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RAPID COMMUNICATION

Skp2 is a novel regulator of LSD1 expression and function in gastric cancer

S-phase kinase-associated protein 2 (Skp2) is a well-characterized oncoprotein localized mainly in the nucleus and cytoplasm. It is an integral component of SCF^{Skp2} E3 ubiquitin ligase complex which confers substrate selectivity to the ligase by specifically targeting a distinct set of proteins destined for proteasomal degradation such as p21, p27, cyclin E, and c-Myc.[1] Skp2 is crucial in a multitude of cellular processes including cell cycle, cell proliferation, apoptosis, differentiation, and survival. However, despite its immense and well-established role in ubiquitin-proteasome system-mediated protein turnover, much is unknown about the function of Skp2 independent of the ubiquitination pathway. Previously, Skp2 has been reported to regulate RhoA gene transcription and the p300 signaling pathway in an E3 ligase-independent manner.[2] Moreover, Skp2 also acts as a cofactor for c-Myc-regulated gene expression.[3,4]

Skp2 is a key oncoprotein in several human cancers mainly through the degradation of Cdk inhibitory proteins (CKIs) and activation of growth and survival pathways.[1] Consistently, we also found that Skp2 served as a positive regulator of gastric cancer cell growth and proliferation, clonogenic properties, and cell cycle progression in gastric cancer. It also promoted *in vivo* tumor growth. Tumor weight and tumor volume were decreased by almost 9-fold and 7-fold under Skp2 knockdown condition, respectively (Fig. S1). These effects are possibly due to Skp2-mediated direct activation of growth and survival pathways such as PI3K/Akt signaling pathways (Fig. S3A–C) or indirect regulation of lysine-specific demethylase (LSD1) expression and posttranslational stability (Fig. 1A–F; Fig. S3D, E, 4A). LSD1 might thus be necessary to coordinate Skp2 functions in gastric cancer through the regulation of Akt activation (Fig. S4B, E).

In addition to its effect on gastric cancer cell phenotypes, Skp2 also regulated LSD1 expression both at the gene and protein levels in gastric cancer (Fig. 1A–F; Fig. S3D, E). The level of LSD1 mRNA expression was significantly decreased by 2.45-fold and 1.73-fold upon Skp2 knockdown in MGC-803 and MKN-45 cell lines, respectively (Fig. 1D, E). Similarly, Skp2 knockout down-regulated LSD1 mRNA expression by 1.63-fold and 1.61-fold compared to the control groups in MGC-803 and MKN-45 cells, respectively (Fig. S3D, E). On the other hand, induction of Skp2 overexpression in MGC-803 cells up-regulated LSD1 mRNA expression by 2.86-fold (Fig. 1F). Taken together, the results suggested that Skp2 is involved in modulating LSD1 expression in gastric cancer. Moreover, Skp2 affected LSD1 demethylase activity on H3 core histone proteins at Lys 4 (H3K4me1 and H3K4me2), especially on mono-methylation of histone H3 proteins (Fig. 1G–I). This effect of Skp2 on LSD1 demethylase activity might have an impact on downstream LSD1 signaling. Furthermore, Skp2 regulated posttranslational LSD1 stability in gastric cancer. Skp2 depletion resulted in reduced LSD1 stability and its half-life was decreased by more than 1.5 h under Skp2 depleted states in MGC-803 cells compared to control groups (Fig. S4A).

Mechanistically, Skp2-mediated LSD1 regulation did not involve its E3 ligase activity. Skp2 and LSD1 are overexpressed in gastric cancer (http://ualcan.path.uab.edu/analysis.html) and correlated positively at the transcript level in STAD (http://gepia.cancer-pku.cn/) (Fig. S2A, B). Moreover, Skp2 has been reported to modulate the activity and stability of transcription factors such as Myc that regulate LSD1 gene expression.[2,3,5] In view of this, we hypothesized that Skp2 might be an indirect regulator of LSD1 expression and function in gastric cancer possibly through p300. Accordingly, the level of p300 mRNA expression was significantly decreased by 2.45-fold and 2.61-fold upon Skp2 knockdown in MGC-803 and MKN-45 cell lines compared to the corresponding controls, respectively (Fig. 1D, E). On the other hand, Skp2 overexpression up-regulated p300 mRNA expression by 1.95-fold in MGC-803 cells (Fig. 1F). Skp2-mediated regulation of LSD1 expression might be mediated through up-regulation of p300 acetyltransferase transcription co-activator in gastric cancer. Mechanistically, Skp2 can recruit p300

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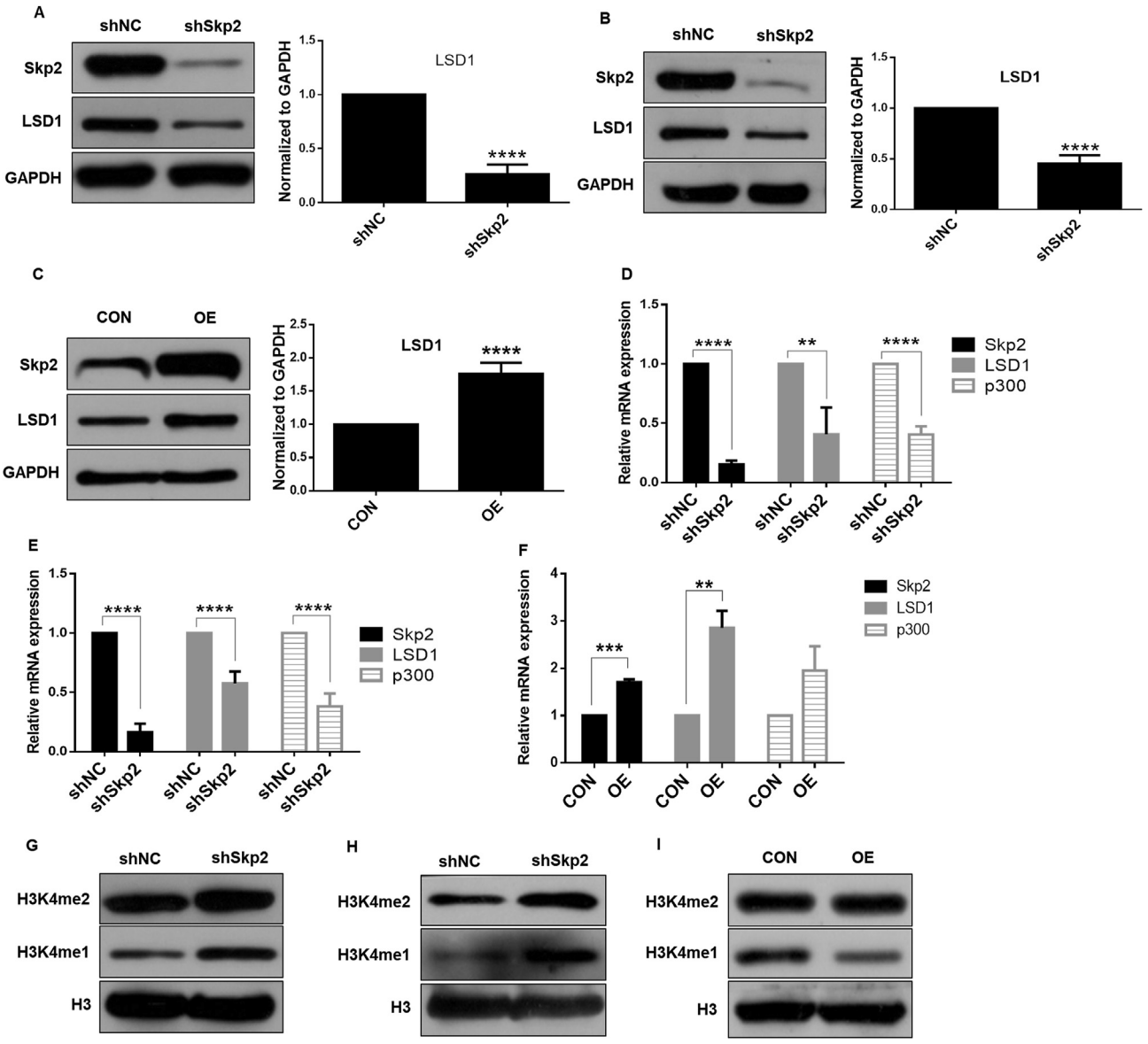

Figure 1 Skp2 regulates LSD1 expression and function in gastric cancer cells. **(A, B)** Western blot analysis of the effect of Skp2 on the expression of LSD1 under Skp2 knockdown conditions in MGC-803 and MKN-45 cell lines, respectively. **(C)** Western blot analysis of the effect of Skp2 on the expression of LSD1 under Skp2 overexpression conditions in MGC-803 cells. Skp2 regulates LSD1 and p300 mRNA expression in gastric cancer. **(D, E)** The relative mRNA expression levels of LSD1 and p300 in MGC-803 and MKN-45 cells, respectively. **(F)** The relative mRNA expression levels of LSD1 and p300 in MGC-803 cells transfected with pIRES2-EGFP-Skp2 (OE) or control empty vector (CON) were assessed by qPCR analysis. **(G, H)** Western blot analysis of the effect of Skp2 on histone protein methylation in Skp2 knockdown MGC-803 and MKN-45 cells. **(I)** Western blot analysis of the effect of Skp2 on histone protein methylation in Skp2 overexpressing MGC-803 cells. ($n = 3$; values are normalized to GAPDH; error bars represent mean ± standard deviation; ****$P < 0.0001$ ***$P < 0.001$ **$P < 0.01$ *$P < 0.05$). shNC, CON, and NC are the corresponding control groups.

acetyltransferase to Myc. Interestingly, c-Myc binds with Skp2 in the nucleus.[3] LSD1 is a downstream target gene of Myc which activates LSD1 gene expression by binding to two non-canonical E-boxes in the proximal promoter region of LSD1 gene. As a Myc co-activator, p300 might be involved in Myc-mediated regulation of LSD1 expression. Consistently, we found that the expression of both LSD1 and p300 mRNA was down-regulated upon Skp2 depletion (Fig. 1D, E) and up-regulated under Skp2 overexpression condition (Fig. 1F) in gastric cancer cell lines. Taken together, Skp2-mediated regulation of LSD1 gene expression might be through up-regulating and/or recruiting p300 to the Myc transcriptional complex which binds to the promoter region of LSD1 and promotes its expression. Skp2 also promoted LSD1 stability in gastric cancer (Fig. S4A).

In conclusion, we discovered a novel Skp2 function in gene regulation independent of its E3 ligase activity, and a new regulatory mechanism of LSD1 expression and activity in gastric cancer. Skp2 regulated LSD1 expression primarily at the gene level possibly through recruiting p300 to Myc,

which binds to the promoter region of LSD1 gene. Skp2 might also regulate posttranslational LSD1 stability. Moreover, Skp2 enhanced LSD1 catalytic activity on mono- and di-methylated H3 core histone proteins at Lys 4. Furthermore, Skp2 regulated LSD1-mediated cancer cell growth and proliferation or at least both oncoproteins might act in concert to regulate the activation of growth and survival pathways such as the PI3K/Akt pathways in gastric cancer. Our findings would shed light on the unforeseen aspects of Skp2 function in cancer and serve as one line of evidence supporting the notion that the role of Skp2 is not limited to the regulation of posttranslational protein stability and activity in cancer but is also involved in regulating gene expression. The discovery of the interaction between Skp2 and LSD1 proteins would be helpful to understand the molecular mechanism of carcinogenesis, and triggers further studies aimed at identifying novel cross-talk mechanisms between Skp2 and other oncogenic cancer signaling pathways that regulate gene expression epigenetically. Overall, owing to the crucial roles both Skp2 and LSD1 playing in cancer development, understanding their interaction at the molecular level would have significance in cancer therapy, especially for drug molecules that target Skp2 and LSD1 pathways.

Author contributions

Moges Dessale Asmamaw performed all the experiments and wrote the draft manuscript. Ying Liu, Xiao-Jing Shi, and Li-Rong Zhang conceived and designed the study. All authors critically reviewed the manuscript and approved the final version for publication.

Conflict of interests

There are no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.01.015.

References

1. Asmamaw MD, Liu Y, Zheng YC, et al. Skp2 in the ubiquitin-proteasome system: a comprehensive review. *Med Res Rev*. 2020;40(5):1920–1949.
2. Chan CH, Lee SW, Li CF, et al. Deciphering the transcriptional complex critical for RhoA gene expression and cancer metastasis. *Nat Cell Biol*. 2010;12(5):457–467.
3. von der Lehr N, Johansson S, Wu S, et al. The F-box protein Skp2 participates in c-Myc proteosomal degradation and acts as a cofactor for c-Myc-regulated transcription. *Mol Cell*. 2003;11(5):1189–1200.
4. Faiola F, Liu X, Lo S, et al. Dual regulation of c-Myc by p300 via acetylation-dependent control of Myc protein turnover and coactivation of Myc-induced transcription. *Mol Cell Biol*. 2005;25(23):10220–10234.
5. Nagasaka M, Tsuzuki K, Ozeki Y, et al. Lysine-specific demethylase 1 (LSD1/KDM1A) is a novel target gene of c-Myc. *Biol Pharm Bull*. 2019;42(3):481–488.

Moges Dessale Asmamaw [a], Li-Rong Zhang [a], Hong-Min Liu [b,c], Xiao-Jing Shi [d,*], Ying Liu [e,f,**]

[a] *Department of Pharmacology, School of Basic Medical Sciences, State Key Laboratory for Esophageal Cancer Prevention and Treatment, Zhengzhou University, Zhengzhou, Henan 450001, China*
[b] *School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou, Henan 450001, China*
[c] *Key Laboratory of Advanced Drug Preparation Technologies, Ministry of Education of China, Zhengzhou, Henan 450001, China*
[d] *Academy of Medical Sciences, Zhengzhou University, Zhengzhou, Henan 450052, China*
[e] *Department of Pharmacy, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China*
[f] *Henan Engineering Research Center for Application & Translation of Precision Clinical Pharmacy, Zhengzhou, Henan 450052, China*

*Corresponding author.

**Corresponding author. Department of Pharmacy, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China.
E-mail addresses: shixiaojing@zzu.edu.cn (X.-J. Shi), 991536786@qq.com (Y. Liu)

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