregulation of the c-myc gene in human cancers, therefore, may be a major contributor to the enhanced tumor glycolysis known as the Warburg effect, which may confer growth advantage to tumor cells deprived of oxygen.

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Figure Legends

Figure 1. (A) Northern analysis of GLUT1 and glycolytic gene expression in non-adherent Rat1a-Myc (R1a-Myc) or Rat1a (R1a) fibroblasts. Vimentin served as a sample loading control. Glucose transporter 1 (GLUT1); phosphosfructokinase (PFK); glyceraldehyde 3-phosphate dehydrogenase (GAPDH); glucose phosphate isomerase (GPI); phosphoglucomutase (PGM); triosephosphate isomerase (TPI); hexokinase (HK); phosphoenolpyruvate carboxykinase (PEPCK). (B) c-Myc protein expression at 3 to 5 days after injection of adenovirus expressing c-Myc. Equal amounts of protein (20 µg/lane) were loaded and Western analysis was performed with the monoclonal anti-Myc 9E10 antibody. (C) Elevated in vivo glycolytic gene expression in c-myc adenovirally transduced murine livers. Numbers at the bottom represent days after injection of adenovirus expressing galactosidase (LacZ) or c-Myc. 18S rRNA is shown as a loading control.

Figure 2. (A) Direct c-Myc targets determined by the use of the Myc-estrogen receptor ligand binding domain (MycER) hybrid protein system. Rat1 fibroblasts expressing the MycER protein were treated with cycloheximide (CHX), 4-hydroxytamoxifen (HT) or both reagents (CHX+TM), and RNAs were collected at the times (hours) indicated at the bottom of the figure. Northern blots were probed with labeled cDNAs of GLUT1 (GT1) or glycolytic genes (for abbreviations see Fig. 1A) indicated along the left sides of the panels of blots. Both 18S rRNA and human ribosomal phosphoprotein 36B4 are shown as loading controls. Note that only GLUT1, PFK and enolase showed elevated expression with both TM and CHX+TM, but not with CHX only. Both GPI and TPI mRNAs are labile with CHX exposure. (B) Northern analysis of GLUT1 expression in a separate induction experiment with MycER cells. The labels are similar to those of Fig. 2A. The top of the figure shows the relative increase in GLUT1 expression (normalized to the loading control 36B4) as a function of time. For the 4 hour time point, an average over three experiments from two...
induction experiments yielded the following relative increase (from zero time point) in GLUT1
expression with standard errors: CHX, 1.3±0.5; TM, 2.7±0.3; CHX + TM, 2.2±0.4. (C) Northern
analysis of enolase, GLUT1 and PFK expression in 4-hydroxytamoxifen (TM) or TM plus
cycloheximide (CHX+TM) treated Rat1a cells lacking the MycER protein. Numbers at the bottom
represents time of exposure. 18S rRNA is shown as a loading control.

Figure 3. Expression of enolase, GLUT1 and PFK mRNAs in wild-type (TGR(WT)), heterozygous
(HET15), or c-myc null (HO15) Rat1 fibroblasts. HO15-MLV represents RNAs from myc null cells
transfected with the empty MLV-LTR expression vector, and HO15-Myc the null cells reconstituted
with an MLV-LTR driven human c-Myc expression vector. Both 18S rRNA and ribosomal
phosphoprotein 36B4 were used as loading controls.

Figure 4. (A) Nuclear run-on studies showing increased transcription of GLUT1 in Rat1a-Myc nuclei
as compared to Rat1a nuclei. Vimentin was used as a control. (B) Northern blot showing expression
of GLUT1 mRNA in untransformed CB33 human lymphoblastoid cells as compared to those with
elevated c-Myc expression: CB33 with ectopic c-Myc expression (CB33-Myc) and Burkitt’s
lymphoma cell lines Ramos and ST486. Phosphoprotein 36B4 and 18S rRNA are shown as loading
controls. Relative GLUT1 mRNA levels are: CB33, 1.0; CB33-Myc, 1.8; Ramos, 11.2; and ST486,
4.3.

Figure 5. c-Myc stimulates glucose uptake in Rat1 fibroblasts. Rat1a and Rat1-MycER cells were
grown under control conditions (-TM) or with 4-hydroxytamoxifen (+TM) which induces c-Myc
activity. Relative glucose uptake (mean ± SD from triplicate experiments) was determined by
measuring intracellular [3H] 2-deoxyglucose (see Methods for details).
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Figure 2, Osthus et al.
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