Regulation of Monocarboxylate Transporter MCT1 Expression by p53 Mediates Inward and Outward Lactate Fluxes in Tumors

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Abstract

The monocarboxylate transporter (MCT) family member MCT1 can transport lactate into and out of tumor cells. Whereas most oxidative cancer cells import lactate through MCT1 to fuel mitochondrial respiration, the role of MCT1 in glycolysis-derived lactate efflux remains less clear. In this study, we identified a direct link between p53 function and MCT1 expression. Under hypoxic conditions, p53 loss promoted MCT1 expression and lactate export produced by elevated glycolytic flux, both in vitro and in vivo. p53 interacted directly with the MCT1 gene promoter and altered MCT1 mRNA stabilization. In hypoxic p53⁻/⁻ tumor cells, NF-kB further supported expression of MCT1 to elevate its levels. Following glucose deprivation, upregulated MCT1 in p53⁻/⁻ cells promoted lactate import and favored cell proliferation by fuelling mitochondrial respiration. We also found that MCT1 expression was increased in human breast tumors harboring p53 mutations and coincident features of hypoxia, with higher MCT1 levels associated with poorer clinical outcomes. Together, our findings identify MCT1 as a target for p53 repression and they suggest that MCT1 elevation in p53-deficient tumors allows them to adapt to metabolic needs by facilitating lactate export or import depending on the glucose availability. Cancer Res; 72(4): 1–10. ©2011 AACR.

Introduction

Among the large family of monocarboxylate transporters (MCT), 4 of them are described as H⁺/lactate symporters capable of bidirectional transport of lactic acid across the plasma membrane (1). MCT1 is the most ubiquitous, MCT2 and MCT3 expressions are limited to some tissues, and MCT4 is usually described as the hypoxia-inducible MCT family member. MCT4 is actually the prototypical MCT responsible for the efflux of glycolysis-derived lactate occurring under hypoxia in tumors (2–4) or in some specialized cells (1, 5–7). In the skeletal muscle, in particular, MCT4 transports lactate outside the cells of white muscle fibres. Interestingly, MCT1 expressed in red oxidative muscle fibres will in turn facilitate the uptake of lactate which will be used after conversion into pyruvate as an alternate carbohydrate fuel source (6, 7). Similar lactate exchange and uptake through MCT1 is reported in the heart (8), red blood cells (9), and even in the liver, to support gluconeogenesis (10). Within tumors also, we and others recently documented that cancer cells could import lactate through MCT1 from the most glycolytic tumor cells (11–14) or tumor-associated fibroblasts (15, 16) to fuel mitochondrial respiration and thereby spare glucose for hypoxic tumor cells.

In many tumor cell lines, characterized by an elevated glycolytic flux even in the presence of oxygen (the Warburg effect; ref. 17), the reported expression of MCT1 supporting lactate influx is thus somehow paradoxical (11, 18). A simple explanation could be that MCT1 can function in the reverse mode according the environmental conditions. One could therefore envision that in tumor cells, under hypoxia, MCT1 could also act as the bona fide hypoxia-induced MCT4 transporter, releasing lactate outside the cell. Because contrary to MCT4, MCT1 is not under the regulation of HIF-1α, other modes of regulation should be involved. Among the possible pathways, P53 seems as a good candidate considering the known hypoxia inducibility of this transcription factor (19) and the already well-characterized links between metabolism and P53 genetic alterations (20, 21). Physiologically, P53 regulates the cell energy balance between glycolysis and oxidative phosphorylations (OXPHOS). p53 can, for instance, promote mitochondrial respiration by increasing the expression of SCO2 (synthesis of cytochrome c oxidase 2, a key regulator of cytochrome c oxidase complex; ref. 22). P53 also restrains the...
glycolytic rate through the expression of TIGAR (TP53-induced glycolysis and apoptosis regulator; ref. 23). Loss of P53 is therefore associated with a stimulation of glycolysis, thereby supporting the Warburg effect. This may require the need to deal with high levels of lactate, a phenomenon yet exacerbated under hypoxic conditions. In this work, we studied the influence of elevated extracellular and intracellular lactate concentrations on MCT1 expression in a variety of tumor cells and examined whether the P53 status could account for a different capacity to handle these changes in lactate homeostasis.

Materials and Methods

Cell models

Human tumor cells were acquired in the last 3 years from the American Type Culture Collection, where they are regularly authenticated. Cells were stored according to the supplier’s instructions and used within 6 months after resuscitation of frozen aliquots. SiHa and HeLa cervix cancer cells, MCF-7 and MDA-MB-231 mammary cancer cells, wild-type P53 HCT116 colon cancer cells (P53WT HCT116), and null-P53-derived cells (P53<sup>−/−</sup> HCT116) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 5 mmol/L glucose or 10 mmol/L sodium lactate or R-3-hydroxybutyrate; all media were prepared from DMEM-based medium powder (Sigma). To establish short hairpin RNA (shRNA)- or cDNA-expressing tumor cell clones, transfection was done as previously described (13). Sequences were the same as documented elsewhere for MCT1 (13) and P53 (24); HPV16 E6 antigen–targeting shRNA was from Santa Cruz. Transfection of prevalidated siRNA (SmartPool mixture of 4 different siRNA per target gene; Dharmacon) targeting HIF-1α, HIF-2α, and NF-κB P65 subunit were carried out as previously reported (13, 25).

Real-time PCR, immunoblotting, and immunostaining

RNA was extracted from a series of 50 needle core biopsies from locally advanced breast tumors collected at time of diagnosis at the Centre Georges François Leclerc (Dijon, France) and PCR amplification was done as previously described (26). Western blotting and immunostaining were done as reported elsewhere (13).

Chromatin immunoprecipitation and PCR

Cells were incubated under normoxic or hypoxic conditions for the suitable period of time. A one-tenth volume of formaldehyde-based crosslinking mix was then added and the reaction was quenched by the addition of 125 mmol/L glycine. An aldehyde-based crosslinking mix was then added and the reaction was quenched by the addition of 125 mmol/L glycine. Immunoprecipitated DNA was extracted and PCR amplification was done using primers designed to probe the MCT1 promoter region.

Statistical analysis

Results are expressed as mean ± SEM. Student t test, 1-way ANOVA (Tukey post-hoc test), were used where indicated. Survival curves were generated using the Kaplan–Meier method, and the significance of differences between dichotomized patient groups was obtained by the Mantel-Cox log rank test. *, P < 0.05 or **, P < 0.01 was considered statistically significant.

Results

MCT1 mRNA expression is influenced by hypoxia but not by extracellular lactate

We previously documented that lactate could enter some tumor cells through the MCT1 transporter to fuel oxidative metabolism (11), whereas in other cells, this transporter was reported to release lactate to the extracellular medium (27). To study the rationale behind this distinct behavior, we examined the influence of either the addition of extracellular lactate or the stimulated production of intracellular lactate in 6 different human cancer cell lines, namely cervix cancer SiHa and HeLa cells, breast cancer MDA-MB-231 and MCF-7 cells, colon cancer P53<sup>WT</sup> HCT116 and P53<sup>−/−</sup> HCT116 cells. We first exposed these cell lines to glucose-containing medium, with or without 10 mmol/L lactate supplementation. Although we observed changes in MCT1 mRNA expression with time in culture, we did not observe any influence of the presence of excess extracellular lactate on MCT1 transcript expression (see white bars in Fig. 1A). By contrast, when we exposed these cell lines to hypoxia (1% O<sub>2</sub>) to force them to produce lactate through glycolysis, we found different patterns of MCT1 mRNA expression according to the time under hypoxia. Whereas MCT1 mRNA expression rapidly increased in hypoxic SiHa cells, P53<sup>−/−</sup> HCT116 cells and, to a lesser extent, in HeLa cells, we observed a reduction in MCT1 transcript expression in response to hypoxia in MCF-7 and MDA-MB-231 (as well as in P53<sup>WT</sup> HCT116 at 48 and 72 hours) (see black bars in Fig. 1A).

MCT1 mRNA expression under hypoxia correlates with the p53 status of tumor cells

The distinct regulation of MCT1 expression under hypoxia in P53<sup>−/−</sup> and P53<sup>WT</sup> HCT116 led us to examine a possible relationship between MCT1 expression and the p53 status of the different cell lines. In human papilloma virus (HPV)-positive SiHa and HeLa cells, we confirmed the lack of basal P53 expression (and thus of any influence of hypoxia on P53 stabilization; Fig. 1B, top panel). In mammary MCF-7 cells known to exhibit a wild-type P53, we could detect a stimulatory effect of hypoxia on P53 stabilization (Fig. 1B, middle panel); in mammary MDA-MB-231 known to exhibit a gain-of-function P53 mutation (28), we indeed found an elevated basal expression of P53 (Fig. 1B, middle panel). In P53<sup>WT</sup> HCT116, we observed a rapid induction of P53 as soon as 24 hours after exposure to hypoxia, whereas P53 remained undetectable in P53<sup>−/−</sup> HCT116 (Fig. 1B, bottom panel).

In vitro and in vivo MCT1 protein expression is regulated by hypoxia in a p53-dependent manner

To further explore the existence of a potential link between the cell P53 status and MCT1 expression, we then focused on WT and P53<sup>−/−</sup> HCT116 cells. We found that after 72 hours of hypoxia, MCT1 protein was significantly decreased in P53<sup>WT</sup> HCT116 but increased in P53<sup>−/−</sup> HCT116 (Fig. 2A). In parallel,
we also examined MCT4 expression in both cell types and found that MCT4 expression was rapidly induced by hypoxia but rapidly returned to basal level, independently of the cell genotype origin (Supplementary Fig. S1). Changes in MCT1 expression were confirmed by immunocytochemistry, the hypoxia-induced decrease in the expression of MCT1 (at the plasma membrane) in P53WT HCT116 cells, contrasting with the increase in expression in P53−/− HCT116 cells (Fig. 2B). We confirmed the parallel P53 stabilization and nuclear translocation in P53WT HCT116 exposed to hypoxia (Fig. 2C). We also examined whether similar alterations in the regulation of MCT1 expression could be observed in vivo in tumor xenografts resulting from subcutaneous injection of P53WT and P53−/− HCT116 cells. We found that MCT1 expression was absent in

Figure 1. Changes in MCT1 mRNA expression in response to hypoxia and lactate according to the P53 status of cancer cells. A, cancer cells were cultured in glucose-containing DMEM (control conditions used for normalization) for increasing periods of time (hours). In some conditions, 10 mmol/L lactate was added at time 0 (white bars) or the plates were placed in a hypoxic 1%-O2 environment (black bars). Bar graphs represent MCT1 mRNA expression expressed as fold-change versus control (**, P < 0.05; ***, P < 0.01; n = 3). B, representative immunoblot analyses of P53 protein expression in corresponding cells maintained under normoxia or 1% O2 hypoxia for the indicated period of time (hours); β-actin was used as loading control. Asterisk represents the P53 splice variant P47. These experiments were repeated 3 times with similar results.
hypoxic regions of P53WT HCT116 tumors as determined by coimmunostaining with antibodies against carbonic anhydrase 9 (CAIX), a well-known hypoxia-induced protein in tumors (ref. 29; Fig. 2D). By contrast, a perfect match between CAIX and MCT1 localization was found in P53−/− HCT116 tumors (Fig. 2D).
Hypoxia-induced MCT1 expression allows P53<sup>−/−</sup> HCT116 cells to release and take up lactate according to the environmental conditions

We next aimed to determine whether changes in MCT1 expression functionally alter the manner for hypoxic tumor cells to handle lactate. We found that 72-hour hypoxia stimulated lactate efflux to a larger extent in P53<sup>−/−</sup> HCT116 cells (white bars, Fig. 3A). Interestingly, silencing MCT1 with a dedicated shRNA (Fig. 3B) barely influenced the transport of lactate in P53<sup>WT</sup> HCT116 but prevented lactate efflux from P53<sup>−/−</sup> cells (black bars, Fig. 3A).

Because we previously showed that MCT1 could facilitate the uptake of lactate by oxidative tumor cells (11), we also used a protocol wherein cells were maintained under hypoxia (1% O<sub>2</sub>) for 6 days and then reoxygenated or maintained under hypoxia for 3 more days without renewing the culture medium (i.e., exposing them to a naturally lactate-enriched glucose-deprived medium). This protocol led to the death of approximately 40% and 50% of P53<sup>WT</sup> HCT116 after 3 days reoxygenation or prolonged hypoxia, respectively (Fig. 3C). By contrast, P53<sup>−/−</sup> HCT116 did survive and significantly proliferate under both conditions (Fig. 3C). Note that P53<sup>−/−</sup> HCT116 proliferated to a larger extent than WT cells during the initial 6 days hypoxic period (compare white bars in Fig. 3C). We further documented that P53<sup>−/−</sup> HCT116 presented a higher relative glucose consumption rate (than WT cells) and importantly that extracellular lactate could subsequently be taken up during the reoxygenation phase to support P53<sup>−/−</sup> cell growth (Fig. 3D). To exclude that the above difference arose from the higher extracellular lactate content in P53<sup>−/−</sup> HCT116 culture medium, we also used a protocol in which the extracellular medium was replaced after 72-hour hypoxia by a glucose-free 10 mM lactate-containing medium. We found that under consecutive hypoxia (1% O<sub>2</sub>), P53<sup>WT</sup> cells stop to grow whereas proliferation of P53<sup>−/−</sup> HCT116 was significantly stimulated (Fig. 3E). Moreover, because MCT1 is also known to efficiently transport ketone bodies (15), we also used hydroxybutyrate-supplemented glucose-free medium and observed a similar stimulation of P53<sup>−/−</sup> cell proliferation (vs. P53<sup>WT</sup> cells; Fig. 3E).

We then examined how hypoxia-induced upregulation of MCT1 in P53<sup>−/−</sup> HCT116 cells could impact cell proliferation if lactate (instead of glucose) was available in the extracellular medium from the beginning of the experiment. We first confirmed that in these conditions, MCT1 expression was upregulated in response to hypoxia (not shown). We found that with increasing time under hypoxia, P53<sup>−/−</sup> HCT116 grew more efficiently in the presence of lactate to reach at 72 hours, a proliferation rate similar to that reached in the presence of glucose (see ratio ~1 in Fig. 3F). By contrast, in P53<sup>WT</sup> HCT116 cells, the capacity to use lactate as energy fuel for proliferation rapidly decreased with time under hypoxia to reach approximately 40% of the rate observed with glucose (Fig. 3F). To determine whether this difference was dependent on the expression of MCT1, we silenced MCT1 as described above (see Fig. 3B). We found that in P53<sup>−/−</sup> HCT116, MCT1 silencing prevented lactate-exposed cells to acquire the capacity to increase their proliferation rate under hypoxia (vs. P53<sup>WT</sup> HCT116; Fig. 3G). We also confirmed that p53<sup>−/−</sup> HCT116 could take up 5-fold more lactate in these conditions than wild-type cells (Fig. 3H). We also found that, under deeper hypoxia conditions (i.e., 0.1% O<sub>2</sub>), lactate failed to be taken up by P53<sup>−/−</sup> HCT116 tumor cells (Fig. 3H), which in turn rapidly died in lactate-containing medium (not shown).

Lactate uptake under hypoxia is used as an alternate fuel for mitochondrial respiration

To determine whether mitochondria were involved in the consumption of lactate under moderate hypoxia, we used 1 μmol/L rotenone to block cell respiration and found that in this condition, lactate failed to support cell survival (Fig. 3I). Cotreatment with glucose to fuel glycolysis, however, completely rescued rotenone-exposed P53<sup>−/−</sup> cells (Fig. 3I). It is noteworthy that in P53<sup>WT</sup> HCT116 cells, glucose only partly rescued proliferation in the presence of 1 μmol/L rotenone, confirming a higher dependency on (respiratory) oxidative metabolism (vs. p53<sup>−/−</sup> HCT116 cells). Because lactate conversion into pyruvate is required to fuel mitochondrial respiration, we also examined the extent of the LDHB expression in both cell genotypes. We found that in P53<sup>−/−</sup> HCT116 cells, LDHB expression remained significantly higher with time under hypoxia than in P53<sup>WT</sup> cells (Fig. 3J). Interestingly, the expression of LDHA, the gene product known to promote the reverse conversion of pyruvate into lactate, was transiently increased in response to hypoxia in both cell genotypes but more rapidly returned to basal level in P53<sup>−/−</sup> HCT116 cells (than in P53<sup>WT</sup> cells; Fig. 3K). We also observed a significantly higher expression of COX4-2 (the cytochrome c oxidase isoform known to support mitochondrial respiration under low O<sub>2</sub>; ref. 30) in P53<sup>−/−</sup> HCT116 cells (Fig. 3L).

The expression and stability of MCT1 mRNA under hypoxia are dependent on the expression of NF-κB and P53

To understand the p53-dependent changes in MCT1 expression in HCT116 cells exposed to hypoxia, we examined possible regulation by major transcription factors known to be induced under low O<sub>2</sub> conditions, namely HIF-1α, HIF-2α, and NF-κB. Whereas the use of siRNA targeting HIF-1α and HIF-2α failed to prevent the increase in MCT1 expression in P53<sup>−/−</sup> cells, silencing of the NF-κB subunit P65 led to a significant reduction in MCT1 expression in these cells (Fig. 4A). Alteration in MCT1 mRNA stability was also evaluated by exposing either cell genotype to hypoxia in the presence of actinomycin D. This experiment revealed that the half-life of the MCT1 mRNA was about the same (i.e., 15 hours) in normoxia for either P53<sup>−/−</sup> cells (Fig. 4B). We then attempted to determine whether P53 could act as a repressor of MCT1 gene expression, via destabilization of MCT1 mRNA. We first confirmed the presence of 2 P53 response elements in positions −382 and −297 of the MCT1 gene (Fig. 4C). We then carried out a chromatin immunoprecipitation (ChIP) experiment using p53 antibodies followed by a PCR reaction using primers located up- and downstream the putative p53-binding regions...
Hypoxia-induced MCT1 expression allows efflux and influx of lactate according to nutrient availability. A, lactate efflux from P53WT and P53−/− HCT116 cells exposed for 72 hours under 1% O2 hypoxia after control shRNA (open bars) or MCT1 shRNA transduction (**, P < 0.01; n = 3). B, representative immunoblot documenting MCT1 silencing in the conditions described in A. C, P53WT and P53−/− HCT116 cell proliferation (expressed as % of cell density) after 6 days under 1% O2 hypoxia (open bars) followed by 3 days of reoxygenation (21% O2; black bars) or 3 more days of hypoxia (hatched bars); **, P < 0.01 (n = 3). D, kinetics of glucose uptake and lactate fluxes (uptake and release) in P53WT and P53−/− HCT116 cells along the 6 days of hypoxia and 3 days of reoxygenation, as detailed in C; **, P < 0.01 (n = 3). E, P53WT and P53−/− HCT116 cell proliferation after 3 days hypoxia in glucose-containing medium and the additional indicated periods of time in glucose-free medium containing 10 mmol/L lactate or hydroxybutyrate; **, P < 0.01 (n = 3). F, ratio of the extents of P53WT and P53−/− HCT116 cell proliferation after the indicated periods of hypoxia in the presence of lactate- versus glucose-containing medium; **, P < 0.01 (n = 3). G, same experiment as in F depicting proliferation ratio (lactate vs. glucose) after 72 hours hypoxia using MCT1-silenced P53WT and P53−/− HCT116 cells; a control shRNA was used as control, **, P < 0.01 (n = 3). H, lactate uptake by P53WT and P53−/− HCT116 cells along the 6 days hypoxia + 3 days reox (**, P < 0.01; n = 3). I, P53WT and P53−/− HCT116 cell proliferation after 3 days hypoxia in glucose-containing medium and the additional indicated periods of time in glucose-free medium containing 10 mmol/L lactate or hydroxybutyrate; **, P < 0.01 (n = 3). J, LDHB mRNA expression in P53WT and P53−/− HCT116 cells exposed to 1% O2 hypoxia for the indicated period of time (**, P < 0.01; n = 3). K, LDHA mRNA expression in P53WT and P53−/− HCT116 cells exposed to 1% O2 hypoxia for the indicated period of time (**, P < 0.01; n = 3). L, COX4-2 mRNA expression expressed as fold changes (**, P < 0.01 vs. control, n = 3).
within the MCT1 promoter region. We found that amplification occurs exclusively from the DNA material immunoprecipitated from hypoxic P53WT HCT116 cells (Fig. 4D, left panel). Finally, we found that the P53 corepressor mSin-3A (19, 31, 32) was also associated with the MCT1 gene in P53WT HCT116 cells, as documented by using anti-mSin3A antibody in ChIP experiments (Fig. 4D, right panel).

**P53 knockin and knockdown experiments document the intimate relationship between P53 and MCT1**

To validate the P53-dependent regulation of MCT1 expression, we first designed experiments to promote P53 reexpression in SiHa cells using a shRNA-dedicated strategy to stably downregulate HPV16-encoded E6 protein. Clones were selected for their expression of P53 in response to a 2 Gy irradiation. 

**Figure 4.** Hypoxia-induced MCT1 expression in P53−/− cells results from mRNA stabilization and NF-κB activation. MCT1 mRNA expression (as determined by qPCR) in P53WT and P53−/− HCT116 cells cultured in normoxia or 1% O2 hypoxia (A) after transfection with control, HIF-1α, HIF-2α, and P65 siRNA or (B) in the presence of 10 μmol/L actinomycin D; ***, P < 0.01, n = 3. C, sequence localization of putative P53-binding sites (framed) within the MCT1 promoter region as identified by in silico analysis at positions −382 to −359 and −297 to −278. Positions of forward and reverse PCR primers for ChIP analysis are also indicated. D, representative pictures of MCT1 promoter PCR products from anti-P53 (left) and anti-mSin3A (right). ChIP experiments conducted on lysates from cells cultured in normoxia (Norm) or 1% O2 hypoxia (Hyp) for 48 hours; negative controls include the use of irrelevant IgG for IP and a condition with magnetic beads alone (Beads). The input as positive control (Input) and a PCR negative control (NTC) are also included.
These cells as well as parental cells were incubated in normoxia or hypoxia, and the reexpression of P53 in E6-silenced SiHa cells was confirmed (Fig. 5A). Importantly, we found that although in normoxia, there was no difference in MCT1 mRNA expression between parental and E6-silenced cells, a significant inhibition of MCT1 upregulation was observed in hypoxic E6-silenced SiHa cells (Fig. 5B).

In parallel, we also silenced P53 in the MDA-MB-231 cell model of gain-of-function P53 mutation. We selected stably transfected cells with p53-targeting shRNA for their incapacity to respond to a 2 Gy irradiation with an upregulation of P53. We confirmed that P53 silencing prevented any increase in P53 expression in response to hypoxia (Fig. 5C). Furthermore, we found that under hypoxia, P53 knocking down strongly induced MCT1 expression in MDA-MB-231 cells (vs. MCT1 downregulation observed in control MDA-MB-231 cells; Fig. 5D). Breast cancer patients harboring p53 mutations in hypoxic tumors express high levels of MCT1, together with a negative outcome

We then aimed to search for a correlation between P53 status and MCT1 mRNA expression in a bank of needle core biopsies collected from breast cancer patients harboring wild-type P53 (n = 26) or a P53 loss-of-function mutation (n = 24; ref. 33). To integrate the hypoxia parameter in this analysis, we also examined the mRNA expression of CAIX reported to be
induced under low pO2 levels. We used the median expression of CAIX as a cut-off to discriminate between "hypoxic" and "normoxic" tumors. In wild-type P53 tumors, the expression level of MCT1 mRNA was not influenced by hypoxia whereas in mutated P53 tumors, MCT1 expression was significantly increased in the hypoxia group (Fig. 5E). Moreover, when we examined overall survival among breast cancer patients with high expression of MCT1 and p53 mutated status, we found a more negative outcome than in patients harboring lower MCT1 expression (Fig. 5F).

Discussion

The p53 tumor suppressor protein prevents cancer development through various mechanisms, including the induction of apoptosis and cell-cycle arrest for the maintenance of genome stability. Recently, links between p53 and tumor metabolism were also elicited (21). In particular, p53 was documented to regulate the metabolic balance between glycolysis and OXPHOS via p53-inducible genes named TIGAR (23) and SCO2 (22). Whereas expression of TIGAR inhibits glycolysis, expression of SCO2 increases mitochondrial oxygen consumption. As a consequence, loss of p53 stimulates glycolysis and impairs the mitochondrial respiratory chain, thereby promoting a switch in ATP production from OXPHOS to glycolysis.

Here, we show that p53 deficiency also promotes the expression of monocarboxylate transporter MCT1 under hypoxic conditions and thereby facilitates the release of lactate resulting from elevated glycolytic flux. When p53 is active, the mirror picture is observed with the repression of MCT1 expression and a lesser facilitation of the shift from oxidative metabolism to the glycolytic pathway. This observation is in line with previous reports documenting that hypoxia-induced P53 has primarily transrepression activity (19, 31, 32). Mechanistically, we showed that the lack of P53 was associated with the stabilization of the MCT1 mRNA transcript in response to hypoxia, suggesting that, when present, P53 could act as a repressor of MCT1 expression. A similar mode of regulation was recently reported for survivin, the mRNA stability of which is controlled by p53 in hypoxia (33). Moreover, our ChIP experiments confirmed the direct interaction of both P53 and the corepressor mSin3A with the MCT1 gene. In addition, we found that in P53-deficient cells, NF-κB expression was critical to support the hypoxia-induced increased in MCT1 abundance. This mode of regulation is enlightened by genetic manipulation of P53 in 2 distinct tumor models. Indeed, in knockin experiments leading to P53 reexpression in SiHa cells (through silencing of HPV-driven repression of P53), we documented a reduced capacity to stimulate MCT1 expression under hypoxia. Yet more strikingly, in knockdown experiments reducing p53 expression in constitutively active P53 mutation–harboring MDA-MB-231 cells, we could document a dramatic upregulation of MCT1 under hypoxia.

Importantly, our data also unraveled an unsuspected advantage for P53−/− cells because the upregulation of MCT1 expression not only offers the possibility to handle the excess intracellular glycolytic lactate but allows cells to survive when glucose is lacking. We indeed found that the increased expression of MCT1 under hypoxic conditions also contributes to the reverse lactate transport into the cells. We recapitulated this condition (i) by maintaining cells under hypoxic conditions then reoxygenating them without renewing the medium (Fig. 3C), (ii) by exposing cells to hypoxic conditions and then switching the medium from a glucose- to lactate-containing (or hydroxybutyrate-) solution (Fig. 3E), and (iii) by exposing cells under hypoxic conditions in the presence of lactate (Fig. 3F). In each of these conditions, the upregulation of MCT1 was associated with a net survival and/or proliferation advantage due to lactate uptake. Fluxuations in tumor oxygenation are known to occur in tumors and are even proposed to participate in the clonal selection of tumor cells (25, 35–37). Reductions in tumor perfusion and nutrients availability can in part be superimposed to the oxygenation status of tumor areas at a given time. In these areas/periods of reduced glucose availability, our data indicate that MCT1 expression in P53−/− tumor cells may provide them with a metabolic plasticity, offering access to lactate as an alternate fuel.

We actually showed that when glucose is lacking, lactate can fuel mitochondrial respiration under moderate hypoxia and thereby ensure cell survival and even proliferation. Three lines of evidence support this finding: (i) when glucose was made unavailable, the mitochondrial respiratory chain inhibitor rotenone or the reduction in pO2 levels to 0.1% prevented the lactate rescue effects (ii) the LDHB/LDHA ratio is more compatible with the conversion of lactate to pyruvate (38, 39) in hypoxic P53−/− cells (than WT p53 cells), (iii) an increase in cytochrome c oxidase isofrom COX4-2 was observed, an adaptation known to optimize the capacity of the respiratory chain to work under hypoxia (30).

In conclusion, our study identified hypoxia-induced MCT1 upregulation as an additional adaptation of P53-deficient tumor cells to the (Warburg type) glycolytic metabolism, that is, an increased capacity to meet the bioenergetic and biosynthetic demands of proliferating cells (40). Our data also unraveled the capacity of tumor cells under a moderate hypoxic environment to take advantage of the overexpression of MCT1 to work in the reverse mode, that is, to take up lactate to fuel mitochondrial respiration after reconversion into pyruvate.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work was supported by grants from the Fonds de la Recherche Scientifique FRS-FNRS, the Télémé, the Foundation against cancer, the J. Maisin Foundation, an Action de Recherche Concertée (ARC 09/14-020 to O. Feron and P. Sonveaux) and the European Research Council FP7/2007-2013 (grant #243188 to P. Sonveaux).

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Received July 22, 2011; revised November 15, 2011; accepted December 5, 2011; published OnlineFirst December 19, 2011.
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Cancer Res Published OnlineFirst December 19, 2011.

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Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-11-2474

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