WWOX loss activates aerobic glycolysis

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Cancer cells undergo reprogramming of glucose metabolism to limit energy production to glycolysis—a state known as “aerobic glycolysis.” Hypoxia-inducible factor 1 (HIF1α) is a transcription factor that regulates many genes responsible for this switch. As discussed here, new data suggest that the tumor suppressor WW domain-containing oxidoreductase (WWOX) modulates HIF1α, thereby regulating this metabolic state.

During malignant transformation cancer cells undergo significant metabolic changes. The change in glucose metabolism is the best-known example of metabolic reprogramming in cancer cells.1 Under aerobic conditions, normal cells process glucose to pyruvate via glycolysis in the cytosol and thereafter to carbon dioxide in the mitochondria, yielding ATP. However, under anaerobic conditions glycolysis is favored and relatively little pyruvate is dispatched to oxygen-consuming mitochondria. In cancer cells, the main source of cellular energy is glycolysis, even in the presence of abundant oxygen. This shift to aerobic glycolysis, termed the Warburg effect, is a hallmark of malignant cells.1-3 The reliance of cancer cells on aerobic glycolysis for ATP production results in high glucose consumption to compensate for the relatively low efficiency of glycolysis. Identifying the molecular mechanisms responsible for the shift to aerobic glycolysis is important for understanding the basic biology of malignant transformation and for designing targeted therapies.

The transcription factor hypoxia-inducible factor 1 (HIF1α) is a known regulator of glycolysis in response to hypoxia.4 Hypoxic microenvironmental stress activates HIF1α, which in turn increases glycolysis and decreases mitochondrial function. HIF1α was first identified because of its response to hypoxia, but recent studies show that HIF1α is transcriptionally regulated under normoxic conditions by oncogene activation and inactivation of tumor suppressors. For example, under normoxic conditions HIF1α accumulates in cancer cells upon activation of oncogenes such as RAS and AKT (also known as protein kinase B [PKB]) or loss of tumor suppressors such as phosphatase and tensin homolog (PTEN) and Von Hippel–Lindau (VHL) (Fig. 1). These observations provide evidence that HIF1α is tightly regulated to ensure proper cellular responses to nutrient and stress conditions. In a recent paper published in Cell Death & Differentiation, we reported that loss of the tumor suppressor WW domain-containing oxidoreductase (WWOX) is associated with enhanced levels and function of HIF1α, resulting in rewiring of cell metabolism and cancer transformation (Fig. 1).5

WWOX is a 46-kDa protein that contains 2 N-terminal WW domains and a central short-chain dehydrogenase/reductase (SDR) domain. Loss of WWOX expression occurs in a variety of tumors and is associated with poor prognosis (see review6). Wwox knockout mice exhibit post-natal lethality, dying by 4 weeks of age as a result of severe metabolic defects, mainly hypoglycemia.7 At the molecular level, WWOX interacts with proline-tyrosine motif-containing proteins via its WW1 domain, thus regulating their localization and transcriptional function.8

The aberrant metabolism phenotype in Wwox knockout mice led us to

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hypothesize that WWOX regulates cellular metabolism. Using primary mouse embryonic fibroblasts (MEF), we demonstrated that targeted WWOX deletion causes increased glucose uptake in a cell-autonomous fashion. Wwox-deficient cells displayed significantly higher levels of lactate and NADPH, while exhibiting low NADH and ATP levels and reduced oxygen consumption. Together, these findings suggest that Wwox-deficient cells use glucose primarily for glycolysis. We further validated the presumed shift to glycolysis using the Metabolon Inc. platform and concluded that absence of WWOX causes a switch toward enhanced glycolysis and reduced mitochondrial respiration, a response usually observed under conditions of oxygen stress.

These findings led us to further hypothesize that WWOX regulates glucose metabolism. Since HIF1α regulates glycolysis and reduces mitochondrial respiration, we assessed HIF1α levels and activity and observed that HIF1α levels are elevated in Wwox knockout cells relative to WT cells and that multiple glucose-related HIF1α target genes are upregulated in the absence of WWOX. Conditions of oxygen stress cause activation of HIF1α, with increased protein levels due to both enhanced protein synthesis and stabilization of the protein. As lack of WWOX mimics an oxygen stress response, we hypothesized that WWOX levels change under hypoxic conditions and indeed found that WWOX expression is downregulated under hypoxia, consistent with recent findings and indicating that WWOX regulates HIF1α under physiological conditions. These observations led us to suggest that lack of WWOX triggers a HIF1α-dependent metabolic switch and that deleting HIF1α in Wwox-deficient cells would increase glucose uptake. As predicted, both genetic and pharmacologic inhibition of HIF1α reversed the glucose uptake phenotype in vitro and in vivo.

How does WWOX affect HIF1α function and glucose metabolism? Our findings revealed that WWOX controls HIF1α function by suppressing its transcriptional function, possibly by sequestering HIF1α from its target sequences. WWOX might also enhance HIF1α hydroxylation and degradation, thereby maintaining proper glucose flux toward mitochondrial respiration and preventing excessive glycolysis. At the biochemical level, WWOX physically interacts with HIF1α through its WW1 domain and functionally decreases HIF1α levels, and hence glucose uptake. In contrast, mutated WWOX, which has impaired interaction with HIF1α, does not affect HIF1α levels or decrease glucose uptake. Furthermore, WWOX-sufficient cells display higher HIF1α hydroxylation than WWOX-deficient cells. Ultimately, WWOX suppresses glycolysis through inhibition of HIF1α.

To determine whether the WWOX–HIF1α association has functional relevance for tumor formation, we investigated the effect of HIF1α expression on WWOX-mediated tumor suppression. Targeted deletion of Wwox in transformed MEFs is associated with enhanced tumorigenesis in mice. Notably, Wwox-deficient cells that are depleted of HIF1α fail to form tumors. Clinically, expression of WWOX is inversely correlated with that of glucose transporter 1 (GLUT1), a direct target of HIF1α, in human breast cancer patients.

In summary, our study provides evidence that the tumor suppressor WWOX acts as a safeguard mechanism to inhibit HIF1α activity under normoxic conditions, guaranteeing glucose flux into mitochondria and the Krebs cycle and thus
preventing the Warburg effect. Together, these data indicate that loss of WWOX activates aerobic glycolysis, a hallmark of cancer cells.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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