The HIF-1-Inducible Lysyl Oxidase Activates HIF-1 via the Akt Pathway in a Positive Regulation Loop and Synergizes with HIF-1 in Promoting Tumor Cell Growth

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Abstract

Adaptation to hypoxia is a driving force for tumor progression that leads to therapy resistance and poor clinical outcome. Hypoxic responses are mainly mediated by hypoxia-inducible transcription factor-1 (HIF-1). One critical HIF-1 target mediating tumor progression is lysyl oxidase (LOX), which catalyzes cross-linking of collagens and elastin in the extracellular matrix, thereby regulating tissue tensile strength. Paradoxically, LOX has been reported to be both upregulated and downregulated in cancer cells, especially in colorectal cancer. Thus, we hypothesized that LOX might regulate expression of HIF-1 to create a self-timing regulatory circuit. Using human colorectal carcinoma cell lines in which HIF-1 and LOX expression could be modulated, we showed that LOX induction enhanced HIF-1 expression, whereas LOX silencing reduced it. Mechanistic investigations revealed that LOX activated the PI3K (phosphoinositide 3-kinase)–Akt signaling pathway, thereby upregulating HIF-1 protein synthesis in a manner requiring LOX-mediated hydrogen peroxide production. Consistent with these results, cancer cell proliferation was stimulated by secreted and active LOX in an HIF-1–dependent fashion. Furthermore, nude mice xenograft assays established that HIF-1 potentiated LOX action on tumor growth in vivo. Taken together, these findings provide compelling evidence that LOX and HIF-1 act in synergy to foster tumor formation, and they suggest that HIF-1/LOX mutual regulation is a pivotal mechanism in the adaptation of tumor cells to hypoxia. Cancer Res; 71(5); 1647–57. © 2011 AACR.

Introduction

The microenvironment of solid tumors, now recognized as fundamental in tumor progression, is exposed to low oxygen tension (hypoxia) as a result of an inadequate and chaotic blood supply. Accordingly, adaptation to hypoxia, traditionally linked to treatment resistance and poor clinical outcome, is a critical driving force in tumor progression (1). A key regulator of the cellular response to oxygen deprivation is the hypoxia-inducible transcription factor-1 (HIF-1), which comprises a constitutively expressed β-subunit and an oxygen-labile α-subunit. In normoxia, HIF-1α is destabilized by prolyl hydroxylation, leading to proteasomal degradation. Under hypoxic conditions, O2 becomes limiting for prolyl hydroxylase (PHD) activity, and ubiquitination of HIF-1α is inhibited. As a result, HIF-1α accumulates, dimerizes with HIF-1β, and activates transcription of target genes involved in angiogenesis, energy metabolism, adaptive survival, or apoptosis (2–4).

Recently, lysyl oxidase (LOX) has been identified as an important regulator of hypoxia-induced tumor progression via an HIF-1α–dependent mechanism in numerous cancer types, such as breast, head and neck, prostate, or renal cell carcinomas (5–7). LOX is a copper-dependent amine oxidase that catalyzes the cross-linking of collagen and elastin in the extracellular matrix (ECM), thereby increasing insoluble matrix deposition and tensile strength (8). LOX has been shown to enhance tumor cell proliferation and invasion (5, 9, 10), particularly by increasing ECM stiffness (11).

Even though LOX is frequently elevated in numerous tumors (12, 13), so far, its role in colon carcinomas has been controversial. Whereas in one study LOX was shown to be silenced (14), LOX upregulation was also described, and was correlated with hypoxia (15). In this type of cancer, HIF-1α is often overexpressed at very high levels, promoting colonic...
tumorigenesis (16, 17) and potentially stimulating LOX expression. However, a functional link between the 2 proteins in colorectal carcinogenesis has never been described.

In this study, we used different human colorectal carcinoma cell lines to evaluate the relationship between LOX and HIF-1α and their respective contributions to carcinogenesis. We first confirmed that LOX is regulated by HIF-1α, and then showed that LOX reciprocally regulates HIF-1α expression. Indeed, LOX enzymatic activity promotes HIF-1α protein synthesis, through the activation of the PI3K/Akt pathway. In addition, our results highlight that HIF-1α potentiates LOX action on tumor cell proliferation both in vitro and in vivo. Finally, we conclude that LOX and HIF-1α are mutually regulated and act synergistically to favor tumor formation.

Materials and Methods

Reagents

All chemicals were obtained from Sigma, unless otherwise specified.

Cell culture and treatments

The human colon cancer cell line LS174Tr was already described (18). Hct116 and HT29 cell lines were obtained from the American Type Culture Collection (ATCC) in 2007. Cell morphology was monitored by microscopy. Their morphological images were maintained in comparison with the original images. The mutation in the ras proto-oncogene in both cell lines was also checked. All cell lines were cultured as described in Supplementary Methods. Hypoxia at 1% O₂ was carried out at 37°C in 95% humidity and 5% CO₂/94% N₂ in an airtight anaerobic incubator (Birnder) or in a sealed Bugbox anaerobic workstation (Ruskinn). When appropriate, cells were treated with indicated concentrations of β-aminopropionitrile (βAPN), catalase, cycloheximide (Chx), cobalt chloride (CoCl₂), and L2Y94002 under normoxic or hypoxic conditions for indicated time. For conditioned-media (CM) experiments, 4 × 10⁴ cells were cultured for 48 hours in normoxia. CMs were collected, centrifuged, and applied onto cells for indicated time. Cell growth determination, protein extraction, and Western blotting are described in Supplementary Methods.

Construction of lentiviral-infected cell lines

All infections were done with lentiviral particles described in Supplementary Methods. Hct116 cells were first infected with pLenti6/V5-D-TOPO (Life Technologies) encoding luciferase (Luc) and selected with 10 μg/mL blasticidin (PAA Laboratories, Inc.). They were then sequentially infected to give the following cell lines. Simple infection: LOX, shLOX, shHif1α, shCtl. Double infections: LOX+/shCtl, LOX+/shHif1α, EV/shCtl, EV/shHif1α, and EV/shLOX. HT29 and LS174Tr cells were single-infected to give LOX+, EV, shLOX, and shCtl cell lines. To induce LOX or EV expression, cells were treated with 0.1 μg/mL doxycycline (Dox) for 2–3 days before experiments.

mRNA analysis

Total RNA (2 μg), extracted with Tri-Reagent (Euromedex), was reverse transcribed with oligo-dT primers and MMLV-RT (Fermentas). Quantitative real-time PCR (qPCR) was done with an iCycler IQ (Bio-Rad) using MESAGREENPlus-Master-Mix (Eurogentec). Gene expression levels were determined by the Ct method and normalized to 36B4 levels.

Nude mice tumorigenicity and bioluminescence imaging

BALB/c nude mice (Charles River) were maintained in accordance with the regional ethical committee (CREA). Cells (1 × 10⁶) were implanted subcutaneously into the flanks of 4–5-week-old females. The drinking water was supplemented with 5% sucrose and 1 mg/mL Dox for empty vector (EV) or LOX induction. For the LOX chemical inhibition study, animals were treated with βAPN (0.2%) in the drinking water. Each week, luciferin (Promega; 100 mg/kg in PBS) was administered subcutaneously 10 minutes before imaging in ventral position in NightOWL (Berthold Technologies). Signal intensity was quantified as a total of photons per second for a 4-minute-period using winLight software (Berthold Technologies). Each week, tumor volumes were measured with calipers, and the volume was determined using the formula $V = \frac{1}{2}L \times W\times D$.

Statistical analysis

Experiments were done in triplicate, and data are presented as mean ± SEM. For the in vivo LOX-induced tumorigenicity assay in Hct116 and in LS174 or HT29 cells, respectively, 24 and 20 tumors of each group were included for the statistical analysis. For the in vivo LOX chemical inhibition study, 16 tumors of each group were included for the statistical analysis. Statistical comparisons of values were made using 2-tailed, paired Student’s t test and significant differences are indicated: ns, nonsignificant; *, P < 0.05; **, P < 0.01; and ***, P < 0.005.

Results

LOX is the main HIF-1α–responsive gene of the LOX-like (LOs) family in colon cancer cells

Overexpression of LOX gene has been described in colorectal cancers (15). We thus first analyzed LOX protein expression by immunohistochemistry with a specific antibody (19) on 19 colorectal samples. As expected, LOX was expressed in the lamina propria of normal colon, a collagen-rich connective tissue. We found that LOX was overexpressed in 79% of colon carcinoma cases tested in association with fibers and/or in epithelial cells, independently of the stage considered (Fig. 1A). We then examined the contribution of HIF-1α on LOX induction in colon cancer cell lines. Hct116 cells were infected with a lentivirus-producing shRNA directed against HIF-1α (shHif1α) or not (shCtl), and silencing efficiency was verified by qPCR and Western blotting (Supplementary Fig. S1A). We then analyzed LOX expression in Hct116 shCtl and shHif1α cultured in normoxia or hypoxia. LOX mRNA was
6-fold increased by hypoxia in shCtl. In contrast, LOX mRNA expression was inhibited by more than 70% in hypoxia as well as in normoxia in cells depleted for HIF-1α (Fig. 1B). Correlating with the mRNA profile, LOX protein was markedly reduced in shHif1α cells cultured in normoxia or hypoxia were analyzed for LOs expression. Fold induction determination was done comparing the LOX level toward shCtl cells in normoxia. B, relative level of LOX mRNA, normalized to 36B4, was determined by qPCR. C, LOX reduction in shHif1α cells was confirmed by Western blotting with Hsp90 as loading control. D, relative level of LOX-like mRNAs was determined by qPCR. N, normoxia; H, hypoxia. ***, P < 0.005; ns, not significant.

Figure 1. LOX is an HIF-1α-responsive gene in colon cancer cells. A, immunohistochemical staining (Supplementary Methods) of LOX in normal colon (b) and in colon adenocarcinomas overexpressing LOX stage II (c–f) or III (g–j). Negative control is shown in a. Scale bar, 3 μm. B–D, total RNA and whole-cell extracts of shCtl and shHif1α cells cultured in normoxia or hypoxia were analyzed for LOs expression. Fold induction determination was done comparing the LOX level toward shCtl cells in normoxia. B, relative level of LOX mRNA, normalized to 36B4, was determined by qPCR. C, LOX reduction in shHif1α cells was confirmed by Western blotting with Hsp90 as loading control. D, relative level of LOX-like mRNAs was determined by qPCR. N, normoxia; H, hypoxia. ***, P < 0.005; ns, not significant.

HIF-1α protein level is dependent on LOX expression in hypoxia

Several studies have documented that reactive oxygen species (ROS) can influence the level of HIF-1 (20–22). Because...
H₂O₂ is a direct by-product of LOX, we hypothesized that LOX activity could modulate the level of HIF-1 and of downstream targets. To study a possible reciprocal effect of LOX on the HIF pathway, we infected Hct116, HT29, and LS174Tr cells with lentivirus containing EV, LOX-expressing vector (LOX⁺), and shRNA interfering with LOX expression (shLOX) or not (shCtl). LOX mRNA and protein expression modulations were checked (Supplementary Fig. S1C). To examine the hypoxic response, cells were plated at low density (30% confluency) to avoid the impact of cell density on HIF-1α stabilization (23) and incubated in hypoxia, and the HIF-1α expression analyzed (Fig. 2A). Interestingly, in Hct116, HIF-1α expression level was reduced by 60% when LOX was knocked down (compare shLOX with shCtl) and augmented 2-fold when LOX was overexpressed (compare LOX⁺ with EV). These data were extended to HT29 and LS174Tr cells in which LOX silencing reduced the level of HIF-1α by 30% and 50%, respectively, whereas LOX overexpression enhanced it 3-fold. We confirmed these data in Hct116 with an independent LOX shRNA sequence and by attenuating LOX expression with a LOX antisense DNA (Supplementary Fig. S2A and B). The LOX effect on HIF-1α expression was not restricted to colon cancer cells, as we obtained the same result in human primary fibroblasts (Supplementary Fig. S2C).

To investigate the functional effect of LOX-induced HIF-1α expression modulations, we evaluated the transcriptional activity of HIF-1. The expression levels of the HIF-1α-dependent targets carbonic anhydrase IX (CAIX) and BNIP3 were
measured by qPCR in shCtl, shLOX, EV, and LOX+ Hct116 cultured under normoxic and hypoxic conditions. As expected, LOX overexpression resulted in an induction of the CAIX and BNIP3 mRNAs, while LOX silencing inhibited them (Fig. 2B). Taken together, these results indicate that LOX regulates HIF-1α expression and HIF-1α-mediated genes transcription, in both normoxia and hypoxia.

**LOX enzymatic activity regulates HIF-1α expression in hypoxia**

Although the generation of H2O2 after LOX activity could represent a major drive in the modulation of HIF-1α, we could not exclude a priori a nonenzymatic action. Indeed, LOX activity could be affected by hypoxia (24). Furthermore, recent studies have shown that the LOX protein displays biological functions independent of its catalytic activity (25, 26). In our model, LOX activity was only slightly decreased in hypoxia, without any changes in intracellular H2O2 and extracellular ROS levels (Supplementary Fig. S3D). We therefore investigated the action of βAPN (8), a specific and irreversible inhibitor of LOX enzymatic activity, on the expression of HIF-1α in hypoxia. Figure 2C shows that the inhibition of LOX activity by increasing concentrations of βAPN in shCtl cells resulted in the diminution of HIF-1α expression. βAPN had no effect in shLOX cells, showing the specific action of the LOX inhibitor.

The βAPN experiment clearly indicates a role of the catalytic reaction of LOX and the subsequent production of H2O2. To validate this interpretation, we treated shCtl and shLOX Hct116 in hypoxia with catalase, which converts H2O2 to molecular oxygen and water, for 16 hours. Treatment with 500 U/mL of catalase diminished HIF-1α expression in both shCtl and shLOX cells. However, the level of HIF-1α after catalase addition was almost the same in shLOX cells as in shCtl, suggesting that generation of H2O2 by LOX is the mediator of alteration in HIF-1α expression (Fig. 2D). Together, these results support the notion that H2O2 generated by LOX activity contributes to the regulation of HIF-1α expression in hypoxia.

**LOX enzymatic activity regulates HIF-1α protein translation in hypoxia through the PI3K/Akt pathway**

As in most of the cellular systems, HIF-1α mRNA is constitutively expressed (27), and HIF-1α induction under hypoxic conditions is regulated at the protein level. HIF-1α is actively degraded by the proteasome under normoxic conditions, while it rapidly accumulates following exposure to low oxygen tension (28, 29). We then investigated how LOX controls HIF-1α induction in Hct116 shCtl, shLOX, EV, and LOX+ after 16 hours in hypoxia. As expected, LOX modulations did not result in significant changes in the expression levels of HIF-1α mRNA (Fig. 3A). We therefore examined the changes in HIF-1α protein stability. Hct116 EV and LOX+ incubated at low density in hypoxia were treated with the protein translation inhibitor Chx. We determined the HIF-1α fold induction by comparing the protein level at 0 and 15 minutes in each cell line. Figure 3B shows that the HIF-1α protein level after 15-minute Chx treatment was the same in EV and LOX+ cells (Fig. 3B). EV and LOX+ cells were then treated with the inhibitor of PHD activity CoCl2. Figure 3C shows that LOX
overexpression still led to an increase in the HIF-1α level when PHDs were inhibited, confirming that LOX was not involved in HIF-1α stabilization. We finally compared the LOX-dependent fold induction of HIF-1α at 21% or 1% O2 and with CoCl2 (Fig. 3D). We found that LOX overexpression amplified the HIF-1α level regardless of the oxygen level and PHD activity. Together, these results show that LOX was not involved in HIF-1α transcription and protein stabilization. Then, LOX might act on HIF-1α protein synthesis.

The PI3K/mTOR pathway is a major positive regulator of HIF-1α protein translation (20, 23, 29, 30). It has been shown recently that the constitutive activation of PI3K in Hct116 is sufficient to induce HIF-1α protein accumulation in hypoxic conditions (31). Accordingly, PI3K inhibition in our Hct116-derived cell lines resulted in significant HIF-1α protein reduction (Supplementary Fig. S3A). To further explore the mechanism by which LOX regulates HIF-1α synthesis, we then tested whether LOX enzymatic activity was involved in activation of PI3K signaling, and we observed that LOX inhibition by βAPN led to a strong decrease in PDK1 and Akt phosphorylation in both EV and LOX+ cells. βAPN treatment had no effect in shLOX cells, further emphasizing the importance of LOX activity on PDK1 and Akt regulation (Fig. 4B). The HIF-1α level after LY294002 addition was almost the same in shCtl as in shLOX cells, and the level in EV as in LOX+ cells, suggesting that the PI3K pathway mediated the LOX effect on HIF-1α expression (Fig. 4C). These results indicate that LOX, by its enzymatic activity and especially via H2O2 generation, regulates HIF-1α expression in hypoxia through the activation of PDK1 and Akt. As LOX is also an HIF-1α-responsive gene, it suggests the existence of a positive regulation loop between LOX and HIF-1α in hypoxia.

**LOX and HIF-1α act in synergy to promote tumor cell proliferation**

Both HIF-1α and LOX are known to promote tumor cell growth and survival (9, 32). We wanted to determine whether the mutual regulation of LOX and HIF-1α could be involved in
colorectal tumor cell growth. To answer this question, we generated Hct116 cells in which the expression levels of both LOX and HIF-1α could be modulated (Supplementary Fig. S3B and C). To compare cell growth in different conditions of LOX and HIF-1α expression, EV and LOX+ Hct116 were infected again with a lentivirus encoding a nontarget shRNA (EV/shCtl; LOX+/shCtl), with a shRNA against HIF-1α (EV/shHIF1α; LOX+/shHIF1α), or against LOX (EV/shLOX). To determine proliferative properties, we analyzed LOX-dependent in vitro cell growth by MTT assay. Hct116 EV/shCtl, EV/shLOX, and LOX/shCtl were plated at low density and incubated under normoxic or hypoxic conditions, and the cell number was determined daily for 5 days. As shown in Figure 5A, LOX overexpression resulted in a 3-fold increase in cell proliferation in hypoxia and a 2-fold increase in normoxia. In contrast, loss of LOX resulted in a weak but significant decrease of cell proliferation especially in hypoxia (25%). Such results were confirmed in LS174Tr and HT29 (Supplementary Fig. S4A and B). This result shows that LOX stimulates the proliferation of colon cancer cells in both normoxic and hypoxic cultures and that hypoxia increases the amplitude of the LOX effect.

As HIF-1α and LOX regulate each other, we tested the effect of HIF-1α modulation on the ability of LOX to accelerate proliferation in normoxic and hypoxic conditions. The proliferation curve of LOX+/shHIF1α cells was similar to that of EV/shCtl in both culture conditions (Fig. 5B), suggesting that HIF-1α silencing strongly reduced the LOX-dependent proliferative advantage. These results show that LOX stimulate proliferation and that this effect is highly potentiated by HIF-1α.

Enhancement of tumor cell proliferation by secreted and active LOX through the PI3K pathway

To determine whether LOX enzymatic activity was involved in the increase of cell proliferation mediated by LOX overexpression, we analyzed the βAPN effect on growth. A significant reduction of proliferation was observed in LOX+/shCtl cells treated with βAPN, leading to a proliferation rate equivalent to EV/shCtl cells (Fig. 5C). Thus, βAPN treatment fully eliminated the LOX-mediated effect on proliferation. Furthermore, βAPN had no effect in EV/shLOX cells (Supplementary Fig. S4C). In addition, LY294002 treatment also completely abolished the growth-promoting effect of LOX (Fig. 5D). This shows that LOX is involved in cell proliferation through its enzymatic activity in a PI3K-dependent manner.

LOX is secreted by hypoxic tumor cells (5, 13). We therefore used LOX+ or shLOX Hct116 exposed to hypoxia to produce CM, CM-LOX, and CM-shLOX. As shown in Figure 5E, shLOX cells cultured in hypoxia and treated with CM-LOX had a significantly higher proliferation rate as compared with cells treated with CM-shLOX (37%). This effect was abolished by βAPN, showing that a soluble and active form of LOX was involved in cell proliferation. We then added CM-LOX on shHIF-1α cells without any change on the proliferation rate of these cells. This suggests that the ability of extracellular active LOX to promote cell proliferation is dependent on HIF-1α.

LOX and HIF-1α potentiate each other to foster tumorigenicity in nude mice

To determine whether LOX and HIF-1α expression correlated with tumor growth in vivo, nude mice were subcutaneously injected with different cell lines (Fig. 6A). LOX and HIF-1α silencing had no or slight effect, respectively, on tumor growth compared with control. However, the tumors resulting from LOX overexpressing Hct116 cells were notably larger (2.6-fold) than control cells. HIF-1α silencing in cells overexpressing LOX strongly reduced tumor development due to forced LOX expression. Bioluminescence imaging of mice at day 35 confirmed the results obtained by tumor volume calculation (Fig. 6B). These results suggest that LOX and HIF-1α act in synergy to favor tumor formation.

As LOX has never been described to promote tumor growth, we subcutaneously injected 2 other colon cancer cell lines modulating LOX in nude mice. As shown in Figure 6C, tumors were respectively 1.7- and 2.7-fold larger in LS174Tr and HT29 cells overexpressing LOX compared to control cells. It confirms that LOX favors the tumorigenic growth of colon cancer cells.

In in vitro assays, we showed that LOX was involved in cell proliferation through its enzymatic activity. We confirmed that it was also the case in vivo. Indeed, βAPN was added in the drinking water in mice subcutaneously injected with EV/shCtl or LOX+/shCtl Hct116. The tumor volume in mice injected with cells overexpressing LOX and treated with βAPN was highly reduced, showing that the inhibition of LOX activity had a drastic effect on tumor growth (Fig. 6D). Taken together, these findings show that LOX, through its enzymatic activity, is involved in tumor formation, and that protumoral LOX effect is enhanced by the HIF pathway. These data emphasize the implication of the LOX/HIF-1α synergy in the context of tumor progression.

Discussion

In this study, we show that LOX is involved in the hypoxic upregulation of HIF-1α and that LOX and HIF-1α potentiate each other to foster tumor progression. LOX is one of the highest HIF-1α-regulated genes (27, 33) and is a direct target of HIF-1α (5). Of the 4 LOX-like proteins described, LOXL2 has also been characterized as an HIF-1α target in the hepatoma cell line (7). In our model, LOXL2 is induced by hypoxia; however, it does not seem to be a direct HIF-1α target (Fig. 1D). LOXL2 is regulated by HIF-2α to a greater extent than is LOX a greater extent than is LOX (34). Such HIF-2α dependency of LOXL2 could perhaps explain why HIF-1α silencing is not sufficient to induce a strong LOXL2 mRNA reduction in our model.

We showed that LOX enzymatic activity upregulates the PI3K/Akt pathway and thus promotes HIF-1α protein synthesis without affecting its stability (Figs. 3 and 4). This result agrees with those published earlier showing that the PI3K/Akt/mTOR pathway increases the rate of HIF-1α protein synthesis (29, 35, 36). Such an activation of the P53/PI3K pathway by LOX is consistent with results recently published, which have shown that LOX modulates breast tumor progression by stimulating PI3K signaling and stiffening the ECM (11).
Given that the PI3K/Akt/mTOR pathway increases HIF-1 expression and activity, it has also been shown that PI3K activity could modulate the HIF-1 target CAIX independently of HIF-1α expression (37, 38). In our model, HIF-1α silencing led to decreased CAIX expression to the same extent in cells with upregulation of the PI3K pathway (LOX+/shHIF1α) and
Figure 6. HIF-1α potentiates LOX-induced tumorigenicity in nude mice. Xenograft growth of noted Hct116 cells (4–5 mice/group, 3 studies) was determined by measuring the tumor volume each week (A) and bioluminescence as mean photon per second at day 35 (B). Representative solid tumors and bioluminescence images at day 35 are shown. C and D, xenograft growth of noted LS174Tr and HT29 cells (5 mice/group, 2 studies) and (D) Hct116 injected in mice that had received βAPN in the drinking water and in those that had not (4–5 mice/group, 2 studies) was determined with the same method. *, P < 0.05; **, P < 0.01; ***, P < 0.005.
in cells with basal PI3K activity (EV/shHIF1α; Supplementary Fig. S3C). In colorectal carcinoma cells, CAIX expression is strictly controlled by HIF-1.

Our results highlighted that LOX and HIF-1α act in synergy to promote colon cancer cell proliferation and tumor formation in nude mice (Figs. 5 and 6). LOX is known to control metastatic growth and dissemination (5), and both LOX and HIF-1α are considered prognostic markers for metastasis and survival (4–6, 39). However, the role of LOX in primary tumor formation remains little explored. Interestingly, the upregulation of LOX expression has recently been described in precancerous lesions of oral squamous cell carcinoma and primary choroidal melanoma (40, 41). These reports support our observation of a role of LOX in early cancer progression. Therefore, deregulation of LOX could underlie the onset as well as progression of tumor and metastasis.

If increased HIF-1 activity is a common feature in cancer pathogenesis (4), the implication of LOX is more controversial. Remarkably, LOX was first identified as a putative ras-related tumor suppressor in transformed fibroblasts (42). Furthermore, several reports describe reduced LOX expression in various epithelial cancer cell lines and their corresponding tumor tissues (12, 43). It is possible that tumorigenic properties can be gained by either LOX up- or downregulation, depending on the cellular environment, as well as by basal LOX protein expression in normal tissue types. Another possible explanation of this controversy may rely on the existence of multiple forms of LOX proteins. Actually, LOX is synthesized as a 50-kDa proenzyme, secreted into the extracellular environment in which it is then processed by proteolytic cleavage to a functional 30-kDa enzyme and an 18-kDa propeptide (LOX-PP; ref. 8). Increasing evidence indicates that it is not the catalytically active extracellular LOX that mediates the suppression of neoplastic transformation, but rather the LOX-PP (26, 44, 45). A recent article reinforces this idea, showing that tumor suppressor function of LOX-PP is impaired in breast cancer by the tumor suppressor in transformed fibroblasts (42, 44, 45).

At this point, one can speculate that LOX may represent a crucial protein allowing both hypoxic response and modulation of the ECM environment and along the way the progression of the neoplastic process. In keeping with this notion, the refinement of HIF inhibitors with combined drugs targeting extracellular LOX may be a productive way to improve cancer therapy.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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The HIF-1–Inducible Lysyl Oxidase Activates HIF-1 via the Akt Pathway in a Positive Regulation Loop and Synergizes with HIF-1 in Promoting Tumor Cell Growth

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