A New Role for PHD in Chemotherapy

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Enhancing therapeutic activity against cancer cells and minimizing toxic effects on normal cells are critical elements in chemotherapy. In this issue of *Cancer Cell*, Leite de Oliveira and colleagues reveal a previously unrecognized role of a prolyl hydroxylase domain in promoting drug delivery to tumors and reducing toxicity in normal organs.

Chemotherapy using cytotoxic agents, such as doxorubicin and cisplatin, to damage cancer cells and inhibit tumor growth remains a major therapeutic modality in cancer treatment. The low selectivity of cytotoxic agents in killing cancer cells over normal cells has been a major challenge and significantly limits the application of these drugs for cancer treatment. This problem is further compounded by the fact that tumors usually have blood vessels that seem to be leaky and tend to cause high interstitial fluid pressure. This abnormal vasculature limits drug delivery to cancer cells and, therefore, reduces the effectiveness of systemic chemotherapy (Carmeliet and Jain, 2011; Heldin et al., 2004). In most cases, the administration of higher drug doses to increase drug concentrations in tumor tissues is not a practical option due to the risk of severe side effects. Although local administration by injecting cytotoxic agents into the tumor or the main blood vessels feeding the tumor may enhance anticancer activity and reduce systemic toxicity, such approaches may only be applied to a small subset of tumors at certain anatomic locations. Clearly, new therapeutic approaches, including novel strategies to normalize the aberrant tumor vessels and developing tumor-specific agents, are needed to improve cancer treatment outcome.

Recent studies suggest that targeting the prolyl hydroxylase domain-containing protein 2 (PHD2) may lead to normalization of blood vessels in tumors (Mazzone et al., 2009) and prevent oxygen-induced microvascular obliteration in the retina (Duan et al., 2011) through HIF-dependent mechanisms. Prolyl hydroxylases are a family of enzymes that catalyze the hydroxylation of proline residues in a variety of proteins and affect multiple biological functions including collagen formation, oxygen sensing, RNA transcription, and NF-κB signaling (Gorres and Raines, 2010). Among the prolyl hydroxylases, PHD2 seems to play a major role in oxygen sensing and blood vessel formation and significantly affects cancer oxygenation and metastasis. It was recently shown that heterozygous deletion of *Phd2* in mice caused an increased expression of HIF-1α and HIF-2α in endothelial cells, leading to the transition of endothelial tip cells to a more quiescent stage and normalization of the endothelial lining of tumor vasculatures (Mazzone et al., 2009). The profound effect of PHD2 on the structure of tumor blood vessel provides the intriguing possibility of targeting this molecule to normalize blood flow in tumor tissues and to increase drug delivery to cancer cells.

In this issue of *Cancer Cell*, Leite de Oliveira et al. (2012) report that reduced PHD2 expression by heterozygous gene deletion could significantly enhance the therapeutic activity of doxorubicin and cisplatin in mice and substantially reduce the toxic side effects of the drugs in normal organs, such as the heart and kidneys. These striking effects seem to be mediated by two separate mechanisms, both linked to the activation of hypoxia inducible factors (Figure 1). First, the decrease in PHD2 activity caused a significant increase in HIF-1α and HIF-2α protein levels in endothelial cells, leading to a normalization of the endothelial lining of blood vessels within tumor tissues. This, in turn, improved blood circulation and reduced interstitial fluid pressure in the tumor, resulting in an increase in the delivery of anticancer agents to the tumor. The authors suggest that this mechanism was mainly mediated through the oxygen-sensing function of PDH2 via HIF-2. In contrast, the blood circulation in normal organs seemed not to be affected by heterozygous deletion of *Phd2*, and there was no increase in drug concentrations in the normal tissues. Importantly, *Phd2* deficiency in normal cells led to HIF-mediated upregulation of several key antioxidant enzymes, including superoxide dismutases, catalase, and glutathione peroxidase-1, which enhanced the antioxidant capacity of normal cells and increased their ability to tolerate the production of reactive oxygen species (ROS) induced by anticancer drugs. The authors showed that the protection of normal organs by *Phd2* heterozygous deletion was likely due to activation of the ROS-sensing mechanism of HIF-1 and HIF-2, which upregulate the expression of antioxidant enzymes.

The significant in vivo antitumor activity of doxorubicin and cisplatin observed in $Phd2^{+/-}$ mice suggests that targeting PDH2 is a potentially promising strategy to improve the overall therapeutic outcome for cancer patients and that the local drug concentrations in tumor tissues may play a major role in determining in vivo drug response. However, other mechanisms, in addition to increased local drug concentrations, could contribute to the striking inhibition of tumor growth by doxorubicin and cisplatin in $Phd2^{+/-}$ mice. For instance, *Phd2* deletion might, through the activation of HIF-1 and HIF-2, affect the energy metabolism and redox regulation in tumor stroma and, thus, potentially impair stromal support for the tumor cells.

Indeed, recent studies suggest that the tumor-stromal cell interaction plays a major role in affecting the ROS status in cancer cells and their drug sensitivity (Zhang et al., 2012; Nakasone et al., 2012). Thus, testing the potential role of PHD2 in modulating tumor-stroma interaction would be an interesting area of future investigation.

It is important to note that the normalization of tumor blood vasculature in *Phd2*$^{+/-}$ mice seem to be mediated, at least in part, by the HIF-driven upregulation of VEGFR1 and VE-cadherin (Mazzone et al., 2009). Thus, suppression of the VEGF pathway might potentially have an opposite and perhaps unfavorable effect. Indeed, inhibition of angiogenesis by blocking VEGF using the humanized monoclonal antibody bevacizumab has been shown to cause a decrease in the delivery of anticancer drugs to the tumor tissues (Van der Veldt et al., 2012). These observations together suggest that caution should be exercised in considering combination of VEGF blocking drugs and traditional chemotherapeutic agents. The proper timing of drug administration would be important in avoiding potential antagonist effect.

The two major consequences of *Phd2* deletion, namely normalization of tumor vasculature and enhancement of antioxidant and detoxification capacity in normal organs, suggest that pharmacological inhibition of PHD2 may be an effective strategy to increase the therapeutic activity and reduce the toxic side-effect of traditional chemotherapeutic agents. However, systemic administration of PHD2 inhibitors might potentially cause an increase in HIF-1 and HIF-2 in cancer cells and, in turn, upregulation of detoxification enzymes and drug resistance. However, the observation by Leite de Oliveira et al. (2012) that silencing of *Phd2* in tumor cells did not significantly alter their expression of antioxidant enzymes and drug response seem to ease this potential concern, although the mechanisms responsible for the major difference between normal cells and cancer cells in their response to PHD2 abrogation remain largely unclear. This is obviously an important area for future study. Furthermore, since there are multiple prolyl hydroxylases that affect many important biological functions, development of specific inhibitors of PHD2 is important for successful pharmacological targeting. In summary, the study by Leite de Oliveira et al. (2012) suggests that PHD2 is a promising therapeutic target and warrants further in vitro and in vivo studies in terms of mechanisms and clinical applications.

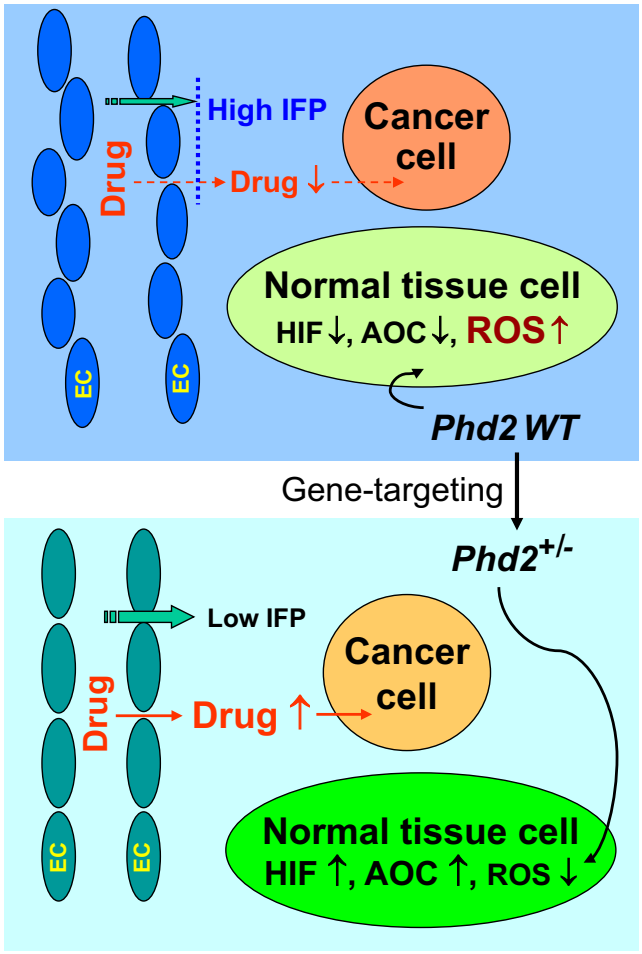

Figure 1. PHD2 Deficiency Increases the Therapeutic Activity of Anticancer Agents and Decreases Side-Toxicity

In mice with wild-type *Phd2*, the blood vessels in tumor tissue have abnormal endothelial lining, are leaky, and often cause high interstitial fluid pressure (IFP), leading to a decrease in drug distribution to cancer cells (upper panel). Heterozygous deletion of *Phd2* promotes normalization of the tumor vasculature and enhances drug delivery to cancer cells (lower panel). A decrease in PHD2 activity also leads to elevated HIF-1α and HIF-2α, resulting in an upregulation of antioxidant enzymes in normal tissue cells, thus, enhancing their antioxidant capacity to detoxify ROS induced by anticancer agents.

PHD2, prolyl hydroxylase domain-2; HIF, hypoxia inducible factor; WT, wild-type; AOC, antioxidant capacity; ROS, reactive oxygen species; EC, endothelial cells; IFP, interstitial fluid pressure.

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