HOX Gene Family Defines Tumor Microenvironment and Drug Sensitive in Hepatocellular Carcinoma

Dan Li

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Tao Yu

Central Hospital of Wuhan: The Central Hospital of Wuhan

Zhi Zeng

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Jie Wu

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Jingjing Han

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Benhong Zhou (✉ benhongzh@whu.edu.cn)

Central Hospital of Wuhan: The Central Hospital of Wuhan <https://orcid.org/0000-0003-1112-1310>

Primary research

Keywords: Hepatocellular carcinoma, TCGA, HOX genes, PD-L1, Drug sensitivity

Posted Date: August 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-806092/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Hepatocellular carcinoma (HCC) pathophysiology is prevalently related with HOX genes. However, the study on associations of extensive HOX genes with tumor microenvironment and drug sensitivity of HCC remains scarce. The data sets of HCC were downloaded from TCGA, ICGC and GEO by bioinformatics method and analyzed. RT-qPCR and IHC were used to verify the expression levels of 10 HOX genes in normal and HCC. Based on unsupervised clustering, two distinct HOX genes patterns has been identified, and a significant shorter OS in clusterB from HCC patients was observed than those in clusterA. Furthermore, based on a computational frame, we divided HCC samples into high and a low HOXscore groups, and significantly shorter survival time in the high HOXscore was observed relative to low HOXscore group using survival analysis. GSEA revealed that the high HOXscore group was more likely to be enriched in cancer-specific pathways. Furthermore, the high HOXscore group was associated with the infiltration of inhibitory immune cells. Besides, the HOXscore has greater prognostic significance than TMB alone and is capable of identifying intermediate subtype group existing with partial TMB functionality in low TMB patients. In response to anti-cancer drugs, the high HOXscore group was more sensitive to mitomycin and cisplatin. Importantly, the HOXscore was associated with the therapeutic efficacy of PD-L1 blockade, suggesting that the development of potential drugs targeting these HOX genes to aid the clinical benefits of immunotherapy. In addition, RT-qPCR and immunohistochemistry showed 10 HOX genes mRNA expression was higher in HCC compared to the normal tissues. Finally, high HOXscore was closely related with poorer overall survival of HCC in an independent validation cohort. Our study provide a comprehensive analysis of HOX genes family in HCC. We revealed the potential function of these HOX genes family in TME and identified their therapeutic liability in targeted therapy and immunotherapy. This work highlights the cross-talk and potential clinical utility of HOX genes family in HCC therapy.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors, and has aroused more and more attention with its high rate of recurrence and distant metastasis[1, 2]. Its recurrence rate is up to 70%, and the 5-year survival rate is less than 60% with successful surgical resection or liver transplantation[3]. Due to the lack of specific symptoms of early HCC, it is difficult to be detected, and 90% of patients are already in the advanced stage at the time of diagnosis[4]. In spite of achieving great progress in prevention, diagnosis, and treatment for this disease recent years, the clinical outcome is still unsatisfactory[5]. There are limited therapeutic methods for HCC. Surgery and percutaneous ablation can achieve long-term control in some early HCC patients. However, since most of the early HCC patients have no obvious symptoms, most of the patients are in the late stage and cannot be resected by radical surgery[6]. Including arterial chemoembolization, radiotherapy, immunotherapy or hormone therapy, the treatment results are poor, and the five-year survival rate of patients is extremely low[7, 8]. Therefore, the search for HCC prognostic markers and drug sensitivity may provide relevant guidance for the survival and treatment of HCC patients.

The pathogenesis of HCC is heterogeneous and complex, which may be a process involving multiple genes and multiple signaling molecules, in which gene mutation, activation of oncogenes and inactivation of tumor suppressor genes play an important role[2]. HOX gene is a family of genes encoding transcription factor (TF), which plays an important role in embryonic development and cell differentiation. The HOX gene family contains a common 120-base-pair DNA sequence that encodes a 61-amino acid peptide called the homologous domain. In the human genome, the HOX gene family contains 39 members, located on four different chromosomes[9]. In recent years, studies have found that the HOX gene family is involved in the development of a variety of tumors[10]. HOX gene can not only be up-regulated as a transcriptional activator in cancer, but also play a role as a transcriptional suppressor. The up-regulation or down-regulation of TF encoded by HOX gene family in cancer is critical to the development and progression of cancer. This abnormal regulation of the HOX gene in cancer suggests that HOX plays a tissue-specific role in tumors[11]. Based on the crucial role in modulating the initiation and progression of cancer, numerous investigators utilized these HOX genes to establish prognostic models for tumor diagnosis or prognosis,, even the guidance of therapeutic strategies. Similarly, HOX genes were recently used to develop an optimal prognostic gene signature for predicting the prognosis of cancer[12, 13].

Thus far, the mechanisms underpinning the role of HOX genes in HCC remain mostly unknown. In this study, based on unsupervised clustering, two distinct HOX genes patterns has been identified, characterized under these two patterns were highly consistent with the three immune phenotypes of tumors. Next, based on a computational frame, we divided HCC samples into high and a low HOXscore groups, and significantly shorter survival time in the high HOXscore was observed relative to low HOXscore group using survival analysis. Besides, the HOXscore has greater prognostic significance than TMB alone and is capable of identifying intermediate subtype group existing with partial TMB functionality in low TMB patients. In response to anti-cancer drugs, the HOXscore group was more sensitive to mitomycin and cisplatin. Importantly, the HOXscore was associated with the therapeutic efficacy of PD-L1 blockade.

Materials And Methods

Data source and preprocessing

The workflow of our study was shown in Figure 1A. HCC RNA-seq data (FPKM value) were obtained from TCGA, ICGC, and GSE76427 database[14], and their clinical information also was downloaded simultaneously. We obtained 725 HCC tissue samples. Considering all data in this study was obtained from PDB Public DataBase, the ethics approval and informed consent were not provided. The detailed clinical characteristics of these patients are summarized in Table 1.

Selection of HOX genes in HCC

Limma packages in R language was used screening the differentially expressed HOX genes in HCC. The default Benjamini-Hochberg false discovery rate (FDR) was employed to adjust the p-value to remove

false positive results. $P < 0.05$ and absolute $\log_2FC > 1$ was considered a criterion for the selection of differentially expressed genes. Differently expressed HOX genes were processed by hierarchical clustering analysis with R package “pheatmap”[15].

Consensus clustering analysis

The cohort was clustered into subgroups according to the consensus expression of HOX genes using “ConsensusClusterPlus” in R. Subsample 80% of samples at each iteration and partition each subsample into up to k (max K = 9) groups by k-means algorithm upon Euclidean distance. This process was repeated for 1,000 repetitions[16]. Sequentially, we selected a perfect clustering result by considering the relative change in area under the cumulative distribution function curve, which was assessed by the proportion of ambiguous clustering (PAC) score, a low value of PAC implies a flat middle segment, allowing conjecture of the optimal K by the lowest PAC. A HOXscore for each patient was calculated according to the following formula: $HOXscore = \sum (PC1_i + PC2_i)$, where i is the TPM value of each screened gene. The overall survival (OS) discrepancy among subclusters was assessed through the Kaplan-Meier method and log-rank test[17]. Principal component analysis (PCA) was used to exhibit the gene expression patterns in the three cohort subclusters using the R package[18]. The differences of clinic pathological features, including age, gender, fustat and stage, were calculated by Mann-Whitney-Wilcoxon test between the subgroups.

Gene set enrichment analysis (GSEA)

In order to further investigate the potential role of HOXscore in HCC, we performed gene enrichment analysis. The HOXscore was divided into high HOXscore group and low HOXscore group based on the median value in samples. The go and kegg standardized pathway gene set obtained from the GSEA website which was used for enrichment analysis. We sequenced 1000 gene sequences and sequenced the enrichment pathways of each phenotype using nominal p value and normalized In review enrichment score (NES). The discovery rate $|NES| > 1$, NOM p-val < 0.05, FDR q-val < 0.25 were considered to be significantly enriched[19].

Evaluation of immunity in stratified three cohorts

We performed the ssGSEA method to calculate the enrichment scores on the basis of metagenes[20]. The reason why we considered the metagene a robust approach was the two main characteristics: (1) the use of a set of genes instead of single genes that represent one immune subpopulation, because the use of single genes as markers for immune subpopulations can be misleading as many genes are expressed in different cell types, and (2) the assessment of relative expression changes of a set of genes in relation to the expression of all other genes in a sample. Referring to the Bindea et al. study, the author incorporate 535 metagenes that represented diverse 24 immune subpopulations: innate immune cells (dendritic cells [DCs], immature DCs [iDCs], activated DCs [aDCs], plasmacytoid DCs [pDCs], eosinophils, mast cells, macrophages, natural killer cells [NKs], NK CD56dim cells, NK CD56bright cells, and neutrophils) and adaptive immune cells (B cells, T cells, T helper cells, T gamma delta [Tgd] cells, T helper 1 [Th1] cells,

Th2 cells, Th17 cells, regulatory T [Treg] cells, CD8+ T cells, T central memory [Tcm] cells, T effector memory [Tem] cells, T follicular helper [Tfh] cells, and cytotoxic cells)[21].

Immunohistochemistry

150 HCC patients tissues and corresponding adjacent tissues were collected to explore the expression of 10 HOX genes in the tissue samples by using immunohistochemical staining (IHC). IHC staining was performed according to the manufacturer's instructions.

Statistical analysis

HOX gene expression and clinical data obtained from TCGA, ICGC, and GSE76427 were combined through perl5.30.0.1. R4.0.3 was used to screen differential HOX genes of HCC, analyze independent prognosis, and search for clinical correlation. In addition, genetic and clinical related data were used to perform univariate Cox regression analysis. The data difference between two groups was calculated by Mann-Whitney-Wilcoxon test. $P \leq 0.05$ was considered as statistical significance.

Result

Identification of HOX genes in TCGA-LIHC patients

In order to visualize the expression pattern of HOX genes between the HCC and normal control, heatmap of 39 HOX genes were obtained in Figure 1A. The significant overexpressions of HOXD9 ($P \leq 0.001$), HOXA13 ($P \leq 0.001$), HOXD8 ($P \leq 0.001$), HOXD3 ($P \leq 0.001$), HOXA10 ($P \leq 0.001$), HOXD4 ($P \leq 0.001$), HOXD1 ($P \leq 0.001$), HOXD10 ($P \leq 0.001$), HOXC9 ($P \leq 0.001$), HOXA3 ($P \leq 0.001$), HOXA11 ($P \leq 0.001$), HOXB8 ($P \leq 0.001$), HOXB9 ($P \leq 0.001$), HOXC6 ($P \leq 0.001$), HOXA9 ($P \leq 0.001$), HOXB7 ($P \leq 0.001$), HOXB13 ($P \leq 0.001$), HOXA6 ($P \leq 0.001$), HOXC4 ($P \leq 0.001$), HOXC13 ($P \leq 0.001$), HOXA7 ($P \leq 0.001$), HOXC8 ($P \leq 0.001$), HOXD13 ($P \leq 0.001$), HOXC11 ($P \leq 0.001$), HOXB3 ($P \leq 0.001$), HOXC10 ($P \leq 0.05$), HOXB2 ($P \leq 0.05$), HOXB5 ($P \leq 0.05$), HOXD11 ($P \leq 0.05$), HOXC12 ($P \leq 0.05$), HOXA5 ($P \leq 0.05$), HOXD12 ($P \leq 0.05$), and HOXA2 ($P \leq 0.05$) in HCC samples were observed relative to normal control samples, while there was no significant difference in HOXC5 ($P = 0.07$), HOXB1 ($P = 0.11$), HOXA1 ($P = 0.49$), HOXA4 ($P = 0.78$), HOXB6 ($P = 0.86$), HOXB4 ($P = 0.95$).

In order to predict the clinical outcomes of HCC with HOX genes, univariate Cox regression analysis was performed to identify the HOX genes that associated with OS in TCGA-LIHC cohort. 11 HOX genes were significantly correlated with OS (Figure 1B). Unreasonably, HOXB1 has no significant between HCC samples and normal control samples, but its has significantly correlated with OS in the univariate Cox analysis, so it was excluded from further study (Figure 1C). A total of 10 prognostic HOX-related DEGs were preserved, and Figure 1D-M show the expression distribution of 10 HOX genes between paired normal and HCC tissues (Table 2).

Unsupervised clustering of 10 HOX genes in the HCC meta-cohort

Next, Three datasets (TCGA-LIHC, LIRI-JP, and GSE76427) with available OS data and clinical information were enrolled into one meta-cohort. Based on the expression similarity of the 10 HOX genes (HOXD13, HOXB1, HOXD11, HOXD10, HOXA6, HOXA7, HOXC8, HOXA11, HOXA9, HOXC6, and HOXD9), the 725 HCC patient cohort was divided into two clusters (cluster A and cluster B) when $k = 2$ was used for consensus clustering analysis (Figure 3A). A significant shorter OS in clusterB from HCC patients was observed than those in clusterA (Figure 3B). To explore functional role of the 2 distinct HOX genes patterns in HCC progression, we performed GSVA analysis, and the results indicated that clusterB was more likely to be enriched in cancer-specific pathways, such as cell cycle, RNA polymerase, RNA degradation, spliceosome, and nucleotide excision repair, and clusterA was more likely to be enriched in cell adhesion molecules, ECM receptpr interction, dilated cardiomyopathy, and hypertrophic cardiomyopathy (Figure 3C). Moreover, the principal component analysis (PCA) analysis of the transcriptional profile between cluster A and cluster B denoted a markedly discrimination each other (Figure 3F). Then, we assessed the associations between the clustering and clinicpathological features. There were significant differences between the cluster A and cluster B for the N, stage, gender and age ($P < 0.05$), and fustat ($P < 0.01$), and the significant overexpressions of 10 HOX genes in cluster B samples were observed relative to cluster A samples (Figure 3D). It has been confirmed that the tumor microenvironment (TME) is correlated with the sensitivity to immunotherapy and HCC prognosis[22], and ssGSEA method was used to calculate the enrichment scores on the basis of metagenes, and was employed in the gene expression data from three datasets to calculate immune score. As shown in figures 3E, the proportion of immune cell in the clusterB group was significantly lower than that in the clusterA group ($P < 0.05$), suggesting that the immune ability of clusterB is suppressed.

Generation of 10 HOX gene signatures and functional annotation

To further investigate the potential biological behavior of each HOX gene pattern, we determined 1417 HOX phenotype-related DEGs using limma package. Next, the GSEA was used to reveal the malignant hallmarks of HCC, and GSEA GO results indicated that immune regulation was significantly associated with HCC (Figure 3G), GSEA KEGG results indicated that metabolism pathway was significantly associated with HCC (Figure 3H). To further validate this regulation mechanism, we consensus clustering of 1417 HOX phenotype-related DEGs, and the 725 HCC patient cohort was divided into three clusters (cluster A, cluster B, and cluster C) when $k = 3$ was used for consensus clustering analysis (Figure 4A). A significant shorter OS in clusterC from HCC patients was observed than those in clusterA and clusterB (Figure 4B). Then, we also observed that there were significant differences between the cluster A, cluster B, and cluster C for the N, stage, gender and age, and fustat ($P < 0.05$) (Figure 4C), and the significant overexpressions of 10 HOX genes in cluster C samples were observed relative to cluster A and B samples (Figure 4D). The above results showed suggested HOXgene played a role in TME, however, these analyses were only based on the patient population and could not accurately predict the pattern of HOX gene in individual patients.

Based on these DEGs, we constructed a set of scoring system to quantify the HOX gene pattern of individual patients with HCC, we termed as HOXscore, and a significant shorter OS in high HOXscore from

HCC patients was observed than those in low HOXscore (Figure 4E), and the positive correlation between HOXscore and immune cell was also observed (Figure 4F). We also used alluvial diagram to showed the relationship between gene cluster, HOX cluster, HOXscore, and fustat, and confirmed this relationship (Figure 4G). We further analyzed the HOXscore value between the HOX clusterA and B group, and significantly higher HOXscore value in the clusterB group was observed relative to clusterA group (Figure 4H). In addition, we analyzed HOXscore value between the gene clusterA, B, and C group, and significantly higher HOXscore value in the clusterC group was observed relative to clusterA and B group (Figure 4I).

Independent prognostic analysis of HOXscore

Next, multivariate Cox regression analysis was performed on clinical factors and HOXscore to determine the independent prognostic value of HOX (Table 3). As shown in figures 5a and 5b, in both the young and old groups, significantly shorter survival time in the high HOXscore group was observed relative to low HOXscore group using survival analysis ($P < 0.05$) (Figure 5A and B). Subsequently, significantly shorter survival time in the high HOXscore group was observed relative to low HOXscore group using survival analysis in the male, female, stage1-2, and stage 3-4 ($P < 0.05$) (Figure 5C-G). Mitomycin and cisplatin are commonly used chemotherapy drugs for HCC, and we found that the IC50 to mitomycin and cisplatin in the high HOXscore group was lower than that in the low HOXscore group, suggesting that the high HOXscore group was more sensitive to mitomycin and cisplatin. Abovementioned evidence demonstrated that the prognostic signature based on HOXscore is reliable in HCC prognosis. To explore functional role of this prognostic signature in HCC progression, we performed GSEA analysis, and the results indicated that the high HOXscore group was more likely to be enriched in cancer-specific pathways (Figure 6A and B).

The HOXscore is better than tumor somatic mutation (TMB) can predict HCC prognosis

It has been confirmed that the abnormality of TMB is correlated with the sensitivity to immunotherapy and HCC prognosis[23]. We further analyzed the HOXscore value between the gene clusterA, B, and C group, and significantly higher HOXscore value in the clusterC group was observed relative to clusterAand B group (Figure 7A). In addition, we analyzed the HOXscore value between the HOX clusterA and B group, and significantly higher HOXscore value in the clusterB group was observed relative to clusterA group (Figure 7B), and a significant shorter OS in high TMB from TCGA-LIHC patients was observed than those in low TMB ((Figure 7C). Next, to evaluate whether HOXscore is better than TMB status can predict HCC prognosis, we divide all patients into H-TMB/H-HOXscore, H-TMB/L-HOXscore, L-TMB/L-HOXscore, and L-TMB/H-HOXscore. The results showed that patients in L-TMB/H-HOXscore had a poor survival as compared with L-TMB/L-HOXscore in TCGA set (Figure 7D), suggest the HOXscore has greater prognostic significance than TMB alone and is capable of identifying intermediate subtype group existing with partial TMB functionality in low TMB patients. Furthermore, we analyzed the somatic mutation between the high and low HOXscore group, and no significant was observed between the two group (Figure 7E).

HOX patterns in the role of PD-1/L1 immunotherapy

HCC is immunogenic and immunosuppressed, thus, the application of immunotherapy is of great significance for the development of THE treatment of HCC. We first analyzed the expression of PD-L1 between high and low HOXscore group, and significantly higher PD-L1 expression in the high HOXscore group was observed relative to low HOXscore group (Figure 8A). Next, we download the immunotherapy data of TCGA-LIHC patients, and the significant therapeutic advantages and clinical response to PD-1/L1 immunotherapy in patients with high HOXscore compared to those with low HOXscore were confirmed (Figure 8B-E). It has been confirmed that the abnormality of MSI is correlated with the sensitivity to immunotherapy. We further analyzed the MSI between high and low HOXscore group, and significantly higher MSI in the high and low HOXscore group was observed relative to low HOXscore group (Figure 8F). In addition, we analyzed the HOXscore value between the MSS, MSI-L, and MSI-H group, and significantly higher HOXscore value in the MSI-H group was observed relative to MSS and MSI-L group (Figure 8G). Abovementioned evidence demonstrated that HOXscore is reliable in HCC PD-1/L1 immunotherapy.

Validation the expression and prognosis of HOXscore in GSE76427

To further validate HOX genes expression in HCC, GSE76427 was used to measure the expression level of HOX genes, and the result showed that compared with normal group, the HOXA6, HOXA7, HOXA9, HOXA11, HOXC6, HOXC8, HOXD10, HOXD11, HOXD13, and HOXD9 level were significantly higher in HCC group. (Figure 9A-J). In addition, significantly shorter survival time in the high HOXscore group was observed relative to low HOXscore group using survival analysis (Figure 9K).

Next, to further validate HOXscore in HCC, we measured the 10 HOX genes protein level by immunohistochemistry, and the result showed that compared with the normal group, the 10 HOX genes protein level was significantly higher in HCC group (Figure 10A). In addition, RT-qPCR was used to detect the 10 HOX genes mRNA expression in HCC. Compared with the normal group, the 10 HOX genes mRNA level was significantly higher in the HCC group (Figure 10B). Additionally, Kaplan–Meier and Cox's proportional hazards regression model survival analysis revealed that patients with high HOXscore group had shorter overall survival in HCC (Figure 10C).

Discussion

Increasing evidence demonstrated that HOX genes took on an indispensable role in embryonic development and cancer. While most studies have focused on a single type of HOX gene, the mutual relationships and functions of multiple types of HOX genes in cancer are not fully understood. Here, we selected 39 HOX genes in this study, and 33 aberrant expression of HOX genes in HCC were observed. 11 HOX genes were significantly associated with OS of TCGA-LIHC cohort. Of these, 10 HOX genes were used to divided HCC patient into two clusters, and a significant OS between the two clusters. The abundance of immune cells in the TME was significantly different between the two HCC subtypes, specifically, the immune ability of clusterB is suppressed. Base on the DEGs between two HCC subtypes, the HCC patient divided into three clusters, and a significant OS between the three clusters.

Simultaneously, based on these DEGs, we constructed HOXscore, and HOXscore is an independent prognostic in HCC. Moreover, HOXscore has greater prognostic significance than TMB alone and is capable of identifying intermediate subtype group existing with partial TMB functionality in low TMB patients. Finally, HOXscore is reliable in HCC PD-1/L1 immunotherapy, and sensitive to mitomycin and cisplatin.

HCC is one of the most popular common forms of liver cancer with the four highest cancer-associated mortality worldwide. Its initiation and progress is a complicated pathomechanism, which involves the epigenetics alterations at the posttranscriptional level[2]. HOX is abnormally expressed in a variety of tumors and is closely related to tumor stage and prognosis[24]. The regulation of HOX gene expression is a major factor affecting the efficacy of HOX in tumors, involving chromosome epigenetic modification and signaling molecules, such as FGF, Wnt, RA and a variety of steroid small molecules[25-27]. Meanwhile, the transcription activity of HOX protein is regulated by cofactors and other transcription factors[28]. HOX regulates the proliferation, apoptosis, migration and invasion of tumor cells by promoting the transcription of target genes, and participates in the occurrence and development of tumors and drug tolerance[29, 30]. The role of HOX in various HCC has been reported. HOXA7 up-regulates the expression of cyclin E1 and CDK2 proteins, thereby promoting the proliferation of HCC cells[31]. HOTAIR gene is located in the HOXC gene cluster and inhibits the expression of HOXD cluster genes such as HOXD10 by recruiting methyltransferase[32]. The expression of HOXD10 was down-regulated in liver cancer tissues and HepG2 cells and promoted the invasion and metastasis of cells. In HCC, the expression of HOXD10 is down-regulated due to promoter methylation, which promotes the survival, migration and invasion of HCC cells. The HOXD10 downstream gene IGFBP-3 induces apoptosis of HCC cells by activating caspase-3 and Caspase-8[33].

Recently, immunotherapies, especially immune checkpoint inhibitor therapy through targeting the PDL1/PD-1 axis, have been focused in the field of HCC therapy[34]. Bewilderingly, less than 20% of cancer patients obtain the beneficial effects following treatment with immune checkpoint inhibitor, whereas the remainders are frustrated. It may be explained that different subtypes in homogeneous cancer exhibit disparate sensitivity to immunotherapy[35]. In HCC, previous study has constructed a prognostic model on the basis of immune signatures, which suggested that the high immunity subtype shows longer OS and more sensitivity to immunotherapy[36]. In our study, the similar tendency was observed that the high HOXscore group with lower immune and stromal scores exhibited shorter OS. Meanwhile, the increased TMB and tumor purity were found in high HOXscore group, which indicated a greater likelihood of malignant behavior. These evidences support that HOXscore can be helpful for guiding the selection of immunotherapeutic strategies in HCC.

There have been many studies on the composition and prognosis of immune cells in the tumor microenvironment. M2 macrophages and proinflammatory M1 macrophages are associated with the growth and spread of tumors and the formation of immunosuppressive microenvironment[37, 38]. High concentrations of CD3⁺ T cells, CD8⁺ cytotoxic T cells, and CD45RO⁺ memory T cells were significantly associated with longer disease-free survival and overall survival[39, 40]. The effect of CD4⁺ regulatory T

cells on tumor has been debated, and studies have shown that there was no effect on survival while other studies showed a negative association with prognosis in head and neck cancer, colorectal cancer, bladder cancer, and ovarian cancer[41-43]. Studies have used CD57 as a phenotypic marker to detect NK cells in liver cancer tissues, showing that THE infiltration of CD57⁺ cells indicates a good prognosis[44]. The role of B cells in tumors is unclear. The spontaneous cancer model in mice suggests that B cells have a carcinogenic effect, which may activate M2 macrophages and promote the early stage of carcinogenesis, and may also promote metastasis by promoting the transformation of resting T cells into regulatory T cells[45, 46]. However, other studies have shown that B cells can act as antigen-presenting cells, inducing CD4⁺T cell-dependent CD8⁺ memory T cells, thereby contributing to the control of tumor invasion and metastasis[47]. In our study, we found most of the immune cell activity is suppressed in HCC with low survival rate, and the positive correlation between HOXscore and immune cell was also observed.

There are several limitations in this study. First, there is a lack of biological verification. Future molecular studies are needed to identify the interactions between 10 HOX genes from our prognostic model. Moreover, all data in this study is obtained from public databases, so future studies with prospective validation are still warranted.

In conclusion, this work is the first to provide a comprehensive analysis of HOX genes family in HCC. We revealed the potential function of these HOX genes family in TME and identified their therapeutic liability in targeted therapy and immunotherapy. This work highlights the cross-talk and potential clinical utility of HOX genes family in HCC therapy, which also provide a wider insight for understanding the HCC.

Declarations

Authors' contributions

Dan Li and Tao Yu designed the study, performed statistical analysis, and drafted the manuscript. Zhi Zeng, Jingjing Han, and Jie Wu helped to draft the manuscript. Benhong Zhou conceived the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Ethics approval and consent to participate

Considering all data in this study was obtained from PDB Public DataBase, the ethics approval and informed consent were not provided.

Consent for publication

The authors agree for publication.

Competing interests

The authors declare that they have no competing interests..

Funding:

This study was supported by the National Natural Science Foundation of China (Grant No. 81803789 to DL, No. 81704023 to TY and No. 81602535 to ZZ), Wu Jieping Medical Foundation (Grant No. 320.6750) and the guidance funding of Renmin Hospital of Wuhan University (Grant No. RMYD2018M79).

References

1. R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2020, CA: a cancer journal for clinicians, 70 (2020) 7-30.
2. A. Villanueva, Hepatocellular Carcinoma, The New England journal of medicine, 380 (2019) 1450-1462.
3. A. Forner, M. Reig, J. Bruix, Hepatocellular carcinoma, Lancet (London, England), 391 (2018) 1301-1314.
4. J. Hartke, M. Johnson, M. Ghabril, The diagnosis and treatment of hepatocellular carcinoma, Seminars in diagnostic pathology, 34 (2017) 153-159.
5. M.S. Grandhi, A.K. Kim, S.M. Ronnekleiv-Kelly, I.R. Kamel, M.A. Ghasebeh, T.M. Pawlik, Hepatocellular carcinoma: From diagnosis to treatment, Surgical oncology, 25 (2016) 74-85.
6. M. Kudo, Y. Kawamura, K. Hasegawa, R. Tateishi, K. Kariyama, S. Shiina, H. Toyoda, Y. Imai, A. Hiraoka, M. Ikeda, N. Izumi, M. Moriguchi, S. Ogasawara, Y. Minami, K. Ueshima, T. Murakami, S. Miyayama, O. Nakashima, H. Yano, M. Sakamoto, E. Hatano, M. Shimada, N. Kokudo, S. Mochida, T. Takehara, Management of Hepatocellular Carcinoma in Japan: JSH Consensus Statements and Recommendations 2021 Update, Liver cancer, 10 (2021) 181-223.
7. F. Piñero, M. Dirchwolf, M.G. Pessôa, Biomarkers in Hepatocellular Carcinoma: Diagnosis, Prognosis and Treatment Response Assessment, Cells, 9 (2020).
8. A. Schlachterman, W.W. Craft, Jr., E. Hilgenfeldt, A. Mitra, R. Cabrera, Current and future treatments for hepatocellular carcinoma, World journal of gastroenterology, 21 (2015) 8478-8491.
9. S. Bhatlekar, J.Z. Fields, B.M. Boman, HOX genes and their role in the development of human cancers, Journal of molecular medicine (Berlin, Germany), 92 (2014) 811-823.
10. A. Paço, S. Aparecida de Bessa Garcia, J. Leitão Castro, A.R. Costa-Pinto, R. Freitas, Roles of the HOX Proteins in Cancer Invasion and Metastasis, Cancers, 13 (2020).
11. S. Fantini, V. Salsi, V. Zappavigna, HOX cluster-embedded micro-RNAs and cancer, Biochimica et biophysica acta. Reviews on cancer, 1869 (2018) 230-247.
12. N. Shah, S. Sukumar, The Hox genes and their roles in oncogenesis, Nature reviews. Cancer, 10 (2010) 361-371.

13. S. Contarelli, V. Fedele, D. Melisi, HOX Genes Family and Cancer: A Novel Role for Homeobox B9 in the Resistance to Anti-Angiogenic Therapies, *Cancers*, 12 (2020).
14. O.V. Grinchuk, S.P. Yenamandra, R. Iyer, M. Singh, H.K. Lee, K.H. Lim, P.K. Chow, V.A. Kuznetsov, Tumor-adjacent tissue co-expression profile analysis reveals pro-oncogenic ribosomal gene signature for prognosis of resectable hepatocellular carcinoma, *Molecular oncology*, 12 (2018) 89-113.
15. M.D. Robinson, A. Oshlack, A scaling normalization method for differential expression analysis of RNA-seq data, *Genome biology*, 11 (2010) R25.
16. M.D. Wilkerson, D.N. Hayes, ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking, *Bioinformatics (Oxford, England)*, 26 (2010) 1572-1573.
17. N. Simon, J. Friedman, T. Hastie, R. Tibshirani, Regularization Paths for Cox's Proportional Hazards Model via Coordinate Descent, *Journal of statistical software*, 39 (2011) 1-13.
18. C.C. David, D.J. Jacobs, Principal component analysis: a method for determining the essential dynamics of proteins, *Methods in molecular biology (Clifton, N.J.)*, 1084 (2014) 193-226.
19. A. Subramanian, P. Tamayo, V.K. Mootha, S. Mukherjee, B.L. Ebert, M.A. Gillette, A. Paulovich, S.L. Pomeroy, T.R. Golub, E.S. Lander, J.P. Mesirov, Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles, *Proceedings of the National Academy of Sciences of the United States of America*, 102 (2005) 15545-15550.
20. D.A. Barbie, P. Tamayo, J.S. Boehm, S.Y. Kim, S.E. Moody, I.F. Dunn, A.C. Schinzel, P. Sandy, E. Meylan, C. Scholl, S. Fröhling, E.M. Chan, M.L. Sos, K. Michel, C. Mermel, S.J. Silver, B.A. Weir, J.H. Reiling, Q. Sheng, P.B. Gupta, R.C. Wadlow, H. Le, S. Hoersch, B.S. Wittner, S. Ramaswamy, D.M. Livingston, D.M. Sabatini, M. Meyerson, R.K. Thomas, E.S. Lander, J.P. Mesirov, D.E. Root, D.G. Gilliland, T. Jacks, W.C. Hahn, Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1, *Nature*, 462 (2009) 108-112.
21. G. Bindea, B. Mlecnik, M. Tosolini, A. Kirilovsky, M. Waldner, A.C. Obenauf, H. Angell, T. Fredriksen, L. Lafontaine, A. Berger, P. Bruneval, W.H. Fridman, C. Becker, F. Pagès, M.R. Speicher, Z. Trajanoski, J. Galon, Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer, *Immunity*, 39 (2013) 782-795.
22. C. Lu, D. Rong, B. Zhang, W. Zheng, X. Wang, Z. Chen, W. Tang, Current perspectives on the immunosuppressive tumor microenvironment in hepatocellular carcinoma: challenges and opportunities, *Molecular cancer*, 18 (2019) 130.
23. C. Guichard, G. Amaddeo, S. Imbeaud, Y. Ladeiro, L. Pelletier, I.B. Maad, J. Calderaro, P. Bioulac-Sage, M. Letexier, F. Degos, B. Clément, C. Balabaud, E. Chevet, A. Laurent, G. Couchy, E. Letouzé, F. Calvo, J. Zucman-Rossi, Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma, *Nature genetics*, 44 (2012) 694-698.
24. S.A. de Bessa Garcia, M. Araújo, T. Pereira, J. Mouta, R. Freitas, HOX genes function in Breast Cancer development, *Biochimica et biophysica acta. Reviews on cancer*, 1873 (2020) 188358.
25. S.S. Hammoud, D.A. Nix, H. Zhang, J. Purwar, D.T. Carrell, B.R. Cairns, Distinctive chromatin in human sperm packages genes for embryo development, *Nature*, 460 (2009) 473-478.

26. R.P. Kapur, M.D. Gershon, P.J. Milla, V. Pachnis, The influence of Hox genes and three intercellular signalling pathways on enteric neuromuscular development, *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society*, 16 Suppl 1 (2004) 8-13.
27. P.B. Pinto, J.M. Espinosa-Vázquez, M.L. Rivas, J.C. Hombría, JAK/STAT and Hox Dynamic Interactions in an Organogenetic Gene Cascade, *PLoS genetics*, 11 (2015) e1005412.
28. F. Memic, V. Knoflach, K. Morarach, R. Sadler, C. Laranjeira, J. Hjerling-Leffler, E. Sundström, V. Pachnis, U. Marklund, Transcription and Signaling Regulators in Developing Neuronal Subtypes of Mouse and Human Enteric Nervous System, *Gastroenterology*, 154 (2018) 624-636.
29. K. Domsch, F. Papagiannouli, I. Lohmann, The HOX-Apoptosis Regulatory Interplay in Development and Disease, *Current topics in developmental biology*, 114 (2015) 121-158.
30. B. Li, Q. Huang, G.H. Wei, The Role of HOX Transcription Factors in Cancer Predisposition and Progression, *Cancers*, 11 (2019).
31. B. Tang, G. Qi, X. Sun, F. Tang, S. Yuan, Z. Wang, X. Liang, B. Li, S. Yu, J. Liu, Q. Huang, Y. Wei, R. Zhai, B. Lei, X. Guo, S. He, HOXA7 plays a critical role in metastasis of liver cancer associated with activation of Snail, *Molecular cancer*, 15 (2016) 57.
32. L. Yang, X. Peng, Y. Li, X. Zhang, Y. Ma, C. Wu, Q. Fan, S. Wei, H. Li, J. Liu, Long non-coding RNA HOTAIR promotes exosome secretion by regulating RAB35 and SNAP23 in hepatocellular carcinoma, *Molecular cancer*, 18 (2019) 78.
33. Y. Guo, Y. Peng, D. Gao, M. Zhang, W. Yang, E. Linghu, J.G. Herman, F. Fuks, G. Dong, M. Guo, Silencing HOXD10 by promoter region hypermethylation activates ERK signaling in hepatocellular carcinoma, *Clinical epigenetics*, 9 (2017) 116.
34. F. Xu, T. Jin, Y. Zhu, C. Dai, Immune checkpoint therapy in liver cancer, *Journal of experimental & clinical cancer research : CR*, 37 (2018) 110.
35. Q. Wu, W. Zhou, S. Yin, Y. Zhou, T. Chen, J. Qian, R. Su, L. Hong, H. Lu, F. Zhang, H. Xie, L. Zhou, S. Zheng, Blocking Triggering Receptor Expressed on Myeloid Cells-1-Positive Tumor-Associated Macrophages Induced by Hypoxia Reverses Immunosuppression and Anti-Programmed Cell Death Ligand 1 Resistance in Liver Cancer, *Hepatology (Baltimore, Md.)*, 70 (2019) 198-214.
36. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma, *Cell*, 169 (2017) 1327-1341.e1323.
37. A. Mantovani, P. Allavena, A. Sica, F. Balkwill, Cancer-related inflammation, *Nature*, 454 (2008) 436-444.
38. V. Bronte, S. Brandau, S.H. Chen, M.P. Colombo, A.B. Frey, T.F. Greten, S. Mandruzzato, P.J. Murray, A. Ochoa, S. Ostrand-Rosenberg, P.C. Rodriguez, A. Sica, V. Umansky, R.H. Vonderheide, D.I. Gabrilovich, Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards, *Nature communications*, 7 (2016) 12150.
39. W.H. Fridman, F. Pagès, C. Sautès-Fridman, J. Galon, The immune contexture in human tumours: impact on clinical outcome, *Nature reviews. Cancer*, 12 (2012) 298-306.

40. W.H. Fridman, L. Zitvogel, C. Sautès-Fridman, G. Kroemer, The immune contexture in cancer prognosis and treatment, *Nature reviews. Clinical oncology*, 14 (2017) 717-734.
41. Y.L. Zhang, J. Li, H.Y. Mo, F. Qiu, L.M. Zheng, C.N. Qian, Y.X. Zeng, Different subsets of tumor infiltrating lymphocytes correlate with NPC progression in different ways, *Molecular cancer*, 9 (2010) 4.
42. M. Tosolini, A. Kirilovsky, B. Mlecnik, T. Fredriksen, S. Mauger, G. Bindea, A. Berger, P. Bruneval, W.H. Fridman, F. Pagès, J. Galon, Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, th2, treg, th17) in patients with colorectal cancer, *Cancer research*, 71 (2011) 1263-1271.
43. M.E. Winerdal, P. Marits, M. Winerdal, M. Hasan, R. Rosenblatt, A. Tolf, K. Selling, A. Sherif, O. Winqvist, FOXP3 and survival in urinary bladder cancer, *BJU international*, 108 (2011) 1672-1678.
44. T. Shiraki, E. Takayama, H. Magari, T. Nakata, T. Maekita, S. Enomoto, Y. Mori, N. Shingaki, K. Moribata, H. Deguchi, K. Ueda, I. Inoue, M. Mizuno-Kamiya, K. Yashiro, M. Iguchi, H. Tamai, Y. Kameyama, J. Kato, N. Kondoh, M. Ichinose, Altered cytokine levels and increased CD4+CD57+ T cells in the peripheral blood of hepatitis C virus-related hepatocellular carcinoma patients, *Oncology reports*, 26 (2011) 201-208.
45. A. Mantovani, B cells and macrophages in cancer: yin and yang, *Nature medicine*, 17 (2011) 285-286.
46. P.B. Olkhanud, B. Damdinsuren, M. Bodogai, R.E. Gress, R. Sen, K. Wejksza, E. Malchinkhuu, R.P. Wersto, A. Biragyn, Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting CD4⁺ T cells to T-regulatory cells, *Cancer research*, 71 (2011) 3505-3515.
47. D.J. DiLillo, K. Yanaba, T.F. Tedder, B cells are required for optimal CD4⁺ and CD8⁺ T cell tumor immunity: therapeutic B cell depletion enhances B16 melanoma growth in mice, *Journal of immunology (Baltimore, Md. : 1950)*, 184 (2010) 4006-4016.

Tables

Table 1. Clinical characteristics of the hepatocellular carcinoma patients used in this study

	TCGA cohort (N=365)	LIRI-JP cohort (N=240)	GSE76427 (N=115)
Age (median, range)	62 (16-90)	69 (31-89)	69 (14-93)
Gender (%)			
Female	119 (32.6%)	65 (27.1%)	22 (19.1%)
Male	246 (67.4%)	175 (72.9%)	93 (80.9%)
AFP (ng/ml)			
≤200	201 (55.1%)	NA	NA
>200	75 (20.5%)	NA	NA
unknown	89 (24.4%)	NA	NA
Grade (%)			
Grade 1	55 (15.1%)	NA	NA
Grade 2	175 (47.9%)	NA	NA
Grade 3	118 (32.3%)	NA	NA
Grade 4	12 (3.3%)	NA	NA
unknown	5 (1.4%)	NA	NA
Vascular Invasion			
Yes	106 (29.0%)	NA	NA
No	205 (56.2%)	NA	NA
unknown	54 (14.8%)	NA	NA
Stage (%)			
I	170 (46.6%)	39 (16.3%)	55 (47.8%)
II	84 (23.0%)	107 (44.6%)	35 (30.4%)
III	83 (22.7%)	73 (30.4%)	21 (18.3%)
IV	4 (1.1%)	21 (8.8%)	3 (2.6%)
unknown	24 (6.6%)	0 (0.0%)	1 (0.9%)
Survival status			
OS days (median)	556	780	669

Table 2. Univariate Cox regression analysis of HOX genes family associated with overall survival in hepatocellular carcinoma

Ensembl ID	Gene Symbol	Genomic location	HR	95% CI	P-value
ENSG00000106006	HOXA6	Chromosome 7: 27,145,396-27,150,603	2.50	1.15-4.43	0.021
ENSG00000122592	HOXA7	Chromosome 7: 27,153,716-27,157,936	2.07	1.47-3.73	0.011
ENSG00000078399	HOXA9	Chromosome 7: 27,162,438-27,175,180	2.29	1.04-4.03	0.038
ENSG00000005073	HOXA11	Chromosome 7: 27,181,157-27,185,232	1.54	1.08-2.20	0.017
ENSG00000197757	HOXC6	Chromosome 12: 53,990,624-54,030,823	1.63	1.13-2.35	0.008
ENSG00000037965	HOXC8	Chromosome 12: 54,008,985-54,012,769	2.04	1.31-3.17	<0.001
ENSG00000128710	HOXD10	Chromosome 2: 176,108,790-176,119,937	2.00	1.43-2.80	<0.001
ENSG00000128713	HOXD11	Chromosome 2: 176,104,216-176,109,754	2.22	1.06-4.64	0.009
ENSG00000128714	HOXD13	Chromosome 2: 176,092,721-176,095,944	2.30	1.55-3.43	<0.001
ENSG00000128709	HOXD9	Chromosome 2: 176,122,719-176,124,937	1.41	1.17-1.70	<0.001

Table 3. Univariate and multivariate Cox regression to identify independent prognosis predictor in both the TCGA cohort and the ICGC cohort

Characteristics	TCGA cohort				ICGC cohort			
	Univariate mode		Multivariate model		Univariate mode		Multivariate model	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Age (≥65 vs <65)	1.02 (1.00–1.04)	0.024	1.03 (1.01–1.05)	0.002	1.18 (0.79–1.78)	0.415	1.54 (0.88–2.68)	0.130
Gender (Male vs Female)	1.41 (0.97–1.98)	0.072	1.38 (0.94–1.87)	0.096	1.05 (0.62–1.77)	0.868	NA	NA
AFP (≥200 vs <200)	0.90 (0.61–1.35)	0.624	1.06 (0.61–1.87)	0.832	NA	NA	NA	NA
TMB (TMB-H vs TMB-L)	1.30 (1.17–1.42)	<0.001	1.31 (1.16–1.48)	<0.001	NA	NA	NA	NA
Tumor grade (G3/4 vs G1/2)	0.87 (0.48–1.59)	0.651	0.90 (0.45–1.90)	0.763	NA	NA	NA	NA
Tumor stage (III/IV vs I/II)	1.48 (1.17–1.86)	<0.001	1.32 (0.85–1.95)	0.215	1.49 (0.60–3.71)	0.385	NA	NA
Vascular invasion (Yes vs No)	1.93 (0.94–3.96)	0.075	1.25 (0.52–2.93)	0.620	NA	NA	NA	NA
Risk (High vs Low)	1.13 (1.09–1.18)	<0.001	1.13 (1.08–1.18)	<0.001	1.81 (1.14–2.87)	0.012	1.70 (1.06–2.71)	0.027

Figures

Figure 1

Flow chart of the steps in the performed analyses.

Figure 2

Landscape of expression and prognosis of 39 HOX genes in hepatocellular carcinoma. (A) Heatmap of the 39 HOX genes expression in hepatocellular carcinoma. The left blue cluster is normal group, and the right red cluster is hepatocellular carcinoma group. (B) Forest plots showing the results of the univariate Cox regression between 11 HOX genes and OS. (C) Venn diagram to identify differentially expressed HOX genes between tumor and adjacent normal tissue that were correlated with OS. (D) HOXD13, (E) HOXD11, (F) HOXD10, (G) HOXA6, (H) HOXA7, (I) HOXC8, (J) HOXA11, (K) HOXA9, (L) HOXC6, (M) HOXD9 expression levels are shown for the hepatocellular carcinoma and normal tissue. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Figure 3

Patterns of HOX and biological characteristics of each pattern. (A) Consensus matrices of the HCC meta-cohort for $k = 2$ base on 10 HOX genes. (B) Survival analyses for the two m6A modification patterns in meta-cohort using Kaplan-Meier curves including 468 cases in HOXcluster-A and 257 cases in HOXcluster-B. The HOXcluster B showed significantly worse overall survival than the HOXcluster-A ($P < 0.001$, Log-rank test). (C) GSEA enrichment analysis showing the activation states of biological pathways in distinct HOX patterns. The heatmap was used to visualize these biological processes, and red represented activated pathways and blue represented inhibited pathways. (D) Unsupervised clustering of HOX in meta-cohort. The HOXcluster, tumor stage, survival status, gender and age were used as patient annotations. Red represented high expression of regulators and blue represented low expression. (E) The abundance of each TME infiltrating cell in three HOX patterns. The upper and lower ends of the boxes represented interquartile range of values. The lines in the boxes represented median value, and black dots showed outliers. The asterisks represented the statistical P value. (F) Principal component analysis for

the transcriptome profiles of two HOX patterns, showing a remarkable difference on transcriptome between different modification patterns. (G) Functional annotation for HOX-related genes using GSEA GO enrichment analysis. (H) Functional annotation for HOX-related genes using GSEA KEGG enrichment analysis. (*P < 0.05, **P < 0.01, ***P < 0.001)

Figure 4

Construction of HOX signatures. (A) Unsupervised clustering of 1417 HOX phenotype-related genes in meta-cohort and consensus matrices for k = 3. (B) Kaplan-Meier curves indicated HOX genomic phenotypes were markedly related to overall survival of 725 patients in meta-cohort, of which 237 cases were in gene cluster A, 288 cases in gene cluster B, and 190 cases in gene cluster C. The gene cluster C showed significantly worse overall survival than the other two gene cluster.(P<0.001, Log-rank test). (C) Unsupervised clustering of overlapping HOX phenotype-related genes in meta to classify patients into different genomic subtypes, termed as HOX gene cluster A-C, respectively. The gene clusters, HOXclusters, tumor stage, survival status, gender and age were used as patient annotations. (D) The expression of HOX genes in three gene cluster. The upper and lower ends of the boxes represented interquartile range of

values. The lines in the boxes represented median value, and black dots showed outliers. The asterisks represented the statistical P value. The one-way ANOVA test was used to test the statistical differences among three gene clusters. (E) Survival analyses for low (618 cases) and high (97 cases) HOXscore patient groups in metacohort using Kaplan-Meier curves ($P < 0.001$, Log-rank test). (F) Correlations between HOXscore and the known immune cells in meta cohort using Spearman analysis. Negative correlation was marked with blue and positive correlation with red. (G) Alluvial diagram showing the changes of HOXclusters, gene cluster, survival status, and HOXscore. (H) Differences in HOXscore among two HOX patterns in meta cohort ($P < 0.001$, Kruskal-Wallis test). (I) Differences in HOXscore among three gene clusters in meta cohort. The KruskalWallis test was used to compare the statistical difference between three gene clusters ($P < 0.001$). (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Figure 5

Kaplan–Meier curves were performed for patients stratified by clinicopathological features in the meta cohort. Impact of prognostic risk on overall survival for patients younger than 65 years old (A) and older than 65 years old (B), for female (C) and male patients (D), for patients in stage1-2 (E) and stage 3-4 (F). (G)Results of the univariate and multivariate Cox regression analyses regarding OS in the meta cohort. (H)The cisplatin sensitivity in high HOXscore and low HOXscore group. (I) The mitomycin sensitivity in high HOXscore and low HOXscore group. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Figure 6

GSEA on the meta cohort to explore mechanisms underlying HOXscore. (A) GSEA GO identifies high and low HOXscore related signaling pathway in HCC. (B) GSEA KEGG identifies high and low HOXscore related signaling pathway in HCC.

Figure 7

Relationship between the HOXscore and tumor somatic mutation (TMB). (A) The relationship between the HOXscore and TMB in three gene clusters in TCGA cohort. (B) Differences in TMB among high and low HOXscore in TCGA cohort. (C) Kaplan-Meier curves indicated TMB markedly related to overall survival of 355 patients in meta-cohort, of which 306 cases were in low TMB, 49 cases in were in high TMB. The high TMB group showed significantly worse overall survival than the low group.($P < 0.001$, Log-

rank test). (D) Kaplan–Meier curve analysis of overall survival is shown for patients classified according to TMB and HOXscore. Statistical analysis was performed using the log-rank test. The waterfall plot of tumor somatic mutation established by those with high HOXscore (E) and low HOXscore (F). Each column represented individual patients. The upper barplot showed TMB, The number on the right indicated the mutation frequency in each gene. The right barplot showed the proportion of each variant type. (*P < 0.05, **P < 0.01, ***P < 0.001)

Figure 8

Relationship between the HOXscore and PD-1/L1 immunotherapy. (A) PD-L1 expression patterns for patients in high- and low-HOXscore groups in the meta cohort. The correlation of HOXscore with clinical response to anti-PD-1 immunotherapy.(B)CTLA4_negative+ PD-1_negative, (C)CTLA4_negative + PD-1_positive, (D) CTLA4_positive + PD-1_positive, (E)CTLA4_positive + PD-1_negative. (F) The proportion of patients with MSI in low or high HOXscore groups. (H) Relationship between the HOXscore and MSI status.

Figure 9

Validation the expression and prognosis of HOXscore in GSE76427. (A) HOXD10, (B) HOXD13, (C) HOXC6, (D) HOXA9, (E) HOXA11, (F) HOXA6, (G) HOXD11, (H) HOXA7, (H) HOXD9 and (H) HOXC8 expression levels are shown for the HCC and normal tissue. (I) Kaplan–Meier curves illustrated that patients with high HOXscore had worse overall survival than those with low HOXscore (P <0.001)

Figure 10

The expression and overall survival of 10 HOX genes in HCC. (A) Representative immunohistochemistry images of 10 HOX genes expression in normal tissues, and HCC tissue. (B) 10 HOX genes mRNA levels are shown for the HCC and normal tissue. (F) Kaplan–Meier analysis of overall survival based on HOXscore in 150 cases of HCC patients.