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Short communication

Prognostic significance of SOX2 for chemotherapeutic patients with oral squamous cell carcinoma

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Oral squamous cell carcinoma (OSCC) is the most common oral cancers worldwide, accounting for over 90% of all oral malignancies [1]. Despite encouraging improvements in therapeutic approaches, including surgical resection, chemotherapy, and radiotherapy, the five-year overall survival rate of OSCC has not been improved significantly over the past decades, mainly due to the high ratio of tumor recurrence and metastasis. OSCC recurrence is largely caused by the failure of chemotherapy [2]. Therefore, the pressing question remains on developing novel biomarkers for predicting the prognosis and guiding chemotherapeutic strategies for patients with OSCC. SOX2 is originally identified as a transcription factor that maintains stem cell pluripotency, known as one of the Yamanaka factors [3]. Following studies demonstrate that aberrant amplification of SOX2 is tightly associated with human cancers. In OSCC, we and others have established the expression pattern and functional roles of SOX2 in tumor progression and metabolic reprogramming [4]. Interestingly, SOX2 was reported to be negatively associated with the sensitivity of OSCC cells to radiation treatment [5]. Thus, we propose that SOX2 may have prognostic significance in chemotherapeutic OSCC patients.

To address this question, we initially enrolled a total of 194 patients. The OSCC samples were used for immunohistochemical analysis of SOX2 levels (Fig. S1A). Results demonstrated a significant correlation between high SOX2 expression and factors such as radiotherapy, chemotherapy, and tumor recurrence (Table S1). The Kaplan-Meier survival analysis further revealed that SOX2 expression was not statistically linked to the overall prognosis of OSCC patients ($n = 194$), although the high SOX2 group displayed a tendency of worse survival (Fig. S1B). Next, we asked whether SOX2 expression was related to the prognosis of chemotherapeutic patients of OSCC. We screened out patients survived over one year that completed chemotherapies ($n = 45$), compared with patients survived over one year without chemotherapies ($n = 61$). Results showed that high SOX2 expression indicated poor prognosis of in these patients (Fig. S1C). The tendency of worse survival in high SOX2 group were supported by the results in The Cancer Genome Atlas (TCGA)-OSCC cohort (Fig. S2).

The effects of SOX2 on the survival were estimated by the Cox proportional hazards regression model, showing significantly difference between high- and low-SOX2 expression subgroups (Table S2). Then the cohort was split into two groups, patients with high- and low- SOX2 expressions. Chemotherapy could not prolong the life-span of patients in high SOX2 expression group ($n = 42$), but was able to extensively improve patients' consequence in low SOX2 expression group ($n = 64$) (Fig. 1A). Moreover, the cohort was stratified into another two groups, patients with or without chemotherapies. In patients receiving chemotherapies ($n = 45$), high SOX2 expression was related to worse survival than the low SOX2 group, whereas in patients not receiving chemotherapies ($n = 61$), high SOX2 expression was not statistically connected to the poor outcomes (Fig. 1B).

Then, we wonder whether SOX2 functionally determines the chemo-sensitivity in OSCC. *In vitro* results showed that SOX2 protein level was high in Cal33 OSCC cells, and low in SCC47 cells (Figs. S3A–C). Afterwards, we applied 5-fluorouracil (5-FU) and cisplatin, the most extensively used chemotherapeutic agents to OSCC cells for cell viability assessment. In the 5-FU group, half-maximal inhibitory concentration (IC_{50}) values of Cal33, Cal27, and SCC47 were 8.62, 2.99, and 1.41 μM, respectively (Fig. S3D). In the cisplatin group, IC_{50} values of these OSCC cell lines 2.90 (Cal33), 2.76 (Cal27), and 1.07 μM (SCC47), respectively (Fig. S3E). To reveal the potential causality of SOX2 and chemo-sensitivity in OSCC cells, we designed to interfere SOX2 in Cal33 cells and examined whether the chemo-sensitivity was increased. As anticipated, SOX2 knockdown dramatically reduced the IC_{50} values of both 5-FU and cisplatin in Cal33 cells (Figs. 1C–E). Moreover, SOX2 overexpression was

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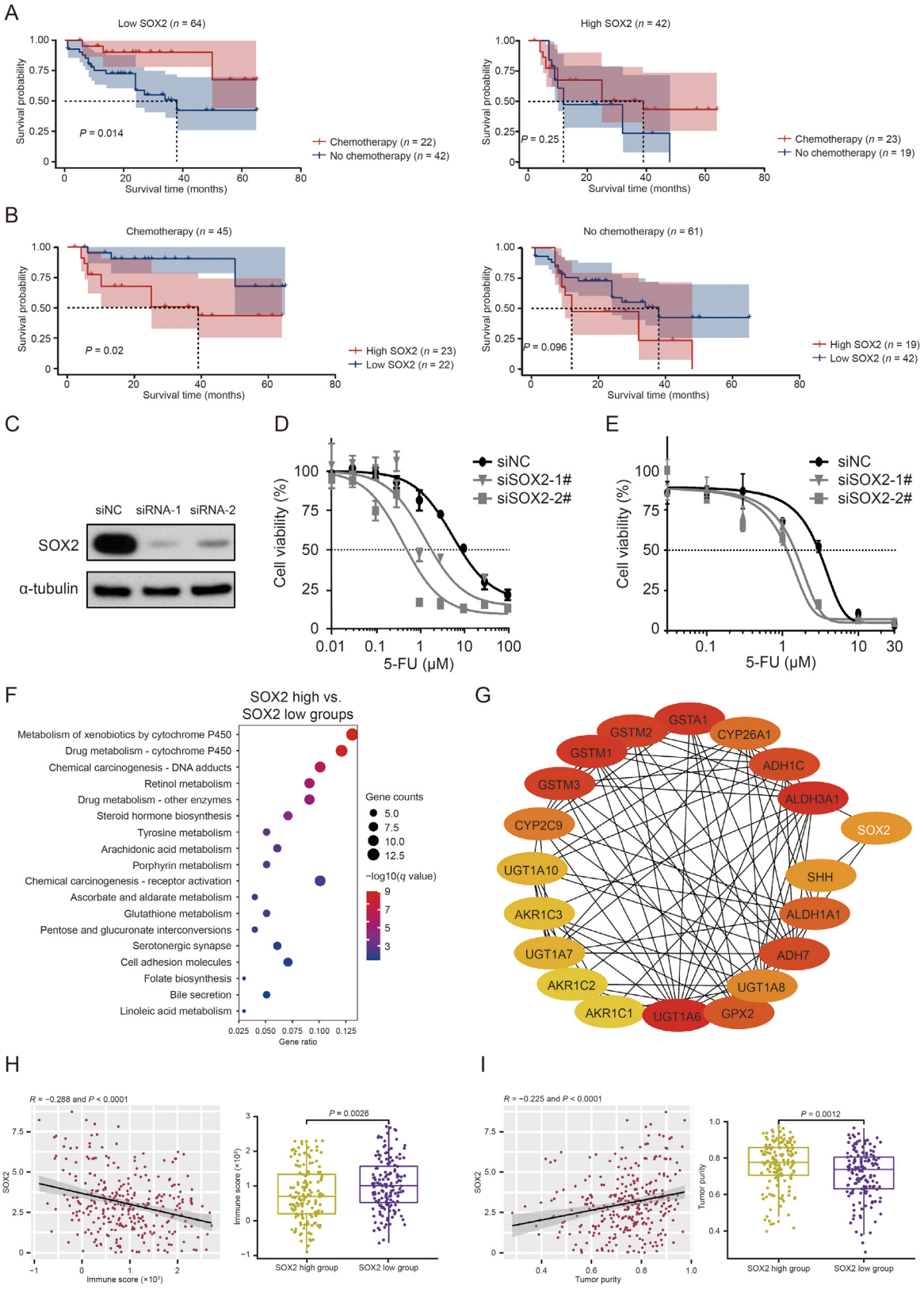

Fig. 1. SOX2 expression determines chemotherapeutic efficacy in oral squamous cell carcinoma (OSCC) patients. (A) Kaplan-Meier analysis showing the overall survival time in groups of different SOX2 expressions with or without chemotherapies. (B) Kaplan-Meier analysis showing the overall survival time in groups of OSCC patients receiving or not-receiving chemotherapies divided by different SOX2 expressions. (C) Western blots showing the reduction of SOX2 expression by small interfering RNA (siRNA) in Cal33 OSCC cells. (D, E) Diagrams showing the cell viability in Cal33 cells with SOX2-1# and 2# knockdown after exposure to different doses of 5-fluorouracil (5-FU) (D) and cisplatin (E). (F) Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showing the functional enrichment of the differentially expressed genes (DEGs) in SOX2 high vs. SOX2 low groups in The Cancer Genome Atlas (TCGA)-OSCC cohort. (G) A network diagram showing the protein-protein interaction (PPI) of the hub genes in the above DEGs. (H, I) Diagrams showing the comparison of immune score (H) and tumor purity (I) in SOX2 high vs. SOX2 low groups in TCGA-OSCC cohort. siNC: negative control siRNA.

sufficient to increase the cell viability of SCC47 cells after treatment with 5-FU (Fig. S4). Finally, we found that *in vivo* tumor growth was dramatically decreased by SOX2 knockdown, and subsequent 5-FU treatment enhanced the tumor suppression (Fig. S5).

To investigate potential mechanisms by which SOX2 regulates the sensitivity of chemo-drugs in OSCC, we divided the patients into high- and low-SOX2 expression groups in TCGA-OSCC cohort. It's noted that the genomic mutations of the two groups were not dramatically different (Fig. S6). Then, differentially expressed genes (DEGs) between the two groups were displayed by the volcano plot (Fig. S7A). The DEGs were found to be enriched in xenobiotic/drug metabolism pathways by both Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) biological process analysis (Figs. 1F and S7B). Then we examined the interactive network of SOX2, and found that multiple subunits of glutathione S-transferase (GSTs), uridine diphosphate (UDP) glucuronosyltransferase (UGTs), and aldehyde dehydrogenase (ALDHs) were interconnected with SOX2, which were reported in chemoresistance (Fig. 1G). Finally, the high SOX2 group had decreased immune scores and increased tumor purity, with abundant inactivated immune cells (e.g., Tregs) (Figs. 1H, 1I, and S8), suggesting that the high SOX2 group had an immunosuppressive tumor microenvironment. The materials and methods are shown in the Supplementary data.

In this study, we provided direct evidence identifying SOX2 as a novel independent prognostic factor for patients receiving chemotherapy for OSCC. We demonstrated that a high expression of SOX2 represents an independent negative prognostic factor for overall survival in patients undergoing chemotherapy for OSCC. Moreover, our work reveals that examining SOX2 expression may provide novel insights into therapeutic options for chemotherapies, to enhance chemotherapeutic efficacy as well as reduce side effects from unnecessary treatments. For example, chemotherapy may lead to exceptionally better prognoses in patients who had low rather than high SOX2 expression. In patients with high SOX2, chemotherapy alone does not have a promising efficacy; however, coupled inhibition of SOX2 may strengthen the chemotherapeutic efficacy. Our study has several limitations. First, its single-cohort nature restricted definitive conclusions regarding the predictive superiority of SOX2 in OSCC chemotherapeutic prognosis over other models. Second, potential selection bias may have arisen from samples sourced from specific clinical centers.

In conclusion, our findings reveal the prognostic value of SOX2 in the chemotherapeutic intervention of OSCC. Examination of tumor SOX2 levels could facilitate a more accurate prognostic analysis compared with conventional methods, providing adjunctive insights to design personalized treatment for patients with OSCC.

Ethics statement

This study was conducted in accordance with the Declaration of Helsinki. All human work was approved by Institutional Review Board approval of the West China Hospital of Stomatology (Approval No.: WCHSIRB-D-2020-040), which led the project involved two other hospitals including the General Hospital of People's Liberation Army (Beijing, China) and Guangdong Provincial Stomatological Hospital (Guangzhou, China). Written informed consent was obtained from all included patients. The animal work was done in accordance with the Animal Care and Use Committee guidelines of West China Hospital of Stomatology, Sichuan University (Chengdu, China) (Approval No.: WCHSIRB-D-2020-019).

CRediT author statement

Xuefeng Zhang: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Funding acquisition, Writing - Original draft preparation; **Hao Xu:** Software, Formal analysis, Investigation; **Tong Zhou:** Investigation, Data curation; **Xiaodong Feng** and **Qianming Chen:** Conceptualization, Funding acquisition, Writing - Reviewing and Editing.

Declaration of competing interest

The authors declare that there are no conflicts of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2023.11.015.

References

[1] A.C. Chi, T.A. Day, B.W. Neville, Oral cavity and oropharyngeal squamous cell carcinoma—an update, CA Cancer J. Clin. 65 (2015) 401–421.
[2] T.Y. Seiwert, J.K. Salama, E.E. Vokes, The chemoradiation paradigm in head and neck cancer, Nat. Clin. Pract. Oncol. 4 (2007) 156–171.
[3] K. Takahashi, K. Tanabe, M. Ohnuki, et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131 (2007) 861–872.
[4] X. Qiu, S. Jiang, Y. Xiao, et al., SOX2-dependent expression of dihydroorotate dehydrogenase regulates oral squamous cell carcinoma cell proliferation, Int. J. Oral. Sci. 13 (2021), 3.
[5] M.-Y. Chou, F.-W. Hu, C.-H. Yu, et al., Sox2 expression involvement in the oncogenicity and radiochemoresistance of oral cancer stem cells, Oral Oncol. 51 (2015) 31–39.