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Perspective

NDRG3-mediated lactate signaling in hypoxia

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Hypoxia is associated with many pathological conditions as well as the normal physiology of metazoans. We identified a lactate-dependent signaling pathway in hypoxia, mediated by the oxygen- and lactate-regulated protein NDRG family member 3 (NDRG3). Oxygen negatively regulates NDRG3 expression at the protein level via the PHD2/VHL system, whereas lactate, produced in excess under prolonged hypoxia, blocks its proteasomal degradation by binding to NDRG3. We also found that the stabilized NDRG3 protein promotes angiogenesis and cell growth under hypoxia by activating the Raf-ERK pathway. Inhibiting cellular lactate production abolishes NDRG3-mediated hypoxia responses. The NDRG3-Raf-ERK axis therefore provides the genetic basis for lactate-induced hypoxia signaling, which can be exploited for the development of therapies targeting hypoxia-induced diseases in addition to advancing our understanding of the normal physiology of hypoxia responses. [BMB Reports 2015; 48(6): 301-302]

Low oxygen (hypoxia) conditions are frequently observed in many aspects of normal physiology such as in cells of exercising muscle or actively growing embryonic/fetal tissues. Hypoxia is also intimately associated with many diseases such as cancer and inflammation. The presence of hypoxia has been positively correlated with poor prognosis of cancer patients. Hypoxic conditions induce various cellular responses in metazoans, including metabolic reprogramming, angiogenesis, and anti-apoptosis, which facilitate adaptation to and survival in the harsh environment. Hypoxia inducible factors (HIFs) play central roles in these processes by controlling the expression of genes involved in diverse aspects of hypoxia responses. However, growing evidence indicates that hypoxia has many aspects that are not explained by HIF-mediated mechanisms alone. For example, the inhibition of HIF-mediated pathways did not always prevent tumor growth. Also, angiogenesis was preserved when *HIF1A* was knocked-out in embryonic stem cells or colon cancer cells. Therefore, a role for other oxygen-regulated pathways that are, similar to HIF pathways, controlled by PHD enzymes has been suggested. However, the HIF-independent aspects of hypoxic signaling remain poorly understood.

Using an immunoprecipitation assay coupled to mass spectrometry, we identified a novel PHD2-binding protein, NDRG3, whose expression is negatively regulated by oxygen at the protein level via the proteasomal pathway. We found that NDRG3 is a bona fide substrate of the PHD2/VHL system, specifically hydroxylated at proline 294 (Fig. 1). In contrast, NDRG3 expression is positively regulated by lactate that accumulates at the later phase of hypoxia when lactate binding inhibits NDRG3 ubiquitination by disrupting its interaction with VHL and subsequently blocks the proteasomal degradation (Fig. 1). However, the mRNA expression of NDRG3 was not dependent on HIF activity.

We predicted the function of *NDRG3* in hypoxia by transcriptome analyses of the cells manipulated for *NDRG3* expression. We then experimentally demonstrated that NDRG3 plays critical roles in the regulation of prolonged hypoxia responses by promoting cell growth and angiogenesis while suppressing hypoxia-induced cell apoptosis. We also showed that hypoxically accumulated NDRG3 physically binds with c-Raf protein and induces its phosphorylation as well as the activation of downstream ERK1/2 signaling. The NDRG3-mediated Raf-ERK pathway activation was critically dependent on lactate accumulation as demonstrated by experiments in which cellular lactate production or flux was genetically or pharmacologically manipulated or when lactate was exogenously provided to lactate-depleted cells. Moreover, the NDRG3-mediated biologic responses in prolonged hypoxia were also dependent on lactate accumulation and Raf-ERK pathway activation.

Lactate is the end product of glycolysis, which is activated at the early stage of hypoxia when HIF-1α up-regulates glycolytic gene expression to induce metabolic adaptation. As a result, increasing amounts of lactate accumulate at later stages of hypoxia. Recent studies reported diverse roles for lactate as an

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Abbreviations: HIF, hypoxia-inducible factor; NDRG3, NDRG family member 3; PHD2, prolyl hydroxylase domain protein 2; VHL, von-Hippel Lindau; LDHA, lactate dehydrogenase A

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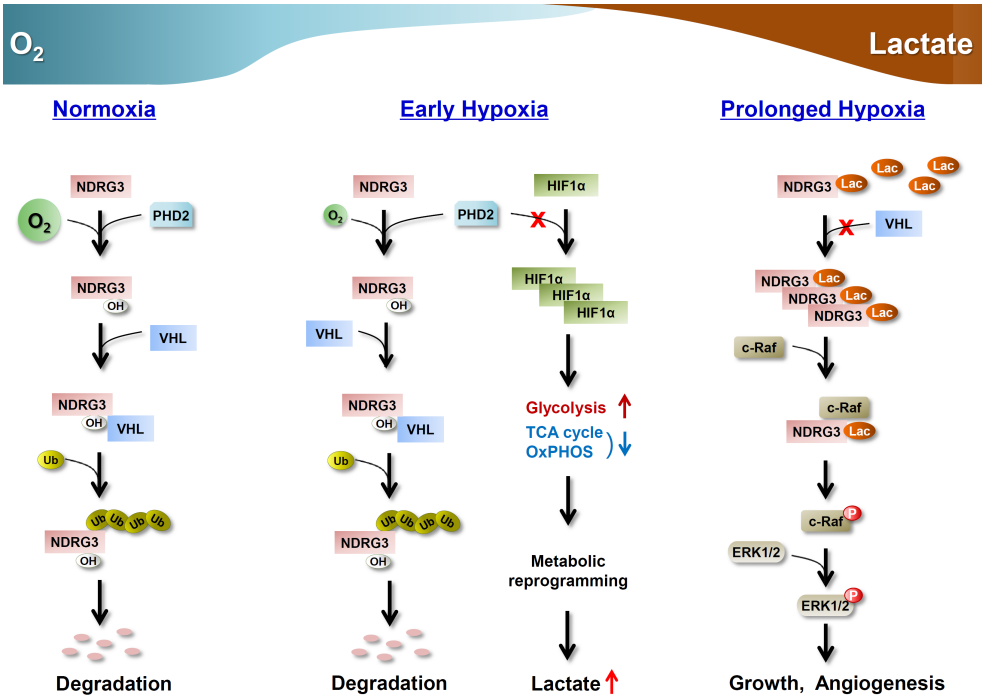

Fig. 1. A scheme outlining the post-translational regulation of hypoxia responses involving HIF-1 α and NDRG3 (Modified from Lee *et al* (2015), Cell, 161(3):595-609).

alternative energy source in oxidative tumors, as an inducer of angiogenesis, and as a modulator of cell motility. In line with these observations, high levels of lactate production and LDHA overexpression are known as negative prognostic marker in cancers. However, the key elements regulating the lactate-induced biologic responses and the underlying molecular mechanisms remain unknown.

Our results demonstrated the roles of lactate signaling in hypoxia as well as its genetic nature, which depends on the NDRG3-c-Raf-ERK1/2 axis. These findings suggest that HIF-1α and NDRG3 form an oxygen-dependent regulatory chain for hypoxia responses, divided into two chronological phases. At the early phase of hypoxia, HIF-1α protein accumulates, regulating the gene expression necessary for early adaptive responses including metabolic reprogramming, while at the later phase, the up-regulated lactate production signals for the accumulation of NDRG3, activating the Raf-ERK pathway to induce responses necessary for coping with prolonged hypoxia. Thus, the lactate signaling and accompanying biologic responses appear to be functionally coupled to the HIF-1α-induced metabolic reprogramming, employing NDRG3 as the critical link. In this regard, it is suggested that portions of the hypoxia responses, especially those occurring at the later phase of hypoxia, that have been so far attributed to HIF-1α, might, in fact, be under the direct control of NDRG3-mediated lactate signaling.

In conclusion, we demonstrated that NDRG3 functions as the hypoxia-inducible lactate sensor, playing key roles in the promotion of hypoxia responses in a HIF-independent manner. The role of NDRG3 in hypoxic lactate signaling implies that the PHD2/VHL system can control both HIF-dependent and -independent hypoxia responses in an oxygen-dependent manner. Therefore, the lactate-NDRG3-Raf-ERK signaling pathway may provide an extended mechanistic clue to the understanding of disorders caused by mutations in *VHL* (hemangioblastoma, renal cell carcinoma, pheochromocytoma, etc) or *PHD2* (familial erythrocytosis-3) as well as the hypoxia-related physiological (differentiation and development, exercise physiology, etc) and pathophysiological responses (cancer, wound healing, inflammation, cardiovascular disorders, altitude sickness, etc). We also suggest that combinatorial targeting of *HIF* and *NDRG3* might prove a highly effective anti-hypoxia strategy, considering the evidence indicating that simple inhibition of HIF may be insufficient for treating hypoxia-induced diseases.

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