Myeloid zinc finger 1 protein is a key transcription stimulating factor of \textit{PYROXD2} promoter

HUILIN LIU$^1$, XINGYAN JIANG$^1$, TAO WANG$^1$, FANG YU$^1$, XINGZHI WANG$^1$, JIAOJIAO CHEN$^1$, XIAOYUAN XIE$^2$ and HANDONG FAN$^1$

$^1$Institute of Aging Research, School of Medicine, Hangzhou Normal University, Hangzhou, Zhejiang 310036; $^2$The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong 510630, P.R. China

Received January 12, 2017; Accepted July 31, 2017

DOI: 10.3892/or.2017.5990

\textbf{Abstract.} Previous studies revealed that \textit{PYROXD2} was more highly expressed in normal liver tissue and liver cell lines than in cancer tissue and cancer cell lines, which indicated that decreased \textit{PYROXD2} expression may be involved in hepatocarcinogenesis. To identify the mechanisms which regulate \textit{PYROXD2} gene transcription, we constructed a series of luciferase reporter plasmids and used them to perform luciferase-based reporter assays with HepG2, Sk-hep1, L02 and 293T cells with the purpose of characterizing the \textit{PYROXD2} reporter region. Our results revealed that the transcription factor myeloid zinc finger 1 (MZF1) is necessary for \textit{PYROXD2} gene transcription and that it functions as a \textit{trans}-activator. DNA binding assays revealed that the MZF1 protein binds to the \textit{cis}-element TGGGGA located in the -320/-312 region of the \textit{PYROXD2} promoter. This promoter had a significantly enhanced activity when the MZF1 protein was overexpressed and a significantly decreased activity when the MZF1 protein expression was silenced. However, MZF1 gene expression was not significantly correlated with \textit{PYROXD2} protein expression in the samples of resected tumor tissues, which revealed that the \textit{PYROXD2} promoter transcription activity was determined by the aggregated effect of numerous transcription factors. This finding may be helpful in understanding the underlying mechanism which regulates the \textit{PYROXD2} expression.

\textbf{Introduction}

\textit{PYROXD2}, also known as YueF, (GenBank accession no. BC006131), was initially identified as a novel hepatitis B virus X-interacting protein (HBx) in studies conducted using a yeast two-hybrid screening system (1,2). As a putative tumor-suppressor protein, the overexpression of \textit{PYROXD2} can cause cell-cycle arrest in the G1 phase, induce cell apoptosis, enhance the expression of p53 and p21WAF1/Cip1, decrease cyclin D1 and pRb expression and suppress the growth of hepatocellular carcinoma (HCC) tumors in nude mice \textit{in vivo} (3). \textit{PYROXD2} is highly expressed in the cytoplasm of normal cells and tissues but is expressed at lower levels in corresponding cancer cells, including liver, lung and renal cell carcinoma and bladder cancer cells (1,2). The biological functions of \textit{PYROXD2} and the mechanism which regulates its expression remain largely unknown.

Myeloid zinc finger 1 (MZF1) is a member of the SCAN-zinc finger (SCAN-ZF) family of transcription factors, and has finger-like molecular structures that bind in a sequence-specific manner into the groove of the DNA (4). MZF1 has been implicated in tumorigenicity and it is thought to mediate the migration and invasion of cancer cells by suppressing the activity of certain gene promoter regions \textit{in vivo} and \textit{in vitro} (5-8). Moreover, higher levels of MZF1 RNA were revealed in a series of human cancer tissue than in normal tissue (5). MZF1 binds with the proteins found in promyelocytic leukemia nuclear bodies (9). Promyelocytic leukemia nuclear bodies strongly influence gene transcription activity and chromosomal structure through their interaction with other factors and their formation is dependent on the oligomerization of promyelocytic leukemia proteins (10,11). The MZF1 protein is a promoter/enhancer binding-type transcription factor, which functions both as a \textit{trans}-activator and a \textit{trans}-repressor. This observation revealed that the relative oncogenic activity of MZF1 is determined by the aggregated effects produced by the increase and decrease in gene expression (12), phosphorylation modifications, SUMOylation modifications, and co-activating and co-repressing molecules (5). The MZF1 protein must become phosphorylated in order to respond to the stimulating effects of transforming growth factor-\(\beta\) (TGF-\(\beta\)) (13), which is a growth factor known to be important for facilitating the migration and invasion of cancer cells and the development of the epithelial-mesenchymal transition phenotype (14). SUMOylation of transcription factors usually requires the participation of co-repressors and may thus mediate certain suppressive processes orchestrated by MZF1 during cellular differentiation and oncolytic processes (15).
In the present study, we examined the expression levels of both PYROXD2 and MZF1 using RT-PCR and western blot analysis. We found increased levels of MZF1 mRNA and protein expression and decreased levels of PYROXD2 mRNA and protein expression in cancer cell lines and HCC tissues compared to the expression levels in normal cell lines and liver tissue. We also sought to identify the cis-elements and transcriptional factors that activate PYROXD2 transcription in liver cancer cells. To accomplish this goal, we performed a deletion analysis of the PYROXD2 gene promoter region, followed by a mutant analysis of that region to identify transcription factors that may regulate PYROXD2 transcription. We then evaluated the influence of MZF1 on PYROXD2 protein expression. Our results revealed that MZF1 is a transcription factor crucial in the regulation of PYROXD2 gene expression. Moreover, an MZF1 gene binding site (TGGGGA) located in the -320/-312 region was significant for the functioning of the PYROXD2 promoter.

Materials and methods

Ethics statement and human tissue preparation. The experiments involving humans were approved by the Ethics Committee, and each study participant provided a signed written informed consent document. All the tissue samples were obtained from the Department of Surgery, Zhejiang Provincial People's Hospital, Hangzhou, China.

Twelve samples of live human HCC tissues and 12 samples of corresponding adjacent normal liver tissues were obtained from 12 HCC patients (2 females and 10 males) and examined by a pathologist. All 12 HCC samples displayed a distinct cellular subtype and all of the adjacent tissue samples appeared to be normal and did not have fibrosis or other non-neoplastic changes. Four tumors were at stage II and eight were at stage III. All tissue samples were immediately dissected into several sections (~100 mg/section), washed with normal saline, frozen in liquid nitrogen and stored at -80°C.

Cell culture and transfection. Liver carcinoma cell lines HepG2, L02, Sk-hepl and 293T were obtained from China Center for Type Culture Collection, Wuhan, China, maintained in our lab and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Gibco, Waltham, MA, USA) at 37°C in a 5% CO₂ atmosphere. Transfections were performed using Lipofectamine 2000 (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions.

Reporter constructs and expression vectors. Polymerase chain reaction (PCR) was used to amplify the full length of the PYROXD2 promoter (~1998/-1) present in the genomic DNA of L02 cells. Subsequently a directional PCR cloning strategy was employed to clone the amplified promoter region into pGL3 basic vectors (luciferase reporter plasmids; Promega, Madison, WI, USA) at locations between the KpnI and the XhoI restriction enzyme sites. The luciferase reporter plasmids were designated as (~1800/-1)-PYROXD2 promoter vector, (~1600/-1)-PYROXD2 promoter vector, (~1400/-1)-PYROXD2 promoter vector, (~1200/-1)-PYROXD2 promoter vector, (~1000/-1)-PYROXD2 promoter vector, (~800/-1)-PYROXD2 promoter vector, (~600/-1)-PYROXD2 promoter vector, (~400/-1)-PYROXD2 promoter vector, (~200/-1)-PYROXD2 promoter vector, (~200/00)-PYROXD2 promoter vector, (~1998/-200)-PYROXD2 promoter vector, (~1998/-400)-PYROXD2 promoter vector, (~1998/-600)-PYROXD2 promoter vector, (~1998/-800)-PYROXD2 promoter vector, (~1998/-1000)-PYROXD2 promoter vector and (~1998/-1200)-PYROXD2 promoter vector, respectively. Two siRNA sequences (GATCCGTACACAAGGGGACCATTC ATTCTTCAAGAGAGAATGAATGGTCCCCTTGTGTATT TTATCAGCTG and GATCCTGCAGGGGACCATTC GGATTTCAAGAGATTACACTACCTGACCGCTTTT TACCGGTA) were cloned into pLVX-U6 between the BamHI and the EcoRI restriction enzyme sites with the purpose of silencing the MZF1 protein expression. A control sequence (GATCCGGCAACCTATGGGTGGGTAATTTTCAAGAGAAATTACCCACCCCATAGGTTGTGCTTTTTT TACCGGTA) was cloned into the same vector at the same restriction enzyme site. The MZF1 gene was cloned into N-3xFlag-CMV (then designated as MZF1-Flag) to force the overexpression of the MZF1 protein. All deletion and mutant constructs were checked using DNA sequencing methods prior to being used in any experiments.

Site-directed mutagenesis. Constructs bearing the mutant promoter variants of PYROXD2 were generated by PCR, with the (~1998/-1)-PYROXD2 promoter vector as a template. Potential transcription factor binding sites were identified using TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html) and the results are listed in Table 1. Site-directed mutagenesis was performed with a KOD-Plus-Mutagenesis kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions. The mutant primers used for site-directed mutagenesis (information provided upon request) were designed and produced by Generay Biotech Co., Ltd. (Shanghai, China). All mutants were verified by sequencing.

Dual-luciferase reporter gene assay. Cells were seeded into 96-well plates and cultured for 12 h, after which they were transfected with luciferase reporter plasmids which had the selected serial PYROXD2 promoter. Subsequently, each sample was co-transfected with 20 ng of Renilla luciferase control vector pGL4.70 (Promega) to monitor the transfection efficiency. The pGL3 vectors were used as controls. The luciferase activity was assessed at 24 h post-transfection using a Dual-Luciferase Reporter Assay system (Promega) according to the manufacturer's instructions. The assay results were assessed with a Varioskan Flash Spectra Scanning Multimode Reader (Thermo Fisher 3001; Thermo Fisher Scientific, Waltham, MA, USA).

DNA binding assay. A DNA binding assay was used to detect the interactions between the MZF1 and the putative promoter core binding DNA sequence (AGGGGA,-320/-312). The biotinylated positive DNA sequence was biotin-TCTCC TCCCCGTGACATCTACTTC-3'. The putative positive DNA duplexes, MZF1 binding duplexes, were created by annealing biotin-TCTCCCTCCTGGCATCTACTTC-3'
antibodies, washed with TBST buffer and then incubated (Roche Diagnostics, Pleasanton, CA, USA). The membranes were incubated with the primary and anti-GAPDH (mouse) (all from Abcam Cambridge, MA, USA) obtained from at least three independent experiments. The DNA binding assays were performed as described by Plotz et al (16) and the MZF1 protein antibody (Abcam, Cambridge, UK) was used to immunoprecipitate the protein-DNA complex.

Real-time PCR. Total RNA was extracted from the cells using TRIzol reagent (Invitrogen) and from liver tumor and normal tissue using the PureLink® RNA Mini kit (Thermo Fischer Scientific) according to the manufacturer's instructions. The extracted mRNA was reverse-transcribed using a Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics, Pleasanton, CA, USA). First Universal SYBR-Green Master Reagent (Roche Diagnostics, Pleasanton, CA, USA) was used to perform comparative Ct analyses with a CFX96™ Real-Time system (Bio-Rad, Hercules, CA, USA). The sense and antisense primers used to detect PYROXD2 mRNA were: 5'-GAAGGTAGATGACAGTAGAGGAGA-3' and 5'-GAGGCATGGCATAAGGTCA-3', respectively. The sense and antisense primers used to detect MZF1 mRNA were: 5'-GAATGCTCATTGGATCAGC-3 and 5'-GGGTGGAATGCACTTGGTGATT-3', respectively.

Western blot analysis. The cells were lysed by incubation in a RIPA buffer (Beyotime Biotechnology, Jiangsu, China) was used to extract total soluble proteins from the L02 cells, according to the manufacturer's instructions. The total soluble proteins were isolated by centrifugation. The proteins were transferred onto nitrocellulose membranes for analysis. The L02 cells were separated by electrophoresis on a 12% SDS gel and the individual protein bands were transferred onto nitrocellulose membranes for analysis using western blotting and standard antibody detection procedures. The proteins were extracted from tissue samples using a T-PER® Tissue Protein Extraction kit (Thermo Fischer Scientific). The primary antibodies used for immunostaining were anti-MZF1 (rabbit), anti-PYROXD2 (rabbit) and anti-GAPDH (mouse) (all from Abcam Cambridge, MA, USA). The membranes were incubated with the primary antibodies, washed with TBST buffer and then incubated with anti-mouse IgG (H+L) conjugate (Dylight™ 800) or anti-rabbit IgG (H+L) conjugate (Dylight™ 800) (both from Cell Signaling Technology, Danvers, MA, USA), depending on which primary antibody was used during the first incubation. The LI-COR® Biosciences Odyssey® Infrared Imaging system (LI-COR Biosciences, Lincoln, NE, USA) was used to detect antibody binding and quantify the individual protein bands.

Statistical analysis. Each data point represents the mean ± SD obtained from at least three independent experiments. The Student's t-test was used to analyze differences between two independent groups; a two-sided P-value <0.05 was considered to indicate a statistically significant difference.

Results

PYROXD2 expression in liver tumor tissue and liver cancer cell lines compared with its expression in normal liver tissue and normal cell lines. Previous studies had found lower levels of PYROXD2 expression in several carcinoma tissues than in corresponding normal tissues. This finding revealed that PYROXD2 plays an important role in tumor suppression (3). In order to further assess the different expression models of PYROXD2, we collected a sample of hepatic carcinoma tissue and a sample of normal liver tissue from each of the 12 liver carcinoma patients who underwent therapeutic surgery. We then used reverse transcription-polymerase chain reaction (RT-PCR) to determine the relative levels of PYROXD2 and GAPDH mRNA expression and western blotting techniques in order to determine the relative levels of PYROXD2 and GAPDH protein expression in each tissue sample. We also examined these expression levels in several liver cell lines.

We obtained the same results observed in previous studies (3). The PYROXD2 protein was highly expressed in the normal hepatic cell line L02 but it was barely detectable in the HepG2 and Sk-hep1 hepatoma cell lines (Fig. 1A). The level of PYROXD2 mRNA expression in the three cell lines, as assessed by RT-PCR, displayed the same pattern as the level of PYROXD2 protein expression (Fig. 1B). The level of PYROXD2 protein expression in normal tissue samples (Levelnormal) and the corresponding HCC tissue samples (LevelHCC) as detected by western blotting (Fig. 1C) were calculated based on the ratio of the gray value for PYROXD2 and the gray value for GAPDH in the same tissue sample (gray-valuePYROXD2/gray-valueGAPDH). By establishing the Levelnormal value as 1, the relative expression levels of PYROXD2 protein in the corresponding HCC tissue could be expressed as the ratio: LevelHCC/Levelnormal. The results of these calculations are illustrated in Fig. 1D. While 9 of the 12 patients had a level of PYROXD2 protein expression in their HCC tissue sample that was 10 to 90% lower than that in their corresponding sample of normal liver tissue, 3 of the 12 HCC patients had a higher level of PYROXD2 expression in their HCC sample than in their normal tissue sample. Furthermore, similar to the trend revealed by the PYROXD2 protein expression, the levels of PYROXD2 mRNA in the samples of normal liver tissue were higher than the levels in the corresponding samples of HCC tissue (Fig. 1E). These results demonstrated that although PYROXD2 was highly expressed in normal liver tissue and cells, its expression was decreased in HCC tissue and certain HCC cell types, a finding which revealed that decreased PYROXD2 expression plays a role in hepatocarcinogenesis.

MZF1 expression in HCC tissue and various liver cell lines. We examined the relative levels of MZF1 protein expression in L02, HepG2 and Sk-hep1 cells using western blotting (Fig. 1A). MZF1 which served as a transcription factor was mainly detected as a component of cell nuclear proteins and exhibited different expression in diverse tissues and cell
The same result was obtained by immunofluorescence analysis (data not shown) and MZF1 also displayed different expression levels in HepG2, L02 and Sk-hep1 cells (Fig. 2B), which indicated that MZF1 was less expressed in the HepG2 cells than in the L02 and Sk-hep1 cells as determined by western blotting. We also examined the endogenous levels of the MZF1 protein and mRNA expression in the 12 resected HCC tissue samples and the 12 corresponding normal tissue samples using western blotting and RT-PCR, respectively. The levels of MZF1 protein and mRNA expression were calculated using the same methods as those used to calculate the levels of PYROXD2 protein and mRNA expression; the results are shown in Fig. 2C-E. The tumors in 6 of the 12 patients had a significantly increased level of MZF1 expression compared with the level in the corresponding normal liver sample; however, the tumors in the other 6 patients had 10-90% lower levels of MZF1 expression than those in the corresponding normal tissue samples.

Sequence AGGGGA (-320/-312) in the PYROXD2 promoter is the main element controlling PYROXD2 expression. We cloned the 1999 base pair (bp) fragment (-1998/-1) of the promoter region of the PYROXD2 gene located upstream of the ATG transcription initiation codon of exon 1, with the purpose of analyzing the promoter and its regulatory elements. A search for potential regulatory motifs which was performed using the TFSEARCH identified the putative transcription factor binding sites and is listed in Table I. Subsequently,
the serial deletion mutants were constructed using strategies and primers (relevant information provided upon request). A nucleotide deletion in the promoter regulatory region could potentially affect the binding of transcription factors and alter the transcription rate of a gene. Promoter activity was determined with a luciferase assay system and the results were normalized by β-galactosidase activity. The absorbance value of a blank control sample was subtracted from each assay result. After the activity of the (-1998/-1)-PYROXD2 promoter was defined as 1, the relative activities of other serial mutant promoters were expressed as the ratio $I_{\text{mutant}}/I_{\text{full length}}$, in which $I$ is the intensity of an absorbance value, the superscript ‘mutant’ signifies a serial mutant promoter and the superscript ‘full length’ signifies the (-1998/-1)-PYROXD2 promoter.

To analyze various characteristics of the PYROXD2 promoter and identify the region most commonly activated, we constructed a series of 200 bp fragment deletions which ranged from base -1998 to base -1. As revealed in Fig. 3A, deletion of bases -1998/-1801 produced only a slight decrease in promoter activity. The most remarkable change in promoter activity occurred when the deletions were produced between the -1600/-1201 and -800/-401 regions.
Liu et al.: MZF1 Mediates PYROXD2 Expression

In which case the promoter exhibited increased activity. In contrast, the (-1998/-1)-PYROXD2 promoter displayed decreased activity when the deletions were produced in both the -1200/-801 and -400/-201 regions. It should be emphasized that the PYROXD2 promoter lost all its activity when the deletions were produced in either the -400/-201 or -200/-1 regions. Our results indicated that cis-elements located in the -400/-1 region constitute the core promoter responsible for basal transcription of the PYROXD2 gene.

Next, we used site-directed mutagenesis to generate a series of mutant reporters based on the (-1998/-1)-PYROXD2 promoter vector, with the purpose of identifying critical cis-elements in the promoter region. As revealed in Fig. 3B and Table I, the different mutants produced different effects on the promoter activity in the three cell lines. Notably, insertion of a mutation into the -320/-312 region

Figure 3. Mutant analysis of the PYROXD2 promoter. The activities of the various mutant promoters were assessed using the dual-luciferase reporter gene assay. The relative promoter activity of each mutant was calculated using the formula: A\text{mutant} = \frac{I_{\text{mutant}}}{I_{\text{full length}}}, in which I indicates the intensity of the absorbance value, the superscript ‘mutant’ signifies a serial mutant promoter and the superscript ‘full length’ signifies the (-1998/-1)-PYROXD2 promoter. (A) Promoter activity of the (-1998/-1)-PYROXD2 promoter with serial 200 bp fragment deletions. (B) Site-directed mutagenesis promoter activities, based on the (-1998/-1)-PYROXD2 promoter sequence. All potential transcription factors and their corresponding binding sites on the PYROXD2 promoter were predicated through the TFS EARCH software online (http://www.cbrc.jp/research/db/TFS EARCH.html). Site-directed mutagenesis was performed based on (-1998/-1)-PYROXD2 promoter vector. Mutant sequences with corresponding primers are available upon request. The vectors which were cloned with the mutant or wild-type promoter of PYROXD2 were transfected into the Sk-hep1, HepG2 and L02 cell lines, respectively. Relative promoter activities were detected at 24 h after transfection using a Dual-Luciferase Reporter Assay system (Promega) according to the manufacturer’s instructions. Each group demonstrated one or more mutants of the transcription factor binding site in PYROXD2 promoter which is listed in Table I. (C) The mutation strategies were used for four putative MZF1 binding sites in the (-1998/-1)-PYROXD2 promoter sequence. (D) The relative transcription activities of four promoters with mutated MZF1 binding sites.
produced a complete loss of promoter activity in all three cell lines (Fig. 3B and Table I). These results indicate that the putative MZF1 binding site (TGGGGA) located in the -320/-312 region of the PYROXD2 promoter may be crucial for the transcription activity of the promoter.

A preliminary sequence analysis of the -1998/-1 domain revealed the presence of four cis-elements that may bind with MZF1. Among them, the -1983/-1975 and -320/-312 regions were non-overlapping, whereas the -757/-749 and -240/-230 regions overlapped with other transcription factors. To assess how the four MZF1 binding cis-elements of the PYROXD2 promoter may affect PYROXD2 gene transcription, we constructed mutant promoters in which only two nucleotides were substituted in the core MZF1 binding sequence. As revealed in Fig. 3C, the substitutions did not affect the binding ability of other overlapping transcriptional effectors. When compared with the effects produced by other mutations, a mutation in the -320/-312 region appeared to produce the largest decrease in luciferase activity (Fig. 3D).

MZF1 is a key trans-acting factor controlling PYROXD2 expression. A separate set of experiments was conducted to elucidate how the -320/-312 region of the PYROXD2 promoter interacts with MZF1 and to determine whether endogenous MZF1 binds to the -320/-312 region of the PYROXD2 promoter. To accomplish these goals, we performed DNA binding assays using primers that spanned the putative MZF1-binding site of the region (Fig. 4). The specific sequence within the -320/-312 region was precipitated from cell lysates by the addition of the anti-MZF1 antibody but not by the addition of the control IgG. Our data markedly indicated that MZF1 binds to the TGGGGA domain in the proximal promoter of PYROXD2.

To further ascertain the involvement of MZF1 in PYROXD2 transcription, we assessed the levels of endogenous PYROXD2 expression in the HepG2 and L02 cells after transfecting them with MZF1 expressing plasmids, or by silencing their endogenous MZF1 expression with siRNA (Fig. 5). Increased endogenous PYROXD2 expression was observed in MZF1 overexpressing the HepG2 and L02 cells, and decreased endogenous PYROXD2 expression was observed in the L02 cells transfected with the MZF1 siRNA (Fig. 5). These findings indicate that MZF1 functions as a key regulator of the PYROXD2 transcription.

Discussion

The tumor-suppressive activity of PYROXD2 and its different expression levels in normal tissues and several corresponding...
In the present study, we collected specimens of cancerous liver tissue and adjacent normal liver tissue from 12 patients, and quantitatively assessed the endogenous levels of MZF1 and PYROXD2 expression in those tissues by western blotting and RT-PCR. We found that the PYROXD2 protein was highly expressed in normal liver tissue and in a normal human liver cell line; however, its expression was either absent or decreased in a large proportion of HCC tissue and hepatocarcinoma cell lines, indicating that decreasing PYROXD2 expression may be involved in hepatocarcinogenesis.

The molecular mechanism which regulates PYROXD2 transcription was not elucidated in previous studies. We sought to examine various characteristics of the PYROXD2 promoter and identify the most commonly activated PYROXD2 promoter region in liver cells. To accomplish this goal, we constructed a series of luciferase reporter plasmids that contained 5' and 3'-deletions in the PYROXD2 promoter, and then used them to perform luciferase-based reporter assays in HepG2 and Sk-hep1 liver cancer cell lines as well as in two normal control cell lines (L02 and 293T). The most remarkable change in the promoter activity occurred when the deletions were produced in both the -400/-199 and -200/-1 regions in which case, there was an almost complete loss of PYROXD2 promoter activity. Our site-directed mutagenesis studies conducted with three different cell lines demonstrated that the putative MZF1 binding site (TGGGGA) located in the -320/-312 region of the PYROXD2 promoter was largely responsible for the loss of PYROXD2 promoter activity. The results of the DNA binding assays also indicated the occurrence of interactions between MZF1 and cis-elements located in the -317/-313 region of the PYROXD2 promoter. Ectopic expression of MZF1 induced an increased expression of PYROXD2; accordingly, silencing of MZF1 inhibited PYROXD2 expression in the same cell lines. These findings suggest that MZF1 is critical for the transcription activity of the PYROXD2 promoter and functions as a trans-activator in regulating PYROXD2 expression. In addition these findings provided novel insights into the mechanism underlying the tumorigenic effect of PYROXD2. Our results indicated that MZF1 was expressed at higher levels in samples of human cancer tissue than in samples of normal tissue, which were consistent with previous studies (5). Based on the previous cellular experiment results, we analyzed the activating function of MZF1 on the PYROXD2 promoter in tissues by comparing the expression level of MZF1 with the PYROXD2 expression level. Notably MZF1 gene expression was not significantly correlated with PYROXD2 expression in samples of resected tumor tissues. The protein expression in human cells is regulated at several levels such as in transcription factors and mRNA stability. Promoter transcription activities were affected by the amount and type of the transcription factor family members and their function antagonism associations. Additively, the untranslated regions (UTR) and the AU-rich elements (ARE) render mRNA unstable in cells and tissues leading to a gradual decrease in protein production (17). Although the present study revealed that the MZF1 gene expression was not significantly correlated with the PYROXD2 expression in samples of resected tumor tissues, we speculated that the main mechanisms were relative to the diversity of the transcription factors involved in the regulation of the PYROXD2 promoter activity and MZF1 is only one of these factors. It should be pointed out that MZF1 is an activating factor of the PYROXD2 promoter, and decreased PYROXD2 expression may contribute to
cancer progression. Therefore, it is important to understand the underlying mechanism that regulates the expression level of PYROXD2 by other transcription factor members and their interaction relationships.

Acknowledgements

The present study was supported by the Natural Science Foundation of Zhejiang province (LQ12C03003), the Natural Science Foundation of China (project nos. 3126021 and 31660240) and the Hangzhou Normal University supporting project (no. PE13002004042).

References