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The prognostic significance of epoxide hydrolases in colorectal cancer

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ABSTRACT

Colorectal cancer (CRC) is a common malignant cancer. Epoxide hydrolases (EHs) are involved in the development of cancer by regulating epoxides, but their relationship with CRC is unclear. We used multiple datasets to confirm the expression of different EPHX family members in CRC tissues, and to explore their association with different clinicopathologic characteristics. The Kaplan–Meier method, correlation analysis and random forest algorithm were used to evaluate the prognostic value of EPHX family members for CRC. Finally, the cell experiment verified function of EPHX4 in CRC. The expressions of EPHX1 and EPHX2 were significantly decreased, while those of EPHX3 and EPHX4 were significantly increased in CRC. The expressions of EPHX family members were correlated with some clinicopathologic features and overall survival. The expressions of the EPHX family were positively associated with CD274, CTLA4, HAVCR2, and TIGIT. EPHX2 and EPHX4 were diagnostic and predictive biomarkers for CRC. EPHX4 promoted the malignant phenotype of CRC cells. Our study firstly elucidated the prognostic significance of EPHX family members in CRC and identified novel diagnostic and prognostic biomarkers for CRC.

1. Introduction

Colorectal cancer (CRC) is a common malignancy of gastrointestinal tract. Currently, approximately 153,020 cases of CRC patients are newly diagnosed in 2023 [1]. In China, the number of newly diagnosed CRC cases is also rising rapidly [2]. Although the treatment of CRC has improved significantly with the rapid development of medical technology, the 5-year survival rate for metastatic CRC patients is approximately 14 % [3]. Besides, the accuracy of the diagnostic results of CRC patients through traditional testing methods (e.g. invasive endoscopic methods) is still not ideal, which has led to the detection of cases at a later stage [4]. Early diagnosis is key to treatment, and reliable biomarkers play an important role in the healthcare management of cancer [5]. Therefore, there is an urgent need to find new diagnostic biomarkers to improve the sensitivity and specification of CRC early detection and prognosis.

Epoxide hydrolases (EHs) are a small family of proteins that were originally thought to be a group of detoxifying enzymes [6]. EHs catalyze the hydrolysis of epoxides by adding active water to form the corresponding vicinal diol [7]. Epoxides are organic ternary oxygen compounds that are produced from endogenous and exogenous compounds through chemical and enzymatic oxidation processes [8]. Studies have reported that changes in levels of epoxides in fatty acids can cause many diseases [9]. Thus, EHs can coordinate key signaling pathways for cell homeostasis [10]. The more studied EHs include EPHX1, EPHX2, EPHX3, and EPHX4 [11]. EPHX1 and EPHX2 are the most well-known EHs. EPHX1 and EPHX2 have been observed to be abnormally expressed in human malignancies, including liver cancer, lung cancer, prostate cancer (PCa), and advanced ovarian cancer [12–16]. In addition, Quercetin can inhibit CRC via up-regulating the expression of EPHX1 and EPHX2 [17]. EPHX3 is mainly expressed in lung, skin and gastrointestinal tissues [11,18]. EPHX3 has been shown to act as a regulator of tumorigenesis in 13 cancers [19]. EPHX4 is highly homologous to EPHX3, and has been found to be highly up-regulated in an independent dataset on primary rectal cancer [18,20]. The above findings indicated that different members of the EPHX family are closely associated with cancers, but there are few studies on the expression and clinical significance of different members of the EPHX family in CRC.

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Here, we validated the expression of different EPHX family members in CRC using multiple independent datasets and investigated the relationship between different EPHX family members and clinical pathological parameters, and assessed their prognostic value. Meantime, the function of EPHX4 in CRC was verified by *in vitro* experiments.

2. Materials and methods

2.1. Datasets collection

The UCSC Xena platform (https://xenabrowser.net/datapages/) was used to download the mRNA sequencing data and corresponding clinical information of The Cancer Genome Atlas (TCGA)-colon adenocarcinoma (COAD)/rectum adenocarcinoma esophageal carcinoma (READ). The mRNA expression data of COAD and READ were combined to obtain the mRNA expression dataset of CRC. A total of 649 COAD/READ samples (48 normal and 601 tumor samples) were collected. Specific sample information is shown in Table S1. Principal component analysis (PCA) was used to examine the randomness of the distribution of the samples in the COAD/READ dataset.

The ICGC dataset (https://www.icgc-argo.org/) provided survival information of CRC patients (57 alive and 14 death). Three independent Gene Expression Omnibus (GEO) datasets (GSE21510, GSE39582, and GSE40967) were downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The GSE21510 dataset provided the gene expression data of the EPHX family members. GSE39582 (19 normal and 556 tumor samples) and GSE40967 (19 normal and 556 tumor samples) provided the mRNA expression data of EPHX1/2/3/4 and clinical and survival information of CRC patients. The detailed information of ICGC, GSE39582 and GSE40967 are shown in Table S2, Table S3 and Table S4, respectively.

2.2. Exploration of the prognostic significance of different EPHX family members

The unpaired *t*-test was used to compare the expressions of different EPHX family members in normal and tumor tissues ($P < 0.05$ together with $|\log_2$ fold change (FC)$| > 0.585$ as thresholds), and compare the EPHX family members expression in CRC patients with different clinicopathological characteristics ($P < 0.05$ together with $|\log_2 FC| > 0.585$ as thresholds). The Kaplan–Meier curves were plotted to distinguish the association between the mRNA expression level of different EPHX family members and CRC patients' overall survival using the *survminer* R package (version '3.3.1'). Subsequently, we evaluated potential relationships between EPHX family members and effective markers of immunotherapy. Immune checkpoint markers are derived from immune checkpoint molecules commonly used in CRC immunotherapy [21,22]. R software *cor.test* (version '3.6.2') was used to evaluate the correlations between EPHX family members and the immune checkpoints biomarkers ($P < 0.05$). Machine learning has gained popularity in several fields in recent years [23–27]. Random forest, as a comprehensive learning algorithm, is combined with decision trees, each of which depends on independent sampling values of random vectors, and all trees have the same distribution in the forest [28]. Here, with EPHX family members as independent predictors, a diagnostic prediction model was established using the random forest algorithm (randomForest version 4.7–1.1) [29]. Briefly, the 'CreateDataPartition' function in R package caret was first used to divide a dataset into training dataset and validation dataset in a ratio of 1:1. Then R package randomForest was used to build a training model based on the training dataset, and validation dataset was employed to verify the performance of the constructed model. To evaluate the performance of the model, the receiver operating characteristic (ROC) was plotted to calculate the area under the curve (AUC) and *P*-value using R package-pROC (version '1.18.4'). EPHX family member with AUC >0.8 and $P < 0.05$ was regarded as an optimal diagnostic biomarker for CRC.

2.3. Cell culture and transfection

Guangzhou Gineo Biotechnology Co., Ltd. provided SW480 and HCT116 cells. These cells were grown in RPMI-1640 medium with 10 % fetal bovine serum (Gibco Company) and 1 % penicillin-streptomycin solution (Sigma-Aldrich), followed by incubation in an incubator (37 °C with 5 % CO_2). Small RNA interfering (siRNA) negative control (NC) and si-EPHX4 were chemically synthesized by Beijing Tsingke Biotech Co., Ltd. The pCMV-EPHX4 overexpression vector was constructed by SignalChem Biotech (Beijing). We used Lipofectamine™ 2000 (Invitrogen) to transfect cells with siRNAs or overexpression vector in line with the manufacturer's recommendations. There was three target sequences for siRNA for EPHX4, and the detailed information is shown in Table S5.

2.4. Quantitative real-time PCR (q-PCR)

TRIzol (Ambion) was employed to isolate total cellular RNA. Then, the PrimeScript™ RT Master Mix (Takara) was utilized to synthesize complementary DNA (cDNA). Subsequently, we performed q-PCR using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad) and SYBR Premix Ex Taq™ II (Takara). The $2^{-\Delta\Delta Ct}$ method was utilized to determine the relative quantification. GAPDH was an internal reference gene. Primer information is shown in Table S6.

2.5. Cell counting kit-8 (CCK-8) assay

CCK-8 assay (DOJINDO) was utilized to measure cell proliferation according to the manufacturer's instructions. Briefly, cells were cultured in RPMI-1640 medium containing 10 % FBS, and seeded in a 96-well plate at 1000 cells/well. According to the time point (Day 1, Day 2, Day3, and Day 4), the original medium in the 96-well plate was removed, 10 μL CCK-8 reagent was added to each well for 2.5 h. Finally, OD value was determined at 450 nm using microplate spectrophotometer.

2.6. Migration assays

The cells without serum medium were inoculated into the upper chamber, and the medium-containing serum was placed in the bottom chamber. After 24 h, 4 % paraformaldehyde was used to fix the migrated cells, and then 0.1 % crystal violet was applied to stain cells for 15 min. Image J was used to quantify the number of migrating cells.

2.7. Statistical analysis

For statistical analysis, IBM SPSS 20.0 software was used to perform the data analysis [30–34]. Draw graphs using GraphPad Prism 8.0 software. Standard Deviation (SD) is an important index to test statistical significance [35]. Data were expressed as mean ± SD, and at least three independent experiments or replicates were performed for each assay. The differences between the two groups were analyzed by Student's *t*-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Differential expression of EPHX family members in CRC and normal tissues

PCA results showed all samples in COAD and READ were randomly distributed (Fig. S1A), and significant separation between tumor and normal samples in the combined COAD/READ dataset (Fig. S1B), indicating that the batch effect of the combined dataset (TCGA-COAD/READ) was not significant and could be used for further analysis. We used multiple datasets to compare the expression of EPHX family members in CRC and normal tissues. TCGA dataset showed that EPHX1

($P \leq$ 2e-16) and EPHX2 ($P \leq$ 2e-16) were down-regulated in CRC, while EPHX3 ($P =$ 1.6e-06) and EPHX4 ($P \leq$ 2e-16) were up-regulated in CRC, comparing with normal tissues (Fig. 1A). Similarly, GSE21510, GSE9582, and GSE40967 datasets demonstrated that compared to normal tissues, EPHX1 ($P < 0.001$) and EPHX2 ($P < 0.001$) were down-regulated in CRC, while EPHX3 ($P < 0.001$) and EPHX4 ($P < 0.001$) were up-regulated in CRC (Fig. 1B–D). These results indicated that EPHX family members are expressed abnormally in CRC.

3.2. Correlation of the EPHX family expression with clinicopathologic features in CRC

For TCGA dataset, patients with stage III/IV characteristic had higher expression of EPHX1 ($P = 0.0143$) and EPHX3 ($P = 0.0423$), and lower expression of EPHX2 ($P = 0.0046$) than patients with stage I/II disease (Fig. 2A); compared with T1/T2 stage patients, the expression of EPHX1 ($P = 0.0157$) and EPHX3 ($P = 0.0004$) was higher in T3/T4 stage patients (Fig. 2B); compared with M0 stage patients, patients with M1 stage had higher of the expression of EPHX3 ($P = 0.031$, Fig. 2C); compared with N0 stage patients, patients with N1/2 stage had increased expression of the expression of EPHX1 ($P = 0.0181$) and EPHX3 ($P = 0.0227$), and decreased expression of EPHX2 ($P = 0.0046$, Fig. 2D). For GSE9582 dataset, EPHX4 was down-regulated in patients with stage 2/3/4 ($P = 0.031$, Fig. 2E), EPHX1 was both up-regulated in patients with T3/T4 stage ($P = 0.013$, Fig. 2F) and patients with M1 stage ($P = 0.013$, Fig. 2G), while EPHX2 was both declined in patients with M1 stage ($P = 0.0042$, Fig. 2G) and patients with N2/N3 stage ($P = 0.038$, Fig. 2H). For GSE40967 dataset, patients with stage 3/4 had lower expression of EPHX2 ($P = 0.0052$, Fig. 2I), patients with T3/T4 stage had higher expression of EPHX1 ($P = 0.011$, Fig. 2J), patients with M1 had higher expression of EPHX1 ($P = 0.017$) and lower expression of EPHX2 ($P = 0.019$, Fig. 2K). There was no obvious relationship between the EPHX family and N stage (Fig. 2L). These findings indicated the expressions of EPHX family members have close correlation with clinicopathologic characteristics.

3.3. Prognostic value of different EPHX family members in CRC patients

As shown by the Kaplan-Meier curve, CRC patients with a high expression level of EPHX1 and EPHX3 had a worse overall survival in TCGA dataset (for EPHX1, $P = 0.028$, Fig. 3A; for EPHX3, $P = 0.036$, Fig. 3C). The expression of EPHX2 didn't influence the overall survival of CRC patients (TCGA dataset: $P = 0.79$, Fig. 3B). CRC patients with a high expression level of EPHX4 had a longer overall survival than low expression patients (TCGA dataset: $P = 0.028$, Fig. 3D). In the GSE40967 dataset, the expressions of EPHX1 and EPHX3 didn't influence the overall survival of CRC patients (for EPHX1, $P = 0.17$, Fig. 3E; for EPHX3, $P = 0.28$, Fig. 3G). Additionally, CRC patients from the high expression level of EPHX2 had significantly better overall survival than patients from the low expression level of EPHX2 (GSE40967 dataset, $P = 0.047$, Fig. 3F). CRC patients with a low expression level of EPHX4 had a longer overall survival than those of high expression patients (GSE40967 dataset: $P = 0.0074$, Fig. 3H).

3.4. Correlation of immune checkpoints with different EPHX family members

Correlation analysis was used to explore the relevance of immune checkpoints to different EPHX family members based on the TCGA dataset. As shown in Fig. 4A–F, EPHX1 expression was significantly positive with CD274, CTLA4, HAVCR2, LAG3, PDCD1, and TIGIT. Similarly, other members' expressions of the EPHX family were positively associated with CD274, CTLA4, HAVCR2, and TIGIT (Fig. 4G–U, Fig. 4X). However, the mRNA expression of EPHX4 was negatively correlated with the levels of LAG3 (Fig. 4V) and PDCD1 (Fig. 4W). These results suggest that tumor immune escape may be involved in EPHX family mediated CRC occurrence.

3.5. EPHX family members as diagnostic and predictive markers

In order to further determine whether EPHX family members can be used as diagnostic prediction markers for CRC, a diagnostic prediction model was established in this study based on the TCGA and GSE40967 datasets. In the TCGA dataset, the diagnostic prediction model

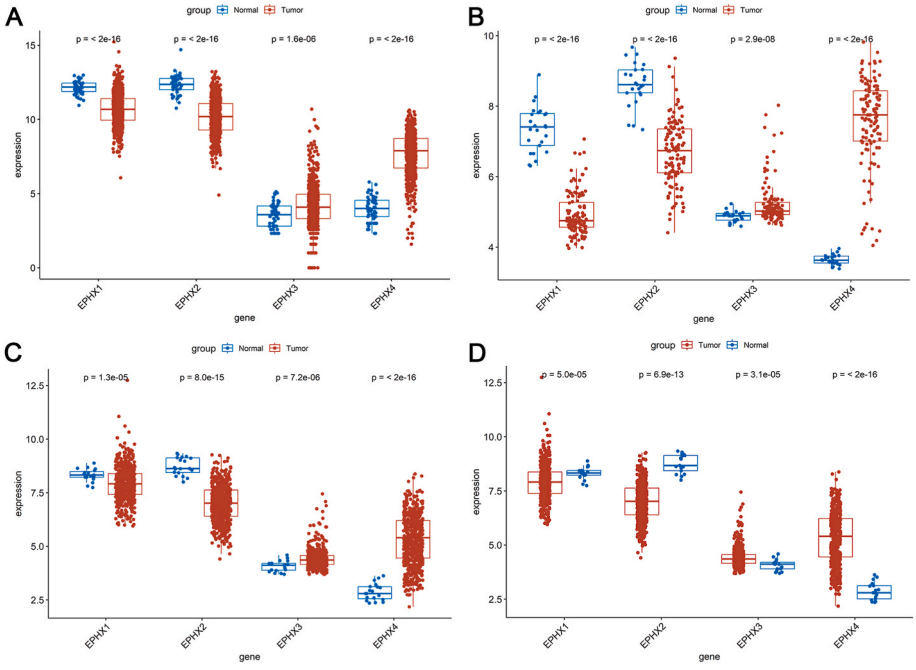

Fig. 1. Comparison the expression of EPHX family members in CRC and normal tissues. (A) TCGA dataset, (B) GSE21510 dataset, (C) GSE9582 dataset, and (D) GSE40967 dataset.

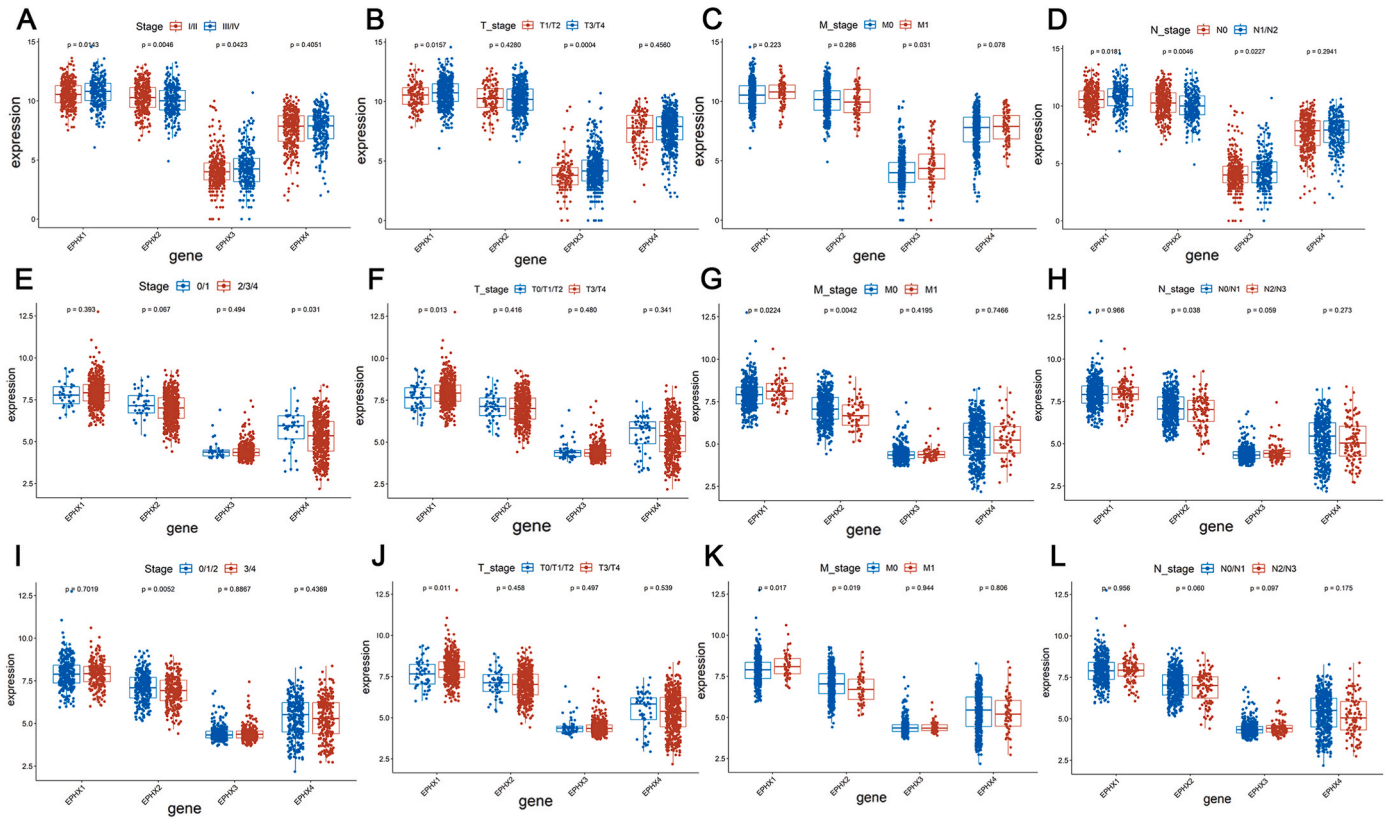

Fig. 2. Association of EPHX family expression with clinicopathologic features in CRC. (A–D) Comparison the expression of EPHX family members in different clinical stage (A), T stage (B), M stage (C) and N stage (D) in TCGA dataset. Comparison the expression of EPHX family members in different clinical stage (E), T stage (F), M stage (G) and N stage (H) in GSE9582 dataset. Comparison the expression of EPHX family members in different clinical stage (I), T stage (J), M stage (K) and N stage (L) in GSE40967 dataset.

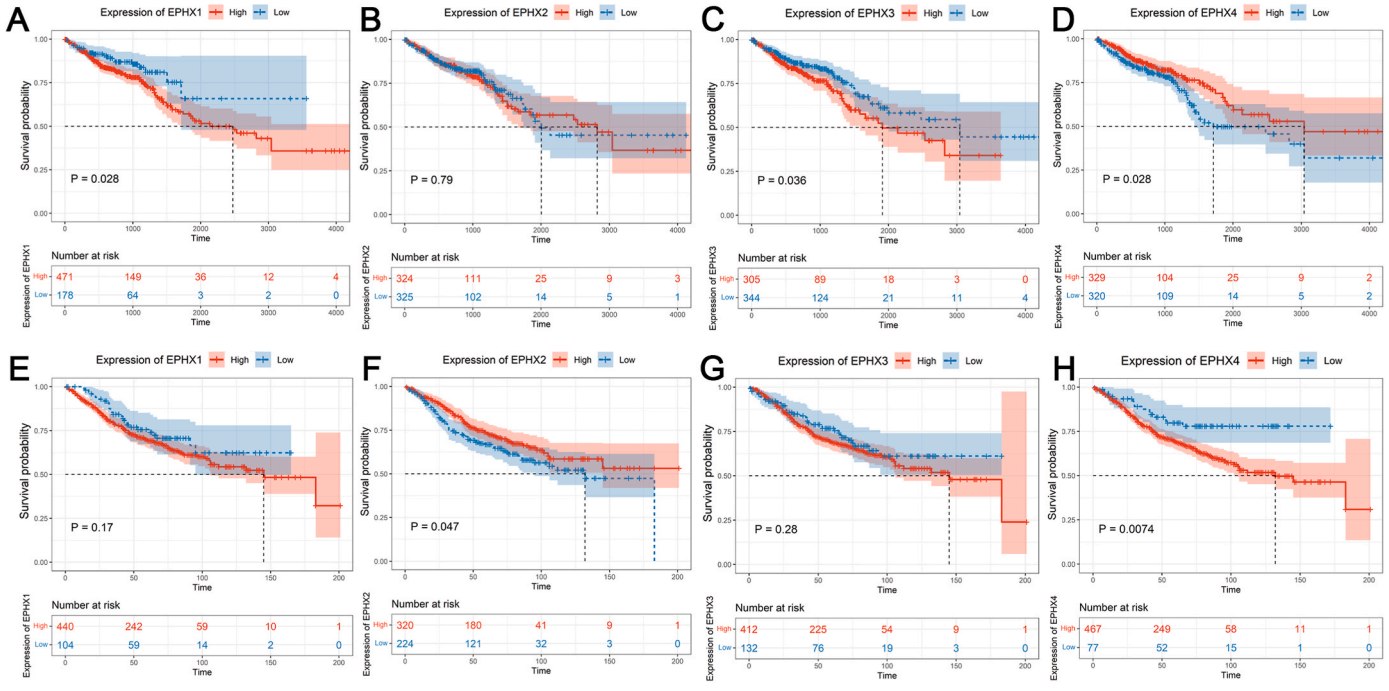

Fig. 3. Survivals analysis of patients with different EPHX1, EPHX2, EPHX3, and EPHX4 expression levels. (A–D) TCGA and (E–H) GSE40967 datasets.

constructed by EPHX1 has good performance (AUC = 0.863, $P < 1e\text{-}04$) as an independent predictor for CRC (Fig. 5A). Similarly, the diagnostic prediction models constructed by EPHX2 (AUC = 0.881, $P < 1e\text{-}04$, Fig. 5B) and EPHX4 (AUC = 0.971, $P < 1e\text{-}04$, Fig. 5D) have great performance as independent predictors for CRC. However, the diagnostic prediction model built with EPHX3 has no diagnostic value (AUC

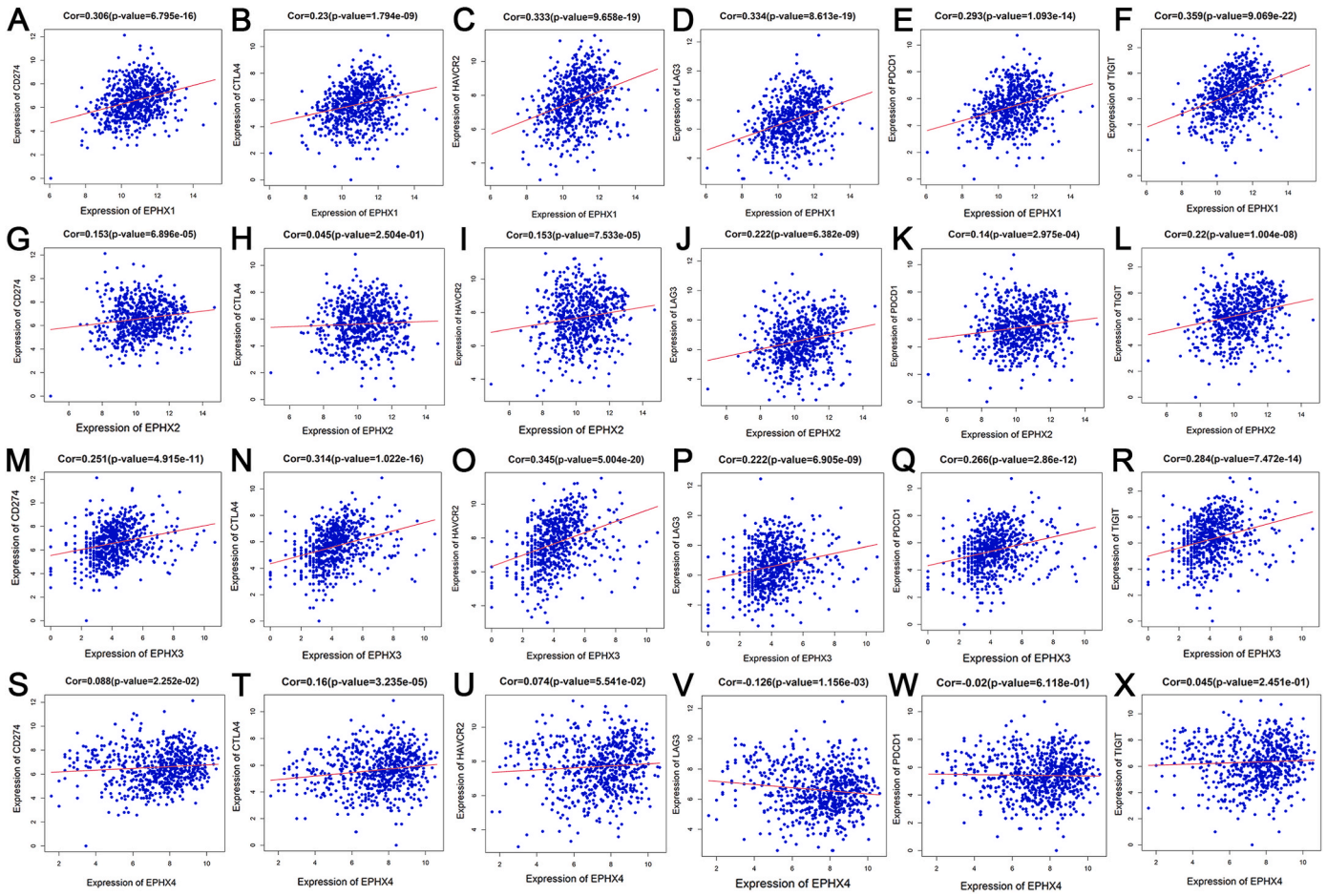

Fig. 4. The analysis of correlations between expression of EPHX family members and expression of immune checkpoints biomarkers on TCGA dataset. (A–F) Correlations of immune checkpoints with expression of EPHX1. (G–L) Correlations of immune checkpoints with expression of EPHX2. (M–R) Correlations of immune checkpoints with expression of EPHX3. (S–X) Correlations of immune checkpoints with expression of EPHX4.

= 0.471, $P = 0.5676$, Fig. 5C). Besides, in the GSE40967 dataset, the diagnostic prediction models constructed by EPHX2 (AUC = 0.915, $P <$ 1e-04, Fig. 5F) and EPHX4 (AUC = 0.869, $P <$ 1e-04, Fig. 5H) still performed well. However, the diagnostic prediction models built with EPHX1 (AUC = 0.346, $P = 0.0956$, Fig. 5E) and EPHX3 (AUC = 0.406, $P = 0.2887$, Fig. 5G) have no diagnostic value. Taken together, the diagnostic prediction models constructed by EPHX2 and EPHX4 had strong applicability and stability for CRC.

3.6. *The role of EPHX4 in CRC cell lines*

The Human Protein Atlas database (https://www.proteinatlas.org/) showed EPHX4 expression was lower in HCT116 cells and higher in SW480 cells (Fig. S2). Therefore, we transfected siRNA-EPHX4 in SW480 cells (Fig. 6A) and EPHX4 overexpression plasmid in HCT116 cells (Fig. 6E) to determine the biological function of EPHX4 in CRC. Because siEPHX4#1 and siEPHX4#2 showed excellent knockout efficiency among 3 siRNAs (Fig. 6A), we choose them for the next step of analysis. As illustrated in Fig. 6B, EPHX4 silencing dramatically suppressed the activity of SW480 cells. Following that, EPHX4 knockdown significantly up-regulated the expression of E-CAD and down-regulated the expression of ZEB2 and SNAI-2 in SW480 cells (Fig. 6C), suggesting that EPHX4 promotes the epithelial-mesenchymal transition (EMT) in CRC cells. Meantime, EPHX4 deficiency decreased the number of migrated cells (Fig. 6D). Consistently, EPHX4 overexpression did not affect cell proliferation of HCT116 cells (Fig. 6F). EPHX4 overexpression significantly increased the expression of ZEB2 (Fig. 6G) and enhanced the migration ability of HCT116 cells (Fig. 6H). These findings demonstrated that EPHX4 acts as an oncogene to facilitate malignant proliferation of CRC.

4. Discussion

EPHX family members are universally expressed in almost all organs and tissues [36]. EPHX family members utilized activated water to catalyze the hydrolysis of epoxides into the corresponding dihydrodiol [37], where epoxides are involved in the development of cancer through interactions with DNA, lipids, and proteins [10]. EPHX family members have been reported to be associated with tumor development and may serve as prognostic markers for certain types of cancer, such as hepatocellular carcinoma (HCC) [38] and PCa [16]. However, the unique role of EPHX family members in CRC remains to be elucidated. Moreover, given the rapid development of bioinformation technology, the computational simulation (in silico) study can extract key information from multiple information repositories more quickly to build digitally-relevant medical models than traditional medical research [39–43]. Therefore, we analyzed the expression patterns and prognostic value of 4 EPHX family members in CRC datasets via bioinformatic methods.

Our results showed EPHX1 and EPHX2 were down-regulated, and EPHX3 and EPHX4 were up-regulated in CRC. Besides, increased EPHX1 and EPHX3 were associated with an increase in disease stage, and increased EPHX2 and EPHX4 were associated with a decrease in disease stage. These findings indicated that EPHX1 and EPHX4 may be

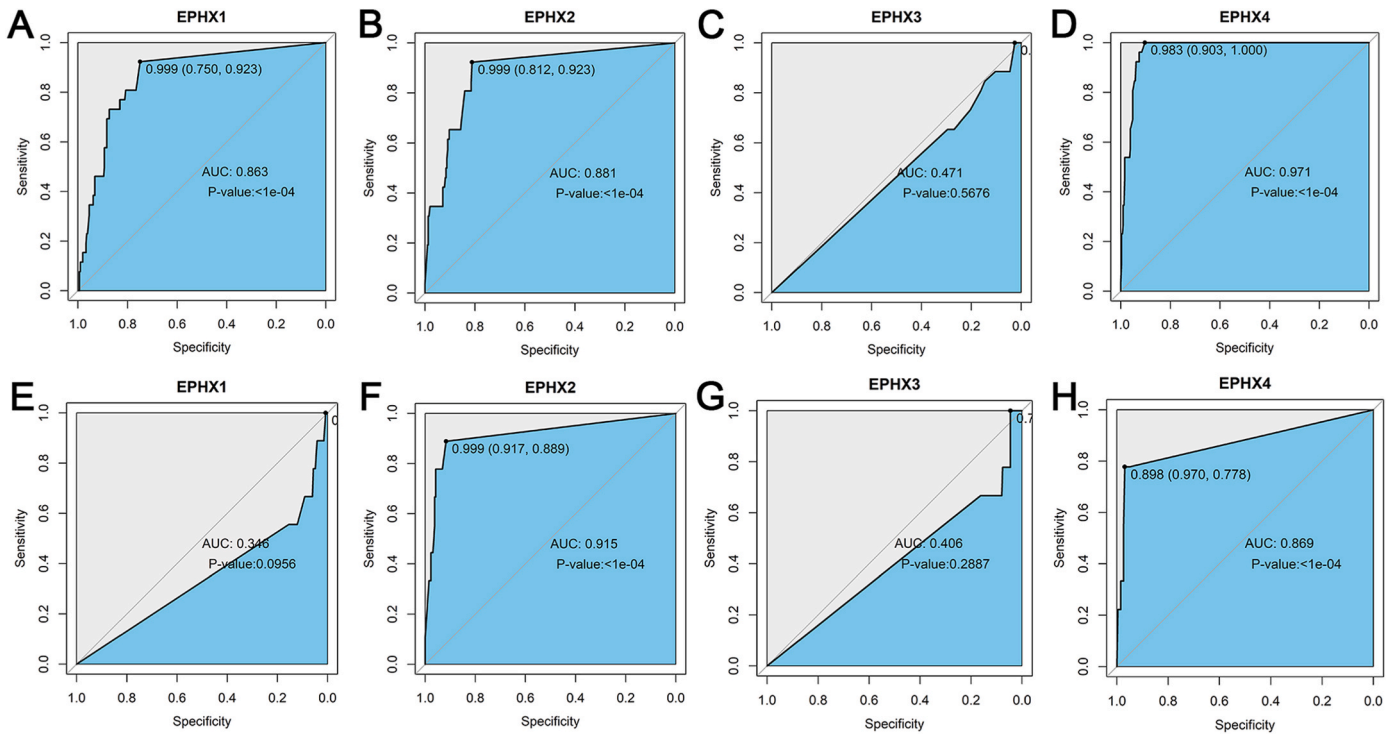

Fig. 5. EPHX family members as diagnostic and predictive markers. (A–D) The diagnosis and prediction model of CRC was constructed based on EPHX1, EPHX2, EPHX3, and EPHX4 in the TCGA dataset. (E–H) The diagnosis and prediction model of CRC was constructed based on EPHX1, EPHX2, EPHX3, and EPHX4 in the GSE40967 dataset.

correlated with CRC progression rather than tumorigenesis. EPHX1 has been demonstrated to regulate the progression of a variety of cancers. For instance, EPHX1 was down-regulated in patients with HCC, and elevation of EPHX1 inhibited malignant activity in HCC cells [38]. EPHX1 His139Arg polymorphism had a potential protective effect on CRC [44]. In our study, CRC patients with up-regulated EPHX1 had poor survival, indicating EPHX1 may be an important risk factor in CRC. Besides, EPHX2 was decreased in PCa, and its deregulation was related to the clinical characteristics of PCa development [16]. EPHX2 depressed colon cancer progression by promoting fatty acid degradation [45]. Similarly, our results showed patients with up-regulated EPHX2 had favorable survival, suggesting EPHX2 was a tumor suppressor gene for CRC. EPHX3 was down-regulated in head and neck squamous cell carcinomas (HNSCC) and could be used as a marker of poor prognosis for HNSCC [19]. Moreover, the risk signature consisted of EPHX3 and other 3-genes was identified as a prognostic tool for colon cancer [46]. Similarly, high expression of EPHX3 was correlated with poor prognosis of CRC patients, demonstrating EPHX3 may be a valuable prognostic biomarker for CRC patients. Surprisingly, our results found CRC patients with increased EPHX4 had better survival in TCGA dataset, while the opposite was true in the GSE40967 dataset. We speculated that this may have something to do with the platform on which the dataset is sequenced. The TCGA dataset was sequenced using second-generation sequencing, while the GSE40967 was sequenced using the array method. Survival analysis of EPHX4 in CRC patients was also performed using additional datasets (ICGC dataset: second-generation sequencing, GSE39582 dataset: array method). Similarly, CRC patients with increased EPHX4 had better survival in ICGC dataset (Fig. S3A), while the opposite was true in the GSE39582 dataset (Fig. S3B). Therefore, the critical role of EPHX4 in CRC needs to be further explored.

Immunotherapy can eliminate cancer cells and produce new antigens, which may have advantages over chemotherapy in suppressing tumor development [47]. Our study showed that the expressions of EPHX family members were positively correlated with CD274, CTLA4, HAVCR2 and TIGIT. CD274, an important immune checkpoint protein, binds to PD-1 on T-Lymphocytes, resulting in reversing the phenotype of aging T cells and normalizing anti-tumor responses [48]. Tumor cells have been found to be able to activate CTLA-4, inactivating activated T cells, thereby achieving immune escape [49]. HAVCR2, a negatively regulated immune checkpoint protein, is involved in the regulation of the immune response and immune tolerance [50]. TIGIT is a newly discovered immunosuppressive molecule that interacts with two ligands, CD155 and CD112 [51]. The expressions of EPHX family members are positively correlated with these immunosuppressive point biomarkers, suggesting that EHs plays an important role in CRC immunotherapy. Furthermore, EPHX4 was negatively correlated with LAG3 and PDCD1. Both LAG3 and PDCD1 (coding for PD-1) are associated with T-cell surface receptors. Recent studies have shown that blocking LAG-3 and PD-1 can lead to co-expression of cytotoxic and depletion gene modules in $CD8^+$ T cells, thus promoting anti-tumor immunity [52]. The findings means that EPHX4 is critical for CRC immunotherapy, and more experiments are needed to explore its mystery.

EPHX4 is a member of the epoxide hydrolase family, which consists of a small number of detoxifying proteins whose main function is to catalyze the addition of a water molecule to an epoxide as a whole reaction within the cell [53]. The abnormal activation of epoxides may lead to mutagenesis, toxicity and carcinogenesis [54]. EPHX4 was found to be primarily present in the brain, but it has also been reported that EPHX4 was expressed in normal colonic epithelium [55]. Furthermore, EPHX4 was elevated in laryngeal cancer specimens and had a worse prognosis [56]. However, previous studies have not clarified the expression level and function of EPHX4 in CRC. Our study showed for the first time that the diagnostic prediction model constructed by EPHX4 had a good predictive performance, and downregulation of EPHX4 inhibited cell growth, EMT, and migration in CRC, suggesting EPHX4 as a putative prognostic biomarker for CRC.

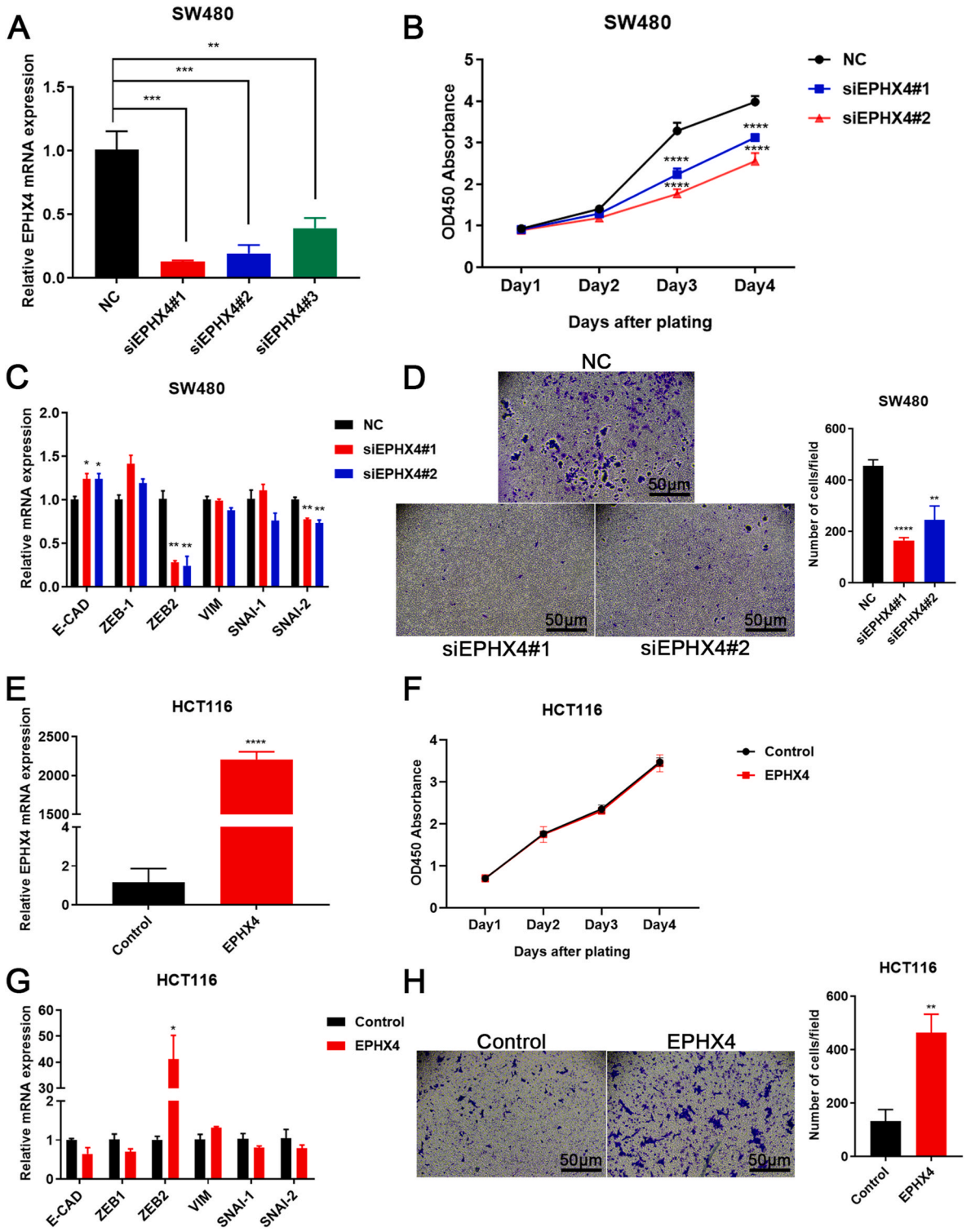

Fig. 6. The role of EPHX4 in CRC cell lines was verified *in vitro*. (A) The knockdown efficiency of EPHX4 was tested in SW480 cells by q-PCR. (B) EPHX4 knockdown reduced the activity of SW480 cells. (C) q-PCR was used to verify the mRNA expression of EMT biomarkers following EPHX4 knockdown in SW480 cells. (D) EPHX4 knockdown inhibited the migration of SW480 cells, scale bar = 50 μm. (E) EPHX4 overexpression vector up-regulated EPHX4 expression in HCT116 cells. (F) EPHX4 overexpression didn't influence the activity of HCT116 cells. (G) EPHX4 overexpression up-regulated ZEB2 expression in HCT116 cells. (H) EPHX4 overexpression promoted the migration of HCT116 cells, scale bar = 50 μm $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, n = 3.

5. Conclusion

Our study firstly demonstrated the prognostic significance of different EPHX family members in CRC and mentioned EPHX4 as a novel prognostic biomarker for CRC, contributing to helping doctors enhance the clinical management of patients with CRC.

6. Limitations

There are undoubtedly some limitations to our study. There are

biases in conclusions between datasets. Validation experiments of clinical samples are missing. EPHX4 was functionally validated using only two cell lines. Therefore, the prognostic role of EPHX4 in CRC will be explored with additional datasets, clinical samples, and cell lines in the future.

CRediT authorship contribution statement

Lichao Cao: Writing – original draft, Conceptualization. **Ying Ba:** Writing – original draft, Conceptualization. **Fang Chen:** Methodology. **Dandan Li:** Validation. **Shenrui Zhang:** Data curation. **Hezi Zhang:** Writing – review & editing, Conceptualization.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

All datasets used in this study are publicly available on the UCSC Xena platform, ICGC and GEO databases.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2024.101912.

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