The Role of *HOX* Genes as Potential Biomarkers in Colorectal Cancer: A Systematic Review

Eirini Martinou (em01023@surrey.ac.uk)
University of Surrey Faculty of Health and Medical Sciences https://orcid.org/0000-0002-7481-5494
Giulia Falgari
University of Surrey Faculty of Health and Medical Sciences
Angeliki Angelidi
Beth Israel Deaconess Medical Center
Izhar Bagwan
Royal Surrey NHS Foundation Trust

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Abstract

Background: Colorectal cancer (CRC) is worldwide the third leading cause of cancer-related death, and despite therapeutic advances, survival remains low. Emerging evidence shows that Homeobox (*HOX*) genes are important in carcinogenesis, and their dysregulation has been linked with metastatic potential and poor prognosis. This systematic review aims to present the current evidence on the role of *HOX* genes as biomarkers in CRC and the impact of their modulation in tumour growth and progression.

Methods: MEDLINE, EMBASE, Web of Science and Cochrane databases were searched by following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement. Eligible studies investigated two research questions: a) the clinicopathological and prognostic significance of *HOX* gene dysregulation in patients with CRC and b) the functional role of *HOX* genes in CRC progression. This study was registered in the international prospective register of systematic reviews (PROSPERO), CRD42020190953.

Results: Twenty-five studies enrolling 3003 patients with stage I-IV CRC, showed that 26 out of 39 *HOX* genes were dysregulated in cancerous versus normal colon. Aberrant expression of HOX proteins was significantly related to tumour depth, nodal invasion, distant metastases, advanced stage and poor prognosis. Twenty-two preclinical studies showed that HOX proteins are crucially related to tumour growth and metastatic potential by affecting cell proliferation and altering the expression of epithelial-mesenchymal transition modulators.

Conclusions: In conclusion, our findings suggest that HOX proteins play vital roles in CRC progression and significantly affect survival. Further research, though, is required to elucidate their potential role as biomarkers in CRC.

Introduction

Colorectal Cancer (CRC) is the most common gastrointestinal malignancy and the third worldwide leading cause of cancer-related death[1,2]. Despite significant advances in diagnostic and therapeutic strategies, the prognosis for CRC patients remains poor, indicating that cancerous cells are not entirely eradicated by current therapies, thus leading to metastatic disease which is the primary cause of cancer-related mortality[3]. CRC arises as a result of the accumulation of genetic and epigenetic changes, which transform normal glandular epithelial cells into invasive carcinomas and eventually progress into metastatic disease[4]. Colorectal carcinogenesis is a complex multistep process involving the dysregulation of oncogenes or tumour suppressor genes related to initiation, progression and resistance to therapy[4,5]. Therefore, there is an urgent need to identify novel biomarkers which could be used to predict prognosis and act as therapeutic targets.

Since the last decades, a lot of attention has been paid to the role of homeobox [*HOX*] genes in cancer[6]. *HOX* genes encode a highly conserved family of homeodomain-containing transcription factors which play essential roles in embryonic development including morphogenesis, organogenesis and differentiation[7]. HOX proteins control various cellular processes by regulating the expression of several downstream target genes; hence they can alter cell behaviour such as proliferation, invasion and migration[7]. The human genome contains 39 *HOX* genes which are classified into four clusters, *HOXA*, *HOXB*, *HOXC* and *HOXD* based on their sequence similarity and chromosomal position, [figure 1][8]. Dysregulation in *HOX* gene expression leads to developmental abnormalities and is associated with an increased incidence of malignant tumours in humans[9,10]. Emerging evidence shows that *HOX* genes can act either as oncogenes or tumour suppressors depending on cancer type. For instance, *HOXA9* has an oncogenic role in acute leukaemia[11] whereas it acts as a tumour suppressor in breast cancer by regulating the expression of Breast Cancer gene 1 (*BRCA1)*[12]. Additionally, *HOXB9* has been shown to inhibit cancer cell proliferation in gastric carcinoma[13] whilst it demonstrates an oncogenic role in breast cancer[14].

Expression of *HOX* genes is dysregulated and often reported to be associated with adverse clinicopathological characteristics and poor survival in various types of cancers[15–17]. However, their differential expression and their role as potential biomarkers in CRC has not been fully explored. To better understand the existing evidence with regards to the prognostic and functional role of *HOX* genes in CRC, the authors performed a systematic review of the current literature. Specifically, this review aims to answer the following research questions: a) What is the clinicopathological and prognostic significance of *HOX* genes dysregulation in CRC and b) What is the functional role of *HOX* genes in CRC progression.

Methods-materials

Search Strategy

A literature search was conducted for eligible studies in the Medline, EMBASE, Web of Science and Cochrane Database search engines in accordance to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[18]. The end date of the retrieval period was the 1st of July. A search strategy was developed in Medline including keywords, MeSH (Medical Subject Headings) and synonyms related to *HOX* genes, colorectal and neoplasms. The strategy was adapted for the other databases using separate algorithms for each search engine (supplementary material, ESM_1). This systematic review was registered in the international prospective register of systematic reviews (PROSPERO) with identification number CRD42020190953.

Eligibility criteria and study selection

Three reviewers (EM, GF and AA) independently selected and identified eligible English language studies based on predefined inclusion criteria according to the research question. Discrepancies between the three reviewers were resolved by discussion or with a 4th author.

For the first research question, studies conducted on individuals over 18 years old with sporadic colorectal adenocarcinoma and reporting on *HOX* genes were included. Specifically, studies reporting at least one of the following criteria were included: (i) studies on *HOX* dysregulation between cancer and normal tissue, (ii) studies on the association of *HOX* genes with clinicopathological characteristics of CRC, (iii) studies reporting outcome measures such as overall survival (OS) and disease-free survival (DFS). Exclusion criteria were studies reporting the dysregulation of *HOX* genes in cell lines (*in vitro*) or animal tissues (*in vivo*). Comparison groups were selected to be the following: cancer to normal colorectal tissue as well as high versus low *HOX* gene expression patient group. Outcomes were defined to be: *HOX* gene dysregulation, tumour depth, lymph node status, metastases, stage of colorectal cancer disease, grade of disease, disease-free survival (DFS) and overall survival (OS) rates.

For the second research question, laboratory-based and animal research studies were included reporting on the effect of *HOX* gene expression in CRC cell growth. The intervention was considered gene expression editing to either suppress or overexpress the *HOX* gene of interest. The intervention group in the included studies consisted of either human colorectal cancer cell lines or animal models which had an altered expression of *HOX* genes. Therefore, the comparison groups were defined as edited versus non-edited human CRC cell lines or animal models. Outcomes were selected to be cell proliferation, cell migration, cell invasion *in vitro* as well as tumour growth and metastases *in vivo.*

Studies such as case reports, editorials, opinions, conference abstracts, reviews and other secondary research studies were excluded. Studies not using the universal *HOX* chromosomal cluster terminology as described in figure 1 for reporting findings were also excluded for both research questions.

Data Extraction, Synthesis and Quality Assessment

For each study, the following details were extracted on publication year, surname of first author, study design, participant characteristics (sample size, gender), study characteristics (intervention and control group, endpoint assays) and outcomes. The quality of each eligible primary study involving human participants was assessed using the National Heart, Lung and Blood Institute (NIH) study quality assessment tools for case studies, available online https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools, (table S1, ESM_2). Preclinical animal studies were assessed using the Systematic Review Centre for Laboratory animal experimentation risk of bias tool (SYRCLE'S), (table S2, ESM_3)[19,20]. For cell line studies, no established quality assessment tool is currently available[19]. The results were summarised narratively according to each research question using a qualitative data synthesis approach.

Results

Study selection

The initial literature search identified 2548 eligible citations. Following duplicate citation removal 1498 studies were screened for eligibility. For research question 1 and 2, a total of 25 and 22 studies met our inclusion criteria respectively and were included in the final analysis. The process of literature retrieval, according to PRISMA guidelines, is shown in detail in figure 2. Study characteristics and findings are presented for each research question separately.

Clinicopathological and prognostic significance of *HOX* dysregulation in CRC

Study characteristics

A total of 3003 patients with stage I-IV CRC between 20 and 90 years old were included in the studies[21–45] All 25 studies were single-centre and were published between 1997 and 2020. Two studies included patients who either received or not neoadjuvant chemotherapy[24,25], 5 studies included only patients without neoadjuvant chemotherapy[23,26,27,29,42] whereas in the remaining studies no information on the chemotherapy status was available. Differential *HOX* gene expression between cancer and normal tissue was reported by 22 studies[21–23,25–28,30–34,36–45]. The analysis assays were real-time quantitative polymerase chain reaction [RT-qPCR], immunohistochemistry [IHC] or western blot [WB]. Four studies included bioinformatics analysis from publicly available RNA sequencing data[22,33,43,45], table 1.

Seventeen studies investigated the association of *HOX* genes with clinicopathological characteristics in patients with CRC[21,23–33,35,40–42,44], table 1. Variables included at least one of the following: Age, sex, tumour depth, lymph node status, metastases, stage of colorectal cancer disease, grade of disease and carcinoembryonic antigen (CEA) levels.

The impact of HOX expression on OS and DFS in patients with CRC was investigated by 12[21–23,25,28–31,33,42–44] and 4[23,24,33,40] studies, respectively, table 1. OS and DFS rates in patients with high versus low expression of the *HOX* gene of interest were compared. Most of the studies used a semiquantitative IHC approach to score the expression levels of HOX proteins, whereas two studies used the median mRNA level as cut-off value[22,33].

Findings

HOX dysregulation and clinicopathological significance in CRC

Twenty-six out of 39 *HOX* genes were identified in this systematic review to be differentially expressed in cancerous versus normal colon tissues with 15 of them being overexpressed and 6 being downregulated, (table 1). Discrepancies were reported between studies for 5 *HOX* genes (*A4, B8, B9, B13* and *D10*)[26–

30,33,36,37,41–43]. Among the dysregulated *HOX* genes and their protein products, several were found to demonstrate potential clinical significance in CRC. Three studies reported that patients with high HOX (B7, B9, C6) protein expression levels demonstrated significantly advanced T status in comparison with patients with low expression, [24,25,31], (table 1). Similarly, regarding N status, 6 studies showed that the percentage of patients with regional lymph node invasion was significantly increased in the high HOX expression group for the following: HOXA3, A9, B8, B9, B13, C6, D1 and D9[21,25,30,32,41,44]. Inverse correlation between HOX expression and nodal invasion was demonstrated only for HOXD10 by Wang *et al.* [26](table 1). Eight studies investigated the presence of metastatic disease according to HOX expression levels[24,25,27–30,33,44]. Two studies by Liao *et al.*[31] and Huang *et al.*[30] for HOXB7 and B9 respectively, reported that significantly more patients had metastatic disease in the high HOX expression group in comparison with the low expression one, (table 1). Eleven studies have reported findings based on the overall stage of disease and level of HOX expression[21,23,27,29–31,33,35,40,42,44]. For HOXA3, A9, B7 and D9, high expression groups correlated with an increased number of patients with advanced disease in comparison with the low expression groups[21,23,31,44]. For HOXB9 studies by Zhan *et al.*[29] and Huang *et al.*[30] reported equivocal findings, (table 1). Most studies reported no significant association between tumour differentiation and HOX expression level, except for D9 where Liu *et al.* reported that high HOXD9 levels were significantly associated to poor differentiation[21]. Studies by Hoshino *et al.*[28] and Zhan *et al.*[29] have reported contradictory results regarding HOXB9 expression levels and its association with CRC differentiation, (table 1). Given the limited available data, it appears that there is no significant association between age, sex and CEA with HOX expression levels.

HOX dysregulation and prognostic significance in CRC

The HOX prognostic role (A3, B7, B8, C6, C11, D9, D10) was investigated by 14 studies[21–25,28–31,33,40,42–44]. Specifically, patients who were characterised by high HOX expression levels had significantly worse survival rates in comparison with the ones with low expression levels. Only two studies by Ji *et al.*[25] and Liao *et al.*[31] conducted multivariate analysis and reported that HOXC6 and HOXB7 are independent prognostic markers in patients with CRC, (table 1). HOXB9 was investigated by 4 studies which reported contradictory results with regards to its positive or negative impact on survival[28–30,42]. In terms of DFS, studies showed that high expression levels of HOXA3, A10, B8 and B9 were significantly associated with worse DFS rates in patients with CRC[23,24,33,40]. HOXA10 and B9 were additionally reported to serve as independent risk factors for worse DFS by Yuan *et al.*[40] and Carbone *et al.*[24] respectively in a multivariate analysis model, (table 1).

Functional role of *HOX* genes in CRC progression

Study characteristics

Twenty-two studies investigated the functional role of *HOX* genes dysregulation in CRC progression[21–23,25,28–31,33,36,39,40,44–53]. All studies were preclinical with 11 having conducted *in vivo* experiments as well as *in vitro.* All *in vitro* experimental studies used various human colorectal cell lines to conduct gain and/or loss of function experiments by altering the gene expression level of the *HOX* gene of interest. *In vivo* studies used nude mice which were subjected to subcutaneous injection of human CRC cell lines with altered or not *HOX* expression level[21,23,25,28–31,33,40,52,53].

Twenty studies investigated the impact of *HOX* genes dysregulation on tumour growth, 8 of which performed additional *in vivo* experiments[21–23,25,28,29,31,33,36,39,40,44–51,53]. The *in vitro* primary outcome as cell proliferation rate over time being measured by relevant assays, (table 2). The *in vivo* primary outcome was tumour growth which was assessed differently in each study by reporting either tumour weight (gr), size (diameter in cm) or volume (mm^3), (table 2).

Ten studies investigated the effect of *HOX* genes differential expression in the metastatic potential in CRC[21,29,30,33,39,45–47,49,52]. Primary outcomes were the percentage of cells that showed invasion and/or migration in the relevant assays between the control versus the intervention group. Secondary outcomes were molecular markers involved in the Epithelial-Mesenchymal Transition (EMT) being reported by 6 studies[21,30,39,45,49,52]. Five studies provided additional results from *in vivo* experiments by assessing the number of lung/liver metastases as primary outcome[21,29,30,33,52], (table 2). One study reported markers involved in angiogenesis and vessel formation *in vivo*[28].

Findings

HOX dysregulation and tumour growth in CRC

Out of the 18 *HOX* genes and their protein products having been investigated to date, 15 were found to have oncogenic properties whereas only 3 were reported to exert tumour suppressive functions. Specifically, loss of function *in vitro* studies showed that knockdown of *HOXA1, A3, A4, A9, A10, B8, C6, C11, C13* and *D3* resulted in reduced proliferation rate of CRC cells[22,23,25,33,40,44,47,48,50,51]. Additionally, overexpression *in vitro* experiments showed that increased levels of *HOXA6, B2, B7* and *D9* resulted in increased proliferation rates indicating the tumour promoting properties of the above gene products[21,31,39,46]. Findings from *in vivo* studies, which have been conducted for *HOXA3, B7, B8, B13, C6* and *D9*, agreed with the functional role observed *in vitro*[21,23,25,31,33,53]. Findings for *HOXB9* by Hoshino *et al.*[28] and Zhan *et al.*[29] reported contradictory results with the former study reporting tumour promoting and the latter showing tumour suppressive properties in CRC.

HOX dysregulation and metastatic potential in CRC

Nine *HOX* genes and their proteins have been reported to affect CRC disease progression *in vitro*[21,29,30,33,39,45–47,49,52]. Knockdown of *HOXA1* and *HOXB8* resulted in a decreased number of invasive cells[33,47]. Similarly, overexpression of *HOXA6, B2* and *D9* led to a significantly increased number of invasive and migratory cells in the intervention group(21,39,46). On the contrary, overexpression of *HOXA5*, *A10* and *D8* resulted in the decreased number of invasive and migratory cells in the intervention group[45,49,52], (table 2). *In vivo* findings agreed with the *in vitro* results regarding HOXA10 as mice who were injected with cells overexpressing A10 developed fewer metastases than the control group indicating a protective role of HOXA10 in CRC progression[52]. On the other hand, altered expression of HOXB8 and D9 showed the metastatic promoting effect of these genes *in vivo*[21,33]. (table 2). The expression of important EMT markers (E-cadherin and Vimentin) known to increase the invasive behaviour of cancer cells facilitating metastases, was altered between the intervention and control group. Specifically, downregulation of E-cadherin with subsequent upregulation of vimentin was observed in the studies investigating metastases-enhancer *HOX* genes (*A6, D9, B9*)[21,30,39] whereas the opposite pattern was observed for the metastases-suppressors ones (*A5, A10, D8*) [45,49,52], (table 2). Three studies reported findings regarding the role of HOXB9 in CRC progression; however the results are contradictory with Zhan *et al.* showing a metastatic promoting function whereas Huang *et al.* reported a tumour suppressive function[28–30].

Discussion

In recent years with the change in people's lifestyle and dietary factors, the incidence of CRC is increasing, especially in the younger population, making this disease a public health burden[54]. Since recurrence and development of distant metastases are the major causes of cancer-related mortality, it is crucial to investigate and discover new molecular markers that contribute to CRC aggressiveness and which may affect survival. We conducted this systematic review to investigate the clinicopathological and prognostic significance of *HOX* genes in CRC and determine the impact of their altered expression in CRC disease progression.

The present systematic review indicates that *HOX* genes become dysregulated in CRC in comparison with normal tissue and are a diverse group of genes as some may favour disease progression, whereas others act as tumour suppressors in CRC. The combination of clinical and preclinical findings of the studies included revealed that *HOXA3, A9, B7, B8, C6, C11* and *D9* were found to be upregulated in CRC tissues[21–23,25,31,33,44]. Their high expression was correlated with adverse clinicopathological characteristics of CRC and worse survival outcomes suggesting an oncogenic role which was supported by the *in vitro* and *in vivo* experimental observations. On the other hand, *HOXB13* and *HOXD10* were found to be downregulated in CRC, and preclinical studies indicated a protective role towards disease progression[26,36]. Among the dysregulated *HOX* genes, most of them favour an oncogenic behaviour promoting disease progression rather than acting as tumour suppressors. Similar findings with our study were reported by a recent systematic review by Jin *et al.* on *HOX* genes in gastric cancer (GC) which demonstrated diversity in the dysregulation profile of *HOX* genes with most of them acting as potential oncogenes and are associated with worse disease characteristics and worse OS[55]. *HOXB9* was the most frequently investigated gene; however, studies (*n*=5) reported contradictory findings regarding its prognostic role in CRC with Carbone *et al*.[24], Hoshino *et al*.[28] and Huang *et al*.[30] showing a negative association with survival outcomes whereas Song *et al*.[42] and Zhan *et al*.[29] reported a positive one. Similarly, experimental findings were also opposing between studies regarding its tumour promoting or suppressive role, highlighting that *HOX* genes may also have a dual role in CRC progression depending on which mechanism is activated that regulates their function[56,57]. For instance, Wan *et al*. identified the acetylation of HOXB9 as an important post-translational modification which caused suppression of transcription of the HOXB9 target gene Jumonji domain-containing protein 6 (*JMJD6*) leading to inhibition of tumour growth and migration in lung adenocarcinoma, *in vitro* and *in vivo*[58]. HOXB9 acetylation was also shown by Song *et al.* to potentially be responsible for the *HOXB9* potential protective role in CRC progression[42,58].

HOX genes contribute to a plethora of functionalities and can be regulated by transcriptional expression, regulating micro-RNAs and post-translational modifications that add complexity in understanding their role[59]. Therefore, the exact mechanism of how *HOX* genes promote CRC growth, invasion and metastasis has not yet been elucidated. *HOX* genes, as conserved developmental genes, have the ability to control various cellular functions responsible for cell survival and in many cancers seem to participate in cell proliferation(6). In CRC Liao *et al*., showed that *HOXB7* could accelerate the transition from G1 to S phase in cell cycle through the activation of PI3K/AKT and MAPK pathways resulting in upregulation of cyclin D1[31]. Additionally, Zhang *et al.* found that *HOXA3* can serve as apoptosis-suppressor for cancer development through regulation of apoptosis-related factors (Bcl-2, caspase 3) and activation of EGFR/Ras/Raf/MEK/ERK pathway[23]. CRC progression to invasive and metastatic disease is characterised by the EMT process, which involves the transition of the stationary cancerous epithelial cells into motile mesenchymal ones enabling them to detach and metastasise[5]. *HOX* genes have been found to play an essential role in the EMT, promoting cell invasion and migration. In CRC, Liu *et al.* reported that *HOXD9* promoted CRC cell invasion and migration through enhancing EMT by upregulating vimentin while downregulating E-cadherin[21]. This study also showed that *HOXD9* might promote cell invasion and migration through the transforming growth factor-beta (TGF-β) pathway, which is an important pathway in the EMT process in CRC[5].

Angiogenesis plays a vital role in the progression of cancer, and various *HOX* genes have been shown to function in promoting the formation of new vessels in solid tumours by upregulating angiogenic genes[6,60]. In CRC, Hoshino *et al.* showed that overexpression of *HOXB9* resulted in upregulation of angiogenic factors such as interleukin 8 (*IL8*) and vascular endothelial growth factor (*VEGF*) both *in vitro* and *in vivo*[28]. *HOXB9* was also found to be of an important clinical significance as patients with high expression levels appeared to respond better to anti-angiogenic therapy with bevacizumab demonstrating longer OS and DFS in comparison with those who had low *HOXB9* levels[28]. Interestingly, Carbone *et al.* reported the same effect of *HOXB9* in the expression of angiogenic factors as Hoshino *et al*., however *in vivo* models showed that *HOXB9* positive nude mice showed resistance to treatment with bevacizumab[24]. Either way, both studies demonstrate that *HOXB9* could serve as a potential marker for selecting treatment with anti-angiogenic chemotherapeutic drugs. The possible synergistic role of *HOX* genes modulation with chemotherapy treatment was shown by Yuan *et al.* for *HOXA10,* which was found to promote tumour progression *in vitro* and knockdown resulted in increased sensitivity to 5-Fluorouracil therapy *in vitro* and *in vivo*[40].

This systematic review is the first to provide cumulative current evidence regarding the role of *HOX* genes in CRC progression, their clinicopathological and prognostic significance. This study outlines the heterogeneity among studies, as many have only investigated a specific *HOX* gene out of the 39 present in the human genome. Since the use of *HOX* genes as future biomarkers in CRC has recently started to attract research interest, further studies are warranted on the subject to fully explore the function of each *HOX* gene. This systematic review showed that few studies had been conducted to date which combine clinical and preclinical data (*in vitro* and *in vivo*) to thoroughly investigate the clinicopathological and functional role of a *HOX* gene in CRC progression. Moreover, studies demonstrated diversity in the study population characteristics included as well as variability in methodological approaches used. For instance, population characteristics varied between studies in terms of neoadjuvant chemotherapy administration. Furthermore, there was no established standardised cut-off point between high and low expression level and inconsistent criteria were used between studies to investigate the clinicopathological and prognostic role of the *HOX* gene of interest. It is worth highlighting the lack of power sample size reporting in both clinical and preclinical studies, as well as sample size information in some studies. For the reasons which include the diversity between studies, the lack of detailed and robust data a meta-analysis could not be conducted.

In conclusion, this systematic review provides the current cumulative evidence that *HOX* genes are dysregulated in patients with CRC and evaluates the association of their differential expression with the clinicopathological characteristics and prognosis. We have observed that altered expression of *HOX* genes affects CRC progression *in vitro* and *in vivo*. Based on these findings, we propose *HOX* genes have potential as biomarkers or therapeutic targets in CRC[61]. Due to the complexity and heterogeneity of the *HOX* gene family, further well-conducted and even larger-scale or multicentre clinical and preclinical studies with robust methodology are needed to elucidate the role of each gene in CRC thus determining the validity of their role as potential biomarkers or therapeutic targets in CRC.

Abbreviations

AKT: protein kinase B

BRCA1: Breast Cancer gene 1

CEA: Carcinoembryonic antigen

CRC: Colorectal Cancer

DFS: Disease Free Survival

EGFR: Epidermal growth factor receptor

EMT: Epithelial Mesenchymal Transition

ERK: Extracellular signal-regulated kinases

HOX: Homeobox

IL8: Interleukin 8

JMJD6: Jumonji domain-containing protein 6

MAPK: mitogen-activated protein kinase

MEK: Mitogen-activated protein kinase

MeSH: Medical Subject Headings

NIH: National Heart, Lung and Blood Institute

OS: Overall Survival

PI3K: Phosphoinositide 3-kinase

PRISMA: Preferred Reported Items for Systematic Reviews and Meta-analyses

PROSPERO: Prospective Register of Systematic Reviews

RNA: Ribonucleic acid

SYRCLE'S: Systematic Review Centre for Laboratory animal experimentation

Declarations

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Tables

Table 1. Included studies reporting on clinicopathological and prognostic significance of HOX genes in CRC. *: p<0.05, **: p<0.01, ***: p<0.001

Author (year)	Gene	Patients (%M)	Age (ys)	Stage	FUP(m) (max)	Sample	Methods	DE (C vs N)	HOX overexpression association with clinicopathological characteristics (positive or negative)						
									Age	Sex	T	N	M	S	G
Liu *et al* [21] (2020)	*HOXD9*	100 (59%)	NR	I-IV	NR	FFPE	IHC	↑ ***	NS	NS	NS	↑ *	NR	↑ *	↑ ***
Cui *et al* [22] (2019)	*HOXC11*	265 (NR)	NR	NR	NR	NR	Data mining	↑ *	NR	NR	NR	NR	NR	NR	NR
Ying *et al* [22] (2019)	*HOXB8*	80 (59%)	NR	I-IV	120	NR	qRT-PCR	↑ *	NS	NS	NR	↑	NR	↑	NS
		510 (NR)	NR	NR	120	NR	Data mining	↑ **	NS	NS	↑	NS	↑	NS	NS
Wu *et al* [22] (2018)	*HOXA6*	16 (63%)	49-80	NR	NR	NR	qRT-PCR	↑ *	NR	NR	NR	NR	NR	NR	NR
Yuan *et al* [40] (2018)	*HOXA10*	85 (58%)	26-80	II-IV	60	FFPE	IHC	↑ ***	NS	NS	NS	NS	NR	NS	NS
Tatangelo *et al* [41] (2018)	*HOXA13*	82 (54%)	50-91	I-IV	NR	FFPE	IHC	↑	NS	NS	NS	NS	NR	NR	NS
	HOXB13							↑	↑	↑	NS	↑ **	NR		
	HOXC13							↑	NS	NS	NS	↑	NR		
	HOXD13							↑	NS	NS	NS	NS	NR		
Song *et al* [42] (2018)	*AcK27-HOXB9*	90 (51%)	24-90	I-IV	73	FFPE	IHC	↓ ***	↑ *	NS	NS	NS	NR	↓ *	NR
Bhatlekar et al [43] (2018)	*HOXA4, HOXD10*	3 (NR)	NR	NR	NR	FT	qRT-PCR/IHC	↑ (NR)	NR	NR	NR	NR	NR	NR	NR
Watanabe et al [44] (2017)	*HOXA9*	231 (58.9%)	NR	I-IV	100	FT FFPE	qRT-PCR/IHC	↑ ***	NS	NS	NS	↑ *	NS	↑ *	NR

Mansour *et al* *(45)* (2017)	*HOXD8*	26 (NR)	30-60	II-IV	NR	FT	qRT-PCR & data mining	↓ *	NR	NR	NR	NR	NR	NR	NR
Zhang *et al* *(23)* (2017)	*HOXA3*	232 (61%)	NR	I-IV	140	FFT	qRT-PCR	↑ **	NR	NR	NR	NR	NR	↑ **	NR
Carbone *et al* *(24)* (2017)	*HOXB9*	58 (53%)	25-84	I-IV	NR	FFPE	IHC	NR	NS	NR	↑ *	NR	↑	NR	NR
Ji *et al* *(25)* (2016)	*HOXC6*	462 (61%)	NR	I-IV	84	FFPE	IHC	↑ ***	NS	NS	↑ ***	↑ ***	NS	NR	NS
Wang *et al* *(26)* (2016)	*HOXD10*	126 (59%)	NR	I-III	NR	FFT	qRT-PCR/IHC	↓ **	NR	NR	NR	↓ **	NR	NR	NR
Shen *et al* *(27)* (2016)	*HOXB8*	30 (63%)	20-90	I-IV	NR	FFT	qRT-PCR/WB	EQ	NS	NS	NS	NS	NS	NS	NS
Hoshino *et al* *(28)* (2014)	*HOXB9*	93 (NR)	NR	II-III	NR	FFT FFPE	qRT-PCR/IHC	↑ (NR)	NR	NR	NR	NR	NR	NR	↑ ***
Zhan *et al* *(29)* (2014)	*HOXB9*	63 (54%)	24-90	I-IV	73	FFPE	IHC	NR	NR	NS	NS	NS	NS	NS	↓ *
Huang *et al* *(30)* (2014)	*HOXB9*	128 (47%)	NR	I-IV	60	FFT FFPE	IHC/WB	↑ *	NS	NS	NS	↑ *	↑ **	↑	NS
Liao *et al* *(30)* (2011)	*HOXB7*	224 (57%)	23-86	I-IV	87	FFT FFPE	qRT-PCR/IHC	↑ (NR)	NS	NS	↑ *	↑	↑ *	↑ ***	NR
Kanai *et*	*All HOX*	40	48-	I-IV	NR	FFT	qRT-PCR	↑*:	NR	NR	NR	↑*	NR	NR	NR

al *(32)* **(2010)**		(68%)	89					A9,B3, B8,B9 ↓**: B2,B13, D1,D3, D4,D8, D12				A3D1			
Cantile et al *(34)* **(2009)**	*HOXD13*	48 (NR)	NR	NR	NR	FFT	qRT-PCR	↑ ***	NR	NR	NR	NR	NR	NR	NR
Groene *et al* *(35)* **(2006)**	*HOXA9*	36 (50%)	NR	II-III	NR	FFT	qRT-PCR	NR	NR	NR	NR	NR	NR	↑ *	NR
Jung *et al* *(36)* **(2005)**	*HOXB13*	53 (NR)	NR	NR	NR	FFT	qRT-PCR	↓ (NR)	NR	NR	NR	NR	NR	NR	NR
Toiyama *et al* *(37)* **(2005)**	*HOXA4*	4	40-68	NR	NR	FT	qRT-PCR	↓ **	NR	NR	NR	NR	NR	NR	NR
Vider *et al* *(38)* **(1997)**	*HOXB5 ,B6,B7,B8,B9,C9*	11 (NR)	NR	NR	NR	FFT	qRT-PCR	↑ (NR)	NR	NR	NR	NR	NR	NR	NR

%M: percentage of male patients, **FUP**: Follow up, **DE**: Differential Expression, **C**: Cancerous tissue, **N**: Normal colon tissue, **T**: Tumour depth, **N**: Lymph node s disease, **S**: Stage, **G**: Grade, **CEA**: Carcinoembryonic antigen, **DFS**: Disease Free Survival, **OS**: Overall Survival, **NR**: Not Reported, **EQ**: Equivocal findings, **FFPE**: FT: Fresh Tissue, **FFT**: Fresh Frozen Tissue, **IHC**: Immunohistochemistry, **WB**: Western Blot, **NS**: Not Significant, **RT-qPCR**: Real Time Quantitative Polymerase **CI**: Confidence Intervals

Table 2. Included studies reporting on the functional role of HOX genes dysregulation in CRC progression. *: p<0.05, **: p<0.01, ***: p<0.001

Figures

Author (year)	Gene	Cell lines	Intervention	Endpoint assays	Outcomes (intervention vs control cell line group)						Nude mice (type, *n*)	Intervention
					PR	CLF	AP	INV	MIGR	Molecular markers		
In Vitro studies												
Cui *et al*[22] (2019)	*HOXC11*	SW480	siRNA (KD)	Proliferation (CCK-8) Flow cytometry	↓ *	NR	↑ *	NR	NR	NR	–	–
Li *et al*[46] (2019)	*HOXB2*	SW620 SW480	Plasmid (OE)	Proliferation (MTT) Wound healing Transwell invasion	↑ ***	NR	NR	↑ **	↑ **	NR	–	–
Wu *et al*[39] (2018)	*HOXA6*	HT29 Caco-2	Plasmid (OE) ShRNA (KD)	Proliferation (CCK-8) Colony formation Transwell invasion/migration Flow cytometry	*HOXA6* (OE) ↑ ***	↑ **	↓ **	*HOXA6* (OE) ↑ ***	↑ ***	*HOXA6* (OE) ↓: PARP, PAX, Casp3, E-cadherin ↑: N-cadherin, Vimentin	–	–
Li *et al*[47] (2018)	*HOXA1*	SW620	siRNA (KD)	Proliferation (CCK-8) Transwell invasion	↓ **	NR	NR	↓ **	NR	NR	–	–
Watanabe *et al*[44] (2018)	*HOXA9*	HCT116, LoVo RKO, LS174T Colo205 Colo201 SW620, LS180 SW83, SW480 HCT15, SW480	SiRNA (KD)	Proliferation (trypan blue)	NS	NR	NR	NR	NR	NR	–	–
Bhatlekar *et al*[48] (2018)	*HOXA4* *HOXA9*	SW480 HT29	siRNA (KD)	Proliferation (WST-1) Colony formation	↓ **	↓ **	NR	NR	NR	NR	–	–
Mansour *et al*[45] (2017)	*HOXD8*	HCT116, DLD-1 HT29, SW620 SW1080	Retrovirus (OE)	Proliferation (MTT) Colony formation Apoptosis Transwell invasion	↓ *	↓ *	↑ *	↓ *	NR	↑: Caspase 6,7, PARP E-cadherin	–	–
Han *et al*[49] (2017)	*HOXA5*	HCT116 HT29	Plasmid (OE)	Proliferation (CCK-8) Colony formation Transwell invasion/migration	↓ **	↓ **	NR	↓ **	↓ **	↓**: Vimentin, Fibronectin ↑**: E-cadherin, a-catenin	–	–
Chen *et al*[50] (2016)	*HOXD3*	RKO	Lentivirus (KD)	Proliferation (MTT) Colony formation Flow cytometry	↓ **	↓ **	↑ **	NR	NR	NR	–	–
Kasiri *et al*[51] (2013)	*HOXC13*	SW480	Antisense nucleotide (KD)	Proliferation (MTT) Flow cytometry	↓ *	NR	↑ (NR)	NR	NR	↓: Cyclin B, D, E	–	–

Jung *et al*[36] (2005)	*HOXB13*	HCT116, LoVo, SW480, Caco-2	Plasmid (OE)	Proliferation (MTT)	↓ (NR)	NR	NR	NR	NR	NR	–	–
In Vitro and In Vivo studies												
Liu *et al*[21] (2020)	*HOXD9*	LoVo, SW116	Lentivirus (KD) Plasmid (OE)	Proliferation (CCK-8) Wound healing Transwell invasion	*HOXD9* (OE) ↑ ***	↑ **	NR	*HOXD9* (OE) ↑ ***	↑ ***	*HOXD9* (OE) ↑: Vimentin, MMP9 ↓: E-cadherin	BALB/c (*n*=NR)	Caecal injection
Ying *et al*[33] (2019)	*HOXB8*	HCT116	Lentivirus (KD)	Proliferation (CCK-8) Colony formation Transwell invasion/migration	↓ **	↓ **	NR	↓ *	↓ **	NR	BALB/c (*n*=24)	Flank SC & spleen injection
Zhang *et al*[23] (2018)	*HOXA3*	HCT116 HT29	siRNA (KD)	Proliferation (MTT) Colony formation Flow cytometry	↓ **	↓ **	↑ ***	NR	NR	↑: Caspase3 ↓: BCL-2, BRAF, MEK, ERK	Nod *n*=10	Flank SC injection
Yuan *et al*[40] (2018)	*HOXA10*	LoVo HT29	Lentivirus (KD)	Proliferation (MTT) Colony formation Flow cytometry	NR	↓ (NR)	↑ (NR)	NR	NR	NR	BALB/c (*n*=10)	Flank SC injection
Ji *et al*[25] (2016)	*HOXC6*	HCT116	Lentivirus (KD)	Proliferation (MTT) Colony formation Flow cytometry	↓ ***	↓ ***	NS	NR	NR	NR	Nu/Nu (*n*=8)	Forelimb SC injection
Sun *et al*[52] (2016)	*HOXA10*	SW480	Lentivirus (OE) siRNA (KD)	Transwell invasion	*HOXA10* (OE) NR	NR	NR	*HOXA10* (OE) ↓ *	NR	*HOXA10* (OE) ↑: E-cadherin ↓: Snail	BALB/c (*n*=10)	Flank SC injection
Hoshino *et al*[28] (2014)	*HOXB9*	HCT116	Plasmid (OE) Lentivirus (KD)	Proliferation (MTT)	*HOXB9* (OE) NS	NR	NR	*HOXB9* (OE) NR	NR	*HOXB9* (OE) ↑: IL8, VEGF, AREG, bEGF, ERG	BALB/c (*n*=8)	Flank SC injection
Zhan *et al*[29] (2014)	*HOXB9*	HCT116	Plasmid (OE) siRNA (KD)	Proliferation (WS-1) Transwell migration/invasion	*HOXB9* (OE) ↓ **	NR	NR	*HOXB9* (OE) ↓ **	↓ **	*HOXB9* (OE) ↑: E-cadherin ↓: Vimentin, Twist, ZEB1, ZEB2, SNAI2	BALB/c (*n*=19)	Flank SC injection

Huang *et al* [30] **(2013)**	*HOXB9*	LoVo SW620	Retrovirus (KD) Plasmid (OE)	Wound healing Transwell invasion/migration	*HOXB9* (KD)			*HOXB9* (KD)		NR	BALB/c (*n*=24)	Orthotopic implantation
					NR	NR	NR	↓ *	↓ *			
Liao *et al* [31] **(2011)**	*HOXB7*	HCT116, Ls174t, SW480, SW620, DLD-1	Plasmid (OE) Retrovirus (KD)	Proliferation (MTT) Colony formation	*HOXB7* (OE)			*HOXB7* (OE)		*HOXB7* (OE)	BALB/c (*n*=10)	Flank SC injection
					↑ *	↑ **	NR	NR	NR	↑: Cyclin D1, GSK3β, ERK, Akt		
Ghoshal *et al* [53] **(2010)**	*HOXB13*	HCT116 RKO	Plasmid (OE)	Proliferation (MTT) Colony formation	↓ **	↓ **	NR	NR	NR	NR	NR	Flank SC injection

PR: Proliferation, **CLF**: Colony Formation, **AP**: Apoptosis, **INV**: Invasion, **MIGR**: Migration, **KD**: Knockdown, **OE**: Overexpression, **ASO**: Antisense Oligonucleotide Reported, **CCK-8**: Cell Counting Kit 8, **MTT**: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, **TV**: Tumour Volume, **TW**: Tumour Weight, **SC**: Subcuta **PARP**: Poly [ADP-ribose] polymerase, **PAX**: Paired Box, **MMP9**: Matrix Metallopeptidase 9, **BCL-2**: BCL2 Apoptosis Regulator, **BRAF**: v-Raf murine sarcoma vira homolog B, **MEK**: Mitogen-Activated Protein Kinase Kinase , **ERK**: Rxtracellular-signal-Regulated Kinase, **IL8**: Interleukin 8, **VEGF**: Vascular endothelial growth Amphiregulin , **bEGF**: Endothelial growth factor beta, **ERG**: ETS transcription factor, **ZEB1**: Zinc Finger E-Box Binding Homeobox 1 , **ZEB2**: Zinc Finger E-Box Bi Homeobox 2 , **GSK3β**: Glycogen Synthase Kinase 3 Beta , **Akt**: AKT Serine/Threonine Kinase 1

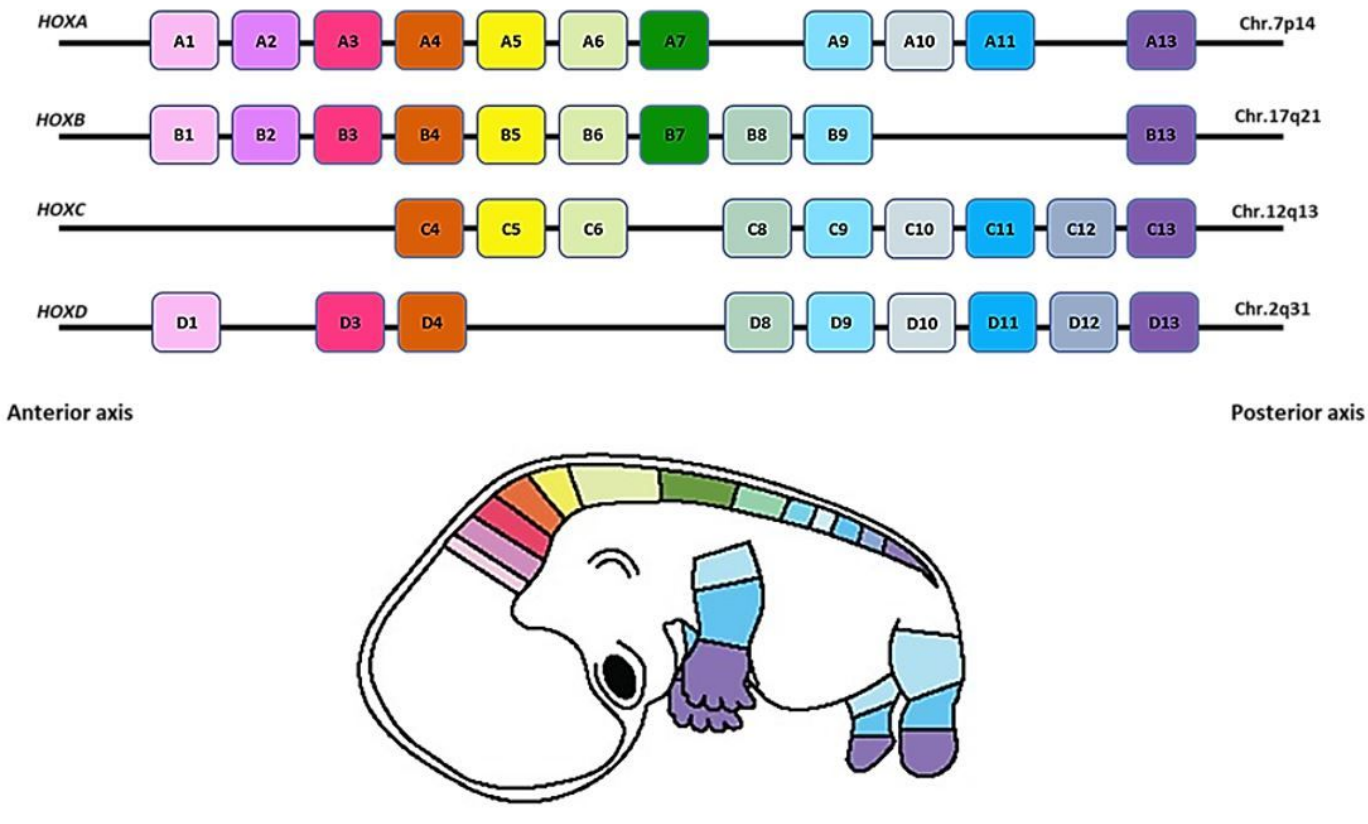

Figure 1

HOX genes in human genome. Adapted from Durston et al. (8). The colour coding represents the correspondence between the genomic order of each HOX gene in the chromosomal cluster with the segmental identity in human embryo, (Microsoft PowerPoint software was used to create this figure)

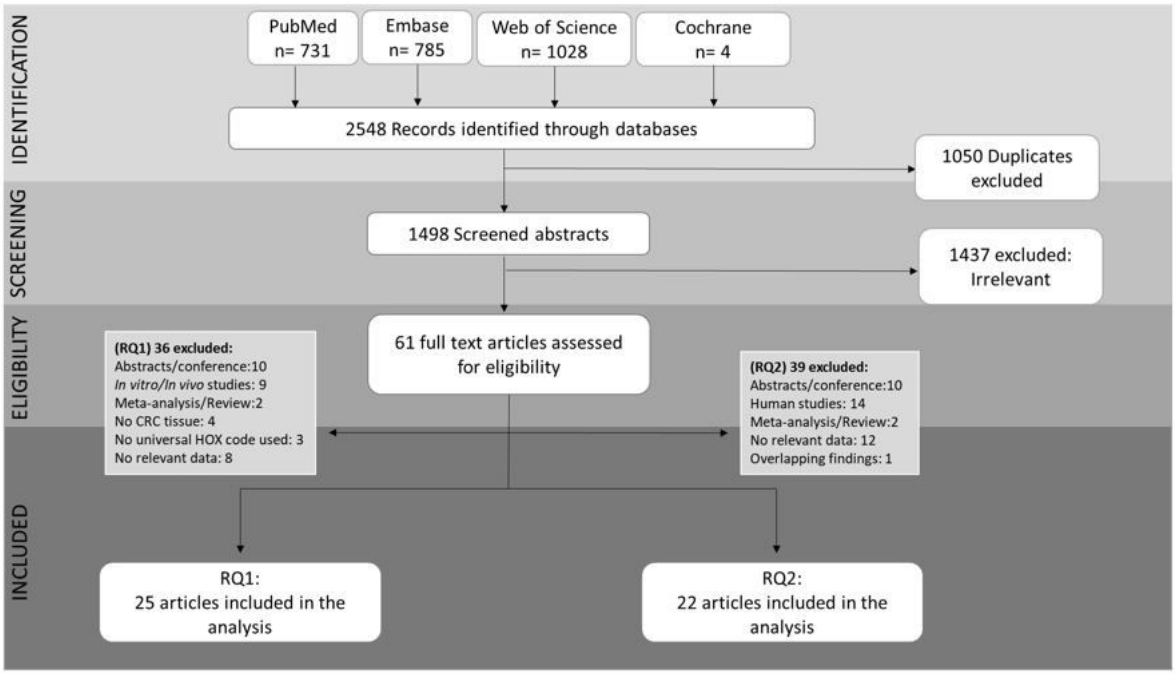

Figure 2

PRISMA flow chart of systematic review article retrieval (Microsoft PowerPoint software was used to create this figure)

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