Commentary

Targeting the non-canonical AKT-FOXO3a axis: A potential therapeutic strategy for oral squamous cell carcinoma

Ying Liu

Institute for Translational Medicine, College of Medicine, Qingdao University, No. 38, Deng Zhou Road, Qingdao, 266021, China

A R T I C L E   I N F O

Article history:
Received 23 September 2019
Accepted 11 October 2019
Available online 21 October 2019

Oral squamous cell carcinoma (OSCC) is the most common form of head and neck cancer, which seriously affects the patient’s quality of life and brings huge financial burden to their families. Although some advances in the strategies of OSCC treatment have been made in recent years, it is still a major public health problem [1]. Currently, surgical resection, radiation, chemotherapy, and immunotherapy are the main treatment modalities for OSCC [2]. However, the side effects of these therapies are also severe. Therefore, continued in-depth research into the molecular mechanisms of OSCC progression is urgently required to provide novel therapeutic strategies for OSCC patients.

Cancer stem cells (CSCs) are a unique subset of cells in a tumor with “stem-like” properties, such as self-renewal ability, differentiation capacity and tumor initiation [3]. Growing evidence reveals that CSCs may be the main cause of therapeutic resistance, metastasis, and recurrence of cancer, including in OSCC [4,5]. Thus, it could be beneficial to investigate the regulation mechanisms of CSCs to develop efficient treatment strategies to improve the prognosis of OSCC patients.

Forkhead box O3a (FOXO3a) belongs to the FOXO transcription factor family and mediates a variety of crucial biological processes, such as proliferation, cell cycle, and carcinogenesis [6]. Its activity can be regulated by multiple types of post-translational modifications (PTMs), including phosphorylation. Phosphorylation mediated by a series of kinases (such as AKT) alters the translocation of FOXO3a in the nucleus and the cytoplasm, which influences the regulation of FOXO3a on its target genes [6]. FOXO3a is normally identified as a tumor suppressor due to its negative role in cancer progression. However, it plays an opposite role in the regulation of CSCs functions across different types of cancer. For instance, overexpression of FOXO3a significantly inhibits CSCs properties, leading to the elimination of CSCs in breast cancer [7], whereas FOXO3a plays an indispensable role in the CSCs maintenance of pancreatic ductal adenocarcinoma [8]. Currently, the effects of FOXO3a on CSC in OSCC have not been reported.

In an article in EBioMedicine [9], Li et al reported the regulation of FOXO3a on the stemness of OSCC. The team first analyzed the correlation between FOXO3a expression and the clinical-pathologic features of OSCC patients. The purpose of these studies was to determine whether FOXO3a expression was associated with the stemness of OSCC. Using 124 OSCC patient samples, Li and colleagues showed that FOXO3a expression was not related to patient age or gender, histological grade, or T or N stage, but it was negatively correlated with the expression of SOX2, which is one of the markers of CSCs in OSCC. To further confirm the correlation of FOXO3a expression with the stemness of OSCC, they examined the effect of FOXO3a on the expression of other stemness and differentiation markers. The data showed that FOXO3a expression was negatively correlated with stemness markers including SOX2, ABCG2, and CD44 and positively correlated with the differentiation marker IVL. These results demonstrate the negative correlation of FOXO3a expression with the stemness of OSCC. Next, the team attempted to detect the regulation of FOXO3a on the stem cell-like properties of OSCC cells. The authors showed that overexpression of FOXO3a inhibited the self-renewal capacity of OSCC cells. Moreover, knockdown of FOXO3a enhanced the chemoresistance of OSCC cells to cisplatin. In vivo, the FOXO3a knock-down mice showed a higher tumor formation rate with OSCC cells than the control mice. Together, these results confirm that FOXO3a negatively regulates the stemness of OSCC.

Transforming growth factor β (TGF-β) is a well-known cytokine involved in inducing the stemness of cancer cells, which mainly acts through the canonical Smad pathway [10]. The authors found that TGFβ could also enhance the stemness of OSCC cells. Therefore, they proposed that FOXO3a may mediate the stemness of OSCC induced by TGFβ. Li and colleagues detected the effect of TGFβ on FOXO3a functions. Results showed that TGFβ treatment...
resulted in phosphorylation, nuclear exclusion and degradation of FOXO3a. Phosphorylation by kinases is considered to be the main cause for translocation of FOXO3a [6]. The authors identified upstream kinases (AKT and ERK1/2) to confirm which one might cause the nuclear exclusion of FOXO3a. They found that AKT, rather than ERK1/2, was phosphorylated and activated by TGFβ. The authors further addressed the importance of the AKT-FOXO3a axis in TGFβ-induced stemness. They found that inhibition of AKT by its inhibitor MK2206 blocked the stemness of OSCC cells induced by TGFβ. These results demonstrated the crucial role of the non-canonical AKT-FOXO3a axis in TGFβ-induced stemness.

One of the main concerns is the pathological role of the non-canonical AKT-FOXO3a axis in OSCC. This must be detected in vivo. Moreover, there are other kinases apart from AKT and ERK1/2 (such as SGK, CK1 and IKK) that can induce the translocation of FOXO3a between the cytoplasm and the nucleus, and these kinases can also be activated by cross-talk of TGFβ. In the newly accepted article by Li et al. only AKT and ERK1/2 were detected as the upstream targets [9]. It will be interesting in the future to investigate the pathological role of the non-Smad axis using animal models or OSCC patient samples and to further confirm whether other kinases also regulate the translocation of FOXO3a.

In summary, the study by Li et al. has added an important layer to the understanding of the AKT-FOXO3a axis in regulating the stemness of CSCs induced by TGFβ in OSCC (Fig. 1). Their results not only elucidated the mechanism of FOXO3a in mediating TGFβ-induced stemness of OSCC but also provided a new direction for the effective treatment of OSCC. Although the translational process from basic research to clinical application is very long and the failure rate is also high, the exciting findings from this study suggest that targeting the non-canonical AKT-FOXO3a axis is a novel potential therapeutic strategy for treating OSCC patients.

**Declaration of Competing Interest**

None.

**References**

[1] Xia B, Hong T, He X, Hu X, Gao Y. A circular RNA derived from MMP9 facilitates oral squamous cell carcinoma metastasis through regulation of MMP9 mRNA stability. Cell Transplant 2019;963689719875409. doi:10.1177/0963689719875409.

[2] Lorenzo-Pouso AL, Pérez-Sayán M, Rodríguez-Zorrilla S, Chamorro-Petronacci C, García-García A. Dissecting the proton transport pathway in oral squamous cell carcinoma: state of the Art and theranostics implications. Int J Mol Sci 2019;20:4222. doi:10.3390/ijms20174222.

[3] Toh TB, Lim JJ, Chow EK. Epigenetics in cancer stem cells. Mol Cancer 2017;16:29. doi:10.1186/s12943-017-0596-9.

[4] Wu HJ, Chu PY. Role of cancer stem cells in cholangiocarcinoma and therapeutic implications. Int J Mol Sci 2019;20:4154. doi:10.3390/ijms20174154.

---

**Fig. 1.** The AKT-FOXO3a axis mediates the stemness of CSCs induced by TGFβ. The non-phosphorylated form of FOXO3a located in the nucleus regulates the expression of genes involved in the stemness of CSCs. TGFβ activates AKT, which phosphorylates FOXO3a, leading to the nuclear exclusion and degradation of FOXO3a.
[5] Prasetyanti PR, Medema JP. Intra-tumor heterogeneity from a cancer stem cell perspective. Mol Cancer 2017;16:41. doi:10.1186/s12943-017-0600-4.

[6] Liu Y, Ao X, Ding W, Ponnusamy M, Wu W, Hao X, et al. Critical role of FOXO3a in carcinogenesis. Mol Cancer 2018;17:104. doi:10.1186/s12943-018-0656-3.

[7] Liu H, Song Y, Qiu H, Liu Y, Luo K, Yi Y, et al. Downregulation of FOXO3a by DNMT1 promotes breast cancer stem cell properties and tumorigenesis. Cell Death Differ 2019. doi:10.1038/s44148-019-0389-3.

[8] Kumazoe M, Takai M, Hiroi S, Takeuchi C, Kadomatsu M, Nojiri T, et al. The FOXO3/PGC-1β signaling axis is essential for cancer stem cell properties of pancreatic ductal adenocarcinoma. J Biol Chem 2017:292:10813–23. doi:10.1074/jbc.M116.772111.

[9] Li K, Yang L, Li JY, Guan CY, Zhang SE, Lao XM, et al. TGFβ induces stemness through non-canonical AKT-FOXO3a axis in oral squamous cell carcinoma. EBioMedicine 2019. https://www.ebiomedicine.com/article/S2352-3964(19)30631-0/fulltext.

[10] Ajani JA, Song S, Hochster HS, Steinberg IB. Cancer stem cells: the promise and the potential. Semin Oncol 2015(42):S3–S17. doi:10.1053/j.seminoncol.2015.01.001.