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	The title is clearly marked: "a retrospective cross-sectional study with in vitro mechanistic validation" clearly distinguishes between the clinical observational part (retrospective cross-sectional study) and the experimental part (in vitro mechanistic validation).
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1	The abstract summarizes the downregulation of miR-185, its association with lymph node metastasis and disease staging, in vitro functional assays, and CTTN-targeted validation. The balance is adequate, but no clear distinction is made between clinical samples and in vitro experiments.
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2-3	The burden of gastric cancer, the role of miR-185 in tumors, and the background of the CTTN and Cdc42-N-WASP signaling pathways have been elucidated.
Objectives	3	State specific objectives, including any prespecified hypotheses	3	Clear hypothesis: miR-185 regulates the Cdc42-N-WASP pathway by targeting CTTN, thereby influencing the malignant phenotype of gastric cancer.
Methods				
Study design	4	Present key elements of study design early in the paper	3	The "Research object" section describes a retrospective cross-sectional study design based on archived tissue samples.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3	The samples were obtained from the Biobank of the Basic Medical College at Jiamusi University; the

				start and end dates of sample collection were not specified.
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	3	Specifically define the inclusion criteria (initial diagnosis, no prior radiotherapy or chemotherapy, complete data) and exclusion criteria (recurrence/metastasis, coexisting other tumors, etc.).
		(*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	3	Cancer and adjacent tissues were paired (58 pairs) and described.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3	Variables such as TNM staging, degree of differentiation, lymph node metastasis, and tumor size were defined; no adjustment for confounding factors was mentioned.
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	*3*	*Pathological diagnosis and TNM staging were performed by the pathology department during surgery, while miR-185 expression was detected via qPCR.*
Bias	9	Describe any efforts to address potential sources of bias	3, 10	Paired adjacent tumor tissues were used as controls; sample size estimation was based on prior studies (effect size d = 0.8); however, measures to control for selection bias and detection bias were not described.
Study size	10	Explain how the study size was arrived at	3	Based on the effect size d = 0.8, α = 0.05, and power = 0.80 reported by Shahabi et al. (2021), a minimum sample size of 26 cases per group was calculated, resulting in a final inclusion of 58 cases (reported).

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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	4, 10	miR-185 was divided into high-expression group and low-expression group based on median values for χ^2 testing; Spearman correlation analysis was employed for continuous/ordinal variables.
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4-5	Described methods including paired t-test, independent samples t-test, one-way ANOVA, Spearman correlation, and χ^2 test; no multivariate regression analysis was performed to control for confounding factors.
		(*b*) Describe any methods used to examine subgroups and interactions	-	Not described
		(*c*) Explain how missing data were addressed	-	Not mentioned
		(*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	4-5	This study was a retrospective cross-sectional study with samples drawn from continuously archived tissues in a biobank. Cancer and adjacent normal tissues were paired samples from the same patient (58 pairs), and this pairing design controlled for confounding factors such as age, gender, and genetic background using intra-individual controls. Samples were collected consecutively, with uniform inclusion/exclusion criteria applied without selective sampling. Data from all 58 patients were complete (no missing values) and no imputation was performed. Due to the small sample size (n=58) and exploratory nature of the study, only univariate analyses (paired tests, Spearman correlation, χ^2 test) were conducted; multivariate regression analysis was not performed. No formal subgroup analyses or sensitivity analyses were carried out.
		(*e*) Describe any sensitivity analyses	-	Not performed
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	3	Only 58 cases were included in the analysis; no screening flowchart or number of exclusions was provided.
		(b) Give reasons for non-participation at each stage	-	Not reported

		(c) Consider use of a flow diagram	-	Not provided
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	3	The study reported the following characteristics: gender (32 males/26 females), age (38–72 years, mean 52.5 ± 9.5 years), TNM stage (Stage I: 12; Stage II: 17; Stage III: 11; Stage IV: 18), degree of differentiation (high/medium-low/un differentiated: 18/19/12/9), and lymph node metastasis (metastasis present in 39 cases).
		(b) Indicate number of participants with missing data for each variable of interest	-	Not reported
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	-	Not applicable
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	-	*Not applicable – The clinical component of this study is a retrospective cross-sectional study, not a cohort study, and lacks follow-up time data.*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	-	*Not applicable – This study is not a case-control study and does not include comparisons between exposure groups (e.g., exposed vs. non-exposed).*
		Cross-sectional study—Report numbers of outcome events or summary measures	*4-6*	*The summary measurements of miR-185 expression in 58 gastric cancer tissues were reported as mean ± standard deviation (SD), with sample sizes (n) and corresponding correlation statistics (correlation coefficient ρ, 95% confidence interval [CI], P-value) presented for each subgroup stratified by clinical-pathological parameters.*
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	4-5	The Spearman correlation coefficient, 95% confidence interval (CI), and P-value were reported; only univariate analysis was performed without multivariate adjustment.
		(*b*) Report category boundaries when continuous variables were categorized	10	Classified into high/low expression groups based on median values (reported)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-	Not applicable

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-	Not reported
Discussion				
Key results	18	Summarise key results with reference to study objectives	13	The key findings have been summarized in the opening and closing paragraphs of the discussion.
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	15	Key limitations include: small sample size (n=58), single-center design, unadjusted for confounding factors with single-factor analysis, absence of in vivo validation despite in vitro experiments, and limited replication trials.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15	Conclusions Caution: "provide a preliminary experimental basis for further research"
Generalisability	21	Discuss the generalisability (external validity) of the study results	15	It was noted that large-scale multicenter studies and in vivo validation are required, but no specific discussion was provided regarding extrapolation.
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	16	List all funding sources (e.g., Heilongjiang Provincial Major Innovation Projects, National Natural Science Foundation of China, etc.); specify the exact role of each sponsor in the research.

*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.