STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	Title: "3-Hydroxyflavone inhibits the cytotoxicity of oral squamous cell carcinoma cells through the AGEs/RAGE/NF-κB signaling pathway" – observational study of OSCC patient tissues and cell lines
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1	Abstract: "Methods: The expression of p65 and miR-142-5p was analyzed in OSCC tissues and Tca8113 cells... Results: OSCC tissues showed increased p65 nuclear translocation and decreased miR-142-5p levels..."
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2-3	Introduction: "Oral squamous cell carcinoma (OSCC) represents a major global health concern... the five-year survival rate remains disappointingly low... the identification of novel therapeutic targets and the development of effective pharmacological agents are urgently needed."
Objectives	3	State specific objectives, including any prespecified hypotheses	3	"This study aimed to determine whether 3-Hydroxyflavone inhibits OSCC cell proliferation and enhances patient outcomes by regulating the miR-142-5p/AGEs/RAGE/NF-κB axis..."
Methods				
Study design	4	Present key elements of study design early in the paper	4	"This study enrolled 83 patients with primary OSCC who underwent surgical resection... During surgery, tumor tissues and adjacent normal tissues were collected."
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure,	4	"The Affiliated Yantai Yuhuangding

		follow-up, and data collection		Hospital of Qingdao University between November 2017 and November 2019."
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4	"Inclusion criteria were: (1) histopathologically confirmed primary OSCC; (2) no prior antitumor treatment; (3) availability of sufficient tumor and adjacent normal tissues; (4) complete clinical records; (5) signed written informed consent. Exclusion criteria were: (1) previous antitumor treatment; (2) recurrent or metastatic OSCC; (3) concurrent other active malignancies; (4) uncontrolled severe systemic diseases; (5) autoimmune or chronic inflammatory diseases; (6) pregnancy or lactation; (7) incomplete clinical or follow-up data."
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4	Not applicable (no matching was performed)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-9	Outcomes: p65 expression (IHC), miR-142-5p expression (RT-qPCR), cell proliferation (MTT, colony formation), patient survival (Kaplan-Meier). Exposures: 3-Hydroxyflavone treatment. Confounders: age, gender, smoking history, alcohol history, BMI, TNM stage (Table 2).
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-9	"Tumor tissues and adjacent normal tissues were collected during surgery." Methods described for: Western blotting (page 6), RT-qPCR (page 6), Immunohistochemistry (page 8), Immunofluorescence (page 8), MTT assay (page 6).

Bias	9	Describe any efforts to address potential sources of bias	4, 8	Exclusion criteria applied to minimize confounding (uncontrolled diabetes, autoimmune diseases). Blinded scoring of IHC by two pathologists (page 8): "Two pathologists independently scored the slides in a blinded manner... If the difference exceeded 2 points, a third senior pathologist conducted a re-evaluation."
Study size	10	Explain how the study size was arrived at	4,	"This study enrolled 83 patients with primary OSCC... Among the 83 tumor tissue samples, 25 with high viability and sufficient volume (≥0.5 cm^3) were selected for primary cell culture." (No formal sample size calculation was performed; all eligible patients during the recruitment period were included.)

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Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8	IHC scoring: "staining intensity (0–3 points) and percentage of positive cells (0–4 points). Composite score ≤4 = low expression; >4 = high expression." Statistical methods: Student's t-test, one-way ANOVA with Tukey's post hoc test, Kaplan-Meier with log-rank test.
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9	"All data were expressed as mean ± SD. Student's t-test was used for two-group comparisons. One-way ANOVA followed by Tukey's post hoc test was used for multiple-group comparisons. Survival analysis was performed using Kaplan-Meier method with log-rank test. $P < 0.05$ was considered significant. GraphPad Prism 8.0 was used."
		(*b*) Describe any methods used to examine subgroups and interactions	9	Subgroup analysis by p65 expression level (low vs. high) was performed for survival analysis (Fig. 5B).
		(*c*) Explain how missing data were addressed	4, 17	Exclusion criterion: incomplete clinical or follow-up data. Discussion mentions limitation: "stable transfection approaches yielded relatively low miR-142-5p expression."
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4, 17	Not applicable (no loss to follow-up reported; all 83 patients completed 80-month follow-up).
		(*e*) Describe any sensitivity analyses	17	Mentioned as limitation: "further validation of its in vivo efficacy and safety in animal models is still required." No formal sensitivity analysis reported.
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4, 11	"83 patients with primary OSCC... Among the 83 tumor tissue samples, 25 with high viability and sufficient volume (≥0.5 cm^3) were selected for primary cell culture, while the remaining samples were used for protein/RNA extraction and histological analysis."

		(b) Give reasons for non-participation at each stage	4	Exclusion criteria applied: "recurrent or metastatic OSCC; concurrent other active malignancies; uncontrolled severe systemic diseases; autoimmune or chronic inflammatory diseases; pregnancy or lactation; incomplete clinical or follow-up data."
		(c) Consider use of a flow diagram	N/A	Not provided (not required by journal)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	14 (Table 2)	Table 2: Clinicopathological characteristics (gender, age, histological grade, BMI, smoking history, alcohol history, primary site, treatment modality, TNM stage) stratified by p65 expression level.
		(b) Indicate number of participants with missing data for each variable of interest	4, 14	"Exclusion criteria: incomplete clinical or follow-up data." Table 2 shows complete data (n=83 for all variables).
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	11, 17	"All patients were followed up for 80 months, with a median follow-up time of (65.37 ± 12.63) months."
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	13 (Fig. 5B), 17	"All patients were followed up for 80 months, with a median follow-up time of (65.37 ± 12.63) months. Kaplan-Meier survival analysis revealed that the overall survival rate at 80 months was higher in the p65 low-expression group (88.06%, 59/67) than in the p65 high-expression group (43.75%, 7/16) (Fig. 5B, $P < 0.01$)."
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A	Not applicable. This study is not a case-control study. It is a cross-sectional study with a cohort follow-up component. No case-control matching was performed.
		Cross-sectional study—Report numbers of outcome events or summary measures	10-13 (Fig. 1-4), 13 (Fig. 5A)	Summary measures of outcomes: (1) Western blot: AGEs, RAGE, p65, and pp65 expression significantly elevated in OSCC tumor tissues compared to adjacent normal tissues (Fig. 1A, $P < 0.01$). (2) RT-qPCR: RAGE and p65 mRNA levels upregulated in OSCC tissues (Fig. 1B, $P < 0.01$). (3) Immunofluorescence: p65 nuclear accumulation observed in OSCC tissues, while predominantly cytoplasmic in normal

				tissues (Fig. 1C). (4) miR-142-5p expression: significantly lower in OSCC tissues than normal tissues, with negative correlation to p65 protein levels (Fig. 3B, P < 0.001). (5) Dual-luciferase assay: miR-142-5p directly targets p65 3′-UTR (Fig. 3C, P < 0.01). (6) MTT and colony formation: 3-Hydroxyflavone (20 μM) significantly inhibited Tca8113 cell proliferation and colony formation (Fig. 4A, 4B, P < 0.05). (7) IHC classification: p65 low-expression group: 67 cases (80.7%); p65 high-expression group: 16 cases (19.3%) (Fig. 5A).
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-13, 14	Unadjusted estimates: Fig. 1-5. Table 2 shows no significant differences between p65 high and low expression groups for all variables (all P > 0.05). No adjusted estimates reported.
		(*b*) Report category boundaries when continuous variables were categorized	8	IHC scoring: "composite score ≤4 = low-expression group; score >4 = high-expression group."
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	13	"80-month overall survival rate: 88.06% (low expression) vs. 43.75% (high expression)"

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10-13, 14	Subgroup analysis by p65 expression level (Table 2, Fig. 5B). Correlation analysis between miR-142-5p and p65 (Fig. 3B).
Discussion				
Key results	18	Summarise key results with reference to study objectives	15	"This study systematically elucidates, for the first time, the molecular mechanism underlying 3-Hydroxyflavone-mediated inhibition of OSCC cell proliferation and improvement of patient prognosis through modulation of the miR-142-5p/AGEs/RAGE/NF-κB signaling axis."
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17	"First, our experiments were conducted primarily in OSCC cell lines... stable transfection approaches yielded relatively low miR-142-5p expression... Second, the association between p65 expression and survival outcomes suggests that p65 regulation may be influenced by both cancer cell-intrinsic factors and microenvironmental components."
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-17	Discussion provides interpretation linking findings to existing literature (pancreatic cancer, prostate cancer, gastric cancer). Concludes that 3-Hydroxyflavone represents a novel therapeutic strategy.
Generalisability	21	Discuss the generalisability (external validity) of the study results	17	"Further validation of its in vivo efficacy and safety in animal models is still required... to advance this discovery toward preclinical and clinical translation."
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18	"This research received no external funding."

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.