Cancer Biology

PA28γ promotes the malignant progression of tumor by elevating mitochondrial function via C1QBP

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eLife Assessment

This work attempts to demonstrate an ATP-independent non-canonical role of proteasomal component PA28γ in the promotion of oral squamous cell carcinoma growth, migration, and invasion. The evidence remains **incomplete** and the work would benefit from further experimental work. The authors have not adequately addressed the reviewers' comments.

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Abstract

Proteasome activator 28γ (PA28γ) plays a critical role in malignant progression of various tumors, however, its role and regulation are not well understood. Here, using oral squamous cell carcinoma (OSCC) as main research model, we discovered that PA28γ interacted with complement 1q binding protein (C1QBP), which is dependent on the N-terminus of C1QBP rather than the known functional domain (amino acids 168-213). Notably, we found that PA28γ can enhance C1QBP protein stability in OSCC. Functionally, PA28γ contributes to the malignant progression of OSCC by affecting mitochondrial morphology and oxidative phosphorylation (OXPHOS) through C1QBP in vitro and vivo. Mechanically, PA28γ upregulate the expression of optic atrophy 1 (OPA1), mitofusin 2 (MFN1), mitofusin 2 (MFN2) and the mitochondrial respiratory complex by C1QBP. Moreover, in a clinical cohort of OSCC patients, PA28γ was positively correlated with C1QBP expression and negatively correlated with prognosis. Therefore, C1QBP is also a potential target for the treatment and prognosis of cancer.

Introduction

Proteasome activator 28γ (PA28γ), also known as REGγ or PSME3, is a well-known non-ATP-dependent proteasome regulator. PA28γ is overexpressed in some malignant tumors, such as thyroid, colon and breast cancer and is significantly associated with poor prognosis (Mao, Liu, Li, & Luo, 2008; Stadtmueller & Hill, 2011). By regulating the stability of key components within various complex signaling pathways, PA28γ impacts the biological behavior of tumor cells. Our previous studies revealed that the expression level of PA28γ in oral squamous cell carcinoma (OSCC) cancer nest tissues is positively correlated with patient prognosis (J. Li et al., 2015; S. Liu et al., 2018). However, its role and regulation are not well understood.

OSCC is the most common oral and maxillofacial malignant tumor(Siegel, Miller, Wagle, & Jemal, 2023; Sung et al., 2021). The pathogenic factors of OSCC include smoking, drinking and viral infection, such as human papilloma virus (HPV). Therapies for OSCC include surgery, radiotherapy, chemotherapy and immunotherapy. Despite the rapid development of imaging, surgery, radiotherapy and immunotherapy in recent years, along with the emphasis on personalized treatment and multidisciplinary cooperation in the treatment of OSCC, the overall survival rate of OSCC patients has not significantly improved (Peres et al., 2019). The main reasons for unsatisfactory treatment efficacy include invasion of surrounding tissue, early lymph node metastasis and a tendency toward recurrence (Lindemann, Takahashi, Patel, Osman, & Myers, 2018). Metabolic reprogramming is a crucial characteristic of tumors, closely associated with malignant behaviors such as tumor growth, invasion, metastasis, and immune escape (Ohshima & Morii, 2021). Current research on metabolic reprogramming in OSCC primarily focused on mechanism of glycolytic metabolism and metabolic shift from glycolysis to oxidative phosphorylation (OXPHOS) of oral squamous cell carcinoma, which lays the groundwork for novel therapeutic interventions to counteract OSCC (Chen et al., 2024; Zhang et al., 2020). Understanding the molecular events that lead to the occurrence and development of OSCC can aid in developing new tumor-targeted therapies, which hold significant clinical application potential.

The complement 1q binding protein (C1QBP) is a crucial protein for maintaining mitochondrial function, particularly in mitochondrial OXPHOS (Ghebrehiwet, Geisbrecht, Xu, Savitt, & Peerschke, 2019; Q. Wang et al., 2022). OXPHOS is essential for the production of adenosine triphosphate (ATP), which is necessary for tumor development. C1QBP has been shown to play a significant role in cancer progression, as influencing tumor growth, invasion and metastasis (Bai et al., 2019; Hou, Lu, Wang, & Yang, 2022; Vendramin et al., 2018). Due to these roles, C1QBP presents a promising therapeutic target for various tumors, including melanoma, breast cancer, and colorectal cancer (Matsumoto & Bay, 2021). In OSCC, enhanced mitochondrial OXPHOS function might also be linked to malignant tumor progression (Vyas et al., 2021; Xiao et al., 2021; Zhu, Liu, Wu, & Wang, 2021), although the underlying mechanism remain unclear.

In this study, we discovered that PA28γ interacts with C1QBP and that PA28γ can stabilize C1QBP. This interaction enhances OXPHOS and promotes the growth, migration and invasion of OSCC cells. Additionally, the expression of PA28γ and C1QBP is increased and positively correlated in OSCC. Therefore, PA28γ and C1QBP are potential targets for the treatment and prognosis of cancer. It is the first study to describe the undiscovered role of PA28γ in promoting the malignant progression of OSCC by elevating mitochondrial function, providing new clinical insights for the treatment of OSCC.

Results

PA28γ interacts with and stabilizes C1QBP

To explore how PA28γ promotes OSCC progression, we conducted a gene[gene interaction analysis using the GeneMANIA database. This analysis identified C1QBP as a significant gene within the interaction network, with the protein-coding gene of PA28γ also playing a key role in the C1QBP interaction network (**Fig. 1A** and Appendix Fig. 1A). Subsequently, pull-down and coimmunoprecipitation analysis demonstrated that both endogenous and exogenous PA28γ and C1QBP could bind to each other (**Fig. 1B, C** and Appendix Fig. 1 B, C). Then, we developed four OSCC cell lines with stable overexpression of PA28γ using a Flag-PA28γ-expressing lentivirus and observed that the protein level of C1QBP was higher in these cell lines compared to control cells (**Fig. 1D**). Notably, PA28γ upregulated C1QBP protein levels in a dose-dependent manner (**Fig. 1E**). To determine whether the upregulated C1QBP was due to increased stability, cells were pretreated with cycloheximide (CHX) to inhibit protein synthesis. This treatment minimally affected C1QBP degradation in the presence of PA28γ (**Fig. 1F, G**). Furthermore, pull-down analysis and structure prediction of biomolecular interactions with AlphaFold 3 revealed that the PA28γ-C1QBP interaction depends on the N-terminus of C1QBP, rather than the known functional domain (amino acids 168-213) that bind to mitochondrial antiviral proteins (Peerschke et al., 2020; Xu, Xiao, Liu, Ren, & Gu, 2009) (**Fig. 1H, I** and Appendix Fig. 1D-H). Therefore, our results illustrated that PA28γ could interacts with C1QBP, in a manner dependent on the N-terminus of C1QBP, and that PA28γ could stabilizes C1QBP.

PA28γ and C1QBP colocalize in mitochondria and affect mitochondrial functions

To further investigate the physical interaction between PA28γ and C1QBP, we conducted an immunofluorescence (IF) assay in two OSCC cell lines, as shown these two proteins were colocalized in the mitochondria (**Fig. 2A**). Remarkably, transmission electron microscopy (TEM) images revealed fewer mitochondrial vacuoles and higher mitochondrial ridge density in PA28γ-overexpressing cells compared to control cells (**Fig. 2B** and Appendix Fig. 2A). IF analysis also showed increased mitochondrial lengths and areas in the PA28γ-overexpressing cells (**Fig. 2C, D**). Following that, we conducted a Cell Mito Stress Test to measure the oxygen consumption rate (OCR) (**Fig. 2E, F**). The results indicated significantly higher basal respiration, maximal OCRs and ATP production in PA28γ-overexpressing cells compared to control cells (**Fig. 2G-I** and Appendix Fig. 2B-D). Considering that mitochondria are the primary site for ROS generation and that ROS play a crucial role in carcinogenesis, we measured ROS levels in OSCC cells. Consistently, ROS levels were significantly higher in PA28γ-overexpressing cells (**Fig. 2J** and Appendix Fig. 2E). In addition, experiments conducted in PA28γ-sh OSCC cells yielded opposite results, further confirming these conclusions (Appendix Fig. 2F-K).

To demonstrate the carcinogenic abilities of PA28γ and C1QBP, we injected 4MOSC2 cells, a mouse OSCC line, with or without Pa28γ overexpression under the surface of the tongue in C57BL/6 mice (Appendix Fig. 3A). After 10 days of normal feeding, the tumors in the Pa28γ-overexpressing groups were significantly larger than those in the control group. Furthermore, via immunohistochemical (IHC) staining revealed that that PA28γ overexpression upregulate C1QBP in vivo (**Fig. 3A-C**). Consistent with the in vitro experiments, ATP production and ROS generation were significantly increased (**Fig. 3D, E**). Additionally, analysis of OSCC xenograft tumor tissue in nude mice revealed that PA28γ's regulation of C1QBP in OSCC cells is independent of the immune system (Appendix Fig. 3B, C). In addition, we established stable Pa28γ-silenced B16 cells, a mouse melanoma cell line, and found that the level of C1qbp protein was decreased in

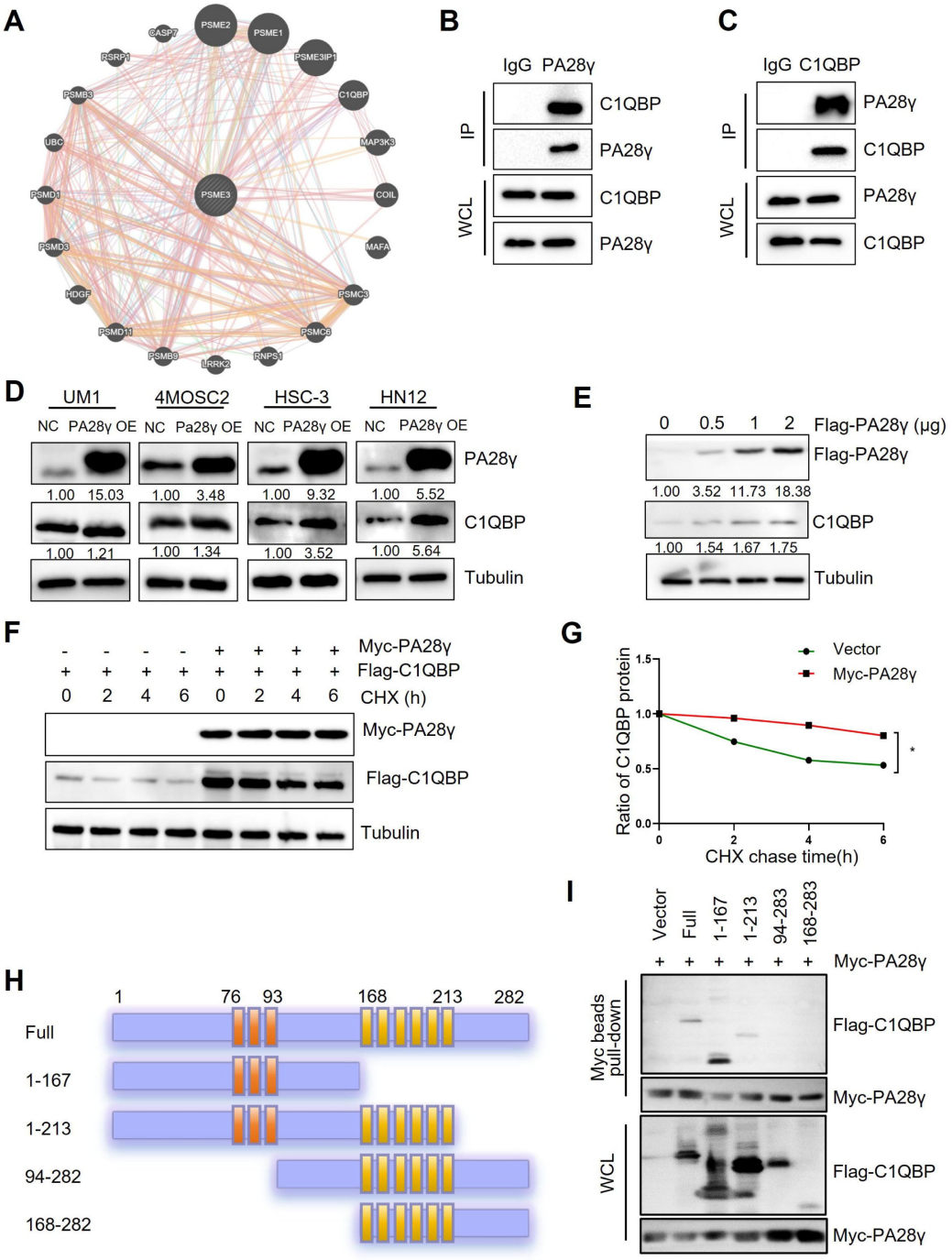

Fig. 1.

The interaction between PA28γ and C1QBP.

(A) The gene network of PA28γ and C1QBP in the GeneMANIA database. **(B, C)** The interaction between endogenous PA28γ and C1QBP in HSC-3 cells was verified via immunoprecipitation. **(D)** Western blot analysis of C1QBP in four OSCC cell lines with PA28γ-overexpressing. **(E)** Western blot analysis of C1QBP in 293T cells transfected with increasing doses of Flag-PA28γ. **(F)** 293T cells transfected with Flag-C1QBP with or without Myc-PA28γ were treated with CHX (100 μg/ml) for the indicated periods of time. **(G)** Quantification of Flag-C1QBP levels relative to tubulin levels is shown (the data are representative of 1 experiment with 3 independent biological replicates, **P*<0.05). **(H)** Full-length C1QBP and truncation with deletion of functional domains. **(I)** Pull-down of 293T cells transfected with Myc-PA28γ and full-length Flag-C1QBP or truncation mutants of functional domains for 36h.

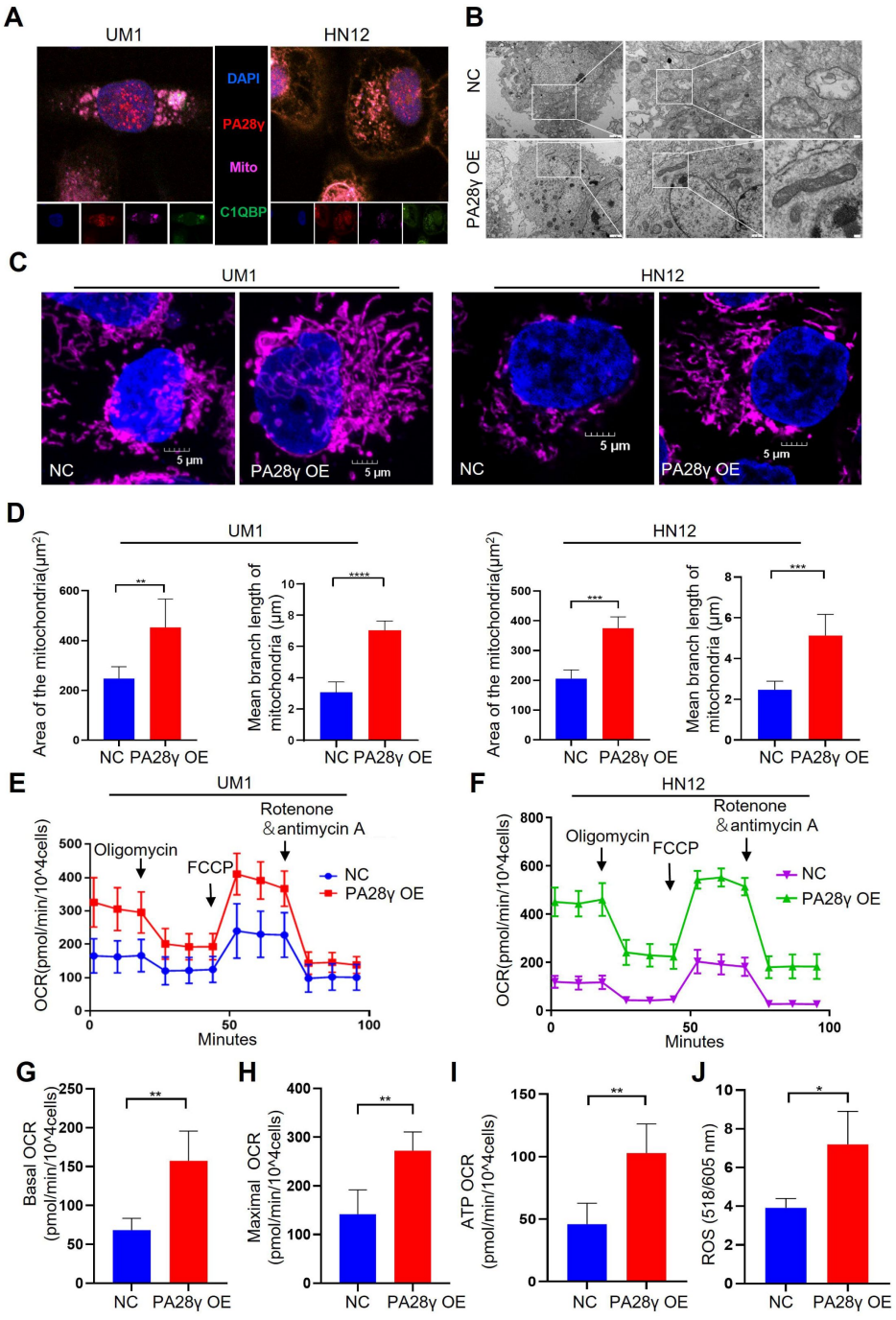

Fig. 2.

PA28γ and C1QBP colocalize in mitochondria and affect mitochondrial functions *in vitro*.

(A) Confocal image of IF in two OSCC cell lines. **(B)** TEM images of PA28γ-overexpressing and control UM1 cells. **(C)** Representative confocal images of mitochondria in two OSCC cell lines. **(D)** The area and mean branch length of mitochondria in two OSCC cell lines were measured by ImageJ (the data are presented as the mean ± SD of 3 independent experiments; **P<0.01, ***P<0.001, and ****P<0.0001). **(E, F)** OCRs of PA28γ-overexpressing and control UM1 and HN12 cells were plotted using a Cell Mito Stress Test Kit (the data are presented as the means ± SDs of 3 independent experiments). **(G-I)** Basal OCRs, maximal OCRs and ATP production of PA28γ-overexpressing and control UM1 cells measured by the Cell Mito Stress Test (the data are presented as the means ± SDs of 3 independent experiments, **P<0.01). **(J)** ROS generation in PA28γ-overexpressing and control UM1 cells (the data are presented as the means ± SDs of 3 independent experiments; * P<0.05).

Pa28γ-silenced B16 cells (Appendix Fig. 3D). Cells with or without Pa28γ silencing were grafted into the flanks of Pa28γ $^{ko/ko}$ and Pa28γ $^{wt/wt}$ C57BL/6 mice by subcutaneous injection (Appendix Fig. 3E). Remarkably, the tumors derived from the silenced Pa28γ cells in Pa28γ $^{ko/ko}$ mice were smaller than those in the other groups (**Fig. 3F-H**). The trend of ATP and ROS was consistent with tumor size (**Fig. 3I, J**), and the protein levels of C1qbp in the tumors were also reduced in the Pa28γ-silenced groups (**Fig. 3K**).

Collectively, these data suggest that PA28γ, which co-localizes with C1QBP in mitochondria, may involve in regulating mitochondrial morphology and function.

PA28γ regulates mitochondrial OXPHOS by upregulating C1QBP

Next, we explored the protein levels of the mitochondrial respiratory chain complex, C1QBP and other mitochondrial functional proteins. We found that the levels of C1QBP, complex I and IV proteins, as well as OPA1, MFN1 and MFN2 proteins, were upregulated in PA28γ-overexpressing OSCC cells (**Fig. 4A, B** and Appendix Fig. 4A, B), These increases could be reversed by C1QBP silencing in PA28γ-overexpressing OSCC cells (**Fig. 4C**).

Consistent with this, the OCRs of C1QBP-silenced PA28γ-overexpressing OSCC cells were markedly lower than those of control cells (**Fig. 4D** and Appendix Fig. 4C-E). Subsequently, we constructed a series of experiments to detect the biological behavior of control and PA28γ-overexpressing OSCC cells with or without C1QBP silencing. C1QBP knockdown significantly attenuated the migration, invasive and proliferation capabilities previously augmented by PA28γ overexpression (**Fig. 4E-H**). These data suggest that PA28γ enhances mitochondrial OXPHOS function through C1QBP.

C1QBP expression was positively associated with PA28γ expression and was associated with a worse prognosis in patients with OSCC or SKCM

To determine the correlation between PA28γ and C1QBP, IHC staining was performed in the clinical cohort of oral mucosa carcinogenesis. The results showed that both C1QBP and PA28γ were notably upregulated during the progression of oral mucosa carcinogenesis (**Fig. 5A, B** and Appendix Fig. 5A), and the levels of PA28γ and C1QBP were positively related (**Fig. 5C**). Similarly, both C1QBP and PA28γ were upregulated in metastatic OSCC tissues (**Fig. 5D, E** and Appendix Fig. 5B), and the levels of PA28γ and C1QBP were consistently positively correlated in OSCC cohort (**Fig. 5F**). Notably, consistent with our previous findings on PA28γ (J. Li et al., 2015), C1QBP or combining PA28γ and C1QBP could be a negative predator in our multicenter OSCC clinical cohort (**Fig. 5G, H**), TCGA HNSC database (**Fig. 5I, J**) and TCGA SKCM database (Appendix Fig. 5C, D).

Discussion

Metabolic reprogramming is one of the hallmarks of malignant tumors and is related to the malignant biological behavior of tumors (Faubert, Solmonson, & DeBerardinis, 2020; Hanahan, 2022; Tsai et al., 2023; Xia et al., 2021). The metabolic phenotype of tumor cells not only differs from that of normal cells but also dynamically changes during tumor progression. OSCC is a common head and neck malignancy that is still a growing global health problem (Johnson et al., 2020). The development of OSCC is a complex and multifaceted process in which metabolic reprogramming appears to be important (B. Liu, Si, Wei, Zhang, & Chen, 2023). However, the mechanism of metabolic reprogramming remains unclear. Excitingly, we found the evidence that PA28γ interacts with and stabilizes C1QBP. We speculate that aberrantly accumulated C1QBP enhances the function of mitochondrial OXPHOS and leads to the production of additional ATP

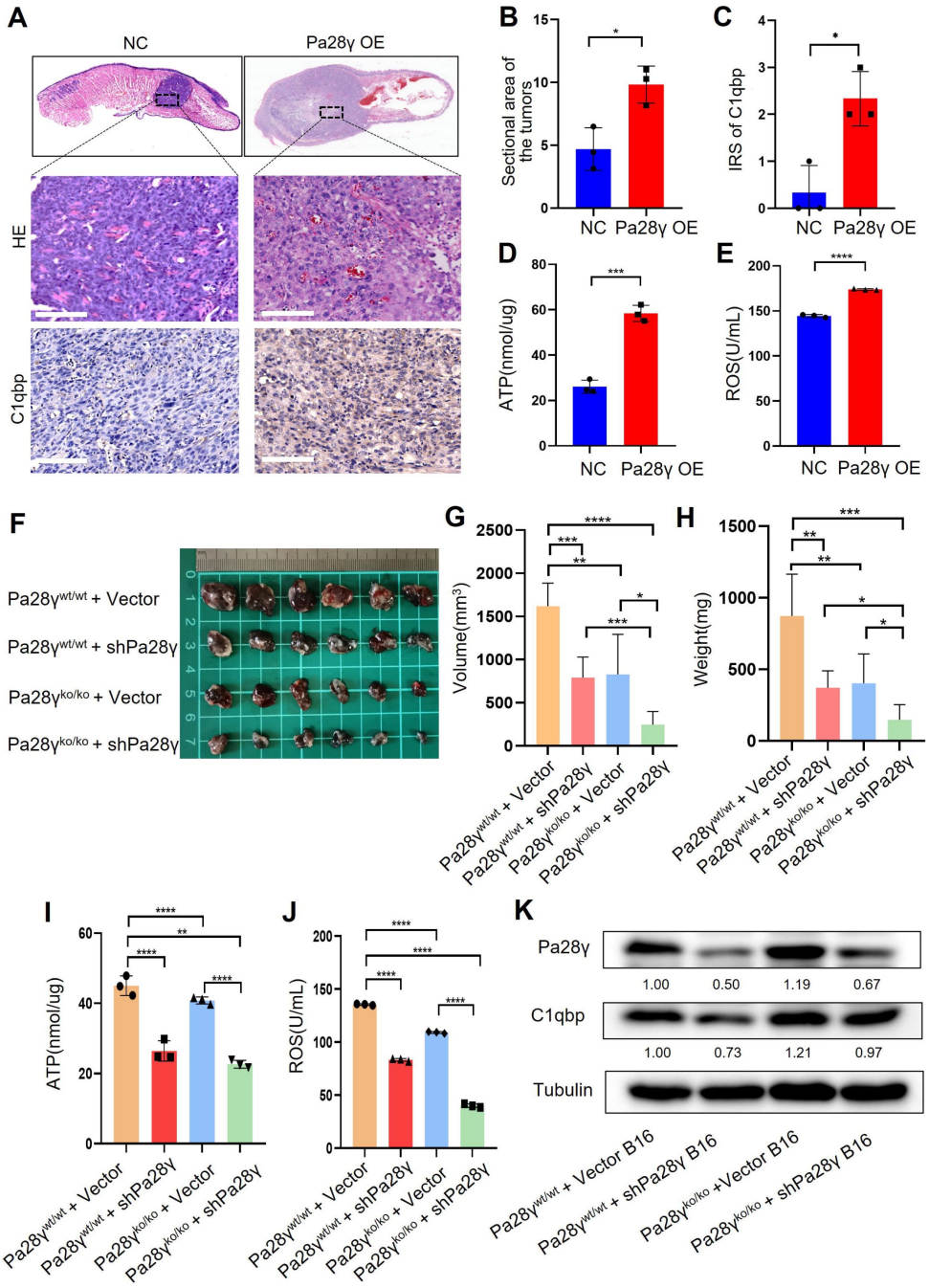

Fig. 3.

PA28γ affect mitochondrial functions *in vivo*.

(A) Representative images of H&E staining and IHC staining of C1qbp of tongue sections from mice (n=3). **(B)** Quantification of the sectional area in tumors from the Pa28γ-overexpressing and control groups (n=3; the data are presented as the means ± SDs of 3 samples; *P<0.05). **(C)** Comparison of the immunoreactive scores (IRSs) of C1qbp antibody staining in the Pa28γ-overexpressing and control groups (n=3, the data are presented as the means ± SDs; *P<0.05). **(D, E)** Quantification of ATP production and ROS levels in tumors from the Pa28γ-overexpressing and control groups (n=3; the data are presented as the means ± SDs of 3 samples; *P<0.05, ***P<0.001, ****P<0.0001). **(F)** Images of the tumors in different groups at the endpoint (n=6). **(G-J)** The volume, weight, ATP and ROS of tumors in different groups at the endpoint (n=6; the data are presented as the means ± SDs of 3 independent experiments; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001). **(K)** Western blot analysis of tumors in different groups.

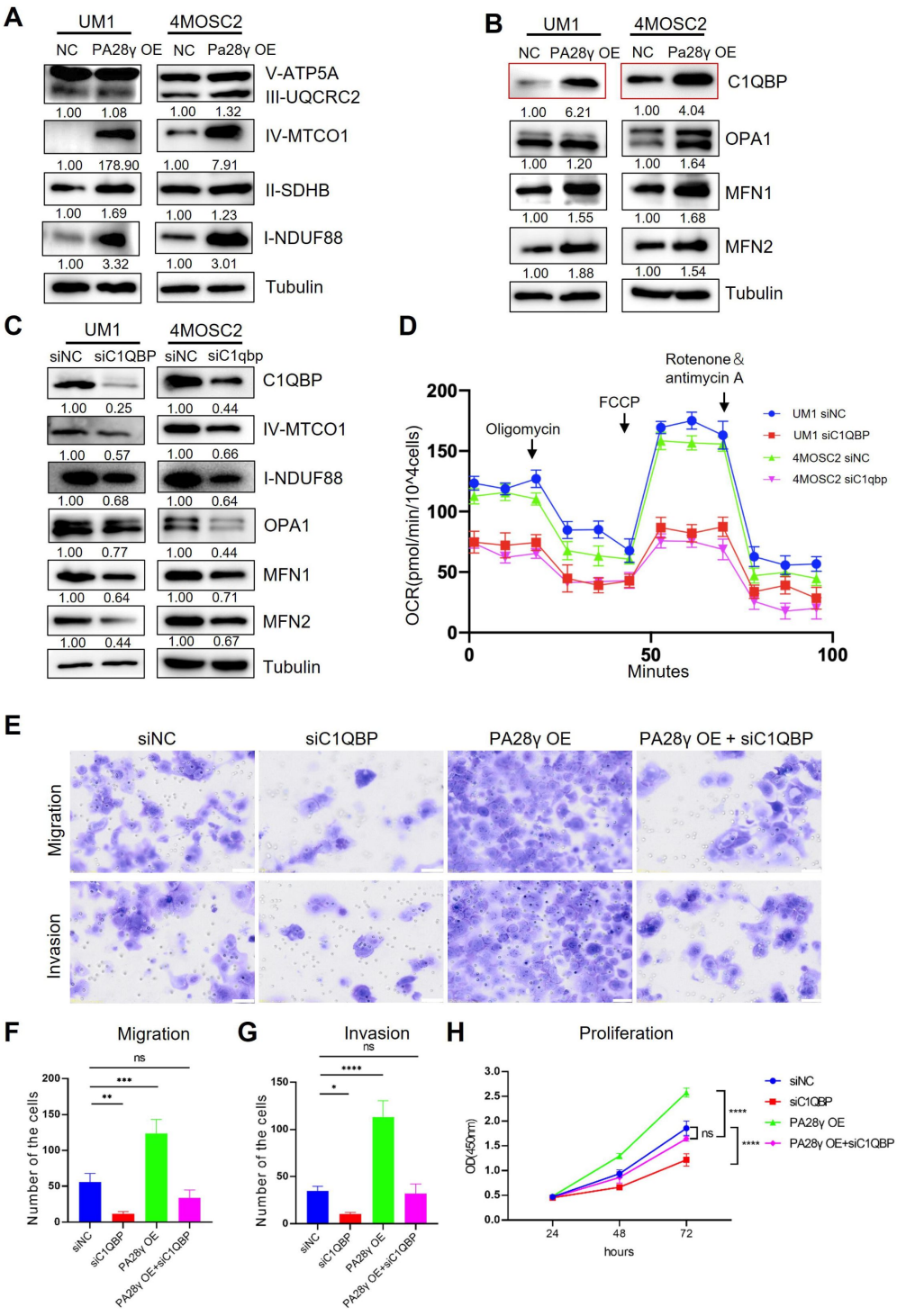

Fig. 4.

PA28γ regulates mitochondrial OXPHOS and cellular biological behavior via C1QBP.

(A, B) Western blot analysis of PA28γ-overexpressing and control UM1 and 4MOSC2 cells. **(C)** Western blot analysis of PA28γ-overexpressing OSCC cells transfected with siNC or siC1QBP. **(D)** OCRs of C1QBP-silenced and control PA28γ-overexpressing UM1 and 4MOSC2 cells (the data are presented as the means ± SDs of 3 independent experiments). **(E-H)** Cell migration, invasion and proliferation in control, C1QBP-silenced, PA28γ-overexpressing and PA28γ-overexpressing + C1QBP-silenced UM1 cells (the data are presented as the means ± SDs of 3 independent experiments; **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001).

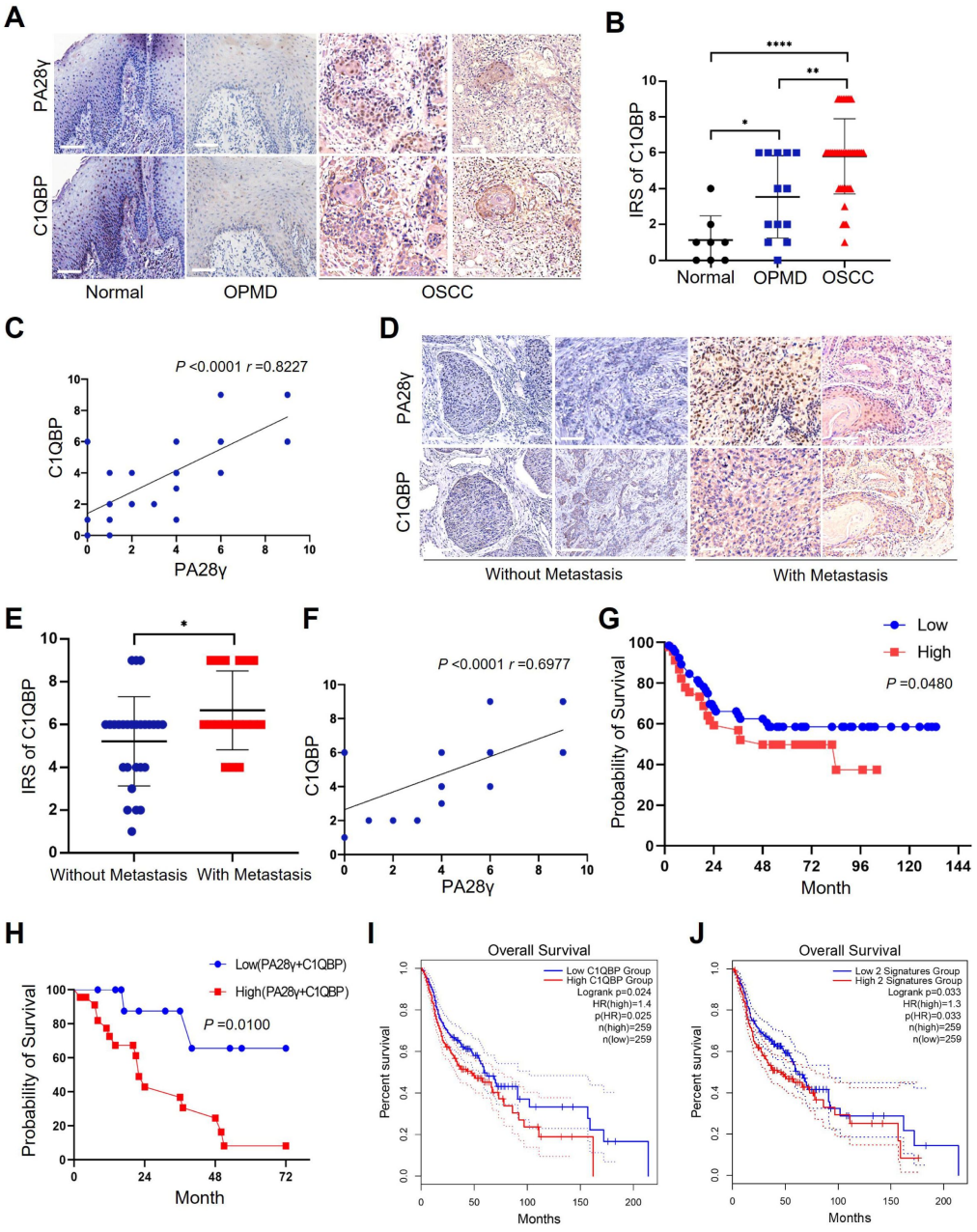

Fig. 5.

The correlation between PA28γ and C1QBP in the carcinogenesis and development of OSCC.

(A) Representative IHC staining of PA28γ and C1QBP in normal (n=8), OPMD (n=13) and OSCC (n=45) samples. **(B)** Comparison of the immunoreactive scores (IRSs) of C1QBP between the normal, OPMD and OSCC groups (the data are presented as the means ± SDs; **P*<0.05, ***P*<0.01, *****P*<0.0001). **(C)** Spearman correlation analysis was used to test the correlation between PA28γ and C1QBP in normal, OPMD and OSCC tissues (*P*<0.0001, *r*=0.8227). **(D)** Representative IHC staining of PA28γ and C1QBP in nonmetastatic (n=27) and metastatic (n=18) OSCC patients. **(E)** Comparison of the IRSs of C1QBP in the nonmetastatic and metastatic OSCC groups (the data are presented as the means ± SDs; **P*<0.05). **(F)** Spearman correlation analysis was used to test the correlation between PA28γ and C1QBP in OSCC tissues (*P*<0.0001, *r*=0.6977). **(G)** Kaplan–Meier analysis of the protein expression of C1QBP in our multicenter OSCC clinical cohort (n=295, *P*=0.0480). **(H)** Kaplan–Meier analysis of both low or high protein expression of C1QBP and PA28γ in our multicenter OSCC clinical cohort (n=295, *P*=0.0100). **(I)** Kaplan–Meier analysis of the protein expression of C1QBP in TCGA HNSC database (n=259, *P*=0.025). **(J)** Kaplan–Meier analysis of both low or high protein expression of C1QBP and PA28γ in TCGA HNSC database (n=259, *P*=0.033).

and ROS by activating the expression and function of OPA1, MNF1, MFN2 and mitochondrial respiratory chain complex proteins. This process results in resulting in mitochondrial fusion and malignant tumor progression (**Fig. 6**). Our study highlights the underlying mechanism by which PA28γ participates in the regulation of OXPHOS by upregulating C1QBP in OSCC.

C1QBP is also called gClqR, p32, p33 and HABP1, can be distributed inside cells, on the cell surface, and can also be secreted extracellularly (Egusquiza-Alvarez & Robles-Flores, 2022). Within cells, C1QBP is primarily located in the mitochondria. The key functions of mitochondrial C1QBP include maintaining OXPHOS, supplying energy to cells, and achieving homeostasis of mitochondrial metabolism (Ghebrehiwet et al., 2019; Q. Wang et al., 2022). C1QBP is highly expressed in cells that require a large amount of energy, such as the heart, skeletal muscle, testis, ovary, small intestine and colon (Feichtinger et al., 2017). Sufficient energy is essential for cancer development, and C1QBP promotes malignant behaviors such as tumor invasion and metastasis by enhancing OXPHOS function in various cancers (Raschdorf et al., 2021; Q. Wang et al., 2022). However, there are no reports on the expression and functions of C1QBP in OSCC. In our study, we found the evidence that PA28γ interacts with and stabilizes C1QBP. As a proteasome activator, PA28γ usually affects the development of tumors by regulating the stability of various important proteins. For example, PA28γ can promote the degradation of p53, p21 leading to cancer progression (J. Liu et al., 2010). In addition, PA28γ can also play as a non-degradome role on tumor angiogenesis. For example, PA28γ can regulate the activation of NF-κB to promote the secretion of IL-6 and CCL2 in OSCC cells, thus promoting the angiogenesis of endothelial cells (S. Liu et al., 2018). However, the function of mitochondrial PA28γ may be differ from that of nuclear PA28γ (Mao et al., 2008). Our study reveals that PA28γ interacts with C1QBP and stabilizes C1QBP at the protein level. Therefore, we speculate that the binding sites of PA28γ and C1QBP may mask the specific post-translational modification sites of C1QBP and inhibit its degradation. For example, the transcriptional coactivator p300 interacts with Smad7 by acetylating two lysine residues in its N-terminus, which stabilize Smad7 and protect it from TGFβ-induced degradation (Grönroos, Hellman, Heldin, & Ericsson, 2002). Overall, the specific mechanism involved in the interaction between PA28γ and C1QBP still needs further exploration.

Considering that C1QBP is a vital protein for maintaining mitochondrial metabolism (Tian et al., 2023), we further investigated the function underlying the malignant progression of OSCC through the interaction between PA28γ and C1QBP in vitro and in vivo. PA28γ and C1QBP colocalized in the mitochondria, and the stabilization of C1QBP by PA28γ enhanced mitochondrial morphology and OXPHOS. This enhancement is crucial for ATP production, and ATP is the primary energy currency of cellular metabolism. Therefore, PA28γ's regulation of OXPHOS may impact cellular energy metabolism. PA28γ has been reported to activate the mTORC1 signaling pathway in hepatocellular carcinoma cells to promote glycolysis and inhibit OXPHOS (Yao et al., 2021). These findings contrast with our study, potentially due to organ heterogeneity or the complexity of metabolic reprogramming.

In the tissue microenvironment of oral lichen planus, a potentially malignant oral disorder, PA28γ in epithelial cells can regulate T cell differentiation (Y. Wang et al., 2024), while PA28γ in CAFs is involved in the crosstalk between stromal cells and tumor cells (Z. Li et al., 2024). This suggests that PA28γ may interact with the tumor immune microenvironment. Our phenotypically similar OSCC xenograft tumor models in both immunocompetent and nude mice indicate that the regulation of mitochondrial OXPHOS by PA28γ localized in tumor cell mitochondria does not entirely depend on the immune system (Z. Wang et al., 2019).

Furthermore, our study reveals that PA28γ can regulate C1QBP and influence mitochondrial morphology and function by enhancing the expression of OPA1, MFN1, MFN2 and the mitochondrial respiratory complex. Mitochondrial fusion, crucial for oxidative metabolism and cell proliferation, is regulated by MFN1, MFN2, and OPA1. The first two fuse with the outer mitochondrial membrane, while the last fuses with the inner mitochondrial membrane

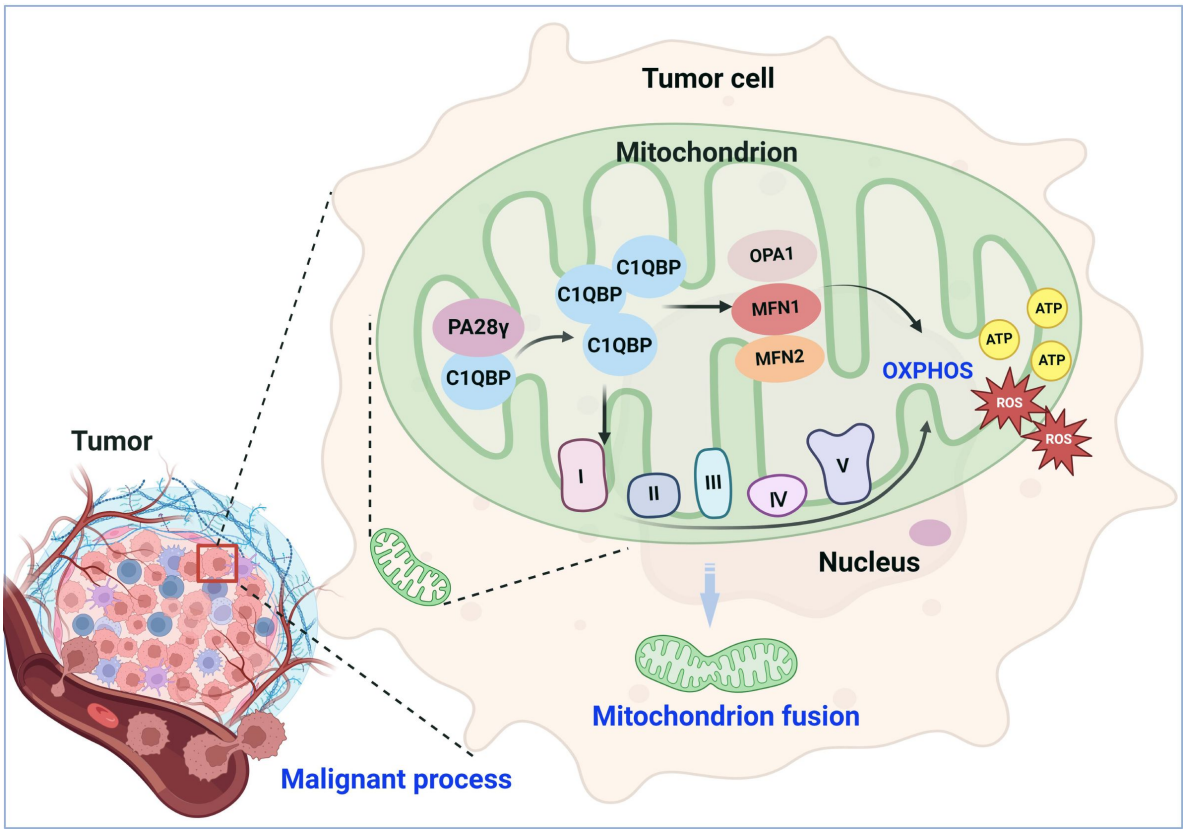

Fig. 6.

Molecular mechanism through which PA28γ interacts with C1QBP in the malignant progression of tumor.

PA28γ can interacts with and stabilize C1QBP, which can activate the expression and function of OPA1, MNF1, MFN2 and mitochondrial respiratory chain complex proteins, resulting in enhanced mitochondrial OXPHOS, mitochondrial fusion and malignant tumor progression.

(Westermann, 2010). OPA1 is an essential inner mitochondrial membrane protein with multiple functions, including regulating mitochondrial fusion, crest morphology, mtDNA stability, and interacting with the mitochondrial respiratory chain to regulate OXPHOS (Del Dotto et al., 2018).

Finally, we analyzed the expression patterns and significance of PA28γ and C1QBP in a clinical cohort. PA28γ and C1QBP were negatively correlated with prognosis in the OSCC clinical cohort. Notably, some studies have shown that OXPHOS is upregulated in various tumors, with some advanced tumors preferring OXPHOS metabolically (Ohshima & Morii, 2021; Qiu, Li, & Zhang, 2023). OXPHOS activity is associated with the recurrence of OSCC (Noh et al., 2023). Additionally, the enhancement of OXPHOS and ATP production by ROS proto-oncogene 1, which localizes to mitochondria in OSCC, promotes the OSCC invasion(Noh et al., 2023; Grimm et al., 2014). These findings are consistent with our results. Our study not only explain the mechanisms and signaling networks underlying the oncogenic potential of PA28γ but also contribute to understanding the possible mechanisms involved in OSCC metabolic reprogramming.

In summary, we found that PA28γ could interact with C1QBP and stabilize C1QBP. This enhances the function of OXPHOS, leading to increased production of ATP and ROS by activating the expression and function of OPA1, MNF1, MFN2 and the mitochondrial respiratory chain complex. This process promotes mitochondrial fusion and malignant tumor progression. The expression of PA28γ and C1QBP was increased and positively correlated in OSCC. High expression of PA28γ and C1QBP was correlated with poor prognosis in OSCC patients, indicating that both factors could serve as potential targets for the treatment and prognosis of OSCC.

Materials and methods

Patients and Follow-up

Our study included 4 cohorts. The first cohort included 8 normal controls, 13 patients with oral potentially malignant disorder (OPMD), and 45 patients with OSCC (Appendix Table 1). These patients visited the West China Hospital of Stomatology in 2021. The normal tissues were obtained from patients who underwent maxillofacial plastic surgery. The second cohort for survival analysis included 295 patients diagnosed with primary OSCC tumors who visited West China Hospital of Stomatology, Peking University Hospital of Stomatology and Guangdong Provincial Stomatological Hospital from 2005 to 2009 (Appendix Table 2). None of the patients had cancer in other organs. These patients received regular follow-up for 1-133 months after the operation. The protocol of this study was approved by the ethics committee of the West China Hospital of Stomatology (Approval number: WCHSIRB-D-2020-046). The third cohort, comprising 518 patients with head and neck squamous cell carcinoma (HNSCC), was obtained from The Cancer Genome Atlas (TCGA) database (Appendix Table 3). The last cohort, comprising 458 patients with skin cutaneous melanoma (SKCM), was obtained from TCGA database (Appendix Table 4).

Laboratory Experiments

All animal studies were approved by the Animal Care and Use Committee for the State Key Laboratory of Oral Diseases (Approval number: WCHSIRB-D-2022-032) in compliance with the Guide for the updated Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 guidelines. Other methods are detailed in the *Supplemental Materials and Methods*, including cell culture and cell-related experiments, animal models, mitochondrial function detection, western blot/immunoprecipitation assays, hematoxylin and eosin (H&E) staining, and immunohistochemistry.

Statistical Analysis

The data were analyzed using GraphPad Prism software version 8.0. The statistical data are expressed as the mean ± standard deviation (SD). Chi-square and Fisher exact tests, Spearman correlation tests, Wilcoxon rank-sum tests, one-way ANOVA and Student's t tests were used to analyze the data. The Kaplan[Meier method was used for survival analysis, and the log-rank test was used to evaluate the prognostic value of C1QBP in OSCC patients. The data are shown as the mean ± standard error, and a value of $P<0.05$ was considered to indicate statistical significance: $P<0.05$*, $P<0.01$**, $P<0.001$***, and $P<0.0001$****. Each experiment was repeated 3 times.

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Additional information

Author contributions

J. Wang, Y. Shi, X. Zeng, J. Li contributed to conception and design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; Y. Wang, Y. Shen, H Liu contributed to data acquisition; S. Sun, Y. Wang contributed to data analysis and interpretation; X. Zhou, Q. Chen contributed to conception and design, critically revised the manuscript. All the authors gave final approval and agreed to be accountable for all aspects of the work.

Conflict of interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Bai Y., Wang W., Li S., Zhan J., Li H., Zhao M., ..., Wang J. (2019) **C1QBP Promotes Homologous Recombination by Stabilizing MRE11 and Controlling the Assembly and Activation of MRE11/RAD50/NBS1 Complex** *Mol Cell* **75**:1299–1314 https://doi.org/10.1016/j.molcel.2019.06.023

Chen J., Zhou Q., Li S., Ling R., Zhao Y., Chen D., ..., Cao Y. (2024) **Metabolic reprogramming driven by METTL1-mediated tRNA m7G modification promotes acquired anlotinib resistance in oral squamous cell carcinoma** *Transl Res* **268**:28–39 https://doi.org/10.1016/j.trsl.2024.01.009

Del Dotto V., Fogazza M., Lenaers G., Rugolo M., Carelli V., Zanna C. (2018) **OPA1: How much do we know to approach therapy?** *Pharmacol Res* **131**:199–210 https://doi.org/10.1016/j.phrs.2018.02.018

Egusquiza-Alvarez C. A., Robles-Flores M. (2022) **An approach to p32/gC1qR/HABP1: a multifunctional protein with an essential role in cancer** *J Cancer Res Clin Oncol* **148**:1831–1854 https://doi.org/10.1007/s00432-022-04001-5

Faubert B., Solmonson A., DeBerardinis R. J. (2020) **Metabolic reprogramming and cancer progression** *Science* **368** https://doi.org/10.1126/science.aaw5473

Feichtinger R. G., Oláhová M., Kishita Y., Garone C., Kremer L. S., Yagi M., ..., Prokisch H. (2017) **Biallelic C1QBP Mutations Cause Severe Neonatal-, Childhood-, or Later-Onset Cardiomyopathy Associated with Combined Respiratory-Chain Deficiencies** *The American Journal of Human Genetics* **101**:525–538 https://doi.org/10.1016/j.ajhg.2017.08.015

Ghebrehiwet B., Geisbrecht B. V., Xu X., Savitt A. G., Peerschke E. I. B. (2019) **The C1q Receptors: Focus on gC1qR/p33 (C1qBP, p32, HABP-1)** *Semin Immunol* **45** https://doi.org/10.1016/j.smim.2019.101338

Grimm M., Cetindis M., Lehmann M., Biegner T., Munz A., Teriete P., ..., Reinert S. (2014) **Association of cancer metabolism-related proteins with oral carcinogenesis – indications for chemoprevention and metabolic sensitizing of oral squamous cell carcinoma?** *Journal of Translational Medicine* **12** https://doi.org/10.1186/1479-5876-12-208

Grönroos E., Hellman U., Heldin C. H., Ericsson J. (2002) **Control of Smad7 stability by competition between acetylation and ubiquitination** *Mol Cell* **10**:483–493 https://doi.org/10.1016/s1097-2765(02)00639-1

Hanahan D. (2022) **Hallmarks of Cancer: New Dimensions** *Cancer Discov* **12**:31–46 https://doi.org/10.1158/2159-8290.cd-21-1059

Hou G., Lu Z., Wang Z., Yang X. (2022) **The Mitochondrial Protein C1QBP Promotes Hepatocellular Carcinoma Progression by Enhancing Cell Survival, Migration and Invasion** *J Cancer* **13**:2477–2489 https://doi.org/10.7150/jca.69379

Johnson D. E., Burtness B., Leemans C. R., Lui V. W. Y., Bauman J. E., Grandis J. R. (2020) **Head and neck squamous cell carcinoma** *Nat Rev Dis Primers* **6** https://doi.org/10.1038/s41572-020-00224-3

Li J., Feng X., Sun C., Zeng X., Xie L., Xu H., ..., Chen Q. (2015) **Associations between proteasomal activator PA28γ and outcome of oral squamous cell carcinoma: Evidence from cohort studies and functional analyses** *EBioMedicine* **2**:851–858 https://doi.org/10.1016/j.ebiom.2015.07.004

Li Z., Sun S., Wang Y., Hua Y., Liu M., Zhou Y., ..., Li J. (2024) **PA28γ coordinates the cross-talk between cancer-associated fibroblasts and tumor cells to promote OSCC progression via HDAC1/E2F3/IGF2 signaling** *Cancer Lett* **594** https://doi.org/10.1016/j.canlet.2024.216962

Lindemann A., Takahashi H., Patel A. A., Osman A. A., Myers J. N. (2018) **Targeting the DNA Damage Response in OSCC with TP53 Mutations** *J Dent Res* **97**:635–644 https://doi.org/10.1177/0022034518759068

Liu B., Si W., Wei B., Zhang X., Chen P. (2023) **PTP4A1 promotes oral squamous cell carcinoma (OSCC) metastasis through altered mitochondrial metabolic reprogramming** *Cell Death Discov* **9** https://doi.org/10.1038/s41420-023-01657-x

Liu J., Yu G., Zhao Y., Zhao D., Wang Y., Wang L., ..., Li X. (2010) **REGgamma modulates p53 activity by regulating its cellular localization** *J Cell Sci* **123**:4076–4084 https://doi.org/10.1242/jcs.067405

Liu S., Liu D., Zeng X., Wang J., Liu J., Cheng J., ..., Chen Q. (2018) **PA28γ acts as a dual regulator of IL-6 and CCL2 and contributes to tumor angiogenesis in oral squamous cell carcinoma** *Cancer Lett* **428**:192–200 https://doi.org/10.1016/j.canlet.2018.04.024

Mao I., Liu J., Li X., Luo H. (2008) **REGgamma, a proteasome activator and beyond?** *Cell Mol Life Sci* **65**:3971–3980 https://doi.org/10.1007/s00018-008-8291-z

Matsumoto K., Bay B. H. (2021) **Role of C1QBP/p32 and its Therapeutic Potential in Breast Carcinoma and other Cancers** *Curr Med Chem* **28**:5048–5065 https://doi.org/10.2174/0929867328666201231124038

Noh J. K., Woo S. R., Kong M., Lee M. K., Lee J. W., Lee Y. C., ..., Eun Y. G. (2023) **Gene signature predicting recurrence in oral squamous cell carcinoma is characterized by increased oxidative phosphorylation** *Mol Oncol* **17**:134–149 https://doi.org/10.1002/1878-0261.13328

Ohshima K., Morii E. (2021) **Metabolic Reprogramming of Cancer Cells during Tumor Progression and Metastasis** *Metabolites* **11** https://doi.org/10.3390/metabo11010028

Peerschke E., Stier K., Li X., Kandov E., de Stanchina E., Chang Q., Adusumilli P. S. (2020) **gC1qR/HABP1/p32 Is a Potential New Therapeutic Target Against Mesothelioma** *Front Oncol* **10** https://doi.org/10.3389/fonc.2020.01413

Peres M. A., Macpherson L. M. D., Weyant R. J., Daly B., Venturelli R., Mathur M. R., Watt R. G. (2019) **Oral diseases: a global public health challenge** *Lancet* **394**:249–260 https://doi.org/10.1016/s0140-6736(19)31146-8

Qiu X., Li Y., Zhang Z. (2023) **Crosstalk between oxidative phosphorylation and immune escape in cancer: a new concept of therapeutic targets selection** *Cell Oncol (Dordr)* **46**:847–865 https://doi.org/10.1007/s13402-023-00801-0

Raschdorf A., Sünderhauf A., Skibbe K., Ghebrehiwet B., Peerschke E. I., Sina C., Derer S. (2021) **Heterozygous P32/C1QBP/HABP1 Polymorphism rs56014026 Reduces Mitochondrial Oxidative Phosphorylation and Is Expressed in Low-grade Colorectal Carcinomas** *Frontiers in Oncology* **10** https://doi.org/10.3389/fonc.2020.631592

Siegel R. L., Miller K. D., Wagle N. S., Jemal A. (2023) **Cancer statistics, 2023** *CA Cancer J Clin* **73**:17–48 https://doi.org/10.3322/caac.21763

Stadtmueller B. M., Hill C. P. (2011) **Proteasome Activators** *Molecular Cell* **41**:8–19 https://doi.org/10.1016/j.molcel.2010.12.020

Sung H., Ferlay J., Siegel R. L., Laversanne M., Soerjomataram I., Jemal A., Bray F. (2021) **Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries** *CA Cancer J Clin* **71**:209–249 https://doi.org/10.3322/caac.21660

Tian H., Chai D., Wang G., Wang Q., Sun N., Jiang G., Zheng J. (2023) **Mitochondrial C1QBP is essential for T cell antitumor function by maintaining mitochondrial plasticity and metabolic fitness** *Cancer Immunol Immunother* **72**:2151–2168 https://doi.org/10.1007/s00262-023-03407-5

Tsai C. H., Chuang Y. M., Li X., Yu Y. R., Tzeng S. F., Teoh S. T., ..., Ho P. C. (2023) **Immunoediting instructs tumor metabolic reprogramming to support immune evasion** *Cell Metab* **35**:118–133 https://doi.org/10.1016/j.cmet.2022.12.003

Vendramin R., Verheyden Y., Ishikawa H., Goedert L., Nicolas E., Saraf K., Leucci E. (2018) **SAMMSON fosters cancer cell fitness by concertedly enhancing mitochondrial and cytosolic translation** *Nat Struct Mol Biol* **25**:1035–1046 https://doi.org/10.1038/s41594-018-0143-4

Vyas A., Harbison R. A., Faden D. L., Kubik M., Palmer D., Zhang Q., ..., Duvvuri U. (2021) **Recurrent Human Papillomavirus-Related Head and Neck Cancer Undergoes Metabolic Reprogramming and Is Driven by Oxidative Phosphorylation** *Clin Cancer Res* **27**:6250–6264 https://doi.org/10.1158/1078-0432.ccr-20-4789

Wang Q., Chai D., Sobhani N., Sun N., Neeli P., Zheng J., Tian H. (2022) **C1QBP regulates mitochondrial plasticity to impact tumor progression and antitumor immune response** *Front Physiol* **13** https://doi.org/10.3389/fphys.2022.1012112

Wang Y., Zhang Q., Deng X., Wang Y., Tian X., Zhang S., ..., Li J. (2024) **PA28γ induces dendritic cell maturation and activates TDcell immune responses in oral lichen planus** *MedComm* **5** https://doi.org/10.1002/mco2.561

Wang Z., Wu V. H., Allevato M. M., Gilardi M., He Y., Luis Callejas-Valera J., ..., Gutkind J. S. (2019) **Syngeneic animal models of tobacco-associated oral cancer reveal the activity of in situ anti-CTLA-4** *Nat Commun* **10** https://doi.org/10.1038/s41467-019-13471-0

Westermann B. (2010) **Mitochondrial fusion and fission in cell life and death** *Nature Reviews Molecular Cell Biology* **11**:872–884 https://doi.org/10.1038/nrm3013

Xia L., Oyang L., Lin J., Tan S., Han Y., Wu N., ..., Liao Q. (2021) **The cancer metabolic reprogramming and immune response** *Mol Cancer* **20** https://doi.org/10.1186/s12943-021-01316-8

Xiao L., Hu Q., Peng Y., Zheng K., Zhang T., Yang L., ..., Liu Y. (2021) **TRAP1 suppresses oral squamous cell carcinoma progression by reducing oxidative phosphorylation metabolism of Cancer-associated fibroblasts** *BMC Cancer* **21** https://doi.org/10.1186/s12885-021-09049-z

Xu L., Xiao N., Liu F., Ren H., Gu J. (2009) **Inhibition of RIG-I and MDA5-dependent antiviral response by gC1qR at mitochondria** *Proc Natl Acad Sci USA* **106**:1530–1535 https://doi.org/10.1073/pnas.0811029106

Yao L., Xuan Y., Zhang H., Yang B., Ma X., Wang T., ..., Zhang B. (2021) **Reciprocal REGγ-mTORC1 regulation promotes glycolytic metabolism in hepatocellular carcinoma** *Oncogene* **40**:677–692 https://doi.org/10.1038/s41388-020-01558-8

Zhang Z., Gao Z., Rajthala S., Sapkota D., Dongre H., Parajuli H., Liang X. (2020) **Metabolic reprogramming of normal oral fibroblasts correlated with increased glycolytic metabolism of oral squamous cell carcinoma and precedes their activation into carcinoma associated fibroblasts** *Cell Mol Life Sci* **77**:1115–1133 https://doi.org/10.1007/s00018-019-03209-y

Zhu Z., Liu Y., Wu D., Wang H. (2021) **Association Between Mitochondrial DNA Copy Number and Head and Neck Squamous Cell Carcinoma: A Systematic Review and Dose-Response Meta-Analysis** *Med Sci Monit* **27** https://doi.org/10.12659/msm.928327

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Reviewer #1 (Public review):

Summary:

The manuscript the authors have tried to dissect the functions of Proteasome activator 28γ (PA28γ) which is known to activate proteosomal function in an ATP independent manner. Although there are multiple works that have highlighted the role of this protein in tumour, this study specifically tried to develop a correlate with Complement C1q binding protein (C1QBp) that is associated with immune response and energy homeostasis.

Strengths:

The observations of the authors hint that beyond PA28y association with proteasome, it might also stabilize certain proteins such as C1QBP which influences the energy metabolism.

Weaknesses:

The strength of the work also becomes its main drawback. That is, how PA28y stabilizes C1QBP or how C1QBP elicits its pro-tumourigenic role under PA28y OE.

In most of the experiments the authors have been dependent on the parallel changes in the expression of both the proteins to justify their stabilizing interaction. However, this approach is indirect at best and does not confirm the direct stabilizing effect of this interaction. IP experiments do not indicate direct interaction and have some quality issues. The upregulation of C1QBP might be indirect at best. It is quite possible that PA28y might be degrading some secondary protein/complex which is responsible for C1QBP expression. Since the core idea of the work is PA28y direct interaction with C1QBP stabilizing it, the same should be demonstrated in more convincing manner.

In all of the assays C1QBP has been detected as doublet. However, the expression pattern of the two bands vary depending on the experiment. In some cases the upper band is intensely stained and in some the lower bands. Does C1QBP isoforms exist and whether they are differentially regulated depending on experiment conditions/tissue types?

Problems with the background of the work: Line 76. This statement is far-fetched. There are presently a number of literatures that have dealt with metabolic programming of OSCC including identification of specific metabolites. Moreover, beyond estimation of OCR, the authors have not conducted any experiments related to metabolism. In the Introduction,

significance of this study and how it will extend our understanding of OSCC needs to be elaborated.

Review of Revised Version:

Although the authors have partly corrected the manuscript by removing the mislabeling in their Co-IP experiments, my primary concern on the actual functional connotations and direct interaction between PA28y and C1QBP still remains unaddressed. As already mentioned in my previous review, since the core idea of the work is PA28y's direct interaction with C1QBP, stabilizing it, the same should be demonstrated in a more convincing manner.

My other observation on the detection of C1QBP as a doublet has been addressed by usage of anti-C1QBP Monoclonal antibody against the polyclonal one used before. C1QBP doublets have not been observed in the present case.

The authors have also worked on the presentation of the background by suitably modifying the statements and incorporating appropriate citations.

However, the authors are requested to follow the recommendations provided to them by the reviewers to address the major concerns.

https://doi.org/10.7554/eLife.101244.2.sa2

Reviewer #2 (Public review):

Summary:

The authors tried to determine how PA28g functions in oral squamous cell carcinoma (OSCC) cells. They hypothesized it may act through metabolic reprogramming in the mitochondria.

Strengths:

They found that the genes of PA28g and C1QBP are in an overlapping interaction network after an analysis of a genome database. They also found that the two proteins interact in coimmunoprecipitation and pull-down assays using the lysate from OSCC cells with or without expression of the exogenous genes. They used truncated C1QBP proteins to map the interaction site to the N-terminal 167 residues of C1QBP protein. They observed the levels of the two proteins are positively correlated in the cells. They provided evidence for the colocalization of the two proteins in the mitochondria and the effect on mitochondrial form and function in vitro and in vivo OSCC models, and the correlation of the protein expression with the prognosis of cancer patients.

Weaknesses:

Many data sets are shown in figures that cannot be understood without more descriptions either in the text or the legend, e.g., Fig. 1A. Similarly, many abbreviations are not defined.

The revision addressed these issues.

Some of the pull-down and coimmunoprecipitation data do not support the conclusion about the PA28g-C1QBP interaction. For example, in Appendix Fig. 1B the Flag-C1QBP was detected in the Myc beads pull-down when the protein was expressed in the 293T cells without the Myc-PA28g, suggesting that the pull-down was not due to the interaction of the C1QBP and PA28g proteins. In Appendix Fig. 1C, assume the SFB stands for a biotin tag, then the SFB-PA28g should be detected in the cells expressing this protein after pull-down by streptavidin; however, it was not. The Western blot data in Fig. 1E and many other figures must be quantified before any conclusions about the levels of proteins can be drawn.

The revision addressed these problems.

The immunoprecipitation method is flawed as it is described. The antigen (PA28g or C1QBP) should bind to the respective antibody that in turn should binds to Protein G beads. The resulting immunocomplex should end up in the pellet fraction after centrifugation, and analyzed further by Western blot for coprecipitates. However, the method in the Appendix states that the supernatant was used for the Western blot.

The revision corrected this method.

To conclude that PA28g stabilizes C1QBP through their physical interaction in the cells, one must show whether a protease inhibitor can substitute PA28q and prevent C1QBP degradation, and also show whether a mutation that disrupt the PA28g-C1QBP interaction can reduce the stability of C1QBP. In Fig. 1F, all cells expressed Myc-PA28g. Therefore, the conclusion that PA28g prevented C1QBP degradation cannot be reached. Instead, since more Myc-PA28g was detected in the cells expressing Flag-C1QBP compared to the cells not expressing this protein, a conclusion would be that the C1QBP stabilized the PA28g. Fig. 1G is a quantification of a Western blot data that should be shown.

The binding site for PA28g in C1QBP was mapped to the N-terminal 167 residues using truncated proteins. One caveat would be that some truncated proteins did not fold correctly in the absence of the sequence that was removed. Thus, the C-terminal region of the C1QBP with residues 168-283 may still bind to the PA29g in the context of full-length protein. In Fig. 1I, more Flag-C1QBP 1-167 was pull-down by Myc-PA28g than the full-length protein or the Flag-C1QBP 1-213. Why?

The interaction site in PA28g for C1QBP was not mapped, which prevents further analysis of the interaction. Also, if the interaction domain can be determined, structural modeling of the complex would be feasible using AlphaFold2 or other programs. Then, it is possible to test point mutations that may disrupt the interaction and if so, the functional effect.

The revision added AlphaFold models for the protein interaction. However, the models were not analyzed and potential mutations that would disrupt the interact were not predicted, made and tested. The revision did not addressed the request for the protease inhibitor.

https://doi.org/10.7554/eLife.101244.2.sa1

Author response:

The following is the authors' response to the original reviews.

> ***Public Reviews:***
>
> ***Reviewer #1 (Public review):***
>
> *Summary:*
>
> *In this manuscript, the authors have tried to dissect the functions of Proteasome activator 28y (PA28y) which is known to activate proteasomal function in an ATP-independent manner. Although there are multiple works that have highlighted the role of this protein in tumours, this study specifically tried to develop a correlation with Complement C1q binding protein (C1QBp) that is associated with immune response and energy homeostasis.*
>
> *Strengths:*

> *The observations of the authors hint that beyond PA28y's association with the proteasome, it might also stabilize certain proteins such as C1QBP which influences energy metabolism.*
>
> *Weaknesses:*
>
> *The strength of the work also becomes its main drawback. That is, how PA28y stabilizes C1QBP or how C1QBP elicits its pro-tumourigenic role under PA28y OE.*
> *In most of the experiments, the authors have been dependent on the parallel changes in the expression of both the proteins to justify their stabilizing interaction. However, this approach is indirect at best and does not confirm the direct stabilizing effect of this interaction. IP experiments do not indicate direct interaction and have some quality issues. The upregulation of C1QBP might be indirect at best. It is quite possible that PA28y might be degrading some secondary protein/complex that is responsible for C1QBP expression. Since the core idea of the work is PA28y direct interaction with C1QBP stabilizing it, the same should be demonstrated in a more convincing manner.*

Thank you very much for the important comments. Using AlphaFold 3, we found that interaction between PA28γ and C1QBP may depend on amino acids 1-167 and 1-213 (Revised Appendix Figure 1D-H), which was confirmed by our immunoprecipitation (Revised Figure 1I). In the future, we will use nuclear magnetic resonance spectroscopy to analyze protein-protein interaction between PA28γ and C1QBP and demonstrate it by GST pull down in vitro experiments.

> *In all of the assays, C1QBP has been detected as doublet. However, the expression pattern of the two bands varies depending on the experiment. In some cases, the upper band is intensely stained and in some the lower bands. Do C1QBP isoforms exist and are they differentially regulated depending on experiment conditions/tissue types?*

Thank you very much for the important comments. We have rechecked the experimental results with two bands, which may have been caused by using polyclonal antibody of C1QBP (Abcam: ab101267). Therefore, we conducted the experiment with monoclonal antibody of C1QBP (Cell Signaling Technology: #6502) and replaced the corresponding images in revised figure (Revised Figure 1E and Revised Appendix Figure 3D).

> *Problems with the background of the work: Line 76. This statement is far-fetched. There are presently a number of works of literature that have dealt with the metabolic programming of OSCC including identification of specific metabolites. Moreover, beyond the estimation of OCR, the authors have not conducted any experiments related to metabolism. In the Introduction, the significance of this study and how it will extend our understanding of OSCC needs to be elaborated.*

Thank you very much for the important comments. Based on your suggestion, we have revised the content and updated the references (“Introduction”, Paragraph 2, Line 13-17 and Paragraph 4, Line 5-8). In addition, we plan to conduct experiments to investigate the regulation of metabolism by PA28γ and C1QBP and update our data in the future.

The modified content is as follows:

“Current research on metabolic reprogramming in OSCC primarily focused on mechanism of glycolytic metabolism and metabolic shift from glycolysis to oxidative phosphorylation (OXPHOS) of oral squamous cell carcinoma, which lays the groundwork for novel therapeutic interventions to counteract OSCC (Chen et al., 2024; Zhang et al., 2020).”

"It is the first study to describe the undiscovered role of PA28γ in promoting the malignant progression of OSCC by elevating mitochondrial function, providing new clinical insights for the treatment of OSCC."

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *The authors tried to determine how PA28g functions in oral squamous cell carcinoma (OSCC) cells. They hypothesized it may act through metabolic reprogramming in the mitochondria.*
>
> *Strengths:*
>
> *They found that the genes of PA28g and C1QBP are in an overlapping interaction network after an analysis of a genome database. They also found that the two proteins interact in coimmunoprecipitation and pull-down assays using the lysate from OSCC cells with or without expression of the exogenous genes. They used truncated C1QBP proteins to map the interaction site to the N-terminal 167 residues of C1QBP protein. They observed the levels of the two proteins are positively correlated in the cells. They provided evidence for the colocalization of the two proteins in the mitochondria, the effect on mitochondrial form and function in vitro and in vivo OSCC models, and the correlation of the protein expression with the prognosis of cancer patients.*
>
> *Weaknesses:*
>
> *Many data sets are shown in figures that cannot be understood without more descriptions, either in the text or the legend, e.g., Figure 1A. Similarly, many abbreviations are not defined.*

Thank you very much for the important comments. We have revised the descriptions in the legend to make it easier to understand.

> *Some of the pull-down and coimmunoprecipitation data do not support the conclusion about the PA28g-C1QBP interaction. For example, in Appendix Figure 1B the Flag-C1QBP was detected in the Myc beads pull-down when the protein was expressed in the 293T cells without the Myc-PA28g, suggesting that the pull-down was not due to the interaction of the C1QBP and PA28g proteins. In Appendix Figure 1C, assume the SFB stands for a biotin tag, then the SFB-PA28g should be detected in the cells expressing this protein after pull-down by streptavidin; however, it was not. The Western blot data in Figure 1E and many other figures must be quantified before any conclusions about the levels of proteins can be drawn.*

Thank you very much for the meticulous review. We have rechecked the experimental results, and we made a mistake in the labeling of the image. Therefore, we have corrected it in the revised figure (Revised Appendix Figure 1B, C). In addition, we have conducted a quantitative analysis of gray values to confirm the results of western blot data are accurate by Image J software.

> *The immunoprecipitation method is flawed as it is described. The antigen (PA28g or C1QBP) should bind to the respective antibody that in turn should binds to Protein G beads. The resulting immunocomplex should end up in the pellet fraction after centrifugation and be analyzed further by Western blot for coprecipitates. However, the method in the Appendix states that the supernatant was used for the Western blot.*

Thank you very much for the careful review. We have corrected it in the revised appendix file ("Supplemental Materials and Methods", Part"Immunoprecipitation assay", Line 4-6).

The modified content is as follows:

The sample was shaken on a horizontal shaker for 4 h, after which the deposit was collected for western blotting.

> *To conclude that PA28g stabilizes C1QBP through their physical interaction in the cells, one must show whether a protease inhibitor can substitute PA28q and prevent C1QBP degradation, and show whether a mutation that disrupts the PA28g-C1QBP interaction can reduce the stability of C1QBP. In Figure 1F, all cells expressed Myc-PA28g. Therefore, the conclusion that PA28g prevented C1QBP degradation cannot be reached. Instead, since more Myc-PA28g was detected in the cells expressing Flag-C1QBP compared to the cells not expressing this protein, a conclusion would be that the C1QBP stabilized the PA28g. Figure 1G is a quantification of Western blot data that should be shown.*

Thank you very much for the meticulous review. We have rechecked the experimental results, and we made a mistake in the labeling of the image. Therefore, we have corrected it in the revised figure. Compared with the control group, the presence of Myc-PA28γ significantly increased the expression level of Flag-C1QBP (Revised Figure 1F). Gray value analysis showed that in cells transfected with Myc-PA28γ, the decay rate of Flag-C1QBP was significantly slower than that of the control group (Revised Figure 1G), suggesting that PA28γ can delay the protein degradation of C1QBP and stabilize its protein level. This indicates that an increase in the level of PA28γ protein can significantly enhance the expression level of C1QBP protein, while PA28γ can slow down the degradation rate of C1QBP and improve its stability. In addition, we plan to conduct experiments to investigate the effects of protease inhibitors and PA28γ mutants on the stability of C1QBP and update our data in the future.

> *The binding site for PA28g in C1QBP was mapped to the N-terminal 167 residues using truncated proteins. One caveat would be that some truncated proteins did not fold correctly in the absence of the sequence that was removed. Thus, the C-terminal region of the C1QBP with residues 168-283 may still bind to the PA29g in the context of full-length protein. In Figure 1I, more Flag-C1QBP 1-167 was pulled down by Myc-PA28g than the full-length protein or the Flag-C1QBP 1-213. Why?*

Thank you very much for the important comments. Immunoprecipitation is a qualitative experiment. Using AlphaFold 3, we found that interaction between PA28γ and C1QBP may depend on amino acids 1-167 and 1-213 (Revised Appendix Figure 1D-H), which was confirmed by our immunoprecipitation (Revised Figure 1I).

> *The interaction site in PA28g for C1QBP was not mapped, which prevents further analysis of the interaction. Also, if the interaction domain can be determined, structural modeling of the complex would be feasible using AlphaFold2 or other programs. Then, it is possible to test point mutations that may disrupt the interaction and if so, the functional effect.*

Thank you very much for the important comments. Based on your suggestion, we have added relevant content to the revised appendix figure. (Revised Appendix Figure 1D-H).

> ***Recommendations for the authors:***
>
> ***Reviewer #1 (Recommendations for the authors):***
>
> *(1) There are a lot of typos in the figure and manuscript that need to be addressed.*

Thank you very much for the important comments. We have corrected the typos in the revised figure and manuscript.

> *(2) Figure 1A: The amount of protein that has been immunoprecipitated is more than the actual amount present in the lysate. The authors should calculate the efficiency of the precipitation to support their results.*

Thank you very much for the important comments. Immunoprecipitation is a qualitative experiment. Moreover, it can enrich specific proteins and their binding partners, increase their concentration in the sample, and thus improve the sensitivity of detection.

> *(3) Figure 1D: The relative expression levels of C1QBP look similar in almost all cell lines except for HN12. It seems that the relation of PA28y with C1QBP is more of a cell type-specific effect. It would be better if the blots were quantified, and the differences were statistically determined.*

Thank you very much for the important comments. We have conducted a quantitative analysis of gray values to confirm the results of western blot data are accurate by Image J software.

> *(4) Figure 1E: How do the authors quantify the expression of the protein in absolute terms? From the methods, it is understood that the flag-tagged construct is stably expressed. Under such conditions, how the authors observed the variable expression of the protein should be elaborated.*

Thank you very much for the important comments. We transfected Flag-PA28γ plasmids at 0ug, 0.5ug, 1ug, and 2ug in 293T cells. After collecting the protein for Western Blot, we found that the protein expression of Flag-PA28γ gradually increased. Moreover, the increased protein expression of C1QBP is consistent with the expression of Flag-PA28γ, which indicated a dose-dependent relationship between the two proteins.

> *(5) Figures 1F, G: The data does not correlate with the arguments presented in the text. The authors propose that interaction with PA28y increases the stability of C1QBP. However, the experiment lacks appropriate controls. Ideally, the expression of C1QBP should be tested in the presence and absence of PA28y. Moreover, the observed difference in expression between lanes 1-4 and 5-8 for myc-PA28y needs to be explained. Are the samples from different sources with variable PA28y expression? Figure 1G quantification for C1QBP does not correlate with the figure presented in F since the expression of the protein in the first four lanes is undetectable.*

Thank you very much for the meticulous review. We have rechecked the experimental results, and we made a mistake in the labeling of the image. Therefore, we have corrected it in the revised figure. Compared with the control group, the presence of Myc-PA28γ significantly increased the expression level of Flag-C1QBP (Revised Figure 1F). Gray value analysis showed that in cells transfected with Myc-PA28γ, the decay rate of Flag-C1QBP was significantly slower than that of the control group (Revised Figure 1G), suggesting that PA28γ can delay the protein degradation of C1QBP and stabilize its protein level. This indicates that an increase in the level of PA28γ protein can significantly enhance the expression level of C1QBP protein, while PA28γ can slow down the degradation rate of C1QBP and improve its stability. In addition, we plan to conduct experiments to investigate the effects of protease inhibitors and PA28γ mutants on the stability of C1QBP and update our data in the future.

> *(6) Appendix Figure 1B: Lane 1 does not express Myc-tagged protein but pull-down has been performed using Myc beads. Then how come flag-C1qbp is getting pulled down in lane 1 if there is no PA28γ? This indicates a non-specific interaction of C1qbp with the substrata under the experimental conditions used. Similarly, in Figure 1C SFB-PA28γ is expressed in both lanes but is reflected only in lane 2 and not in lane 1 even when pull-down is being performed using SFB beads, again reflecting the non-specificity of the interactions shown through immunoprecipitated.*

Thank you very much for the meticulous review. We have rechecked the experimental results, and we made a mistake in the labeling of the image. Therefore, we have corrected it in the revised figure (Revised Appendix Figure 1B, C).

> *(7) Figure 2A: Figure 2A the co-localization of P28γ with C1QBP in mitochondria is not very convincing. The authors are urged to provide high-resolution images for the same along with quantification of co-localization coefficients.*

Thank you very much for the important comments. We plan to obtain high-resolution images of co-localization of PA28γ with C1QBP in mitochondria and add the quantification analysis. We will update our data in the future.

> *(8) Figure 2C: Mitochondria dynamics is an interplay of multiple factors. From the images, it seems that PA28γ OE elevates mitochondria biogenesis in general which is having an umbrella effect on mitochondria fusion/fission and OCR. Images also do not convincingly indicate changes in mitochondrial length. The role of PA28γ on mitochondria dynamics requires further justification. However, the presented data does not underline whether the changes in mitochondria behaviour are a consequence of PA28γ and C1QBP interaction. Correlating higher mitochondria respiration with ROS generation is a far-fetched conclusion since, at present, there are multiple reports that suggest otherwise.*

Thank you very much for the important comments. We plan to knock out the interaction regions between PA28γ and C1QBP (like amino acids 1-167 and 1-213) to confirm whether PA28γ affects mitochondrial function through C1QBP and update our data in the future.

> *(9) Line 157: The presented data does not substantiate the claims made that Pa28γ regulates mitochondrial function through C1QBP.*

Thank you very much for the important comments. Based on your suggestion, we have made some modifications to make it more accurate (“Results”, Part “PA28γ and C1QBP colocalize in mitochondria and affect mitochondrial functions”, Paragraph 3, Line 1-2).

The modified content is as follows:

“Collectively, these data suggest that PA28γ, which co-localizes with C1QBP in mitochondria, may involve in regulating mitochondrial morphology and function.”

> *(10) Line 159: From the past data it is not very clear how PA28γ upregulates C1QBP, hence the statement is not well supported. The presented data indicates the presence of a functional association between the two proteins.*

Thank you very much for the important comments. We detected the expression of C1QBP in two PA28γ-overexpressing OSCC cells (UM1 and 4MOSC2) and found an increase in C1QBP expression (Revised Figure 4B). Based on the results of the protein levels of the mitochondrial

respiratory chain complex and other mitochondrial functional proteins, we believe that PA28γ regulates mitochondrial function by upregulating C1QBP.

> *(11) Figure 4A, B: Given the mitochondrial role of C1QBP, the lesser levels of mitochondrial proteins upon C1QBP silencing are expected. Does it get phenocopied upon PA28y silencing? Similarly, all the subsequent mitochondrial phenotypes in D should be seen in a PA28y-depleted background.*

Thank you very much for the important comments. We plan to detect the mitochondrial protein expressions and OCRs of PA28γ-silenced OSCC cells. We will update our data in the future.

> *(12) Line 198: The presented data do indicate a functional association between these two proteins but it does not provide a solid evidence for the same.*

Thank you very much for the important comments. Based on your suggestion, we have made some modifications to make it more accurate (“Discussion”, Paragraph 1, Line 9-10).

The modified content is as follows:

“Excitingly, we found the evidence that PA28γ interacts with and stabilizes C1QBP.”

> *(13) Line 218-220: In this work, the authors highlight the non-degradome role of PA28y and hence, this fact should be treated appropriately in discussion in line with the presented data.*

Thank you very much for the important comments. Based on your suggestion, we have added relevant content to the revised manuscript (“Discussion”, Paragraph 2, Line 16-19).

The modified content is as follows:

“In addition, PA28γ can also play as a non-degradome role on tumor angiogenesis. For example, PA28γ can regulate the activation of NF-κB to promote the secretion of IL-6 and CCL2 in OSCC cells, thus promoting the angiogenesis of endothelial cells (S. Liu et al., 2018).”

> *(14) Line 236-240: Although the authors' statement on organ heterogeneity being the cause for getting the contrasting result is justifiable but here there is no direct evidence of PA28y involvement in regulation of OXPHOS and its impact on cellular metabolism (glycolysis, metabolic signalling, etc).*

Thank you very much for the important comments. Based on your suggestion, we have made some modifications to make it more accurate (“Discussion”, Paragraph 3, Line 7-9).

The modified content is as follows:

“Therefore, PA28γ's regulation of OXPHOS may impact cellular energy metabolism.”

> *(15) Line 249: No conclusive data supporting this statement.*

Thank you very much for the important comments. Based on your suggestion, we have made some modifications to make it more accurate (“Discussion”, Paragraph 5, Line 1-3).

The modified content is as follows:

“Furthermore, our study reveals that PA28γ can regulate C1QBP and influence mitochondrial morphology and function by enhancing the expression of OPA1, MFN1, MFN2 and the mitochondrial respiratory complex.”

> ***Reviewer #2 (Recommendations for the authors):***
>
> *(1) The images shown in Figure 2A need to be quantified before the conclusion about the mitochondrial colocalization of the two proteins can be drawn. In Figure 2B and Appendix Figure 2A, the mitochondrial vacuoles and ridge should be indicated for general readers, and quantification should be performed before the conclusion is drawn.*

Thank you very much for the important comments. We will update our data in the future.

> *(2) The OCR data from two cell lines are shown in Figure 2E and F. Which is which? The sentence, "The results indicated ... compared to control cells" in lines 130-132, was confusing; perhaps, it would be clear if "were significantly greater" could be deleted.*

Thank you very much for the important comments. We have re-labeled the Figure 2E and F to make it clearly (Revised Figure 2E, F). Based on your suggestion, we have deleted the words in revised manuscript. (“Results”, Part “PA28γ and C1QBP colocalize in mitochondria and affect mitochondrial functions”, Paragraph 1, Line 9-11).

The modified content is as follows:

“The results indicated significantly higher basal respiration, maximal OCRs and ATP production in PA28γ-overexpressing cells compared to control cells (Fig. 2G-I and Appendix Fig. 2B-D).”

> *(3) Figures 4E-H show the migration, invasive, and proliferation capabilities of the cells. Which for which?*

Thank you very much for the important comments. We have re-labeled the Figure 4F-H to make it clearly (Revised Figure 4F-H).

> *(4) In the Discussion, lines 198-201, it states that "C1QBP enhances ... function of OPA1, MNF1, MFN2..." What is the evidence? In lines 222-224, it says that "the binding sites ... may mask the specific ... modification sites". Please justify. In lines 253-254, "fuse" and fuses" are misleading, Did the authors mean "localize" and "localizes"?*

Thank you very much for the important comments. Based on your suggestion, we have made some modifications to make it more accurate (“Discussion”, Paragraph 1, Line 9-13, Paragraph 2, Line 20-23, and Paragraph 5, Line 3-6).

The modified content is as follows:

“Excitingly, we found the evidence that PA28γ interacts with and stabilizes C1QBP. We speculate that aberrantly accumulated C1QBP enhances the function of mitochondrial OXPHOS and leads to the production of additional ATP and ROS by activating the expression and function of OPA1, MNF1, MFN2 and mitochondrial respiratory chain complex proteins.”

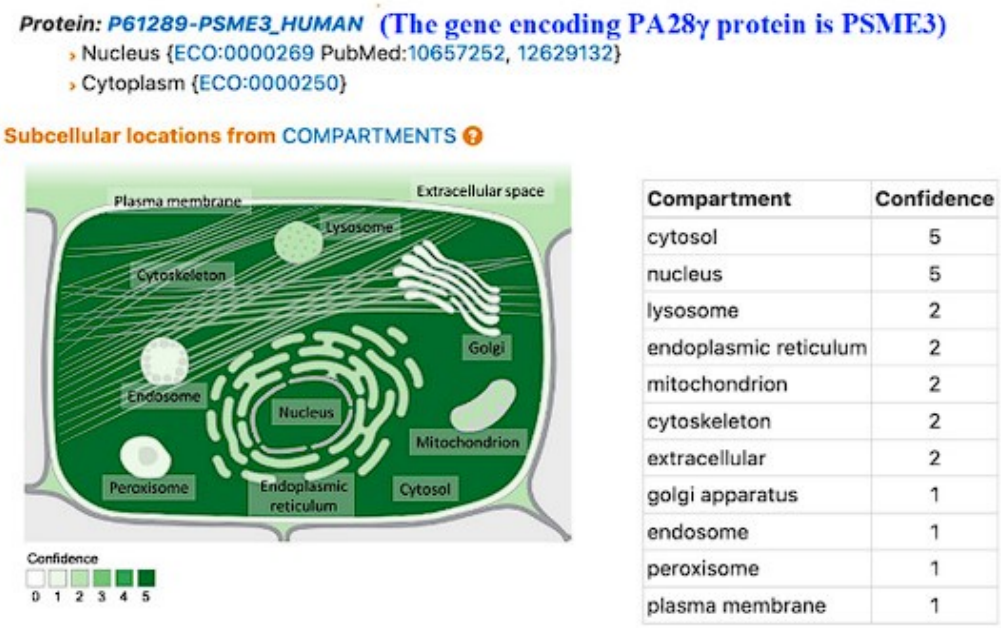
“Our study reveals that PA28γ interacts with C1QBP and stabilizes C1QBP at the protein level. Therefore, we speculate that the binding sites of PA28γ and C1QBP may mask the specific post-translational modification sites of C1QBP and inhibit its degradation.”

“Mitochondrial fusion, crucial for oxidative metabolism and cell proliferation, is regulated by MFN1, MFN2, and OPA1. The first two fuse with the outer mitochondrial membrane, while the last fuses with the inner mitochondrial membrane (Westermann, 2010).”

(5) Figure 6 was not referred to in the text. In this figure, PA28g and C1QBP are located in the inner membrane and matrix. Has this been determined? What is the blue ovals that are intermediaries of PA28g/C1QBP and OPA1/MFN1/MFN2?

Thank you very much for the important comments. According to our immunofluorescence assay (Figure 2A), PA28γ is in both the nucleus and cytoplasm. A recent study has demonstrated that PA28γ can shuttle between the nucleus and cytoplasm, participating in various cellular processes. Furthermore, GeneCard information indicates that the subcellular localization of PA28γ includes the nucleus, cytoplasm and mitochondria (Author response image 1). In this article, we mainly focus on the functions of PA28γ and C1QBP located in the cytoplasm. Therefore, figure 6 mainly displays PA28γ and C1QBP in the cytoplasm. Based on your suggestion, we have made some modifications to make it more accurate in revised figure (Revised Figure 6).

Author response image 1.



Compartment	Confidence
cytosol	5
nucleus	5
lysosome	2
endoplasmic reticulum	2
mitochondrion	2
cytoskeleton	2
extracellular	2
golgi apparatus	1
endosome	1
peroxisome	1
plasma membrane	1

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