Homeobox proteins are potential biomarkers and therapeutic targets in gastric cancer: a systematic review and meta-analysis

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Research article

Keywords: homeobox proteins, gastric cancer, prognosis, clinicopathological characteristics, meta-analysis

DOI: https://doi.org/10.21203/rs.3.rs-25253/v3

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Abstract

**Background:** An increasing number of studies have described the aberrant expression of homeobox (HOX) proteins in gastric cancer (GC), which is critically associated with the prognosis and clinicopathological characteristics of GC. This study was conducted to investigate the clinical value and potential mechanisms of HOX proteins in GC.

**Methods:** A comprehensive search of PubMed, EMBASE, Web of Science and Cochrane Library was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement. The pooled hazard ratio (HR) with its 95% confidence interval (95% CI) and the pooled odds ratio (OR) with its 95% CI were used to assess the effects of HOX protein expression on the prognosis and clinicopathological features of GC, respectively.

**Results:** Nineteen studies involving 3775 patients were selected for this study. Heterogeneity among HRs of overall survival (OS) was markedly high ($I^2=90.5\%$, $p=0.000$). According to the subgroup analysis, increased expression of HOX proteins in the downregulated subgroup was associated with a good prognosis for patients with GC (pooled HR: 0.46, 95% CI: 0.36-0.59, $I^2=3.1\%$, $p=0.377$), while the overexpression of HOX proteins in the upregulated subgroup correlated with a reduced OS (pooled HR: 2.59, 95% CI: 1.79-3.74, $I^2=73.5\%$, $p=0.000$). The aberrant expression of HOX proteins was crucially related to the TNM stage, depth of tumour invasion, tumour size, lymph node metastasis, distant metastasis, vascular invasion, histological differentiation and Lauren classification in patients with GC. In addition, the molecular mechanisms by which HOX proteins regulate the tumorigenesis and development of GC were also explored.

**Conclusions:** HOX proteins play vital roles in GC progression and might serve as prognostic markers for GC. Novel therapeutic strategies targeting HOX proteins are promising for GC prevention and therapy.

Background

Gastric cancer (GC) is one of the most common cancers. Although the incidence of GC is decreasing, it remains the sixth most common malignancy and accounts for 8.2% of global cancer-related deaths, according to the most recent global cancer statistics reported in 2018 (1). GC is highly heterogeneous, and patients with GC are usually diagnosed at advanced stages. Despite significant developments in surgical techniques and adjuvant therapy, the overall prognosis of patients with GC is still poor. Therefore, novel molecular markers of GC that are exploitable for predicting the prognosis and effectiveness of therapy must be identified.

Homeobox (HOX) genes encode a highly conserved family of 39 transcription factors that are divided into four clusters (HOXA, HOXB, HOXC, and HOXD). HOX proteins play crucial roles in the early development of embryos and organs, including vertebrae, external genitalia, and the gastrointestinal tract and central nervous system (2). Because embryogenesis and tumorigenesis share similar characteristics, such as growth and differentiation, the deregulation of HOX proteins has been observed in abnormal development and malignancy (3). HOX proteins function as oncogenes or tumour suppressors, depending on the context (4). An increasing number of HOX proteins have been investigated in various tumours, including acute myeloid leukaemia (5), breast cancer (6), lung cancer (7), and digestive tract neoplasms (8-10). Currently, diverse HOX proteins have been implicated in the carcinogenesis and development of GC. Nevertheless, the roles of HOX proteins in GC remain controversial.

Therefore, considering the inconsistent results of current findings, we conducted this systematic review and meta-analysis to explore the prognostic and clinicopathological value of HOX proteins in GC and summarized the molecular mechanisms by which HOX proteins regulate tumorigenesis and development of GC.

Methods

This study was conducted according to the PRISMA guidelines (11).

Search strategies

Two researchers (XJ and LD) independently performed a comprehensive literature search of PubMed, EMBASE, Web of Science and Cochrane Library through March 6, 2020. The following MeSH terms and text words were used in combination: "genes, homeobox" or "gene, homeobox" or "homeobox gene" or "homeobox genes" or "genes, homeotic" or "gene, homeotic" or "homeotic gene" or "hox genes" or "gene, hox" or "genes, hox" or "hox gene" or "genes, homeo box" or "gene, homeo box" or "homeo box gene" or "homeo box genes" or "homeotic genes" or "homeo box sequence" or "homeo box sequences" or "sequences, homeo box" or "homeo boxes" or "sequence, homeo box" or "homeo box sequence" or "homeobox sequences" or "sequence, homeobox" or "sequences, homeobox" or "homeoboxes" or "homeo box" or "homeo box" or "hoxa1" or "hoxa5" or "hoxa9" or "hoxa10" or "hoxa11" or "hoxa13" or "hoxb5" or "hoxb7" or "hoxb8" or "hoxb9" or "hoxb13" or "hoxc6" or "hoxc9" or "hoxc10" or "hoxd4" or "hoxd9" or "hoxd10" or "hoxd13" or "stomach neoplasms" or "neoplasm, stomach" or "stomach neoplasm" or "neoplasms, stomach" or "gastric neoplasms" or "gastric neoplasm" or "neoplasm, gastric" or "neoplasms, gastric" or "cancer of stomach" or "stomach cancers" or "gastric cancer" or "cancer, gastric" or "cancers, gastric" or "gastric cancers" or "stomach cancer" or "cancer, stomach" or "stomach cancers" or...
cancers, stomach" or "cancer of the stomach" or "gastric cancer, familial diffuse". The references of eligible studies in this field were also searched manually. Two investigators (XJ and LD) reviewed the titles and abstracts of studies and retrieved studies that met the inclusion criteria for full-text evaluation.

Selection criteria

All authors jointly determined the selection criteria. The following inclusion were established: (1) GC was pathologically and histologically confirmed; (2) HOX protein expression was detected using immunohistochemical (IHC) staining in GC tissues and paired noncancerous mucosa; (3) studies evaluated the correlation between HOX protein expression and the outcome of GC; and (4) studies supplied sufficient information for calculating the HR with its 95% CI for survival and the OR with its 95% CI for clinicopathological parameters. The following exclusion criteria were used: (1) overlapping or duplicate data; (2) reviews, letters, case reports, conference abstracts, retracted articles, editorials, and full texts not published in English; (3) studies of cancer cells, animal models or irrelevant studies; and (4) studies without adequate information for calculating HRs and 95% CIs.

Quality assessment

Two researchers (YM and JW) assessed the quality of studies using the Newcastle-Ottawa Quality Assessment Scale (NOS) (12). The NOS consists of three aspects of evaluation: selection, comparability and outcomes between the case group and control group. Studies that scored ≥6 points were considered high quality. Any disagreement was resolved by discussion.

Data extraction

Two investigators (XJ and LD) independently reviewed all included studies and extracted the available data. The following information was collected: (1) publication details, including the first author’s name, publication year and origin of the studied population; (2) characteristics of the studied population, including HRs with 95% CIs and clinicopathological features. In the studies that reported HRs from both univariate and multivariate models, we extracted the HR from the latter model that had been adjusted for potential confounders. If the HR was not reported, it was extrapolated using Engauge Digitizer v.11.1 software, Tierney’s spreadsheet (13), and (3) a cut-off value.

Statistical analysis

HR and its 95% CI from each study were used to calculate the pooled HR and its 95% CI. The heterogeneity of the combined HR was determined using Cochran’s Q test and Higgins’ I-squared statistics. A p value<0.05 was considered significant. We used the random effects model if heterogeneity was observed ($I^2 \geq 50\%$). The fixed effects model was used in the absence of heterogeneity ($I^2 < 50\%$) (14). A subgroup analysis was conducted based on the expression level of HOX proteins in patients with GC. The sensitivity analysis was conducted to evaluate the stability of the results, after excluding each study. Publication bias was assessed using the Begg’s funnel plots and Egger’s tests, and a p value<0.05 was considered significant. Statistical analyses were performed using Stata statistical software (version 15.0).

Results

Literature search

Our search strategy preliminarily identified 329 potential records. One hundred seventy-three articles remained after the removal of duplicated studies. Forty-eight of these studies were removed after perusing the titles and abstracts. Then, reviews, editorials, letters, conference abstracts, retracted articles, full texts not published in English, and studies of cancer cells or animal models were excluded. Subsequently, 18 studies lacking insufficient data were rejected. Finally, 19 studies including 3775 patients with GC were included in this analysis. The selection process is shown in Fig. 1.

Study characteristics

All included studies were conducted in China, Japan and Korea and were published between 2012 and 2019. These studies involve the following HOX proteins: HOXB9 (15), HOXD10 (16), HOXA5 (17, 18), HOXA10 (19-21), HOXA13 (22, 23), HOXC6 (24), HOXB7 (25, 26), HOXA1 (27), HOXA9 (28), HOXC9 (29), HOXC10 (30), HOXD4 (31), HOXA11 (32) and HOXD9 (33). These studies explored the prognostic value of HOX protein expression for determining OS or disease-free survival (DFS) and the correlation between the expression of HOX proteins and clinicopathological characteristics of patients with GC. HOX expression at the protein level was detected using immunohistochemical staining. All included studies divided HOX protein expression into high (positive) and low (negative) groups, but the cut-off value was slightly different among these studies. A detailed description of the characteristics of the included studies is provided in Table 1.

Correlation of HOX protein expression with the prognosis
This meta-analysis included a total of 19 articles containing 14 HOX proteins. HOXB9, HOXD10 and HOXA5 were expressed at low levels in GC and acted as tumour suppressors. In contrast, HOXA13, HOXC6, HOXB7, HOXA1, HOXC9, HOXC10, HOXD4, HOXA11 and HOXD9 were expressed at high levels and functioned as tumour promoters in patients with GC. In addition, HOXA10 expression was increased in GC, but its role in modulating the prognosis of GC was controversial. In a pooled analysis including all studies with data on prognostic effect of HOX proteins in GC, considerable heterogeneity among pooled HRs for OS was observed. A subgroup analysis stratified by the expression level was performed, and the results revealed different trends between the downregulated subgroup and the upregulated subgroup. High expression of HOX proteins in the downregulated subgroup was associated with a good prognosis for patients with GC (pooled HR: 0.46, 95% CI: 0.36-0.59, I^2=3.1%, p=0.377), while the overexpression of HOX proteins in the upregulated subgroup correlated with a shorter OS (pooled HR: 2.59, 95% CI: 1.79-3.74, I^2=73.5%, p=0.000) (Fig. 2A). The explanation for the high level of heterogeneity of the upregulated subgroup might be that HOXA10 had different prognostic values in the existing studies. The result of the analysis of the upregulated subgroup after excluding HOXA10 suggested that overexpressed HOX proteins significantly indicated a poor prognosis (pooled HR=3.03, 95% CI: 2.45-3.74, I^2=16.5%, p=0.283) (Fig. 3). DFS was reported in 6 studies analysing 5 HOX proteins. HOX5 expression was associated with an increased DFS in patients with GC (pooled HR=0.46, 95% CI: 0.23-0.91). In contrast, HOXA13, HOXA10, HOXB7 and HOXA1 expression was associated with a decreased DFS in patients with GC (pooled HR=3.77, 95% CI: 2.61-5.45) (Fig. 2B).

**Correlation of HOX protein expression with clinicopathological characteristics**

Seventeen studies with 2899 patients were included to detect the relationship between HOX protein expression and GC tumour stages. As shown in Fig. 4A, increased expression of HOXB9 and HOXD10 was significantly correlated with a lower TNM stage (HOXB9: OR=0.22, 95% CI: 0.12-0.41, HOXD10: OR=0.21, 95% CI:0.14-0.31), while increased expression of HOXA13, HOXB7, HOXA1, HOXA9, HOXC9, HOXC10, HOXA11 and HOXD9 was notably associated with an advanced TNM stage (I^2=92.6%, p=0.000). Due to the high level of heterogeneity, we performed a subgroup analysis based on the expression levels of HOX proteins. The heterogeneity of the upregulated group was decreased but still at a high level (I^2=75.8%, p=0.000) (Fig. 4B). A subsequent analysis showed that this study of HOXA10 contributed a considerable amount of heterogeneity (data not shown). In addition, the inconsistency of the scoring systems regarding HOX protein expression levels in the included studies was also one of the main sources of heterogeneity. The pooled analysis of the relationship between HOX protein expression and the depth of tumour invasion showed that HOXD10 indicated a low T category (HOXD10: OR=0.20, 95% CI: 0.09-0.41), while HOXA13, HOXC6, HOXB7 and HOXA1 were related to a high T category (HOXA13 (2013): OR=4.18, 95% CI: 1.75-10.01; HOXA13 (2018): OR=1.90, 95% CI: 1.08-3.35; HOXC6: OR=3.55, 95% CI: 1.11-11.31; HOXB7 (2015): OR=3.44, 95% CI: 1.32-8.95; HOXB7 (2017): OR=10.14, 95% CI: 4.36-23.58; and HOXA1: OR=2.03, 95% CI: 1.18-4.38) (Fig. 5A). We pooled 11 studies including 2087 patients and found that HOXD10, HOX5 and HOXC10 were associated with a small tumour size (HOXD10: OR=0.37, 95% CI: 0.25-0.54; HOX5 (2018): OR=0.20, 95% CI: 0.07-0.55; HOX5 (2019): OR=0.23, 95% CI: 0.08-0.67; and HOXC10: OR=0.38, 95% CI: 0.15-0.98), while the overexpression of HOXA10, HOXB7 and HOXD4 was associated with an increased tumour size (HOXA10 (2015): OR=2.39, 95% CI: 1.40-4.09; HOXB7 (2017): OR=2.60, 95% CI: 1.61-4.20; and HOXD4: OR=2.71, 95% CI: 1.28-5.74) (Fig. 5B). Similarly, considering the differences in the expression of different HOX proteins in GC, the heterogeneity was significantly reduced by conducting a subgroup analysis of the HOX protein expression level (Fig. 5C). Sixteen studies with 3509 patients reported that HOXB9 and HOXD10 were factors predicting unfavourable lymph node metastasis in patients with GC (HOXB9: OR=0.35, 95% CI: 0.19-0.63 and HOXD10: OR=0.24, 95% CI: 0.16-0.37), and the overexpression of HOXA13, HOXA9, HOXC10, HOXD4 and HOXD9 correlated with the presence of lymph node metastasis (HOXA13 (2013): OR=2.38, 95% CI: 1:02-5.54; HOXA13 (2018): OR=2.38, 95% CI: 1.39-4.09; HOXA1: OR=2.45, 95% CI: 1.49-4.04; HOXA9: OR=2.68, 95% CI: 1.23-5.83; HOXC10: OR=6.18, 95% CI: 2.22-17.18; HOXD4: OR=5.53, 95% CI: 2.55-12.02; and HOXD9: OR=23.11, 95% CI: 6.04-88.49) (Fig. 6A). The results of the pooled analysis revealed that HOXD10 was not conducive to the distant metastasis of GC (HOXD10: OR=0.34, 95% CI: 0.19-0.60), but that HOXC10 and HOX111 promoted distant metastasis of GC (HOX111: OR=5.55, 95% CI: 1.42-21.61 and HOX111: OR=19.02, 95% CI: 1.07-337.91) (Fig. 6B). In addition, the upregulation of HOXB7 promoted vascular invasion in patients with GC (HOXB7 (2017): OR=5.12, 95% CI: 3.18-8.23) (Fig. 6C). Moreover, HOXB9, HOXD10, HOX5 and HOXC9 were factors contributing to good or moderate histological differentiation (HOXB9: OR=0.17, 95% CI: 0.09-0.33; HOXD10: OR=0.66, 95% CI: 0.44-0.99, HOX5 (2018): OR=0.26, 95% CI: 0.10-0.68; and HOXC9: OR=0.28, 95% CI: 0.11-0.71), and overexpression of HOX13, HOX1A, HOX9 and HOX9 was related to a poorly differentiated status of GC (HOX9 (2013): OR=2.41, 95% CI: 1.02-5.67; HOX13 (2018): OR=1.84, 95% CI: 1.06-3.18; HOX1A: OR=2.37, 95% CI: 1.41-4.00; HOX9: OR=4.98, 95% CI: 2.12 11.70; and HOX9: OR=14.63, 95% CI: 4.81-44.43) (Fig. 7A). Additionally, HOXD10 and HOXB7 correlated with the intestinal phenotype of GC (HOXD10: OR=5.02, 95% CI: 3.34-7.57 and HOXB7 (2017): OR=6.27, 95% CI: 3.81-10.31) (Fig. 7B). None of the HOX proteins included in the pooled analysis exhibited significant associations with the age (Fig. 8A), sex (Fig. 8B) or tumour location (Fig. 8C). Additionally, the relationships between HOX5, HOXA10, HOX111 and HOXB7 expression and clinicopathological characteristics were all explored in more than one study. As shown in Fig. 9, HOX5 expression predicted a small tumour size (OR=0.22, 95% CI: 0.10-0.45) (Fig. 9A). A correlation between HOX10 expression and clinicopathological features was not observed (Fig. 9B). The overexpression of both HOX13 (Fig. 9C) and HOXB7 (Fig. 9D) was significantly associated with advanced tumour stages (HOX13 (2013): OR=2.31, 95% CI: 1.44-3.71 and HOXB7: OR=3.48, 95% CI: 2.28-5.32) and high T categories (HOX13: OR=2.62, 95% CI: 1.23-5.60 and HOXB7: OR=6.05, 95% CI: 2.08-17.57), and HOX13 was also related to lymph node metastasis (OR=2.38, 95% CI: 1.51-3.75) and a poor differentiation status (OR=1.99, 95% CI: 1.25-3.15).
Sensitivity analysis

A sensitivity analysis was performed to verify the robustness of our results. As shown in Fig. 10, the pooled HR was not significantly altered when each study was removed, which confirmed the reliability of overall results for the OS of patients with GC.

Publication bias

Begg’s test and Egger’s test were performed to evaluate publication bias. The results did not reveal substantial publication bias (Fig. 11: Begg’s test: p=0.576, Egger’s test: p=0.166).

Mechanisms by which homeobox proteins regulate gastric cancer

In Table 2 and supplementary Fig. 1, we summarize the molecular mechanisms by which the HOX proteins included in this study modulate carcinogenesis and the development of GC (15-57). HOXB9 inhibits GC progression via the AKT and NF-κB pathways (34). HOXD10 suppresses the migration and invasion of GC cells through insulin-like growth factor binding protein-3 (IGFBP3) and Rhoc-AKT pathway (36, 39). HOXA5 suppresses GC by inhibiting the G1-S transition in cells (17). HOXA13 promotes GC development via the TGF-β, ERK1/2, and MDM2-p53-MRP1 pathways and Wnt/β-catenin signalling (23, 41-43). HOXC6 enhances invasive and metastatic abilities of GC cells by upregulating the expression of MMP9 via activating ERK pathway (45). HOXA1 increases the proliferation of GC cells by upregulating cyclin D1 expression (27). HOXB7 mediates GC cell malignancy by activating the AKT/MAPK signalling, the Src-FAK pathway, the PIK3R3/AKT pathway and the epithelial mesenchymal transition (EMT) (26, 49, 50). The miR-182/HOXA9 axis is implicated in RUNX3-mediated GC development (51). In addition, HOXC9 contributes to GC progression by inducing the EMT, MMP2 expression and stem cell-like properties (29). HOXC10 activates the ATM/NF-κB pathway and the MAPK signalling, functioning as an oncogene in GC (30, 54). HOXD4 increases the proliferation and invasion of GC cells by upregulating c-Myc and cyclin D1 (31). HOXD9 activates RUFY3, increasing the proliferation, migration and invasion of GC cells (33). However, the effects of HOXA10 and HOXA11 on GC carcinogenesis and development are controversial.

Discussion

GC is a leading cause of cancer-related mortality. Currently, radical gastrectomy combined with adjuvant chemotherapy is the most effective treatment for GC. Nevertheless, many patients with GC are usually diagnosed at an advanced stage, and the opportunity for radical surgical resection is lost. Based on the current situation, the identification of factors that are helpful for improving the prediction accuracy and curative effect of therapies for GC is very important. Most of the HOX genes are generally activated and expressed during embryogenesis, and many of these proteins are aberrantly expressed during tumorigenesis. HOX proteins affect GC cell proliferation, invasion, migration, apoptosis and other physiological or pathological processes associated with the prognosis and clinicopathological characteristics, but the results are controversial. We conducted this study to further clarify the effects of HOX proteins on the prognosis and clinicopathological characteristics of GC and describe the molecular mechanisms by which HOX proteins regulate tumorigenesis and the development of GC. The present systematic review and meta-analysis included 19 eligible studies enrolling 3775 patients. In the pooled analysis of the effects of HOX proteins on the GC prognosis, HOXB9, HOXD10 and HOXA5 were correlated with a good prognosis for patients with GC, while HOXA13, HOXC6, HOXB7, HOXA1, HOXC9, HOXC10, HOXD4, HOXA1 and HOXD9 were related to a poor prognosis. However, Kato et al. identified positive HOXB9 expression in GC as a marker of a poor prognosis. Unfortunately, the study by Kato and colleagues was not included in this meta-analysis because of the lack of an analysis of HOXB9 expression in paired noncancerous mucosal tissues (58). The relationship between HOX protein expression and various clinicopathological features of GC were also analysed in this study. The results revealed correlations between the expression of HOX proteins and the TNM stage, T category, tumour size, lymph node metastasis, distant metastasis, vascular invasion, histological differentiation, and Lauren classification in patients with GC. Based on the results of the meta-analysis described above, we speculated that HOX proteins might predict the prognosis of patients with GC, which was also confirmed in each included original study. Therefore, we inferred that the combined detection of the expression of various HOX proteins might provide a novel perspective for predicting the prognosis of patients with GC. Currently, some clinicopathological parameters, such as age, sex, tumour stage, depth of invasion, lymph node metastasis, distant metastasis, and resection margins, have been proven to be prognostic indicators of GC (59, 60). At the same time, several molecules are under investigation as predictors of survival, such as gene mutations, DNA methylation, RNAs, and proteins (61). Regrettably, many studies have only explored the individual relationship between clinicopathological characteristics or molecular markers and the prognosis of patients with GC, although a few studies have established a prognostic model (62). Bria et al. combined clinicopathological parameters (sex, age, Lauren classification, stage, margins, grade, site, size, and resected nodes) with molecular markers (HER2, FHIT, and APC) to construct a risk stratification of GC, establishing a scientific model to determine its prognosis. In addition, the authors conducted a large prospective validation with a larger sample size to eliminate all sources of bias in the retrospective study (63). GC is highly heterogeneous, and even similar clinical and pathological features result in different outcomes, suggesting that a more reasonable classification system is needed for predicting the GC prognosis and effectiveness of therapy. A novel classification system with four molecular subtypes was developed by The Cancer Genome Atlas (TCGA) (64). Sohn et al. developed a scoring system (TCGA risk score) based on TCGA to predict the prognosis and adjuvant chemotherapy outcomes in patients with GC, which was validated as an independent prognostic factor for GC in multivariate Cox regression analyses (65). Analogously, Lin
et al. established a novel prognosis scoring system based on TCGA and Gene Expression Omnibus to predict the prognosis of patients with GC, which comprised signatures of tumour protein-coding genes (P), tumour noncoding genes (N) and immune/stroma cells in the tumour microenvironment (M) (PMC score). Furthermore, the combination of PNM scores with American Joint Committee on Cancer (AJCC) staging significantly increased its predictive value (66). In addition, Tahara et al. investigated the prognosis and clinicopathological characteristics of GC by combining genetic and epigenetic abnormalities. The CpG island methylator phenotype (CIMP) and TP53 hot spot mutation status (R175, G245, R248, R273, and R282) were sufficient to predict the prognosis and clinicopathological features of GC. Among these features, patients with the CIMP-TP53 hot spot+ subtype presented the worst overall survival (67). Moreover, Ooi et al. selected three oncogenic pathways (NF-κB, Wnt/β-catenin, and proliferation/stem cells) by analysing a GC pathway heatmap and combined them to predict its prognosis, which was validated in vitro (68). The development of GC is determined by both genetic and environmental factors, which has been confirmed in mouse models. Microbial infections, particularly Helicobacter pylori and Epstein-Barr virus, are one of the most important environmental factors and have been confirmed as a prognostic factor for GC (69, 70). Although H. pylori infection is the strongest risk factor for GC, very few H. pylori-infected populations develop GC. This outcome is attributed to the duration of infection, strain type and host genetic signatures (71). The crucial effects of genetic factors on GC development have been revealed using progress in genetic technology, including the construction of genetically engineered mice via recombinant DNA technology to achieve molecular overexpression or deficiency, as well as gene mutations, clarifying the pathogenesis of GC and the interactions between various factors. For example, INS-GAS transgenic mice on the FVB genetic background that overexpress gastrin develop intramucosal carcinomas with submucosal and intravascular invasion in less than 1 year when infected by Helicobacter felis (H. felis) or H. pylori, with males showing a higher prevalence than females, indicating sex differences in GC tumorigenesis (72, 73). However, INS-GAS mice on a C57BL/6 background infected with H. felis do not progress to GC (74). Surprisingly, gastrin knockout mice (GAS -/- mice) are also confirmed to be susceptible to GC and exhibit antral GC, in contrast to INS-GAS mice, which develop corpus cancers (75). Moreover, GAS -/- mice are more susceptible to antral cancer induced by MNU, a gastric carcinogen used in mouse models, compared to WT mice on the same genetic background (76). Taken together, these studies reveal important roles of genetic signatures in the development of gastric cancer, and external factors such as infection are also indispensable. Thus, the establishment of a comprehensive and detailed scoring system containing the most basic clinicopathological parameters, molecular markers, gene expression profiles, microbial infections, etc., might be more accurate in predicting the prognosis of patients with GC than a single factor. Our manuscript analysed the effects of various HOX proteins in GC development aimed to predict the prognosis and provide therapeutic targets for GC. The results of this meta-analysis recommend the inclusion of HOX proteins in the model predicting the prognosis of patients with GC. Several limitations of this study, further large-scale prospective and high-quality studies are required to confirm the potential value of HOX proteins in GC.

**Conclusions**

This systematic review and meta-analysis firstly generalized and evaluated the significance of HOX proteins in modulating the prognosis and clinicopathological characteristics of GC. We also summarized the molecular mechanisms by which HOX proteins mediate tumorigenesis and the development of GC. Based on these finding, HOX proteins might serve as biomarkers and therapeutic targets for GC. Considering the limitations of this study, further large-scale prospective and high-quality studies are required to confirm the potential value of HOX proteins in GC.

**Abbreviations**

AJCC American Joint Committee on Cancer

AKT protein kinase B

ATM ataxia telangiectasia mutated

CI confidence interval

CIMP CpG island methylator phenotype

DFS disease-free survival

EMT epithelial mesenchymal transition

ERK extracellular regulated protein kinases
FAK focal adhesion kinase
GC gastric cancer
*H. felis* helicobacter felis
HOX homeobox
HR hazard ratio
IGFBP3 insulin-like growth factor binding protein-3
IHC immunohistochemical
MAPK mitogen-activated protein kinase
MDM2 murine double minute 2
MRP1 multidrug resistance-associated protein 1
NF-κB nuclear factor-kappa B
NOS Newcastle-Ottawa Quality Assessment Scale
OR odds ratio
OS overall survival
PIK3R3 phosphoinositide-3-kinase, regulatory subunit 3
RhoC ras superfamily of GTP-binding protein
RUFY3 RUN and FYVE domain containing 3
TGF-β transforming growth factor-β

**Declarations**

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Availability of data and materials**
All data generated or analysed in this study are included in this published article.

**Competing interests**
The authors declare no conflict of interests.

**Funding**
Not applicable.

**Authors’ contributions**
XJ designed the research, searched the literatures, extracted and analysed the data, and wrote the manuscript. LD searched the literatures, extracted and analysed the data. YM and JW conducted literatures quality assessment. XJ, LD, YM, JW, HY, YJ, XZ and ZL established selection criteria. All authors read, reviewed and approved the final manuscript.

**Acknowledgements**
We would like to thank all researchers for their contributions to this study.

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### Tables

| First author | Year | Country | HOX protein | Expression | Sample size (high/low) | Cut-off value | Survival | Survival analysis | HR availability | NOS score |
|--------------|------|---------|-------------|------------|------------------------|---------------|----------|-------------------|----------------|-----------|
| Sha et al (15) | 2013 | China | HOXB9 | Downregulated | 190(86/104) | IS:4 | OS | U, M | Text | 7 |
| Wang et al (16) | 2015 | China | HOXD10 | Downregulated | 436(242/194) | IS:4 | OS | U | Kaplan-Meier curves | 7 |
| Peng et al (17) | 2018 | China | HOXA5 | Downregulated | 81(29/52) | IS:4 | OS | U, M | Text | 8 |
| Wu et al (18) | 2019 | China | HOXA5 | Downregulated | 124(60/64) | median value | OS, DFS | U | Kaplan-Meier curves | 7 |
| Sentani et al (19) | 2012 | Japan | HOXA10 | Upregulated | 749(221/528) | percentage of stained cancer cells=10% | OS | U, M | Text | 7 |
| Han et al (22) | 2013 | China | HOXA13 | Upregulated | 132(103/29) | IS:3 | OS, DFS | U, M | Text | 7 |
| Lim et al (20) | 2013 | Korea | HOXA10 | Upregulated | 57(29/28) | compare to the staining smooth muscle | OS | U | Kaplan-Meier curves | 7 |
| Zhang et al (24) | 2013 | China | HOXC6 | Upregulated | 161(76/85) | IS:2 | OS | U | Kaplan-Meier curves | 8 |
| Han et al (21) | 2015 | China | HOXA10 | Upregulated | 264(159/105) | IS:2 | OS, DFS | U, M | Text | 6 |
| Tu et al (25) | 2015 | China | HOXB7 | Upregulated | 96(66/30) | IS:2 | OS, DFS | U, M | Text | 8 |
| Yuan et al (27) | 2016 | China | HOXA1 | Upregulated | 264(144/120) | IS:3 | OS, DFS | U | Text | 6 |
| He et al (26) | 2017 | China | HOXB7 | Upregulated | 330(195/135) | IS:4 | OS | U | Kaplan-Meier curves | 8 |
| Ma et al (28) | 2017 | China | HOXA9 | Upregulated | 128(88/40) | IS:4 | OS | U, M | Text | 8 |
| Han et al (23) | 2018 | China | HOXA13 | Upregulated | 264(186/78) | IS:3 | OS, DFS | U, M | Text | 6 |
| Peng et al (29) | 2018 | China | HOXC9 | Upregulated | 95(68/27) | IS:4 | OS | U, M | Text | 7 |
| Yao et al (30) | 2018 | China | HOXC10 | Upregulated | 73(38/35) | IS:4 | OS | U, M | Text | 7 |
| Liu et al (31) | 2019 | China | HOXD4 | Upregulated | 127(68/59) | IS:7 | OS | U, M | Text | 7 |
| Wang et al (32) | 2019 | China | HOXA11 | Upregulated | 114(58/56) | IOD/Area=0.31 | OS | U, M | Text | 6 |
| Zhu et al (33) | 2019 | China | HOXD9 | Upregulated | 90(55/35) | IS:3 | OS | U | Kaplan-Meier curves | 6 |
Table 1 Characteristics of the included studies

OS: overall survival; DFS: disease free survival; U: univariate analysis; M: multivariate analysis; IS: immunostaining score; IOD: integrated option density; NOS: Newcastle-Ottawa Scale.

| HOX proteins | Expression | Upstream | Downstream | Pathways | Reference |
|--------------|------------|----------|------------|----------|-----------|
| HOXB9        | Down-regulated | NA       | NA         | ↓ cells proliferation, migration and invasion; ↑ MET; AKT and NF-κB pathway | (15,34,35) |
| HOXD10       | Down-regulated | miR-10b, miR-92b-3p | IGFBP3 | ↓ cells proliferation, migration and invasion; AKT pathway; RhoC pathway | (16,36-39) |
| HOXA5        | Down-regulated | miR-196a | NA         | ↓ cells G1-S transition, proliferation and colony formation; ↓ angiogenesis | (17,18) |
| HOXA10       | Up-regulated | NA       | miR-196b-5p, BCL2 | ↑ cells viability, proliferation, colony information, migration and invasion; ↓ apoptosis; ↓ tumor metastasis; JAK1/STAT3 signaling; HOXA10/miR-196b-5p axis; ↓ cells growth, motility and invasive activity; | (19-21,46-48) |
| HOXA13       | Up-regulated | IncRNA HOTTIP, DHRS2, cadherin17 | ↑ cells proliferation, migration and invasion; ↑ EMT; TGF-β pathway, ERK1/2 pathway, Wnt/β-catenin pathway, MDM2-p53-MRP1 pathway; chemotherapy resistance to 5-FU | (22,23,40-43) |
| HOXC6        | Up-regulated | IncRNA HOTAIR | NA | ↑ cells proliferation, colony formation, migration and invasion; ↑ ERK signaling; | (24,44,45) |
| HOXB7        | Up-regulated | NA       | NA         | ↑ cells G1-S transition, proliferation, migration and invasion; ↑ EMT; ↓ apoptosis; AKT/MAPK pathway; Src-FAK pathway; PIK3R3/ AKT pathway | (25,26,49,50) |
| HOXA1        | Up-regulated | NA       | NA         | ↑ cells proliferation, invasion and migration; ↑ cyclin D1 | (27) |
| HOXA9        | Up-regulated | miR-182  | NA         | ↑ cells proliferation, migration and invasion; ↑ tumor progression | (28,51) |
| HOXC9        | Up-regulated | miR-26a  | NA         | ↑ EMT and stem cell-like phenotypic acquisition; ↑ tumor metastasis | (29) |
| HOXC10       | Up-regulated | miR-136  | CST1       | ↑ cells migration and invasion; ↑ tumor growth and peritoneal metastasis; ATM/NF-kB pathway; MAPK signaling | (30,52-55) |
| HOXD4        | Up-regulated | NA       | NA         | ↑ cells proliferation, migration and invasion; ↑ c-Myc and cyclinD1 | (31) |
| HOXA11       | Controversial | STAT3    | STAT3      | Wnt pathway | (32,56,57) |
| HOXD9        | Up-regulated | NA       | RUFY3      | ↑ cells proliferation, invasion and migration; ↑ tumorigenesis and metastasis | (33) |

Table 2 Action mechanisms of HOX proteins in gastric cancer

↓: inhibit; ↑: promote; NA: not available; AKT: protein kinase B; ATM: ataxia telangiectasia mutated; BCL2: B cell lymphoma-2; CST1: cystatin 1; DHRS2: dehydrogenase/reductase 2; ERK: extracellular regulated protein kinases; FAK: focal adhesion kinase; IGFBP3: insulin-like growth factor binding protein-3; JAK1: janus kinase 1; MAPK: mitogen-activated protein kinase; MDM2: murine double minute 2; MET: mesenchymal epithelial transition; MRP1: multidrug resistance-associated protein 1; PIK3R3: phosphoinositide-3-kinase, regulatory subunit 3; RhoC: ras superfamily of GTP-binding protein; Src: steroid receptor coactivator; RUFY3: RUN and FYVE domain containing 3; 5-FU: 5-fluorouracil

Figures
Figure 1

Flow diagram of this systematic review and meta-analysis.
Figure 2

Subgroup analysis of OS (A) or DFS (B) by HOX protein expression in GC.
Figure 3

Subgroup analysis of OS by HOX protein expression in GC (excluded HOXA10).
Figure 4

Forest plots of the pooled analysis for the association between the HOX protein expression and TNM stage (A), TNM stage subgroup analysis (B).
Figure 5

Forest plots of the pooled analysis for the association between the HOX protein expression and T categories (A), tumour size (B), tumour size subgroup analysis (C).
**Figure 6**

Forest plots of the pooled analysis for the association between the HOX protein expression and lymph node metastasis (A), distant metastasis (B), vascular invasion (C).
### Figure 7

Forest plots of the pooled analysis for the association between the HOX protein expression and histologic differentiation (A), Lauren classification (B).
Figure 8

Forest plots of the pooled analysis for the association between the HOX proteins expression and age (A), sex (B), tumour location (C).
Figure 9

Forest plots of the pooled analysis for the association between HOX protein expression and clinicopathological characteristics: HOXA5 (A), HOXA10 (B), HOXA13 (C), HOXB7 (D).
Figure 10

Sensitivity analysis of the included studies on OS.
Figure 11

Tests for publication bias of OS: Begg's test (A), Egger's test (B).

Supplementary Files

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