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ORIGINAL ARTICLE

Involvement of miR-30b in kynurenine-mediated lysyl oxidase expression

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Abstract

Microenvironment components profoundly influence the propensity of cancer metastasis through regulating key molecules controlling metastasis. Lysyl oxidase (LOX) contributes to extracellular matrix (ECM) remodeling, and finally promoting bone metastasis in breast cancer. Kynurenine (Kyn), a microenvironment component, is capable of regulating the biological behaviors of cancer cells, such as promoting node metastasis in vivo. However, it is still unclear whether Kyn controls the generation of LOX. In the current study, a significant increase of migration in the Kyn (30, 50, 100, 200, and 500 μM) group was detected compared with that in the control group in 95D cells in vitro. Subsequently, we demonstrated that 50 μM Kyn not only substantially upregulated the mRNA and secreted levels of LOX rather than cytoplasmic LOX, but also markedly reduced the level of miR-30b at the same time. Furthermore, the direct interaction between LOX mRNA and miR-30b was also confirmed by dual-luciferase assay system. Most importantly, not only was Kyn-induced increase of LOX mRNA significantly attenuated on miR-30b mimics treatment, but also Kyn-mediated the upregulation of the mRNA, and secreted levels of LOX were distinctly strengthened on miR-30b inhibitor treatment. These results suggest that miR-30b is involved in Kyn-mediated LOX expression.

Keywords Kynurenine · Lysyl oxidase · miR-30b · Metastasis

Introduction

Molecular constituents regulated by the microenvironment components in both the primary cancer and distant tissue profoundly influence the propensity of cancer metastasis. Lysyl oxidase (LOX) is a copper-dependent amine oxidase, and catalyzes the covalent cross-linking of collagen and elastin, which leads to the extracellular matrix (ECM) remodeling [18], and finally influencing the metastasis of cancer cells [15]. LOX has been wrongly considered as a tumor suppressor in the past few decades. However, recent evidences show that the tumor suppressor activity of LOX lies not within the active enzyme itself, but within the propeptide domain following extracellular cleavage from the proenzyme [22, 31]. Furthermore, it has been demonstrated that the LOX propeptide achieves its suppressing effects by reentering the cell following cleavage [12]. However, the mRNA and protein levels of LOX are upregulated in invasive or metastatic breast cancer and melanoma cell lines, and the invasion of them can be prevented, in vitro, by the addition of β-aminoproprionitrile, a nonspecific small molecule inhibitor of LOX activity [9], or by using LOX-targeting antibodies [1]. More importantly, data provided by Cox et al showed that the bone metastasis of breast cancer was driven by LOX [5]. These data suggest that active LOX promotes cancer cells to metastasize.

Kynurenine (Kyn), an endogenous substance in microenvironment, is generated through catalyzing tryptophan degradation by indoleamine 2,3-dioxygenase (IDO) during infection, inflammation, tumor or oxidative stress, or by tryptophan 2,3-dioxygenase under physiological conditions in vivo [13, 19, 28]. It has been reported that the 3-year survival rates of

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diffuse large B cell lymphoma patients undergoing R-CHOP therapy is higher if their serum levels of Kyn are below 1.5 μM [29], which suggests that there is a close relationship between Kyn and cancer metastasis. Indeed, high level of Kyn is strongly positively related to lymph node metastasis, tumor size, parametrial invasion, and poor disease-specific survival in cervical cancer in vivo [11]. Therefore, it is important to further decoding the mechanism of Kyn-mediated metastasis of cancer cells.

miRNAs are endogenous small non-coding RNAs. They can regulate gene expression in the post-transcriptional stage by interacting with the 3′-UTR of the target mRNA [2]. Growing evidences have demonstrated that aberrantly expressed miRNAs can act as oncogenes or tumor suppressors in various types of malignancies [10]. Data from Chen et al showed that miR-30b and collagen triple helix repeat containing 1 (Cthrc1) were downregulated and upregulated respectively in non-small cell lung cancer (NSCLC), which was related to tumor differentiation and tumor node metastasis (TNM) stage [3]. Moreover, miR-30b inhibited NSCLC invasion and migration by targeting Cthrc1 [3]. Similarly, miR-30b-suppressing metastasis in hepatoma cells was detected by regulating Snail [25], in gastric cancer cells by targeting eukaryotic translation initiation factor 5A2 [26], and in colorectal cancer by controlling SIX homeobox 1 (SIX1) [30].

In the present paper, we demonstrated that 50 μM Kyn not only significantly upregulated the mRNA and secreted levels of LOX, but also markedly reduced the level of miR-30b in 95D cells. Moreover, LOX mRNA, a novel target of miR-30b, was also confirmed. Notably, although Kyn mediated the increase of secreted LOX was not completely abrogated upon miR-30b mimics treatment, it was distinctly strengthened upon miR-30b inhibitor treatment. These results suggest that miR-30b plays key roles in Kyn-induced LOX expression.

Materials and methods

Cell culture

95D cells, a human lung cancer cell line, were purchased from Beijing Chuanglian North Carolina Biotechnology Research Institute and cultured in 1640 medium (Hyclone) supplemented with 10% fetal bovine serum (Gibco), 100-μg/ml streptomycin and 100-U/ml penicillin. The HEK 293T cells were obtained from the Kunming Institute of Zoology, Chinese Academy of Sciences and grown in high-glucose DMEM medium (Hyclone) supplemented with 10% fetal bovine serum (Gibco), 100-μg/ml streptomycin and 100-U/ml penicillin. All cells were maintained at 37 °C in an atmosphere of 5% CO_2–95% air.

Migration assay

Migration assays were conducted in transwell with 8-μm-pore filter inserts (Corning). Cells (1×10^4/well) were seeded in the upper chamber. The lower chambers were filled with DMEM medium containing selected concentrations of Kyn. After 16 h, the inserts were removed, and the cells that migrated to the lower chamber were fixed. The cells were stained with 0.1% crystal violet (Solarbio), and captured by a microscope (× 64).

Cell transfection

The transfection experiments in this paper were performed by the Lipofectamine™ 2000 reagent (11668-019, Invitrogen), according to the manufacturer's recommendations.

Real-time PCR

95D cells were administered with Kyn (K8625-100MG, Sigma-Aldrich) (30, 50, 100, 200, and 500 μM), miR-30b mimics (GenePharma), miR-30b inhibitor (GenePharma), 50 μM Kyn + miR-30b mimics, 50 μM Kyn + miR-30b inhibitor or without for 16 h. The non-treated cells were considered the control group. Total RNA was extracted by Trizol reagent (Invitrogen) or RNeasy kit (Qiagen). Subsequently, EasyScript First-Strand cDNA Synthesis SuperMix (Transgen Biotech) was used to synthesize cDNA. CFX96TM real-time system (BIO-RAD) was used to perform real-time PCR analysis. Amplification of cDNA was performed using the TransStart Tip Green qPCR SuperMix (Transgen Biotech) as suggested by the manufacturer's instructions. Gene names and primer sets were listed in Table 1. The real-time PCR cycling conditions were as follows: after an initial denaturation and enzyme activation at 95 °C for 3 min, the amplification was performed by denaturation at 95 °C for 15 s, annealing at 56.5 °C for 15 s, and extension at 72 °C for 30 s. The levels of LOX and miR-30b were normalized to GAPDH and small nuclear RNA U6 respectively, in order to control for differences in RNA loading, quality, and cDNA synthesis. Relative gene expression data were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Western blot analysis

Total cellular protein samples of 95D cells were harvested in RIPA lysis buffer (Pierce) supplemented with PMSF (Solarbio). The non-administered cells were considered the control group. In brief, samples were loaded per lane of SDS-PAGE gels, electrophoresed, and then transferred onto PVDF membranes (Millipore) using a wet transfer technique. Rabbit anti-LOX antibody (1:1000) (17958-1-AP, proteintech) and mouse anti-β-actin antibody (1:5000) (sc-

81,178, Santa Cruz Biotechnology) were used as the primary antibodies, respectively. Immunoreactivity was detected by incubation with HRP-labeled anti-rabbit/mouse IgG antibody (1:5000; 1:5000) (ab6721, abcam; sc-2005, Santa Cruz Biotechnology) respectively, and visualized using an ECL chemiluminescence kit. Quantitation of LOX expression was done by densitometry and normalized with loading controls (β-actin).

Enzyme-linked immunosorbent assay

Cell culture supernatants were collected from 95D cells. The non-stimulated cells were considered the control group. A human lysyl oxidase ELISA kit (HM10997, Bio-Swamp) was used to detect secreted LOX, according to the manufacturer's instructions. The OD at 450 nm was read using an ELISA plate reader (SYNERGY4, BioTek).

Plasmid construction and luciferase reporter assay

The 3′-UTR fragments containing the predicted LOX-miR-30b binding sites were amplified by PCR amplification kit (Takara), and the mutant fragments with mutant sites located in the complementary sequences of miR-30b seed region were obtained by the PCR site-directed mutagenesis. Next, they were cloned into the pmirGLO dual-luciferase miRNA target expression vector (Promega) using a DNA ligation kit (Takara). The recombinant plasmids—pmirGLO-LOX (pmirGLO-L) and pmirGLO-LOX mutant (pmirGLO-L mt)—and the pmirGLO vector were extracted from LB culture using the PureYield Plasmid Midiprep System (Promega). The related PCR primer sets were listed in Table 1. HEK 293T cells were plated in 24-well plates at the concentration of 2×10^5 cells/well. MiR-30b (20 pmol) and each plasmid (0.4 μg) were co-transfected into the HEK 293T cells that were used as a vehicle cell to detect the interaction between miR-30b and predicted target. Luciferase activity was measured using the dual-luciferase assay system (Promega) with SYNERGY4 (BioTek). Firefly luciferase activity was normalized to Renilla luciferase activity to adjust for variations in transfection efficiency among experiments.

Statistical analysis

All the experiments are repeated three times, and data are expressed as the mean ± standard error of mean. Statistical analyses were performed using the professional statistical computer software, SPSS. Differences between groups were determined using an unpaired *t* test. $P < 0.05$ was considered statistically significant.

Results

Promoting migration by Kyn in vitro

Considering that Kyn promotes node metastasis in vivo, and the complex regulation among systems, organs, and tissues, it needs to be clarified whether Kyn can induce cancer cells to metastasize. So, we firstly studied the effects of Kyn on migration using Transwell in vitro. As shown in Fig. 1, compared with the control group, the count of cells into the lower chambers through 8-μm-pore filter inserts is markedly increased in the Kyn (30, 50, 100, 200, and 500 μM) group in vitro, in a

Table 1 Sequences of primers used in this study

Gene name		Sequences (5′-3′)
miR-30b	RT	GTCGTATCCAGTGCAGGGTCCGAGGT
		ATTCGCACTGGATACGACAGCTGAGT
	Forward	GCCGACTGTAAACATCCTACAC
	Reverse	GTGCAGGGTCCGAGGT
snRNA U6	RT/reverse	AACGCTTCACGA ATTTGCGT
	Forward	CTCGCTTCGGCAGCACA
LOX[1]	Forward	GCCGACCAAGATATTCCTGGG
	Reverse	GCAGGTCATAGTGGCTAAACTC
GAPDH	Forward	TGTTGCCATCAATGACCCCTT
	Reverse	CTCCACGACGTACTCAGCG
LOX[2]	Forward	ACGAGCTC[3]ATCAGTGCCTGGTGTTCT
	Reverse	CGCTCTAGA[4]TTCACGGCTGCCTTATGT
	Mutant forward	CCCTATATAAAAAGTATTTAAAAAATTAGTAGATAA
	Mutant reverse	CTAATTTTTTAAATACTTTTTATATAGGGATGT

[1] Primers for real-time PCR; [2] primers for plasmid construction; [3] the site of restriction endonuclease *Sac I*; [4] the site of restriction endonuclease *Xba I*

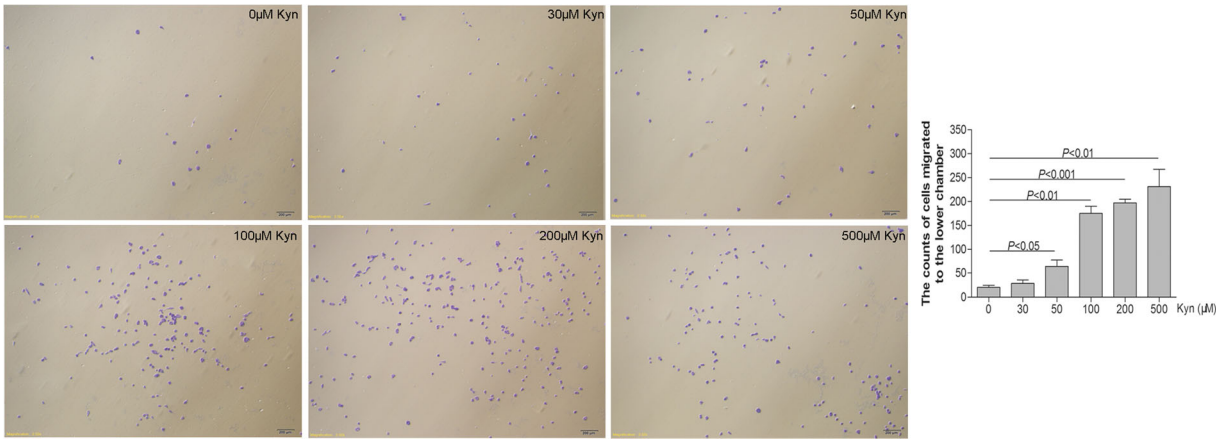

Fig. 1 Effect of Kyn on cell migration. 95D cells were seeded in Transwell inserts. Indicated doses of Kyn were supplemented in 1640 medium placed in the lower chambers. Cell migration was determined as described in the experimental procedures. Results were shown as average ± SEM, $n = 3$. Unpaired t test was used and $P < 0.05$ was considered statistically significant

dose-dependent manner. The results strongly suggest that Kyn promotes the migration of 95D cells.

Upregulating the mRNA and secreted levels of LOX by Kyn in 95D cells

Because LOX can drive metastasis in breast cancer cells [5], real-time PCR, ELISA, and Western blot were performed to detect the effects of Kyn on LOX. As shown in Fig. 2 a and b, compared with the control group, the mRNA and secreted levels of LOX were significantly upregulated by 50 μM Kyn, which indicated that Kyn enhanced the LOX expression. Nevertheless, no significant differences in the mRNA and secreted levels of LOX were detected between the control group and the other selected doses of Kyn (30, 100, 200, and 500 μM) group. Surprisingly, the selected concentrations of Kyn (30, 50, 100, 200, and 500 μM) in the current paper failed to alter the level of cytoplasmic LOX protein (Fig. 2c).

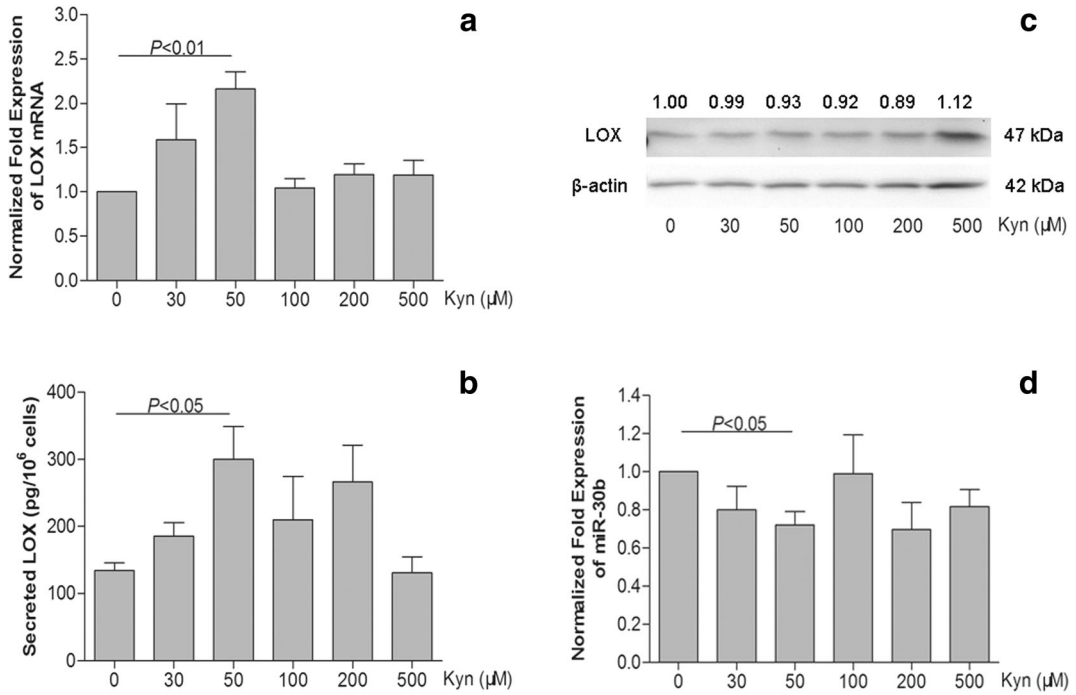

Fig. 2 Effect of Kyn on LOX and miR-30b expression. 95D cells were administered with the indicated concentrations of Kyn for 16 h. Real-time PCR (**a**), ELISA (**b**), and Western blot (**c**) were performed to analyze the mRNA, secreted, and cytoplasmic levels of LOX respectively, as described in the experimental procedures. The miR-30b expression in 95D cells was detected by real-time PCR (**d**). Results were shown as average ± SEM, $n = 3$. Unpaired t test was used and $P < 0.05$ was considered statistically significant

Attenuating miR-30b expression by Kyn

A significant negative correlation in gene expression level between miR-30b and LOX mRNA in clear cell kidney carcinoma (300 samples; $r = -0.38104$; $P = 8.39395e{-}12$), colon and rectal adenocarcinoma (299 samples; $r = -0.42154$; $P = 2.60847e{-}14$), urothelial bladder cancer (229 samples; $r = -0.37855$; $P = 3.2404e{-}09$), and lung adenocarcinoma (441 samples; $r = -0.37427$; $P = 4.12282e{-}16$) was presented by webpage (starBase v2.0). Moreover, it has been reported that miR-30b expression is significantly downregulated in NSCLC [32]. Therefore, the effects of Kyn on the miR-30b expression were analyzed. As shown in Fig. 2d, the level of miR-30b in the 50 μM Kyn group was decreased compared with that in the control group, which confirmed that Kyn inhibited miR-30b expression. However, there were no significant differences in miR-30b expression between the control group and the other selected doses of Kyn (30, 100, 200, and 500 μM) group.

LOX mRNA, a novel target of miR-30b

As described above, since Kyn (50μM) may downregulate miR-30b level and upregulate LOX expression at the same time, is miR-30b targeting LOX directly? Thus, we further analyzed the interaction between miR-30b and LOX mRNA using luciferase reporter assay. The web-accessible miRNA database search programs (Targetscan and MiRanda) predicted one miRNA recognition element (MRE) for miR-30b within the LOX 3′-UTR (Fig. 3a), which indicated that LOX mRNA might be a candidate target for miR-30b. The UCSC blat tool (http://genome.ucsc.edu) was conducted to analyze the conservation status of the MRE among animals. The results showed that it was conserved in human, chimp, rhesus, mouse, rat, pig, cat, dog, and elephant (Fig. 3b). Figure 3 c shows a schematic map about the position and sequences of the primers used for PCR site-directed mutagenesis and amplification of wild/mutant LOX 3′-UTR sequences. After co-transfection in HEK 293T cells for 48 h, luciferase reporter assay was performed to analyze the direct interaction between miR-30b and LOX mRNA. As shown in Fig. 3d, luciferase activity in the pmirGLO-L+miR-30b group was distinctly lower than that in pmirGLO+miR-30b group, whereas there was no significant difference in luciferase activity between pmirGLO-L mt+miR-30b group and pmirGLO+miR-30b group, which demonstrated that LOX mRNA was a novel target of miR-30b.

Involvement of miR-30b in Kyn-mediated increase of LOX

The transfection efficiency of miR-30b mimics and the miR30b inhibitor was analyzed using real-time PCR (Supplementary Fig. 1). The mRNA and cytoplasmic levels of LOX in the miR-30b mimics group were significantly downregulated (Fig. 4a, c) compared with that in the control group. On the contrary, the mRNA, cytoplasmic and secreted levels of LOX in the miR-30b inhibitor group were markedly higher than that in the control group (Fig. 4a–c). These data further confirmed that LOX mRNA was a novel target of miR-30b. Additionally,

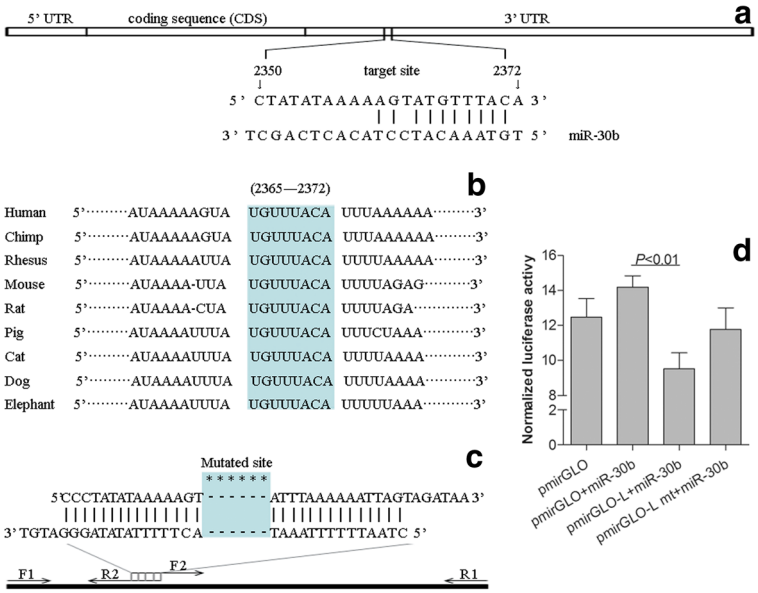

Fig. 3 The direct interaction between miR-30b and LOX mRNA. The position of predicted MRE located in LOX 3′-UTR and the pairing status of miR-30b and predicted MER sequences (**a**). Sequence alignment of predicted MRE in human, chimp, rhesus, mouse, rat, pig, cat, dog, and elephant (**b**). The position and sequences of the primers used for PCR site-directed mutagenesis and amplification of wild/mutant LOX 3′-UTR was presented in schematic map (**c**). Effect of miR-30b expression on LOX promoter was analyzed by luciferase assay (**d**), and plotted as average ± SEM, $n = 3$. Unpaired t test was used and $P < 0.05$ was considered statistically significant

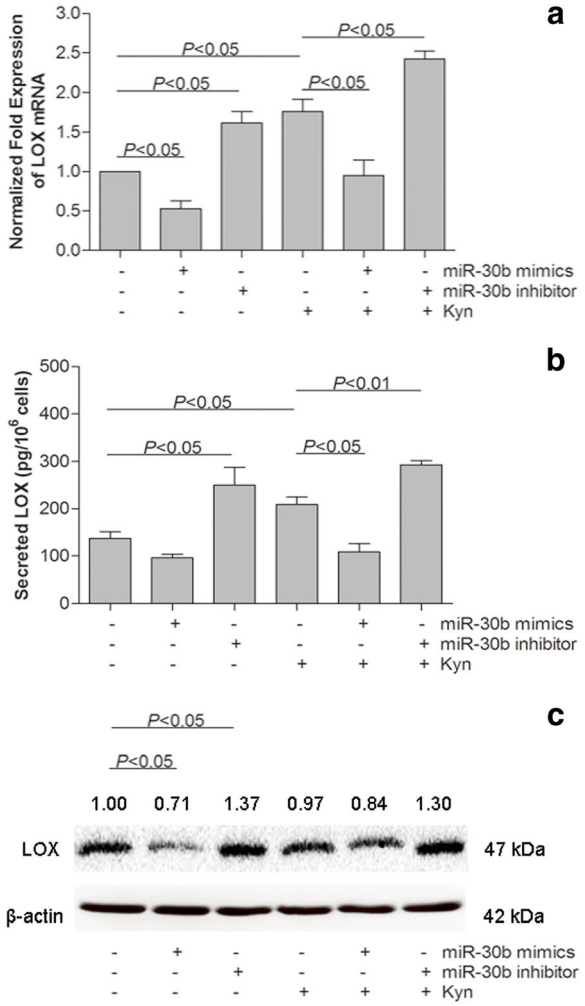

Fig. 4 Effect of miR-30b on LOX expression induced by Kyn. 95D cells were administered with the indicated procedures for 16 h. Real-time PCR (**a**), ELISA (**b**), and Western blot (**c**) were performed to analyze the mRNA, secreted, and cytoplasmic levels of LOX respectively, as described in the experimental procedures. Results were shown as average ± SEM, $n = 3$. Unpaired t test was used and $P < 0.05$ was considered statistically significant

although miR-30b mimics tended to decrease the gene expression of LOX when co-administrated with 50 μM Kyn (Fig. 4a–c), only mRNA and secreted levels of LOX were markedly downregulated (Fig. 4a, b). Notably, Kyn-induced increase of LOX, including the mRNA and secreted levels, was substantially strengthened on miR-30b inhibitor treatment (Fig. 4a, b), which strongly indicated that miR-30b was involved in Kyn-mediated upregulation of LOX.

Discussions

Cancer metastasis is often associated with high mortality because it is difficult to treat surgically or with conventional chemotherapy and radiation therapy. Recent studies have demonstrated that molecular constituents regulated by microenvironment components in cancer cells play key roles in metastasis [6]. Therefore, it is important to understand the effects of microenvironment on metastasis.

Kyn, a microenvironment component, is upregulated due to the high expression of IDO, an enzyme catalyzing tryptophan into Kyn, in cancer tissues [14]. This suggests that Kyn is probably responsible for some biological response of cancer cells. Indeed, the concentration of Kyn in serum is negatively related to the 3-year overall survival rates in clinical studies [29]. However, this was only a phenomenon observed in vivo. Whether Kyn has a significant impact on cancer metastasis in fact still needs to be further confirmed. In the current paper, we firstly demonstrated that Kyn promoted 95D cells, a lung cancer cell line with strongly invasive ability, migration in vitro, which was the same as we expected.

It has been confirmed that ECM reduction in the primary tumor has a high impact on invasion and metastasis [24], which indicates that ECM plays key roles in cancer metastasis. Besides, the alterations in the composition of the tumor ECM, like stiffness and architectural properties, are also altered due to the overexpression of ECM-remodeling enzymes such as the lysyl oxidase [20]. In the present paper, we demonstrated that Kyn (50 μM) markedly upregulated the mRNA and secreted levels of LOX, which revealed that Kyn might fasten the ECM remodeling and ultimately promote the cancer metastasis. Surprisingly, the selected dose of 50 μM Kyn failed to alter the cytoplasmic level of LOX protein. In fact, LOX is synthesized as an approximately 50 kDa proenzyme, secreted, and cleaved by procollagen-C-proteinase (BMP-1) into a 32 kDa mature, active enzyme and a 18 kDa propeptide [27]. The molecular weight of cytoplasmic LOX observed by Western blot in this paper was approximately 47 kDa, which corresponded with the proenzyme of LOX. Therefore, the results from cytoplasmic and secreted LOX are not contradictory. In addition, it has been reported that aryl hydrocarbon receptor, that functions as receptor for Kyn, significantly attenuated the level of miR-96 [23], which is negatively correlated with the expression of BMP-1 in six kinds of malignant tumors (http://starbase.sysu.edu.cn/pcMirTargetInfo.php?database=hg19&name=hsa-miR-96-5p&targetGene=BMP1&autoId=241534). So, it can be speculated that Kyn enhances the expression of BMP1 and accelerates the maturation of LOX.

Considering the negative correlation between LOX mRNA and miR-30b in clinical specimens, we further analyzed the effect of Kyn on miR-30b expression, and found that Kyn (50 μM) significantly reduced miR-30b level in 95D cells. However, other selected doses of Kyn (30, 100, 200, and 500 μM) failed to alter markedly the level of miR-30b. The possible reasons are as follows. Firstly, Kyn induced DRE-luciferase activity in glioma cells, with a concentration giving half-maximal response of 36.6 μM [21]. Therefore, we speculate the reason that 30 μM Kyn fails to cause significant changes of miR-30b/LOX maybe largely contribute to low

concentration. Additionally, when the concentration of Kyn is higher than 50 μM, the increase of Kyn-mediated DRE-luciferase activity gets in the relative plateau stage [21], which is partly accordance with the "tide" model, tight feedback between costimulatory factors and coinhibitory factors maintains that the responses induced by stimulants are tuned and return to baseline [33]. So, the phenomenon that higher concentrations of Kyn (100, 200, and 500 μM) fail to cause the significant differences of miR-30b/LOX maybe partly contribute to coinhibitory factors that play a leading role in the effects of Kyn (100, 200, and 500 μM) on miR-30b/LOX expression.

Next, we studied how miR-30b affects LOX expression. We found that the expression of cytoplasmic LOX was markedly reduced when miR-30b level increased. On the contrary, cytoplasmic LOX was markedly upregulated by decreasing miR-30b expression. These results were in accordance with the mechanism of miRNAs. The data from this study demonstrated that LOX mRNA is a novel target of miR-30b. Interestingly, no substantial changes in secreted LOX when miR-30b expression was increased. However, significant enhancement of secreted LOX was detected when miR-30b expression was decreased. This indicates that the effects of miR-30b on the secretion of LOX are related to the range of miR-30b variation. Because the level of miR-30b is higher than that of the complementary chain, which is similar to miR-30b inhibitor in base sequences, more obvious effects are detected when miR-30b is downregulated by miR-30b inhibitor. In additional, although there is the greater specificity between miRNAs and their targets, some miRNA may target hundreds of different mRNAs [17]. For example, besides LOX, it has been reported that the key transcriptional factors controlling the B cell terminal differentiation, Bach2 [7, 8] and Bcl6 [4], are also the targets of miR-30b. Downregulation of miR-30b will increase the expression of Bach2 and Bcl6, which suppresses immune responses induced by antigens, and facilitates cancer cells to evade immune responses and metastasize. Considering the principle of energy saving in body, these data suggest that miR-30b plays key roles in the process of Kyn-mediated increase of LOX expression.

Lastly, the contribution of miR-30b to the upregulation of LOX expression induced by Kyn was analyzed. miR-30b mimics tended to reduce Kyn-mediated increase of LOX, whereas miR-30b inhibitor significantly increased the Kyn-mediated upregulation of LOX, which suggested that miR-30b is involved in the process of Kyn-induced enhancement of LOX. These results are consistent with previous data showing that upregulation of miR-30b is favorable for the therapeutic actions produced by trastuzumab in breast cancer [16], and by the finding that forced expression of miR-30b inhibits the migration and invasion of colorectal cancer cells in vitro via its target gene SIX1, an epithelial-mesenchymal transition promoting gene [30]. Taken together, our data indicate that miR-30b plays important roles in Kyn-induced increase of LOX expression in 95D cells.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Baker AM, Bird D, Lang G, Cox TR, Erler JT (2013) Lysyl oxidase enzymatic function increases stiffness to drive colorectal cancer progression through FAK. Oncogene 32:1863–1868
2. Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136:215–233
3. Chen S, Li P, Yang R, Cheng R, Zhang F, Wang Y, Chen X, Sun Q, Zang W, Du Y, Zhao G, Zhang G (2015) MicroRNA-30b inhibits cell invasion and migration through targeting collagen triple helix repeat containing 1 in non-small cell lung cancer. Cancer Cell Int 15:85
4. Chang CC, Zhang QY, Liu Z, Clynes RA, Suciu-Foca N, Vlad G (2012) Downregulation of inflammatory microRNAs by Ig-like transcript 3 is essential for the differentiation of human CD8(+) T suppressor cells. J Immunol 188:3042–3052
5. Cox TR, Rumney RM, Schoof EM, Perryman L, Høye AM, Agrawal A, Bird D, Latif NA, Forrest H, Evans HR, Huggins ID, Lang G, Linding R, Gartland A, Erler JT (2015) The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase. Nature 522:106–110
6. Crotti S, Piccoli M, Rizzolio F, Giordano A, Nitti D, Agostini M (2017) Extracellular matrix and colorectal Cancer: how surrounding microenvironment affects cancer cell behavior? J Cell Physiol 232:967–975
7. Duan Z, Duan Y, Lei H, Hu N, Shi J, Shen D, Wang X, Hu Y (2014) Attenuation of antigenic immunogenicity by kynurenine, a novel suppressive adjuvant. Hum Vacc Immunother 10:1295–1305
8. Duan ZQ, Shi JD, Wu MN, Hu NZ, Hu YZ (2016) Influence of miR-30b regulating humoral immune response by genetic difference. Immunol Res 64:181–190
9. Erler JT, Bennewith KL, Nicolau M, Dornhöfer N, Kong C, Le QT, Chi JT, Jeffrey SS, Giaccia AJ (2006) Lysyl oxidase is essential for hypoxiainduced metastasis. Nature 440:1222–1226
10. Farazi TA, Hoell JI, Morozov P, Tuschl T (2015) MicroRNAs in human cancer. Adv Exp Med Biol 774:1–20
11. Ferns DM, Kema IP, Buist MR, Nijman HW, Kenter GG, Jordanova ES (2015) Indoleamine-2,3-dioxygenase (IDO) metabolic activity is detrimental for cervical cancer patient survival. Oncoimmunology 4:e981457
12. Guo Y, Pischon N, Palamakumbura AH, Trackman PC (2007) Intracellular distribution of the lysyl oxidase propeptide in osteoblastic cells. Am J Phys Cell Phys 292:C2095–C2102
13. Heyes MP, Saito K, Major EO, Mlistien S, Markey SP, Vickers JH (1993) A mechanism of quinolinic acid formation by brain in inflammatory neurological disease. Attenuation of synthesis from L-tryptophan by 6-chlorotryptophan and 4-chloro-3-hydroxyanthranilate. Brain 116:1425–1450
14. Hoshi M, Ito H, Fujigaki H, Takemura M, Takahashi T, Tomita E, Ohyama M, Tanaka R, Saito K, Seishima M (2009) Indoleamine 2, 3-dioxygenase is highly expressed in human adult T-cell leukemia/

lymphoma and chemotherapy changes tryptophan catabolism in serum and reduced activity. Leuk Res 33:39–45
15. Hou X, Du H, Quan X, Shi L, Zhang Q, Wu Y, Liu Y, Xiao J, Li Y, Lu L, Ai X, Zhan M, Yuan S, Sun L (2018) Silibinin inhibits NSCLC metastasis by targeting the EGFR/LOX pathway. Front Pharmacol 9:21
16. Ichikawa T, Sato F, Terasawa K, Tsuchiya S, Toi M, Tsujimoto G, Shimizu K (2012) Trastuzumab produces therapeutic actions by upregulating miR-26a and miR-30b in breast cancer cells. PLoS One 7:e31422
17. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433:769–773
18. Li SY, Yan JQ, Song Z, Liu YF, Song MJ, Qin JW, Yang ZM, Liang XH (2017) Molecular characterization of lysyl oxidase-mediated extracellular matrix remodeling during mouse decidualization. FEBS Lett 591:1394–1407
19. Mellor AL, Munn DH (1999) Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation? Immunol Today 20: 469–473
20. Nishioka T, Eustace A, West C (2012) Lysyl oxidase: from basic science to future cancer treatment. Cell Struct Funct 37:75–80
21. Opitz CA, Litzenburger UM, Sahm F, Ott M, Tritschler I, Trump S, Schumacher T, Jestaedt L, Schrenk D, Weller M, Jugold M, Guillemin GJ, Miller CL, Lutz C, Radlwimmer B, Lehmann I, von Deimling A, Wick W, Platten M (2011) An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. Nature 478:197–203
22. Palamakumbura AH, Vora SR, Nugent MA, Kirsch KH, Sonenshein GE, Trackman PC (2009) Lysyl oxidase propeptide inhibits prostate cancer cell growth by mechanisms that target FGF-2-cell binding and signaling. Oncogene 28:3390–3400
23. Rogers S, de Souza AR, Zago M, Iu M, Guerrina N, Gomez A, Matthews J, Baglole CJ (2017) Aryl hydrocarbon receptor (AhR)-dependent regulation of pulmonary miRNA by chronic cigarette smoke exposure. Sci Rep 7:40539
24. Schapers RF, Pauwels RP, Havenith MG, Smeets AW, van den Brandt PA, Bosman FT (1990) Prognostic significance of type IV collagen and laminin immunoreactivity in urothelial carcinomas of the bladder. Cancer 66:2583–2588
25. Sun X, Zhao S, Li H, Chang H, Huang Z, Ding Z, Dong L, Chen J, Zang Y, Zhang J (2016) MicroRNA-30b suppresses epithelial-mesenchymal transition and metastasis of hepatoma cells. J Cell Physiol 232:625–634
26. Tian SB, Yu JC, Liu YQ, Kang WM, Ma ZQ, Ye X, Yan C (2015) MiR-30b suppresses tumor migration and invasion by targeting EIF5A2 in gastric cancer. World J Gastroenterol 21:9337–9347
27. Wang TH, Hsia SM, Shieh TM (2016) Lysyl oxidase and the tumor microenvironment. Int J Mol Sci 18(1).
28. Watanabe Y, Fujiwara M, Yoshida R, Hayaishi O (1980) Stereospecificity of hepatic L-tryptophan 2,3-dioxygenase. Biochem J 189:393–405
29. Yoshikawa T, Hara T, Tsurumi H, Goto N, Hoshi M, Kitagawa J, Kanemura N, Kasahara S, Ito H, Takemura M, Saito K, Seishima M, Takami T, Moriwaki H (2010) Serum concentration of L-kynurenine predicts the clinical outcome of patients with diffuse large B-cell lymphoma treated with R-CHOP. Eur J Haematol 84: 304–309
30. Zhao H, Xu Z, Qin H, Gao Z, Gao L (2014) MiR-30b regulates migration and invasion of human colorectal cancer via SIX1. Biochem J 460:117–125
31. Zhao Y, Min C, Vora SR, Trackman PC, Sonenshein GE, Kirsch KH (2009) The lysyl oxidase propeptide attenuates fibronectin-mediated activation of focal adhesion kinase and p130Cas in breast cancer cells. J Biol Chem 284:1385–1393
32. Zhong K, Chen K, Han L, Li B (2014) MicroRNA-30b/c inhibits non-small cell lung cancer cell proliferation by targeting Rab18. BMC Cancer 14:703
33. Zhu Y, Yao S, Chen L (2011) Cell surface signaling molecules in the control of immune responses: a tide model. Immunity 34:466–478