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## Myoglobin tames tumor growth and spread

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**Tumor growth is accompanied by tissue hypoxia, but does this reduced oxygen availability promote further tumor expansion, resulting in a vicious cycle? In this issue of the *JCI*, Galluzzo et al. report that increasing oxygen tension in tumor cells by ectopically expressing the oxygen-binding hemoprotein myoglobin indeed affects tumorigenesis (see the related article beginning on page 865). Tumors derived from cells transfected with myoglobin grew more slowly, were less hypoxic, and were less metastatic. These results will spur further mechanistic inquiry into the role of hypoxia in tumor expansion.**

Tumorigenesis involves not only cell autonomous genetic alterations that result in activation of oncogenes and loss of tumor suppressor genes but also adaptation of neoplastic cells to the tumor microenvironment. Although the activation of oncogenes and their products MYC, AKT, PI3K, and RAS as well as the loss of p53 and VHL tumor suppressors have been linked to altered tumor metabolism, it has become apparent that the hypoxic tumor microenvironment, which activates the low oxygen-sensing HIF transcription factors, also plays a key role in tumor metabolism and tumorigenesis (1). It is well documented that new blood vessels recruited into a growing tumor mass are disorganized (2), often culminating in vascular “dead-ends” rather than providing the canonical microvasculature characteristic of normal tissue, in which arterioles are connected to venules via a capillary bed. Hence, tumors endure significant hypoxia that is distributed heterogeneously within a tumor mass, and areas of tumor hypoxia could fluctuate with time (3). In this regard, whether tumor

hypoxia itself contributes to tumor progression has been a matter of debate rather than of experimentation.

### Transduction of myoglobin to improve tumor oxygenation

In this issue of the *JCI*, Galluzzo et al. (4) present an innovative and elegant approach to addressing the question of whether hypoxia is a side effect of or a key player in tumor progression. The authors used lentiviral vector-mediated transduction to achieve expression of myoglobin (Mb) in human lung carcinoma cells as a genetic tool to prevent tumor hypoxia. Like its molecular relative hemoglobin, Mb — a cytosolic hemoprotein present in skeletal and heart muscle (5) — reversibly binds O<sub>2</sub> and thus facilitates O<sub>2</sub> transport from the blood to mitochondria during periods of increased metabolic activity or serves as an O<sub>2</sub> reservoir under hypoxic conditions (Figure 1). Transduced cancer cells expressing Mb showed no signs of altered cellular proliferation in vitro, but, surprisingly, in vivo xenograft growth was severely diminished after injection of transduced cells into mice. The data reported by Galluzzo and coworkers are quite impressive: presence of Mb resulted in a 5-fold decrease in tumor expansion compared with controls. Furthermore, the expression of Mb suppressed both local and distal metastatic

spread, led to enhanced cancer cell differentiation, and reduced the degree of abnormal vascularization. The authors also report that the expression of HIF-1 $\alpha$ , which is generally accepted as a master regulator of the cellular hypoxic response, was downregulated in Mb-expressing cancer cells. The data suggest that the beneficial effects of the presence of Mb within cancer cells are the result of improved O<sub>2</sub> delivery to the tumor, which “calms” the tumor’s craving to expand.

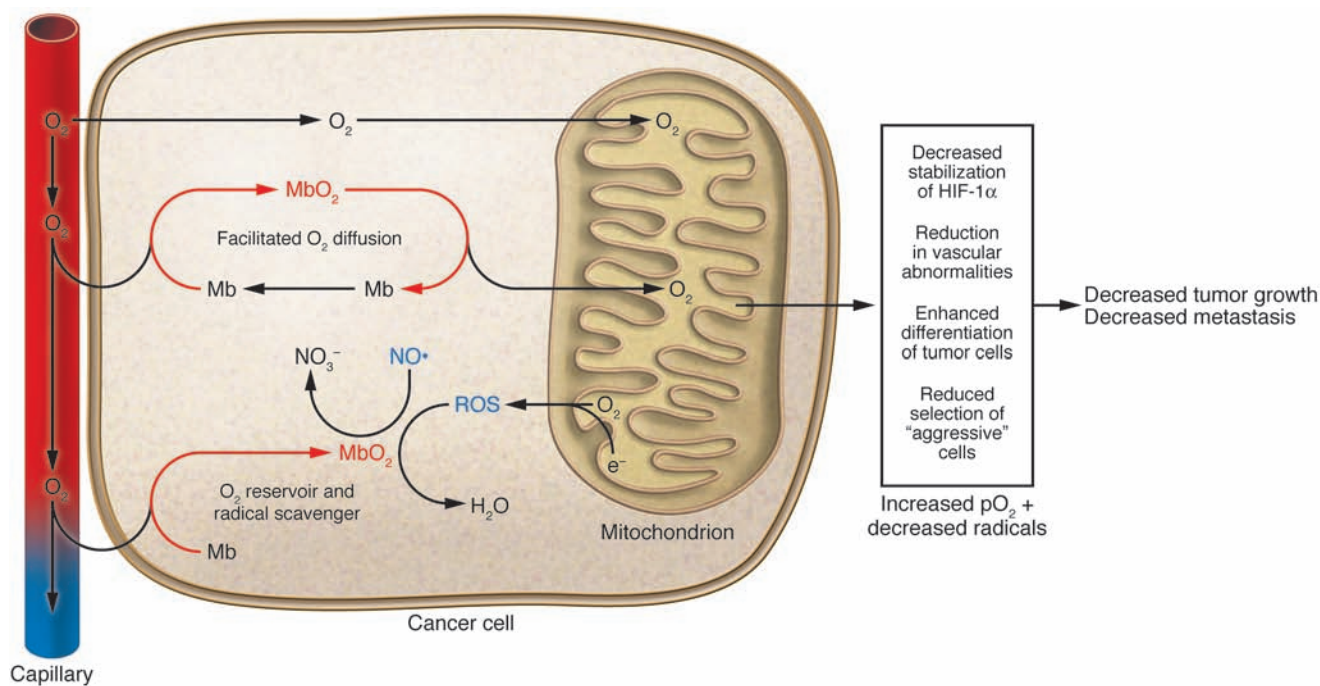
### Globins, oxygen, and beyond

Beyond the initial finding of Mb’s O<sub>2</sub>-binding properties — set into a physiological context especially by the seminal work of Beatrice and Jonathan Wittenberg — within the last decade, the field of globin biology was invigorated, particularly by the generation of gene-deficient mutants but also by the discovery of two new members of the globin family, cytoglobin (Cb) and neuroglobin (reviewed in refs. 5–8). Based on this recent work, the role of Mb in muscle physiology has been reassessed and its scope of function has been considerably extended beyond oxygen storage and delivery to also include a role as an important scavenger of NO and ROS, signaling molecules involved in cellular oxidative stress (Figure 1). Appreciating the diversity of Mb’s properties, Galluzzo et al. (4) used mutated forms of Mb unable to bind O<sub>2</sub> to demonstrate that the inhibition of tumor progression is primarily caused by improved O<sub>2</sub> delivery. However, it should be kept in mind that structural alterations at Mb’s heme-binding pocket will also alter the in vivo kinetics for all other events taking place at this site, including radical reactions. It is, therefore, tempting to speculate that these newly uncovered properties of Mb may

**Conflict of interest:** The authors have declared that no conflict of interest exists.

**Nonstandard abbreviations used:** Cb, cytoglobin; Mb, myoglobin.

**Citation for this article:** *J. Clin. Invest.* **119**:766–768 (2009). doi:10.1172/JCI38796.

**Figure 1**

Possible interactions of Mb within the cancer cell and the metabolic and functional consequences on tumor growth and spread. Tumor progression is well known to be associated with hypoxia, which itself gives rise to enhanced stabilization of the master regulator HIF-1 $\alpha$  and also to an augmented release of ROS, caused by an increased leakage of the mitochondrial electron transport chain. Due to its high  $O_2$  affinity, Mb may serve as an  $O_2$  store and facilitate  $O_2$  transport from capillaries to mitochondria even under conditions of limited  $O_2$  availability. As a consequence, the ectopic expression of Mb within cancer cells carried out by Galluzzo et al. (4) leads to an improved  $O_2$  supply to the tumor, as reflected by the enhanced tissue  $O_2$  partial pressure ( $pO_2$ ) reported in this study. Additionally, Mb can reduce oxidative and nitrosative stress in cancer cells by detoxification of ROS to water and of NO radicals ( $NO\cdot$ ) to nitrate ( $NO_3^-$ ), respectively. Together, these result in an environment of enhanced aerobic mitochondrial respiration and reduced levels of free radicals, favoring efficient degradation of HIF-1 $\alpha$ , a key player in the progression of tumor metabolism to a more aggressive phenotype (15). As demonstrated by Galluzzo et al. (4), the metabolic environment created by the presence of Mb in cancer cells furthermore implicates cell differentiation, a reduction in vascular abnormalities, and a reduced selection of aggressive cells, which, in turn, retards tumor growth and metastatic spread.

contribute or even account in a synergistic manner for the inhibition of tumor growth and spreading. Although the role of NO in cancer is far from clear (9), there is evidence that, especially during the early phase of tumor development, NO may contribute to tumor promotion and progression by stabilizing HIF-1 $\alpha$  (10), thereby mimicking hypoxia and thus initiating a genetic program that helps the tumor to survive and grow, in part by stimulating angiogenesis. Since HIF-1 $\alpha$  is similarly stabilized by enhanced ROS levels (11), scavenging of these species not only reduces the nitrosative and oxidative stress but also prevents activation of a signal cascade that would prepare the ground for tumor expansion.

In addition to the functions mentioned by Galluzzo and coworkers (4), a lesser known property of Mb is its ability to bind fatty acids. Therefore, Mb has been suggested as serving as an intracellular fatty-acid transporter (12) working in support of

the well-known fatty acid-binding protein, which is generally assumed to be the major player in fatty-acid delivery to mitochondria. Interestingly, fatty-acid binding of Mb depends on its oxygenation state, in that conformational changes caused by binding of  $O_2$  promote the interaction of Mb with fatty acids (13). Although the functional relevance of fatty-acid binding to Mb remains to be explored, the simultaneous delivery of  $O_2$  and fatty acids to mitochondria would clearly favor true aerobic respiration. According to this hypothesis, the presence of Mb would diminish the need of tumor cells to rely on inefficient aerobic glycolysis and thereby prevent a metabolic state that has been proposed to facilitate the expansion of cancer cells (14).

When these data are considered together, it seems likely that, in concert, the multifaceted properties of Mb create an environment characterized by improved aerobic mitochondrial respiration and low levels of

free radicals. This results in enhanced degradation of HIF-1 $\alpha$ , considered to be a master regulator in the progression of tumor metabolism to a more aggressive phenotype (15). As a consequence, the presence of Mb within cancer cells favors cell differentiation, reduces tumor expansion, and suppresses metastatic spread, as convincingly demonstrated by the comprehensive investigations reported in this issue by Galluzzo et al. (4). In this context, it is noteworthy that – as also mentioned by the authors – the newest member of the globin family, Cb, has been recently identified as a tumor suppressor (16). However, since the specific functions of Cb are still a matter of intense debate (8), the underlying mechanisms of this effect have yet to be scrutinized. Nevertheless, these observations are exciting per se, and it will be absorbing to follow how these relationships can be transferred into a clinical context or utilized for future drug design or therapy.



## commentaries

### Oxygen availability and tumors: a tricky relationship

The findings of Galluzzo et al. (4) not only provide additional insights into the role of the tumor microenvironment and oxygen in tumorigenesis, but they also raise additional questions regarding our understanding of oxygen metabolism and tumorigenesis. For example, it has been documented that high-altitude populations, subject to chronic hypoxia, tend to have a lower frequency of cancer (17). Is this because there is an adaptation that increases oxygen transport into tissues (e.g., enhanced Mb expression; ref. 18) or because oxygen deprivation is antitumorigenic? In addition, it is known that animals with larger body mass tend to have a lower incidence of cancer development (19), and yet the tissues from these animals have lower specific metabolic rates and oxygen consumption (20). Notwithstanding these intriguing nuances, the work of Galluzzo and coworkers clearly provides additional food for our collective thought on tumor metabolism and tumorigenesis.

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