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RESEARCH

Association of lysyl oxidase expression with clinicopathological features in colorectal adenocarcinomas

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Abstract

Purpose Colorectal adenocarcinoma (CRC) is one of the leading causes of cancer-related mortality worldwide. Within the tumor microenvironment, neoplastic cells, along with tumor-promoting fibroblasts, contribute to the progression of CRC. Lysyl oxidase (LOX), an enzyme involved in this process facilitates collagen cross-linking within the extracellular matrix and plays a crucial role in remodeling the tumor microenvironment (TME) and promoting metastasis through epithelial-mesenchymal transition (EMT). This study investigates LOX expression in both tumor cells and the tumor stroma in relation with clinicopathological features in CRC patients.

Method Immunohistochemical staining of LOX proteins was performed on tissue microarrays from colorectal tumor samples taken from resection specimens. LOX expression was quantified in tumor cells and stroma. The correlation between the expression of LOX and clinicopathological parameters was analyzed.

Results A positive correlation was observed between peritumoral stromal LOX expression and LOX expression in the tumor epithelium. High expression of LOX in tumor cells was significantly associated with poorer progression-free survival (PFS) among patients. Low tumor budding was observed in tumors with low stromal LOX expression.

Conclusion The current study indicates that LOX may be an important contributor to CRC progression. The findings of this series, in which LOX expression correlated with tumor budding and survival, support a contribution for LOX to EMT and metastasis. Furthermore, LOX expression in both the tumor cell and stromal compartment may add information to improve prognosis in CRC management. These findings, however, have to be validated in further studies, as does also the investigation of LOX as a potential therapeutic target in CRC.

Keywords Colorectal adenocarcinoma · Epithelial-mesenchymal transition · Lysyl oxidase · Tumor stroma · Prognosis

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Introduction

Mutations observed in colorectal carcinomas also promote tumorigenesis by interfering with host immune responses or altering interactions with the stroma. Stroma is one of the most important components of tumor microenvironment and it consists of the extracellular matrix, fibroblasts, and vascular structures [1]. In CRC, the tumor microenvironment is associated with tumor behavior. Activated fibroblasts in the tumor periphery play a critical role in tumor progression through several mechanisms, including altering the cancer microenvironment, facilitating EMT, and modulating the cancer stem cell phenotype. Epithelial-mesenchymal transformation is a mechanism closely associated with tumor invasion, migration, and metastatic spread [2]. LOX is a copper-dependent amine oxidase mainly involved in the cross-linking of collagen and the formation of the compact structure of the extracellular matrix. It is also thought to contribute to metastatic spread by decreasing the expression of E-cadherin and increasing vimentin, which play an important role in EMT [3]. A histopathological finding known as tumor budding is believed to have a close relationship with EMT [4]. It is associated with advanced stage, high tumor grade, the presence of a lymphovascular invasion, a lymph node, and distant metastases [5].

This study aims to investigate the relationship between LOX expression in tumor cells and peritumoral stroma in colorectal adenocarcinoma and prognostic parameters as well as the significance of LOX expression as an immunohistochemical pathological prognostic biomarker.

Material–methods

Selection of cases

In our study, 101 cases with resection material diagnosed as colorectal adenocarcinoma were included and evaluated retrospectively. Histological subtype, grade, tumor budding, and presence of lymphovascular invasion were evaluated. Overall survival was calculated as the time from the date of initial diagnosis to the date of death or last follow-up. In stage IV patients, the period from the date of diagnosis to the date of the first progression or the date of the last follow-up was calculated as "progression-free survival."

Tumor compartments and budding

Although carcinomas have different differentiations, they contain parenchymal epithelial component and adjacent host stroma consisting mainly of fibroblasts and extracellular matrix (Fig. 1). Tumor budding was classified according to the International Tumor Budding Consensus Conference (ITBCC) proposed scoring system that classified tumor budding as low for 0–4 buds, medium for 5–9, and high for 10 or more at the invasive edge at 20 × magnification [6].

Immunohistochemical method

For each tumor, two 3 mm-diameter areas that best represent the tumor and the infiltrative margin of the tumor were identified. These areas were removed from the block with the Quick-Ray® Manual Tissue Microarrayer, and a new tissue microarray (TMA) block was created. Sections obtained from TMA blocks were immunohistochemically stained with LOX [EPR4025] rabbit monoclonal antibody in Roche Ventana Ultra devices.

LOX expression was evaluated in the tumor and adjacent stroma. The intensity of cytoplasmic LOX expression in the epithelial component of the tumor was grouped as negative, 1 +, 2 +, and 3 +, and the expression percentages were evaluated. Expression percentages were scored as 0 (0%), 1 (0–25%), 2 (25–50%), 3 (50–75%), and 4 (75–100%). The final score was calculated by multiplying the expression percentages by the intensity scores. The group with a final score of ≤ 4 was considered to have low LOX expression, and the group with > 4 was considered to have high LOX expression

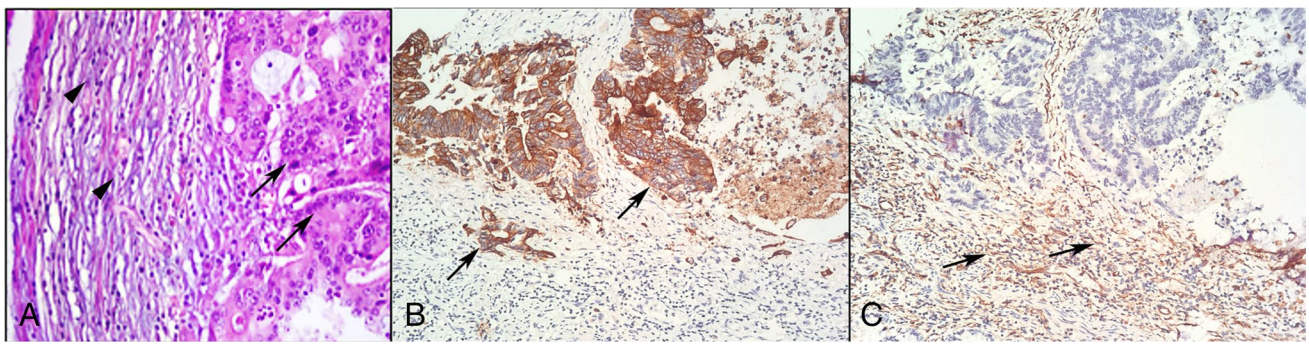

Fig. 1 Demonstration of tumor cells (arrows) and adjacent stroma (arrowheads), Hematoxylin & Eosin (**A**), epithelial component of the tumor (arrows), staining with cytokeratin18 (**B**), stroma of the tumor (arrows), staining with vimentin (**C**)

[7]. Stromal LOX expression is evaluated as mild, moderate, and strong according to the intensity of staining (Fig. 2).

Statistical analysis

Statistical analyses were performed with the help of the SPSS version 23.0 program. Mean, standard deviation, and median values were used to present descriptive analyses. Categorical variables were compared by the Pearson Chi-Square Test. The Mann Whitney *U* Test was used for non-normally distributed (nonparametric) variables between two groups and the Kruskal Wallis Test was used for variables between more than two groups. The Spearman Correlation Test was used in the analysis of measurable data with each other. Survival analysis (Kaplan–Meier) was also performed to estimate the life expectancy of patients after treatment. *p*-values below 0.05 were considered statistically significant results.

Findings

Demographic and clinicopathologic findings

The mean age of the 101 patients included in the study was 61.84 ± 11.39 years, and the age range was between 32 and 85 years. 65 (64.4%) of the patients were male and 36 (35.6%) were female. Twenty-eight (27.7%) of the patients died. A four-step classification system was used for tumor grading. According to this system, 46 (45.5%) were categorized as well differentiated, 40 (39.6%) as moderately differentiated, 11 (10.8%) as poorly differentiated, and 4 (3.9%) as indifferentiated.

According to the pathological tumor stage, 7 (6.9%) cases were pT2, 61 (60.4%) were pT3, and 33 (32.7%) were pT4. According to the staging at the time of diagnosis, 3 (2.97%) patients were stage I, 16 (15.84%) were stage II, 25 (24.75%) were stage III, and 57 (56.44%) were stage IV.

Correlation of LOX expression and clinicopathologic features

In the evaluation of LOX expression in the epithelial component of the tumor, low expression was found in 54 cases (54.55%) and high expression in 45 cases. Two cases could not be evaluated due to technical reasons. LOX expression in the epithelial component was significantly correlated with LOX expression in the stroma ($p = 0.001$). A moderate positive correlation ($r = 0.578$) was observed between LOX expression in the stroma adjacent to the tumor and LOX expression in the tumor epithelium (Table 1).

The relationship between LOX expression in the tumor cells and other clinicopathologic parameters was analyzed (Table 2). No significant association was found between tumoral LOX expression and pathological parameters and overall survival. When evaluated according to stages, a significant correlation was found between LOX expression and progression-free survival in stage IV patients ($p = 0.004$). High LOX expression was associated with a decrease in progression-free survival (Fig. 3).

When LOX expression in the stroma was evaluated, mild expression (1 +) was observed in 35 cases (35.35%), moderate expression (2 +) in 21 cases (21.21%), and strong expression in 7 cases (7.07%). No expression was detected in 36 cases (36.36%). The relationship between LOX expression in the stroma and other clinicopathologic parameters was

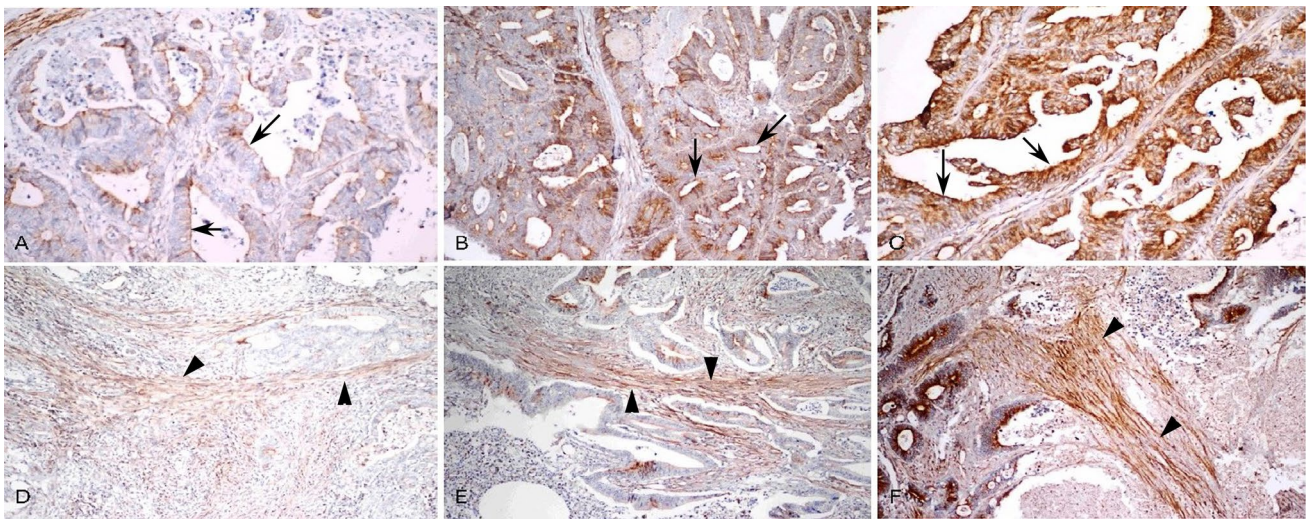

Fig. 2 Mild (**A**), moderate (**B**), and strong (**C**) cytoplasmic LOX expression in tumor cells (arrows). Mild (**D**), moderate (**E**), and strong (**F**) LOX expression in tumor stroma (arrowheads)

Table 1 Relationship between LOX expression in tumor and stromal LOX expression

	LOX Tumor				*p*	r
	Low *n* (%)		High			
LOX stroma						
Negative	27	(50.94)	8	(17.78)	0.001	0.578
1+	17	(32.08)	18	(40.00)		
2+	9	(16.98)	12	(26.67)		
3+	0	(.00)	7	(15.56)		

analyzed. In this study, a significant correlation was found between LOX expression in tumor stroma and tumor budding ($p=0.007$). Low levels of budding were observed in tumors with low stromal LOX expression and those without LOX expression (Table 3).

Discussion

As in many solid tumors, metastatic disease is an important cause of mortality and morbidity in colon carcinomas [8]. New prognostic parameters that can predict metastases or prognosis are crucial for understanding the different course of cases at the same stage, addressing treatment resistance, and managing the disease effectively. Epithelial-mesenchymal transformation is considered an important step in the metastatic potential of the tumor. Tumor budding is a prognostic parameter that has gained importance in recent years and is thought to be a histological finding of epithelial mesenchymal transformation [9, 10].

Tumor progression is a highly complex process, and both neoplastic cells and the surrounding stroma are believed to play active roles in it. Fibroblasts surrounding the tumor, known as cancer-associated fibroblasts (CAFs), are also involved in this process by secreting lysyl oxidase (LOX) and producing collagen [11]. In a study on liver metastasis of gastric cancers, LOX expression from CAFs in the liver metastasis environment was found to promote the formation of a suitable environment for the tumor, and excessive LOX expression was thought to be associated with poor prognosis

Table 2 Relationship between tumoral LOX expression in the epithelial component of the tumor and clinicopathological parameters

		LOX tumor				*p*
		Low		High		
		n	%	*n*	%	
Budding	Low	29	(53.70)	23	(51.11)	0.604
	Modarate	16	(29.63)	11	(24.44)	
	High	9	(16.67)	11	(24.44)	
Infiltration pattern	Infiltrative	47	(87.04)	40	(88.89)	0.779
	Expansive	7	(12.96)	5	(11.11)	
Tumor type	Classic	36	(66.67)	37	(82.22)	0.536
	Mucinous	8	(14.81)	4	(8.89)	
	Serrated	3	(5.56)	2	(4.44)	
	Signet ring	3	(5.56)	1	(2.22)	
	Micropapillary	2	(3.70)	1	(2.22)	
	Meduller	2	(3.70)	0	(0.00)	
Differentiation	G1	28	(51.85)	16	(35.56)	0.057
	G2	17	(31.48)	23	(51.11)	
	G3	5	(9.26)	6	(13.33)	
	G4	4	(7.41)	0	(0.00)	
Stage	I	1	(1.85)	2	(4.44)	0.502
	II	7	(12.96)	9	(20.00)	
	III	12	(22.22)	12	(26.67)	
	IV	34	(62.96)	22	(48.89)	
Lymphovascular invasion	Negative	17	(31.48)	13	(28.89)	0.780
	Positive	37	(68.52)	32	(71.11)	

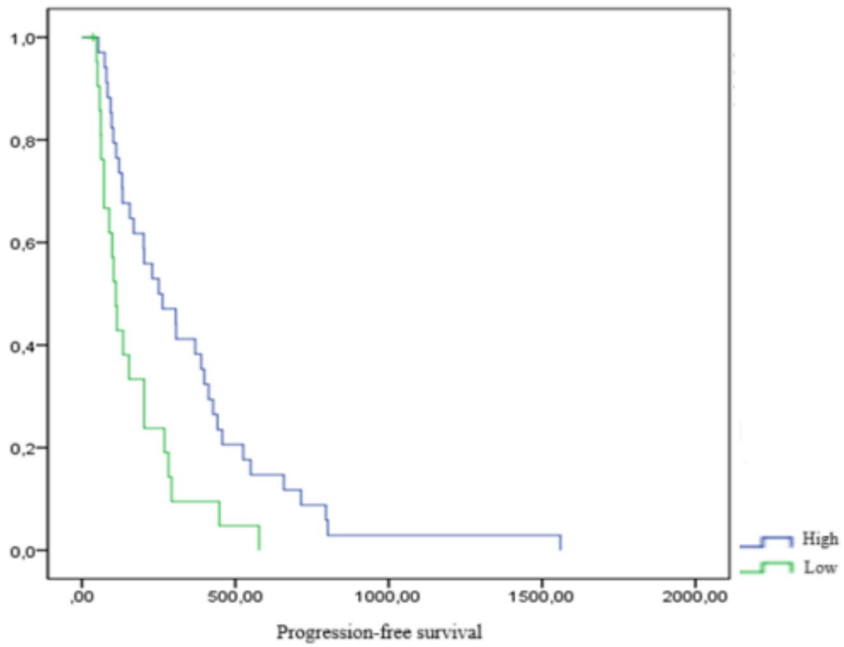

Fig. 3 Progression-free survival in stage IV patients according to LOX expression

Table 3 Relationship between stromal LOX expression and clinicopathological parameters

		Stromal LOX expression								*p*
		Negative		Mild		Modarate		Strong		
		n	%	*n*	%	*n*	%	*n*	%	
Tumor budding	Low	24	(66.67)	22	(62.86)	4	(19.05)	2	(28.57)	0.007
	Modarate	8	(22.22)	5	(14.29)	11	(52.38)	3	(42.86)	
	High	4	(11.11)	8	(22.86)	6	(28.57)	2	(28.57)	
Infiltration pattern	Infiltrative	31	(86.11)	29	(82.86)	21	(100.00)	6	(85.71)	0.277
	Expansive	5	(13.89)	6	(17.14)	0	(0.00)	1	(14.29)	
Differentiation	G1	18	(50.00)	18	(51.43)	7	(33.33)	1	(14.29)	0.480
	G2	13	(36.11)	11	(31.43)	11	(52.38)	5	(71.43)	
	G3	3	(8.33)	4	(11.43)	3	(14.29)	1	(14.29)	
	G4	2	(5.56)	2	(5.71)	0	(0.00)	0	(0.00)	
Stage	I	1	(2.78)	1	(2.86)	1	(4.76)	0	(0.00)	0.584
	II	4	(11.11)	7	(20.00)	4	(19.05)	1	(14.29)	
	III	6	(16.67)	9	(25.71)	5	(23.81)	4	(57.14)	
	IV	25	(69.44)	18	(51.43)	11	(52.38)	2	(28.57)	
Lymphovascular invasion	Positive	12	(33.33)	13	(37.14)	5	(23.81)	0	(0.00)	0.222
	Negative	24	(66.67)	22	(62.86)	16	(76.19)	7	(100.00)	

[12]. In a study examining the extracellular matrix in colon tumors, CAFs were shown to support FAK phosphorylation and EMT development through LOX, as well as provide an environment conducive to distant metastasis [13]. In this study, a positive correlation was found between stromal LOX expression and tumor budding. In the literature, there are few studies exploring the relationship between LOX expression in tumor stroma and budding in colon cancers.

Recently, many studies have indicated that LOX expression contributes to metastatic spread by supporting many pathways involved in tumor progression, such as epithelial mesenchymal transformation [13–15]. When evaluating the effect of LOX expression on prognosis and histopathological parameters, increased LOX expression has been observed in poorly differentiated tumors, patients with lymph node metastasis, and patients at advanced stages [15]. In a study conducted in colorectal cancers with lung and liver metastases, increased LOX mRNA expression was found in colorectal carcinomas compared to normal mucosa, and nuclear expression of LOX was found to be associated with lung/liver metastasis, high CEA level, mucinous

tumor type, and poor survival [7]. In colorectal cancers, LOX expression was detected in tumor cells metastasizing to the bone marrow by Reynaud et al. [16]. In the study conducted by Baker et al. in colorectal cancers, increased LOX expression was found in tumors compared to normal mucosa, in metastatic tumors compared to primary tumors, and in stage II cases compared to stage I [17]. In studies addressing the role of LOX expression in tumor progression, it has been suggested that LOX may affect tumor cell mobility through SRC activation, while LOX inhibition may reduce cancer progression [17]. Ras activation in colon cancers was found to increase LOX expression through activation of the PI3K-Akt-HIF-1a pathway, and Ras inhibitor use was shown to reduce cell migration and lung metastasis by decreasing LOX expression [18]. A recent study in colon cancers examined cancer-associated LOX interactions and found that this interaction participates in ECM organization, degradation, and cross-linking, cell-ECM interactions mediated by integrin and non-integrin receptors, protein folding, chaperone activity, organ-blood vessel development, cellular response to stress, and signal transduction [19].

The hypoxic environment in tumor triggers the activation of hypoxia-inducible factor (HIF). HIF binds to hypoxia-response element (HRE) and induces cellular proliferation and angiogenesis via EGF (epidermal growth factor) and VEGF (vascular endothelial growth factor). HIF-activated HRE binds to the LOX promoter region as a transcription factor and triggers LOX expression [3, 20]. In poorly differentiated breast cancer cell lines, exogenous expression of mature LOX has been found to facilitate Src and FAK activation, which play critical roles in tumor motility and EMT [21]. In a study conducted on colon cancers, it has been shown that CAFs support FAK phosphorylation and EMT development, activate the FAK signaling pathway, and provide an environment for the development of distant metastasis [13]. Chemoresistance is a major obstacle in many tumors. In studies developing chemoresistant triple negative breast tumors in vivo, hypoxia-induced ECM remodeler, lysyl oxidase (LOX), has been identified as an important inducer of chemoresistance. Inhibition of LOX has been shown to increase drug penetration by reducing collagen cross-linking and fibronectin assembly, inhibiting FAK/Src signaling, and causing chemotherapy sensitivity in cell culture, chemoresistant xenografts, and tumor organoid models [22]. Some molecules targeting LOX are being developed. In our study, a decrease in progression-free survival was observed in tumors with high LOX expression in stage IV patients. It was thought that LOX expression in both tumor cells and stroma may be involved in chemoresistance, tumor progression, and poor survival outcomes. Many new parameters are emerging to determine the biological behavior of the disease and guide treatment in colorectal cancers, in addition to the existing prognostic markers. Studies are needed to closely examine the stromal component and the parenchymal features of the tumor histopathologically and to evaluate the impact of these histopathological features on prognosis.

The current study may have the following limitations: First, our understanding of a mechanistic role of LOX in tumor progression is limited by the lack of functional assays, as only immunohistochemical evaluation of LOX expression is analyzed; second although the small sample size was adequate for preliminary analysis, results could not be solely generalized based on these findings.

Conclusion

This study underlines the potential prognostic value of LOX expression in colorectal adenocarcinoma, especially concerning its association with tumor budding and survival outcomes. The observed correlation of high LOX expression with reduced progression-free survival, especially in advanced-stage patients, suggests that LOX may serve as a useful indicator for CRC prognosis. Furthermore, the association of stromal LOX expression with tumor budding underlines its possible role in modifying the tumor microenvironment and promoting invasion and metastasis. Although these findings are encouraging, further studies are required to establish LOX as a biomarker and explore its potential use as a therapeutic target in cancer management.

Author contribution A.D.Ç: Writing – original draft, Methodology, Visualization, Software. S.K: Data curation. D.Ç: Writing – review & editing. MT: Formal analysis A.A.A:Supervision. H.T.D: Writing – review & editing, Conceptualization.

Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval The study was approved by the approved by the Clinical Research and Ethics Committee of Ankara Bilkent City Hospital (decision number: E1-20–1287) and performed in accordance with the Declaration of Helsinki.

Competing interests The authors declare no competing interests.

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