

RESEARCH HIGHLIGHT

Another road leads to HIF-1 activation: implications for prostate cancer progression

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Asian Journal of Andrology (2012) 14, 241–242; doi:10.1038/aja.2011.163; Published online: 21 November 2011

Hypoxia has been identified as a common environmental stress factor associated with therapeutic resistance and metastasis in human cancers. A major player in regulating the response to hypoxia is the transcriptional factor hypoxia-inducible factor 1 alpha (HIF-1 α). Although HIF-1 α is well known to be stabilized under hypoxia, the regulatory mechanism of HIF-1 α signaling in malignant cells is not fully understood. In a recent paper, Yuan *et al.*¹ identified a novel pathway that acts as a ‘vicious cycle’ to amplify HIF-1 activation in prostate cancer cells, hence suggesting potential biomarkers that may predict progression and poor prognosis in human prostate cancer (**Figure 1**).

In prostate cancer, the upregulation of HIF-1 α represents an early event in tumorigenesis² that is highly correlated with the risk of metastases.³ At the molecular level, the HIF-1 α protein rapidly accumulates under anaerobic conditions due to blockage of proteasomal degradation, and functionally induces an array of genes involved in tumor angiogenesis, invasion, metabolism and survival.⁴ HIF-1 can also be activated in an O₂-independent manner as a consequence of aberrant activation of oncogenes or inactivation of tumor suppressor genes (**Figure 1**). In addition to these regulatory mechanisms, an alternative pathway for HIF-1 α activation is reported by Yuan *et al.*¹ Namely, hypoxia is able to induce an HIF-1-dependent positive feedback loop mediated by the destruction of promyelocytic leukemia (PML), a short half-life tumor suppressor protein whose abnormal degradation is often observed in human cancers.

Based on the previous findings that loss of PML can result in HIF-1 α upregulation⁵ and that an adaptor protein of Cullin 3 ubiquitin ligase called KLHL20 is involved in PML assembly and degradation,⁶ the goal of this study was to investigate the impact of hypoxia on the regulation of PML degradation mediated by KLHL20 in prostate cancer cells. The authors first demonstrated that hypoxia was able to induce PML degradation and *KLHL20* gene expression, both of which were HIF-1-dependent. Because PML was a direct substrate of the KLHL20–Cullin 3 ligase, subsequent studies focused on identifying PML upstream contributors that would facilitate PML degradation. The authors observed that PML could be phosphorylated by cyclin-dependent kinases 1 and 2, which could be further modified by the peptidyl-prolyl isomerase Pin1. This in turn resulted in the promotion of the recruitment of PML to KLHL20 and PML decay, suggesting that cyclin-dependent kinase 1/2 and Pin1 are required for PML degradation. Interestingly, these phenomena occurred under both hypoxic and normoxic conditions, suggesting the existence of the pathway irrespective of the tumor environment.

How does hypoxia-induced KLHL20-mediated PML destruction impact HIF-1 α abundance? Yuan *et al.*¹ monitored HIF-1 α expression by silencing the KLHL20–PML pathway. Data suggest that *KLHL20* gene knockdown result in decreased HIF-1 α and its target gene expression under hypoxic conditions. This effect can be reversed by PML gene ablation, implying that hypoxia-/HIF-1-dependent KLHL20-mediated PML loss may further activate HIF-1 signaling. This positive feedback induction of HIF-1 α is partially due to the restoration of the mammalian target of rapamycin (mTOR) pathway, an observation that is in agreement with a previous finding that PML inhibits HIF-1 α through mTOR

repression.⁵ In line with HIF-1 α alteration, knockdown of both KLHL20 and PML, but not KLHL20 alone, maintained prostate cancer cell epithelial–mesenchymal transition, migration, survival and resistance to chemotherapy; phenotypes involved in disease progression and metastasis. These *in vitro* data infer that the KLHL20–PML pathway sustains tumor growth and angiogenesis *in vivo*. Interestingly, another article recently showed that HIF-1 stabilization leads to an enhanced epithelial–mesenchymal transition phenotype through the HIF-1-dependent vascular endothelial growth factor-mediated autocrine pathway in prostate cancer cells.⁷ Together, these two studies highlight a central role of HIF-1 signaling driving prostate cancer cell metastasis.

Hypoxia-associated gene products have been suggested as potential prognostic indicators of conventional anticancer therapies^{8,9} or predictors for high Gleason grade⁷ in prostate cancer patients. By analyzing the expression profiles of components of the HIF-1–KLHL20–PML pathway in 79 patients, Yuan *et al.*¹ found higher expression of HIF-1 α , Pin1 and KLHL20, and lower expression of PML in tumors compared to benign prostate hyperplasia, though cyclin-dependent kinase 1/2 expression was not shown. In addition, a positive correlation between HIF-1 α and KLHL20, as well as a negative correlation between PML and HIF-1 α , KLHL20 or Pin1 was also found. Notably, the molecular signature of high HIF-1 α , KLHL20 and Pin1, and low PML strongly correlated with Gleason score, indicating a possible predictive value when applying this signature as a biomarker. Taken together, these patient data provide clinical support for the notion that deregulation of the HIF-1–KLHL20–PML signaling axis does exist in prostate cancer and is associated with tumor progression.

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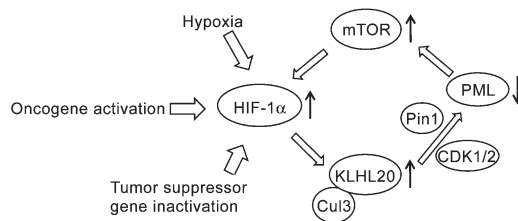


Figure 1 HIF-1 α regulation in cancer. HIF-1 α can be activated in both O₂-dependent and -independent manners. In addition, Yuan *et al.*¹ propose an alternative pathway that may amplify HIF-1 signaling in prostate cancer. CDK, cyclin-dependent kinase; Cul3, Cullin 3; HIF-1 α , hypoxia-inducible factor 1 alpha; mTOR, mammalian target of rapamycin; PML, promyelocytic leukemia.

Despite the significant findings of Yuan *et al.*,¹ several questions remain. Will alterations in the HIF-1–KLHL20–PML pathway modulate the metastatic potential of tumor cells *in vivo*? Since PML is known to exist in normal cells,¹⁰ how specific will it or its associated molecules be when serving as a biomarker for cancer? What proportion of HIF-1 α detected under hypoxic conditions is a consequence of stimulating this pathway? Unraveling these questions will go a long way to validate the role of the HIF-1–KLHL20–PML pathway in prostate cancer and other cancer types.

In summary, Yuan *et al.*¹ proposed an alternative means of HIF-1 activation based on a hyperactivated HIF-1 α –Pin1–KLHL20–PML–mTOR–HIF-1 α positive feedback loop

that could maximize HIF-1 activity in response to aberrant tumor microenvironments (Figure 1). Their study uncovers a potential mechanism for the aggressive behavior of advanced prostate cancer and suggests that interventions in this pathway, such as HIF-1 α targeting and PML restoration, may offer possible treatment strategies to impair disease progression. Additionally, Yuan and colleagues' findings imply that mTOR inhibitors may have utility in impeding HIF-1-associated functions by suppressing HIF-1 α synthesis in this setting.

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