

# Intermittent Hypoxia Furthers the Rationale for Hypoxia-Inducible Factor-1 Targeting

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## Abstract

**Hypoxia-inducible factor-1 (HIF-1) stabilization is a pivotal event in the response to hypoxic stress. A study in the December 15, 2006 issue of *Cancer Research* shows that HIF-1 stabilization occurs more robustly as a result of intermittent hypoxia compared with chronic hypoxia. The findings of this study suggest that intermittent hypoxia might influence the efficacy of radiotherapy by more strongly affecting the growth and survival of vascular endothelial cells. This finding offers additional encouragement to efforts to target HIF-1 for cancer therapy.** [*Cancer Res* 2007;67(3):854–5]

## Background

The subject of intermittent hypoxia has been of interest for more than 20 years. Yamaura and Matsuzawa (1) were the first investigators to provide indirect evidence for intermittent hypoxia based on their direct observation of tumor regrowth in skinfold window chambers after radiation. Their observations indicated that tumor regrowth tended to occur more prevalently in regions where there was a high incidence of intermittent vascular stasis. Because hypoxic cells are more resistant to radiation than aerobic cells, they speculated that these acutely hypoxic cells might be the most radioresistant cells and therefore responsible for tumor regrowth. Brown (2) provided the first radiobiological evidence for the importance of intermittent hypoxia when he selectively killed hypoxic cells with a hypoxic cytotoxin and then observed reemergence of radiobiological hypoxia 24 h later. Subsequent studies by Hill and Chaplin (3) and Pigott et al. (4) clearly showed that instability in perfusion was ubiquitous in several preclinical tumor lines as well as in human tumors.

We have focused our efforts in attempting to determine the underlying cause for intermittent hypoxia. Importantly, we showed that intermittent hypoxia is not due to vascular stasis, but rather instability in red cell flux (5–7). There are two dominant time scales. When observing  $pO_2$  continuously, we observe fluctuations of one to three cycles per hour (Fig. 1A and C). Similar kinetics have been recently reconfirmed in spontaneous canine tumors (8). Chronic observation of hemoglobin saturation in skinfold window chambers has revealed a more prolonged time scale, with fluctuations occurring on a day-to-day basis (Fig. 1A and B; ref. 9). Bennewith and Durand (10) showed mismatch in hypoxia marker drug binding when a

chronically administered hypoxia marker drug was compared with an acutely administered drug. It is likely that events with this longer time scale are related to vascular remodeling and adaptation in response to continued angiogenic stimulus (11). One of the potential consequences of instability in oxygenation might be oxidative stress, which occurs as a result of hypoxia-reoxygenation injury.

The consequences of hypoxia-reoxygenation injury-induced oxidative stress on tumor and endothelial cell survival were not defined until recently. Moeller et al. (12, 13) have published two pivotal articles illustrating that reoxygenation after treatment with radiation ( $2 \times 5$  Gy) leads to a significant increase in the level of reactive oxygen species, and that this even is accompanied by concomitant stabilization of hypoxia-inducible factor (HIF)-1 $\alpha$  under aerobic conditions. Stress granules, which are aggregates of protein-mRNA complexes (including HIF-1 mediated genes), form under hypoxic conditions and disassemble on reoxygenation, further contributing to up-regulation of HIF-1-regulated genes. Reoxygenation after radiation revealed HIF-1-mediated increases in vascular endothelial growth factor (VEGF) levels and protection of vascular endothelium against radiation injury. Administration of superoxide dismutase (SOD) mimetic compounds after radiation treatment eliminated reactive oxygen species and led to significant vascular damage, vascular rarefaction, and tumor growth delay. The effects of HIF-1 $\alpha$  up-regulation on tumor cell survival postirradiation were more complex; in p53 wild-type tumors, HIF-1 $\alpha$  up-regulation stimulated apoptosis and decreased clonogenic survival, whereas in p53 null tumors, HIF-1 up-regulation had no effect on tumor cell survival (13).

## Key Advances

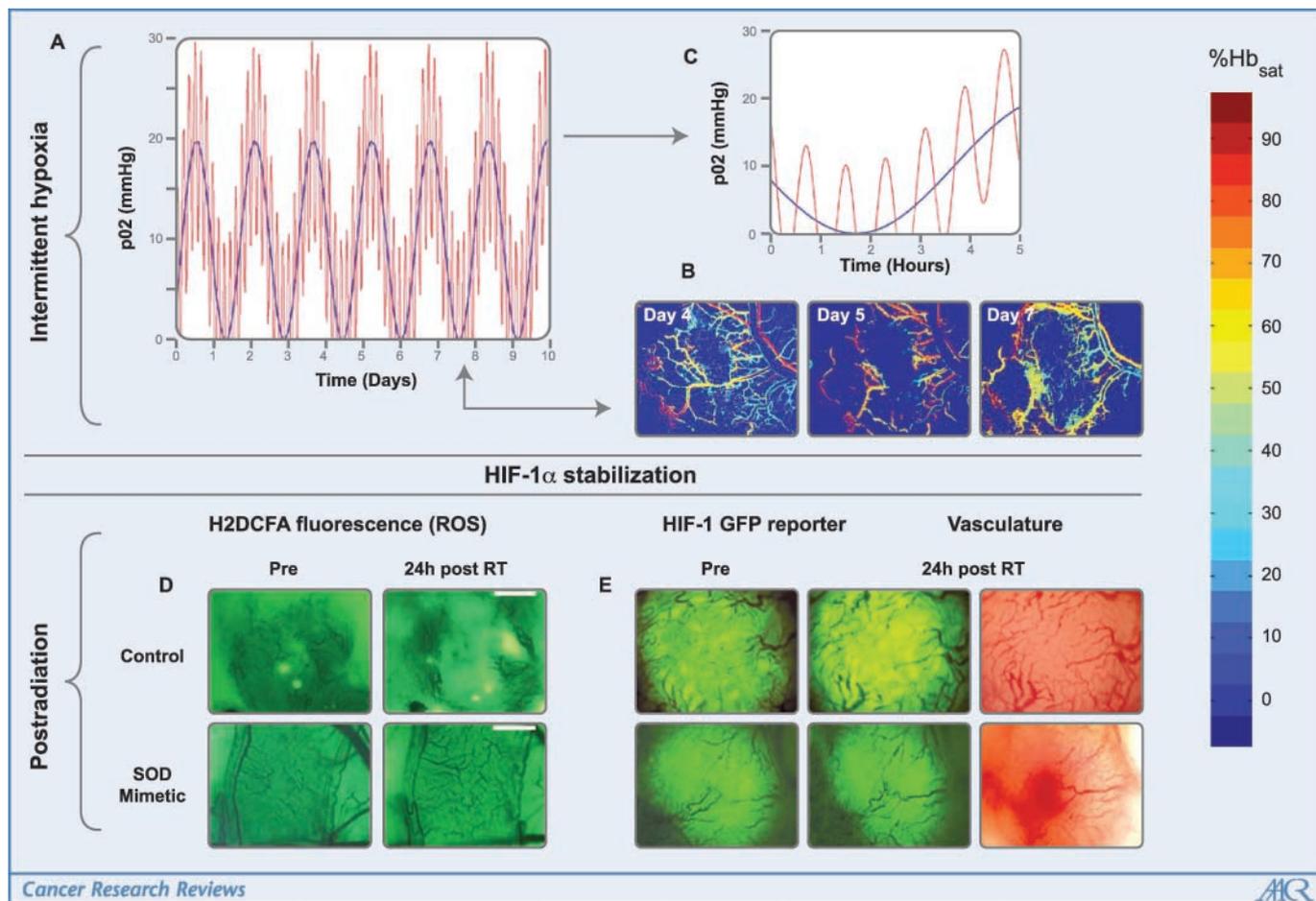
In the December 15, 2006 of *Cancer Research*, Martinive et al. (14) report that intermittent hypoxia can precondition pro-survival pathways in vascular endothelium of tumors by up-regulating HIF-1 $\alpha$  followed by VEGF. *In vitro* studies showed that intermittent hypoxia was more potent than chronic hypoxia at increasing HIF-1 $\alpha$  activity and protecting endothelial cells against radiation killing or serum starvation. In addition, intermittent hypoxia stimulated *in vitro* proangiogenic behavior. Induction of intermittent hypoxia *in vivo* resulted in resistance to radiation-induced growth delay, as compared with controls. This work is extremely important because it puts in context the influence of intermittent hypoxia in governing treatment resistance at a level quite distinct from pure radiobiological radioresistance.

## Future Directions

HIF-1 $\alpha$  stabilization occurring as a consequence of intermittent hypoxia may be an important cause for radiotherapy (and

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**Figure 1.** Intermittent hypoxia and reoxygenation postradiotherapy both contribute to HIF-1 $\alpha$  stabilization, partially due to increased formation of reactive oxygen species. Temporal fluctuations in tissue  $pO_2$  occur on two overlapping time scales (note that fluctuations depicted in the figure are idealized). Although kinetics of the more rapid time scale is relatively well known, fluctuations on the slower time scale are not well characterized. **A**, fluctuations over days occur as a result of angiogenesis and remodeling of tumor vasculature (blue line). **B**, hemoglobin saturation fluctuations observed in window chamber tumor over successive days illustrate the daily fluctuation in oxygen delivery to tumor. Note the higher hemoglobin saturation on day 5, relative to days 4 and 7. Changes in vascular network structure over this time frame, permitting better oxygenated blood to enter the tumor on day 5, are probably responsible for alterations in hemoglobin saturation [modified from the study by Sorg et al. (9)]. **C**, fluctuations on a time scale of one to three times per hour occur as a result of fluctuations in red cell flux in vascular networks (red line). The overall set point for these fluctuations will be influenced by the general oxygenation state of the tissue (blue line), as depicted in (A) and (B). **D**, reoxygenation occurring after radiation exposure increases levels of reactive oxygen species, stabilizes HIF-1 $\alpha$ , and promotes vascular maintenance, relative to animals treated with a SOD mimetic compound [E; modified from the study by Moeller et al. (12)].

possibly chemotherapy) treatment resistance. Fortunately, there is considerable interest in the development of drugs that can inhibit HIF-1 promoter activity (15). As such, HIF-1 is an important target to be considered in combination with cytotoxic therapies.

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