78685_Auto_Edited-check.docx

WORD COUNT	4188	TIME SUBMITTED	28-SEP-2022 10:45AM
		PAPER ID	91016069

Name of Journal: *World Journal of Gastrointestinal Oncology*
Manuscript NO: 78685
Manuscript Type: ORIGINAL ARTICLE

Basic Study

Comprehensive analysis of the tumor suppressive role and prognostic value of the sine oculis homeobox homolog family in colorectal cancer

Fang ZX *et al*. SIXs in CRCs

Abstract

BACKGROUND

Several genes, important for development, are reduced or silenced in adulthood, and their abnormal expression has been related to the occurrence and development of malignant tumors. Human sine oculis homeobox homolog (SIX) proteins belong to the homeobox family and play important roles in the development of different organs. Importantly, SIXs are predicted to have chromatin-binding and DNA-binding transcription factor activity, and reported roles in cancers. However, a comprehensive analysis of SIXs in colorectal cancers (CRCs) has not been performed.

AIM

To explore the expression pattern of 6 SIXs in CRCs and their relationship with the clinicopathological parameters of CRC patients, as well as investigate the potential utilization of SIXs as novel prognostic indicators in CRCs.

METHODS

The expression level of SIXs in normal tissues of different organs and related cancerous tissues was analyzed in the Human Protein Atlas. Kaplan-Meier Plotter and GEPIA2 were used to analyze the prognostic values of SIXs. To analyze the potential signaling pathways with SIX family involvement, LinkedOmics was used to perform Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses of SIX4-related genes. Subsequently, immunohistochemical experiments were performed on CRC tissues and adjacent normal tissues, and we examined the SIX4 expression level in 87 pairs of patients in tissue microarrays. The relationship between SIX4 and clinicopathological parameters in CRC patients was tested using the X2 test and Fisher's exact probability to verify the results of the database analysis.

RESULTS

The RNA levels of SIX1-4 and SIX6 were relatively low in normal human tissues, while SIX5 was highly expressed at both the RNA and protein levels. However, the protein level of SIX4 was found to be elevated in various malignancies. In CRC tissues, SIXs 1, 2 and 4 were elevated in cancer tissues compared with adjacent normal tissue. Among all SIXs, a high level of SIX4 was found to be associated with poor overall and disease-free survival in patients with CRC. For different clinicopathological parameters, increased SIX4 expression was positively correlated with advanced CRC. The top 50 SIX4-related genes were involved with oxidative phosphorylation and the respiratory chain signaling pathway.

CONCLUSION

Current results provide a comprehensive analysis of the expression and prognostic values of SIX family members in CRC. Among different SIXs, SIX4 plays an oncogenic role in CRC to promote the development of malignancy. In CRC, SIX4 mRNA and protein expression is higher than that in normal tissues, and associated with shorter CRC patient survival, suggesting that SIX4 may be a potential therapeutic target for treatment of CRC patients.

Key Words: Sine oculis homeobox homolog; Colorectal cancer; Development; Treatment

Fang ZX, Li CL, Wu Z, Hou YY, Wu HT, Liu J. Comprehensive analysis of the tumor suppressive role and prognostic value of the sine oculis homeobox homolog family in colorectal cancer. *World J Gastrointest Oncol* 2022; In press

Core Tip: This study systematically analyzes the expression pattern and prognostic value of sine oculis homeobox homolog (SIX) family members in colorectal cancer (CRC). It was found that expression of SIX4 in CRC tissues positive correlated with the

development of CRC, and negative correlated with overall survival. SIX4 can be a novel and potential therapeutic target for CRC.

INTRODUCTION

Although the study of early human development is limited due to the small number of samples, it is well known how the genome activates or silences transcriptional programs to govern organ formation[1]. Gerrard *et al*[2] focused on histone modifications during human organogenesis, and found that key developmental gene sets are actively repressed outside of the appropriate organ. Interestingly, the abnormal expression of organogenesis-related genes at inappropriate developmental stages has been reported in different diseases, especially malignant tumors[3].

Human sine oculis homeobox homolog (SIX) proteins comprise a group of six family members that act as key regulators of organogenesis in kidney, limb, eye, brain and craniofacial structure[4-7]. Defects in these genes result in hypoplastic disorders, such as autosomal dominant deafness type 23, branchiootic syndrome type 3, holoprosencephaly type 2, branchiootorenal syndrome type 2, and isolated microphthalmia with cataract type 2[8-12].

Colorectal cancer (CRC) is ranked third in incidence among malignant tumors worldwide, and is the second leading cause of cancer-related mortality[13]. In China, the annual average increase of new CRC cases has been estimated to be 4.2%[14]. Although gender and regional differences are reported to be the prognostic factors for CRC patients[15], the etiology of CRC oncogenesis and development is still complex and unclear. The high mortality of patients with CRC and the limitations of traditional tumor-node-metastasis (TNM) staging emphasize the need to explore key genes closely associated with CRC development and prognosis[16].

Unsurprisingly, abnormal SIX levels have been reported to participate in the regulation of human cancers. Several studies report that mutations or aberrant expression of the SIX family play an important role in colorectal tumorigenesis through multiple processes, such as transformation, proliferation, angiogenesis, migration and

metastasis[17-19]. Song *et al*[20] showed that SIX1 is highly expressed in CRC patients who have short overall survival (OS), and enhances proliferation and migration of CRC cells through activation of Wnt/β-catenin signaling. Human SIX2 is higher in non-metastatic CRC, and targeting this gene can modulate CRC metastasis and immunity, thereby improving the survival of CRC patients[18]. In glioblastoma, where SIX3 is transcriptionally silenced by DNA hypermethylation, SIX3 functions as a tumor suppressor[21], and SIX4 promotes development and progression in CRC through the PI3K-AKT signaling pathways[17]. SIX5 is an important paralog of SIX4 and promotes lung adenocarcinoma (LUAD) progression through transcriptional activation of LINC01468 and its downstream pathways[22], and SIX5 cooperates with hypoxia-induced EYA3 and P3000 to mediate tumorigenesis and cancer progression[19]. However, SIX6 is poorly studied in CRC than in other tumor types. Hence, the current study conducted a comprehensive evaluation of the potential roles of SIX family members and provides novel therapeutic targets for further investigation.

MATERIALS AND METHODS

The expression levels of SIXs in normal tissues and different types of cancers

The Human Protein Atlas (HPA) (https://www.proteinatlas.org/), a rich resource database with more than 5000 types of human protein expression data and high-definition images of human normal and cancerous cells[23], was used to obtain and analyze the mRNA and protein levels of SIX family members in normal tissues and different types of cancers. RNA expression levels were evaluated using consensus normalized expression (NX) combined with three transcriptome datasets (HPA, GTEx and FANTOM5), as described before[24]. RNA expression was divided into four levels: Not detected ($NX < 1$), low expression ($1 \leq NX < 15$), medium expression ($15 \leq NX < 30$), and high expression ($NX \geq 30$)[24]. Similarly, protein expression was also categorized into four groups: Negative (-), low expression (+), medium expression (++) and high expression (+++). TCGA datasets were evaluated through the Tumor Immune Estimation Resource (TIMER2.0) online resource (http://timer.cistrome.org/) for pan-

cancer analysis[25], and the UCSC Xena database (https://genome-cancer.ucsc.edu/)[26] for colon and rectal adenocarcinomas (COAD and READ) and related normal tissues.

The prognostic values of SIXs in patients with CRC

Kaplan-Meier Plotter (http://kmplot.com/analysis/), a dataset containing gene expression and survival of cancer patients[27], was assessed for the prognostic value regarding OS of SIXs in patients with CRC. For disease-free survival (DFS) analysis, GEPIA2 (http://gepia2.cancerpku.cn/) information, containing 9736 tumors and 8587 normal samples[28], was used to detect the correlation between SIX levels and DFS in CRC patients.

Signaling pathways involving SIX4

LinkedOmics (http://www.linkedomics.org), a multi-omics dataset that includes data from 32 TCGA tumor types and 10 clinical proteomic tumor analysis cohorts[29], was used to predict potential SIX4-related genes and explore their related signaling pathways through Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analyses. The LinkFinder module in the LinkedOmics dataset was applied to analyze the correlation between the expression level of SIX4 and clinicopathological parameters of CRC patients.

Patient information and ethics statement

A tissue microarray with 87 matched primary colon cancer tissues and their corresponding adjacent normal colon tissue samples, and 6 extra samples of cancer cases were purchased from the Shanghai OUTDO Biotech Company (Shanghai, China). This study was approved by the Ethics Committee of Shantou University Medical college (SUMC-2022-045).

Immunohistochemistry

Immunohistochemical (IHC) staining of SIX4 was performed as described previously[30]. Deparaffinization in xylene, hydration in graded alcohols, and epitope retrieval by microwaving in EDTA (Fuzhou Maixin Biotechnology Development Co. LTD, Fuzhou, China) was conducted sequentially on the tissue microarray slide. After blocking endogenous peroxidase with 3% H_2O_2, the slide was incubated with anti-SIX4 antibody (dilution: 1:400, bs-17503R, Bioss, China,) at 4 °C overnight. Stained tissues were mounted by nuclear counterstaining with hematoxylin for visualization.

The sections were visualized and evaluated independently under a bright-field microscope (PerkinElmer Vectra, PerkinElmer, United States) by two investigators with no prior knowledge of the patient information. For evaluating SIX4 expression, the staining intensities with colorless, light yellow, brown yellow and dark brown were labeled as 0, 1, 2 and 3, respectively, while the percentage of positive cells corresponding to 0%, 1%-25%, 26%-50%, 51%-75% and 76%-100% were recorded as 0, 1, 2, 3 and 4, respectively. The final staining score for SIX4 expression was calculated as the sum of staining intensity and percentage of positive cells, and divided into low (0-4) and high (5-7) expression groups.

Statistical analyses

SPSS 25.0 statistical software was used to do the statistical analyses. The relationship between expression level of SIX4 in 87 paired CRC and paracancerous tissues was performed using the χ^2 test. A total of 93 case numbers from the tissue microarrays were recruited to analyze the relationship, between SIX4 and clinicopathological parameters of CRC patients, using the χ^2 or Fisher's exact probability test. To investigate the prognostic value of SIX4 in CRC patients, the Kaplan-Meier survival curve and log-rank test were used. The difference was considered statistically significant at $P < 0.05$.

RESULTS

Expression of SIXs in normal tissues

As shown in Table 1, the results from the HPA database revealed that in normal tissues, SIXs 1-4 and SIX6 are expressed at relatively low levels, except for SIX1 in the testis, prostate, and uterine cervix, and SIX5 RNA and protein are highly expressed in thyroid gland, stomach, colon/rectum, kidney, urinary bladder, prostate, endometrium and breast tissues. The protein levels of SIXs 2-4 have yet to be examined.

Expression of SIXs in different types of malignant tumors

To explore the different expression patterns of SIXs in normal and malignant tissues, the expression levels of SIXs in different types of malignant tumors was collected from the HPA (Table 2). Interestingly, the levels of SIXs 1-3 and SIX6 were still relatively low in different types of cancers, except for SIX1 in prostate, cervix uterine and breast cancers. It was found that the RNA levels of SIX5 were low, but the protein levels of SIX5 were quite high in cancers of the thyroid gland, prostate, ovary, and breast, and medium in cancers of the colon/rectum, liver, and endometrium. Although there is still no data on the protein level of SIXs 2 and 3 up to now, the protein level of SIX4, has been evaluated in a series of studies and found to be high in cancers of the thyroid gland, breast and skin, medium in cancers of the colon/rectum, liver, prostate and uterine cervix, and low in cancers of the lung, pancreas, kidney, urinary bladder, testis, ovary, endometrium, and lymph node.

To confirm the above findings, the TIMER2.0 with TCGA datasets were used to evaluate the expression of SIXs in different cancerous tissues and corresponding normal tissues (Figure 1). SIX1 and SIX4 were highly expressed in almost all types of cancer tissues compared with their corresponding normal tissues ($P < 0.001$), except for SIX1 in head and neck squamous cell carcinoma, kidney renal papillary cell carcinoma (KIRP) and thyroid carcinoma (THCA), and SIX4 in kidney renal clear cell carcinoma, KIRP and THCA. In contrast, almost no difference was found in the expression of SIX6 in cancers, except for LUAD and lung squamous cell carcinoma ($P < 0.05$). The expressions of SIXs 2, 3 and 5 were inconsistent in the different types of cancer tissues compared with their corresponding normal ones.

SIXs 1, 2 and 4 are highly expressed in CRC tissues compared with normal tissues

The expression patterns of SIXs in CRC were verified with the UCSC Xena database (Figure 2). In a TCGA colon and rectal cancer cohort (n = 736), the expression of SIXs 1, 2 and 4 in CRC tissues was higher than that in normal tissues (P < 0.001), while no significant difference was found for the levels of SIXs 3 and 6.

Prognostic value of SIXs in CRC patients

To estimate the prognostic function of SIXs in CRC patients, OS and DFS were analyzed. CRC patients with high SIX4 levels had poor OS, with hazard ratio (HR) = 2.28 (1.04-4.99), predicting a potential oncogenic role for SIX4 in CRC development (Figure 3). Although no statistical significance was found for other SIXs, CRC patients with high SIX1 or SIX5 tended to display poor OS and the HR was predicted to be 2.11 (0.96-4.6) and 2.54 (0.94-6.86), respectively.

As shown in Figure 4, only the SIX4 expression level was found to be associated with progression of CRC. High levels of SIX4 in CRC patients predicted short DFS, indicating that the expression of SIX4 might promote the recurrence or relapse of CRC. Interestingly, CRC patients with high SIX1 or SIX5 also tended to have short DFS, but were without statistical significance. Up to now, no results have been collected for SIX6.

SIX4-related signaling pathways

The above results indicate SIX4 could have a potential oncogenic role in CRC, so further investigation was conducted to delineate potential roles. Potential SIX4-related genes were collected through LinkedOmics (Figure 5A), and the top 50 positively- and negatively-correlated genes were recruited for KEGG and GO analysis to identify related signaling pathways (Figures 5B and 5C).

In Figure 6, the top 50 positively- and negatively-correlated genes were analyzed. KEGG analysis revealed that SIX4-related genes were mainly involved in oxidative phosphorylation, peroxisome, pyruvate metabolism, and carbon metabolism pathways

(Figure 6A). For biological process annotation, SIX4-related genes were associated with mitochondrial respiratory chain complex assembly, mitochondrial gene expression, NADH dehydrogenase complex assembly and tricarboxylic acid metabolic process (Figure 6B). In cellular component analysis, respiratory chain was the significant category for SIX4-related genes (Figure 6C), whereas for molecular function, structural constituent of ribosome and rRNA binding were found to be associated with SIX4-related genes (Figure 6D).

Relationship between SIX4 mRNA level and clinicopathological parameters of CRC patients

Using the LinkFinder module in the LinkedOmics dataset, expression of SIX4 was found to be positively related to tumor size and lymph node metastasis in CRC, meaning that the progression of CRC may be driven by high expression of SIX4 (Figures 7A and 7B). However, no association was found between SIX4 expression and CRC patient metastasis status (Figure 7C). Importantly, considering the progression of CRC, the expression of SIX4 was positively correlated with the stage of CRC patients, consistent with the results for tumor size and lymph node status (Figure 7D).

The etiological and demographic information of CRC patients

The tissue microarray included 93 colon cancer patients, 87 of whom had corresponding adjacent tissue (Table 3). There were 44 male patients and 49 female patients. The average age of the patients was 67.7 ± 9.8 years old. Among the pathological stages, stage II patients accounted for the largest proportion (82.8%). Most patients did not have lymph node metastasis. During long-term follow-up, 45 of the patients died of CRC.

SIX4 is highly expressed in CRC compared with adjacent normal tissues

As an important transcription factor involved in development, SIX4 was predicted to be subcellularly located mainly in the nucleus, but also in the cytosol. IHC staining

confirmed the subcellular location of SIX4 (Figure 8). The protein level of SIX4 was consistently high in CRC tissues compared with adjacent normal tissues ($P < 0.0001$) (Table 4).

SIX4 protein tends to promote lymph node metastasis in CRC patients

Further analysis of the expression pattern of SIX4 was conducted in 93 CRC patients (Table 5). However, no statistical significance was found between SIX4 levels and clinicopathological parameters, such as the onset age, gender, primary tumor characteristics and lymph node status ($P > 0.05$). Although no difference was found, the percentage of patients with high SIX4 levels tended to be increased at advanced pathological stage, while high expression of SIX4 tended to promote lymph node metastasis, which is similar to the results from analysis of mRNA SIX4 levels.

SIX4 protein level is a predictor of poor OS for CRC patients

Survival analysis, based on the expression level of SIX4, was also conducted in 93 CRC patients (Figure 9). Unsurprisingly, high levels of SIX4 predicted poor OS for patients with CRC, suggesting that the SIX4 level is negatively correlated with the survival of CRC patients ($P = 0.0263$).

DISCUSSION

The SIX family members were originally identified as members of the homeobox family, SIX sub-family, and are required for organogenesis during human development. Previous reports have demonstrated that members of the SIX family not only regulate progenitor cell proliferation, and differentiation, but also contribute to tumorigenesis[31]. In this study, bioinformatics was used to comprehensively analyze the expression and clinical significance of the SIX family in CRC.

Members of the SIX family are homologs of the *Drosophila sine oculis*, *optix* and *Dsix4* genes, which play important roles in organ formation and mesoderm derivatives in *Drosophila*[32]. Interestingly, the genes encoding SIXs are located at three loci: 14q23.1 for

SIXs 1, 4 and 6, 2q21 for SIXs 2 and 3, and 19q13.32 for SIX5, although they can be divided into three subgroups (SIXs 1 and 2, SIXs 3 and 6, and SIXs 4 and 5) based on their SIX-type homeodomains and SIX domains[33]. All SIX proteins are predicted to have DNA-binding transcription factor and chromatin-binding activity[34]. SIX1 has been reported to be a strong factor for many diseases, with important roles in tumorigenesis[35]. Jiang *et al*[36] showed that SIX1 is highly expressed in gastric cancer (GC) patients who have short OS, and is the target by which the ginsenocide Rh4 suppresses metastasis of GC through inhibition of the SIX1-stimulated transforming growth factor (TGF)-β/Smad2/Smad3 signaling pathway. The TGF-β/Smad2/3 signaling pathway also has been found to be the downstream target of SIX2, which is strongly expressed in hepatocellular carcinoma (HCC) and negatively related to the prognosis of HCC patients[37]. In glioblastoma, where SIX3 is transcriptionally silenced by DNA hypermethylation, SIX3 functions as a tumor suppressor. Elevated expression of SIX4 has been found in human HCC, and to be positively correlated with loss of tumor encapsulation, microvascular invasion, higher TNM stage, and poor prognosis[38]. SIX5 is an important paralog of SIX4 and promotes LUAD progression through transcriptional activation of LINC01468 and its downstream pathways[22]. In T-cell acute lymphoblastic leukemia (T-ALL), SIX6 has been shown to belong a relevant regulatory transcription factor in T-ALL to regulate gene networks[39]. However, research about the function of the SIX family members is still limited, especially for the novel family members.

The present study systematically analyzes the expression pattern of SIXs in normal and cancerous tissues. It is interesting that the expression level of SIXs is relatively low in a majority of organs, indicating they are relatively silent or suppressed in adults. In contrast, the expressions of SIXs 1, 2, and 4 were found to be higher in CRC than in corresponding normal tissues, based on analysis of the UCSC Xena database, while the expression levels of SIXs 3 and 6 were not significantly different. Further survival analysis, using Kaplan-Meier Plotter, showed that the OS and DFS of CRC patients with high SIX4 expression are significantly worse, and there is no significant difference in

other members. Previous studies simply demonstrated that SIX4 promotes the development of CRC cells by activating the PI3K-AKT pathway at the cellular level. To better understand the functional mechanism of SIX4, we used the LinkedOmics database to show that SIX4 expression is positively correlated with clinical stage, T stage and N stage in CRC, and also that the top 50 SIX4-related genes are involved in oxidative phosphorylation and respiratory chain signaling pathways, suggesting that SIX4 is involved in promoting the growth and metastasis of CRC.

CRC threatens the health and life quality of patients, and needs more specific therapeutic targets and novel treatment strategies[40]. Using different databases, the expression SIX4 was found to be increased in CRC tissues compared with adjacent normal ones. As a transcription factor, abnormal increases in SIX4 may enhance the expression of downstream oncogenes, such as AKT, YAP1 and c-MET[38,41]. SIX4 promotes activation of the STAT3 signaling pathway in breast cancer, and plays an important role in EMT. Furthermore, SIX4 has been reported to play a carcinogenic role in CRC by activating the Akt signaling pathway[17]. KEGG and GO annotation predicted SIX4 and its correlated genes to be involved in oxidative phosphorylation, respiratory chain and metabolism, which are also related to classical signaling pathways, including AKT and YAP1. Therefore, the Akt signaling pathway is extremely likely to be the mechanism of SIX4-enhanced development of CRC.

The expression of SIX4 was also evaluated in clinical trials to provide solid evidence for the use of SIX4 in clinical testing. Significantly, lymph node metastasis was found to be positively associated with SIX4 level in CRC patients. The development of CRC may be at least partially due to high SIX4 expression and its transcription of downstream genes, subsequently promoting the migratory ability of tumor cells through the lymph node. As a solid tumor, the recurrence and relapse of CRC could severely affect patient survival[42]. The present study demonstrates that CRC patients with low SIX4 have longer survival, suggesting a novel therapy to inhibit or suppress SIX4 activity would be useful in prolonging CRC patient survival. This study provides the first comprehensive analysis of the potential role and prognostic value of the SIX family in

CRC. However, the current study is limited to analyzing the expression and clinicopathological parameters of SIXs in CRC, and the sample size is relatively limited, so further in-depth analysis will be conducted in future studies. Although SIX4 may be involved in oxidative phosphorylation, respiratory chain activity and metabolism, further studies on these can be investigated.

CONCLUSION

Among the SIX family members, SIX4 was found to be a tumor-promoting factor in the intestinal tract, and could be a prognostic indicator and treatment target for patients with CRC. Further investigation of novel therapeutic strategies targeting SIX4 in CRC patients could provide important potential for treatment of CRC.

ARTICLE HIGHLIGHTS

Research background

Human sine oculis homeobox homolog (SIX) protein belongs to the homeobox family, which plays an important role in different developmental organs, and the abnormal expression of most development-related genes is closely related to the occurrence and development of malignant tumors. However, there is no studies have comprehensively analyzed SIXs in colorectal cancer (CRC).

Research motivation

SIXs has been found to be closely related to the occurrence and development of cancer. However, there are few studies on SIXs in CRC. SIXs may become a new potential prognostic indicator of CRC.

Research objectives

To comprehensively analyze the expression and the prognosis of SIX family in CRC tissues, and explore the potential role of SIX family as a new prognostic indicator of CRC.

Research methods

In this study, the RNA and protein expression levels of the SIX family in CRC were analyzed by various online databases and immunohistochemical, and then the relationship between the SIX family and the prognosis of CRC was further analyzed. In order to better understand the mechanism of SIX4, the positive correlation between SIX4 expression and T/N/M stage of CRC was analyzed. Then, the relationship between SIX4 mRNA level and clinicopathological parameters in CRC patients was analyzed.

Research results

The expression levels of SIXs in most organs were relatively low in the Human Protein Atlas. UCSC Xena database analysis showed that the expression levels of SIX1, SIX2 and SIX4 in CRC were higher than those in corresponding normal tissue. Further survival analysis, Kaplan-Meier Plotter showed that the relation between poor overall survival and disease-free survival of CRC patients and high SIX4 expression were significantly. Using the LinkedOmics database, the expression of SIX4 was positively correlated with the clinical stage, T stage and N stage of CRC, and the top 50 SIX4-related genes were involved in oxidative phosphorylation and respiratory chain signaling pathway, suggesting that SIX4 was involved in promoting the growth and metastasis of CRC.

Research conclusions

As a member of the SIX family, SIX4 plays a role in promoting tumor development in the intestine, which may serve as a potential prognostic indicator and therapeutic target for CRC patients. Therefore, targeting SIX4 may serve as a new therapeutic strategy for CRC patients, providing important potential for the treatment of CRC.

Research perspectives

This is the first comprehensive analysis of the potential role and prognostic value of the SIX family in CRC. However, the current research is limited, and future studies need to further explore the oxidative phosphorylation, respiratory chain activity and metabolism of SIX4 in CRC.

78685_Auto_Edited-check.docx

ORIGINALITY REPORT

4%
SIMILARITY INDEX

PRIMARY SOURCES

1	Ze-Xuan Fang, Chun-Lan Li, Wen-Jia Chen, Hua-Tao Wu, Jing Liu. "Potential of six-transmembrane epithelial antigen of the prostate 4 as a prognostic marker for colorectal cancer", World Journal of Gastrointestinal Oncology, 2022 Crossref	79 words — 2%
2	www.frontiersin.org Internet	17 words — < 1%
3	Runjie Sun, Michael Pisano, Yi Gao, Fumou Sun, Jin Shi, Jie Xu, Xueyan Dong, Wei Zheng, Yanyu Zhang, Xiaoqi Sun, Xing Cui. "A new point mutation in SIX1 causes Branchio-oto syndrome, thymoma and pure red cell aplasia via mitochondrial apoptosis", Research Square Platform LLC, 2021 Crossref Posted Content	16 words — < 1%
4	www.nature.com Internet	15 words — < 1%
5	onlinelibrary.wiley.com Internet	13 words — < 1%
6	www.dovepress.com Internet	13 words — < 1%
7	eprints.whiterose.ac.uk Internet	

12 words — < 1%

8 link.springer.com
Internet

12 words — < 1%

9 www.termedia.pl
Internet

12 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	< 12 WORDS
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 12 WORDS