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Research Article

Stable Overexpression of MZF1 Does Not Alter Proliferation, Viability, or Stemness in HEK293T Cells

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Abstract

Myeloid Zinc Finger 1 (MZF1) is a member of the SCAN domain-zinc finger (SCAN-ZFP) transcription factor family. With a structure comprising five distinct domains, it regulates various transcriptional processes. Numerous studies suggest that MZF1 has bi-functional roles, acting either as an oncogene or a tumor suppressor. However, its overexpression has also been associated with negligible changes in cellular functions, an aspect that remains underexplored. Additionally, although the HEK293T cell line is widely used to investigate molecular mechanisms, the role of MZF1 in these cells has not been reported. In this study, we assessed the effects of MZF1 overexpression on some cellular functions in these cells. Using transposon-mediated gene transfer, we generated stably expressing cell lines and assessed their cellular functions, including cell proliferation, cell viability, and cell stemness. Our findings show that following stable expression of MZF1, the number of cells compared to controls did not reveal a significant difference during 6 time-points over 72 hours. Also, the MTT read absorbance, and as a consequence, cell viability did not change in the MZF1 overexpressing cell line compared to control cells (0.49 against 0.48). By performing the colony formation assay, we did not find any significant changes in the number of colonies or the number of cells in each colony. Also, our soft agar assay did not reveal statistically significant changes in the size and number of colonies between the two groups of MZF1-overexpressing cells and control cells. Taken together, our data provide evidence that the stable overexpression of MZF1 does not alter cellular functions of proliferation, viability, and stemness in HEK293T cells.

***Keywords*:** MZF1, HEK293T cell line, cell proliferation, cell viability, cell stemness

Introduction

Myeloid Zinc Finger 1 (MZF1) is also known as MZF1A, MZF1B, ZFP98, ZSCAN6, or ZNF42. *MZF1* is a single-copy gene and is located a few kilobases from the subtelomeric repeat region of chromosome 19q13.4, making it vulnerable to degradation as a result of telomeric erosion (Hoffman et al., 1996, Hromas et al., 1991). It consists of six exons and, as a result of alternative splicing and due to two distinct transcriptional start sites, generates three transcript variants (Murai et al., 1997, Peterson and Morris, 2000). MZF1 protein belongs to the SCAN domain-zinc finger transcription factor (SCAN-ZFP) family (Peterson et al., 2006) and is composed of five distinguished domains.

The first domain of MZF1 is the acidic domain, which includes the regulatory SUMO and phospho-sites, and is involved in the interaction with other transcription factors (Brix et al., 2019, Lee et al., 2016, Yue et al., 2019). The second highly conserved SCAN domain (Williams et al., 1999), is essential for MZF1 SUMOylation and its localization to promyelocytic leukemia nuclear bodies (PML NBs) (Noll et al., 2008). The third domain of MZF1 is the transcriptional activation domain (TAD), which, similar to the acidic and SCAN domains, is involved in the transcriptional activation of MZF1 (Brix et al., 2019, Ogawa et al., 2003). The fourth and fifth domains of MZF1 consist of 13 highly conserved zinc fingers in its C-terminal region, which provide the possibility of a stronger binding to DNA, other proteins, or RNA (Hromas et al., 1991, Morris et al., 1994).

Originally, the expression of MZF1 was detected in the peripheral blood cells of a patient with chronic myeloid leukemia (Hromas et al., 1991). After a few years, it was uncovered that MZF1 promotes a leukemic phenotype as a repressor of myeloid differentiation and hematopoietic development (Perrotti et al., 1995). Currently, MZF1 is implicated

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in different types of cancers by affecting proliferation, differentiation, migration, and apoptosis (Brix et al., 2020, Eguchi et al., 2015, Lin et al., 2023, Qian et al., 2024).

Numerous studies imply bi-functional roles for MZF1, either as an oncogene (Chen et al., 2014, Eguchi et al., 2019, Yue et al., 2015) or as a tumor suppressor (Lee et al., 2017a, Li et al., 2023, Tian et al., 2025, Wu et al., 2022). Although most studies focus on the bi-functional roles of MZF1, no study has concentrated on or discussed the lack of MZF1's impact on cellular functions. In addition, despite the extensive use of the HEK293T cell line in evaluating molecular mechanisms, there are no reports on the role of MZF1 in these cells. In contrast to cancer cells, the HEK293T cell line is immortalized through the expression of the SV40 large T antigen. Additionally, the growth properties of HEK293T cells are not as deregulated as those of cancer cells. In this study, we stably overexpress MZF1 in the HEK293T cell line by transposon-mediated gene transfer and assess its effects on cellular functions of proliferation, viability, and stemness. Our findings indicate that MZF1 stable overexpression in the HEK293T cell line does not alter cell proliferation, cell viability, and cell stemness.

Materials and Methods

Cell culture, transfection, and transposon-mediated generation of cell lines

The Human Embryonic Kidney (HEK) 293 T cells were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) containing 10% heat-inactivated fetal bovine serum (FBS; Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA) at 37˚C in a humidified atmosphere with 5% CO_2.

To obtain MZF1-overexpressing cells, 24 h before transfection, around 7×10^4 HEK293T cells were seeded in each well of a 24-well plate. Each well was co-transfected with the pHD_4091 plasmid that contained a transposon cassette of CAG promoter-FLAG.MZF1 and EF1α promoter-puromycin resistance gene (PuroR)-IRES-EGFP (Figure 1A), pHD_3501 (encoding transposase), and 2 µl of 1 mg/ml of 25 KDa branched Polyethylenimine (bPEI25; Sigma-Aldrich, USA). To generate control cells, cells were transfected with bPEI25, pHD_3501, and pHD_4090 plasmids (encoding only PuroR and EGFP).

Immunocytochemistry

To confirm the expression of the MZF1 protein in FLAG-MZF1-overexpressing cells, we carried out an immunocytochemistry (ICC) assay by utilizing the anti-Flag antibody. In a 6-well tissue culture plate, the control and FLAG-MZF1 overexpressing cell lines were cultured on 1% gelatin-coated glass coverslips at 37°C in a humidified 5% CO_2 incubator. When they reached 50-60% confluency, the culture medium of each well was removed, and cells were washed with warm 1X D-PBS for 5 min at room temperature. The ICC procedure was followed by fixation with 4% paraformaldehyde in 1X D-PBS for 10 min, washes with 1X D-PBS (three times, 5 min each time), permeabilization with 0.5% Triton X-100 (Merck, Darmstadt, Germany) in 1X D-PBS for 5 min at room temperature, washes with 1X D-PBS (three times, 5 min each), blocking with 3% BSA for 1 h at room temperature, and overnight incubation in the anti-FLAG tag primary antibody (Cat. No. 100233-MM01, 1:200, Sino Biological Inc., Beijing, China) in 1X D-PBS at 4°C. The next day, cells were washed with 1X D-PBS (three times, 5 min each) and incubated for 2 hours at room temperature in anti-mouse IgG-HRP (Cat. No. 7076P2, 1:500, Cell Signaling Technology, Danvers, Massachusetts, USA) in 1% BSA. Then, cells were washed with 1X D-PBS (three times, 5 min each) and incubated with 1% 3,3′-diaminobenzidine (DAB, Cat. No. D8001-1G; Sigma-Aldrich, St. Louis, Missouri, USA) for 5 min. In the end, the coverslips were mounted on slides, and images were captured by a Nikon Eclipse Ts2 inverted microscope (Nikon Co., Tokyo, Japan).

Evaluation of Proliferation Rate

A total of 25×10^3 cells were placed in each well of a 24-well tissue culture plate and cultured for 12, 24, 36, 48, 60, and 72 h at 37 °C in a humidified 5% CO_2 incubator. At each time point, cells were detached using trypsin/EDTA solution (Biowest, France). Resuspended cells in the DMEM medium were mixed with 0.4% trypan blue dye to mark dead cells. Cells were counted using a Neubauer hemocytometer, and the average number of live cells from four replicates at each time point was calculated. Then, proliferation plots were generated using the number of cells at each time point.

MTT assay

A total of 7×10^3 cells were seeded in each well of a 96-well plate (in 9 technical and 3 biological replicates) and incubated for 48 h. Afterward, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide; Sigma-Aldrich, USA) with a final concentration of 0.5 mg/ml was

added to the culture medium and incubated at 37°C in the dark. After 3 hours, the purple formazan precipitates could be observed under a microscope. At this time, the medium in each well was replaced with 100µl of DMSO. The plate was incubated at room temperature in the dark for 15 min. The absorbance was measured by an Epoch 2 microplate reader (BioTek Instruments Inc., USA) at 570 nm.

Colony formation assay and DAPI staining

The MZF1-overexpressing and control cells were seeded at a density of 2400 cells per well of a 6-well tissue culture plate in 2 technical replicates. The cells were cultivated at 37 °C in a humidified 5% CO_2 incubator, with the addition of fresh culture medium every 3 days. Nine days after plating, when cells produced enough large colonies, the culture medium was aspirated and the colonies were rinsed with 1X D-PBS for 5 min. Then, the colonies were fixed in 1 ml of 2% paraformaldehyde in 1X D-PBS at room temperature for 30 min. Then, cells were washed with 1X D-PBS 3 times (5 min each). To distinguish each cell in every colony, we stained the nuclei using antifade + DAPI (1 µg/ml, Cat. No. S-8016, DENAzist Asia Co., Iran). Colonies in both groups were counted and photographed under an inverted microscope.

Soft agar assay

Each well of a 6-well tissue culture plate was covered by 2 ml of an agarose-medium (bottom) layer, prepared by mixing 12 ml of DMEM/F12 supplemented with 5% horse serum and 1% penicillin/streptomycin, and 3 ml of 0.3% 2-hydroxyethyl agarose (Sigma, USA). The plate containing the bottom layer was incubated in a laminar hood for 15 min and then in a refrigerator for 1 hour. Afterward, the top layer consisting of 0.6% 2-hydroxyethyl agarose and 2×10^5 cells was placed on top of the bottom layer. Before incubating the plate in the incubator, the plate was placed in the refrigerator for 10 minutes to speed up the solidification. The culture process was continued for 21 days at 37 °C in a humidified 5% CO_2 incubator. Once a week, a feeder layer (1 ml of a solution containing 1 ml of 0.3% 2-hydroxyethyl agarose and 9 ml of full DMEM/F12 medium) was overlaid on the top layer. After 3 weeks, the colonies were fixed and stained in one step by using a mixture of 1 ml of 4% paraformaldehyde in 1X D-PBS and 1 ml of 0.5 % methylene blue in 50% ethanol at room temperature for 30 min. The washing step was performed by 1X D-PBS thrice (5 min each). The colonies were counted and photographed under an inverted microscope. The area of the colonies was analyzed using Fiji software (Schindelin et al., 2012) .

Results

Generation of cells with stable overexpression of MZF1

To evaluate the effects of MZF1 on cellular functions, we used transposon-mediated gene transfer to generate cells that stably overexpress the MZF1 transcription factor. To this end, we cloned the MZF1 coding sequence fused to the FLAG tag under the control of a CAG promoter along with two selection markers (puromycin resistance gene and EGFP) under the control of an EF1α promoter, in a transposon cassette (Figure 1A). Co-transfection of this construct along with the piggyBac transposase led to the insertion of the transposon in the genome of HEK293T cells. These cells were able to stably express FLAG-MZF1 and EGFP, and were resistant to puromycin sulfate (Figure 1B). A similar construct without the FLAG-MZF1 coding sequence was used to generate a control cell line. The overexpression of FLAG-MZF1 was confirmed by immunocytochemical staining of cells compared with the control group (Figure 1Bd).

The stable overexpression of MZF1 did not affect the cell proliferation and cell viability of HEK293T cells

The proliferation rate of MZF1-overexpressing cells, assessed at 6 time-points over 72 hours, showed no difference compared to control cells (Figure 2A). To assess whether the overexpression of MZF1 may influence cell proliferation in a colony formation assay, the number of colonies was counted and analyzed in two groups of MZF1-overexpressing and control cells. Although the appearance and size of colonies in the MZF1-overexpressing cells were different from the control cells (Figure 2B), the average number of colonies in the two groups and the average number of cells within colonies (218.61 versus 218.24) were statistically similar in both groups (Figure 2C).

Similarly, the stable overexpression of MZF1 did not change the MTT read absorbance in these cells (with a median of 0.49) compared to control cells (with a median of 0.48). Therefore, these data demonstrate that MZF1 overexpression did not alter the metabolic activity and cell survival of HEK293T cells (Figure 2D).

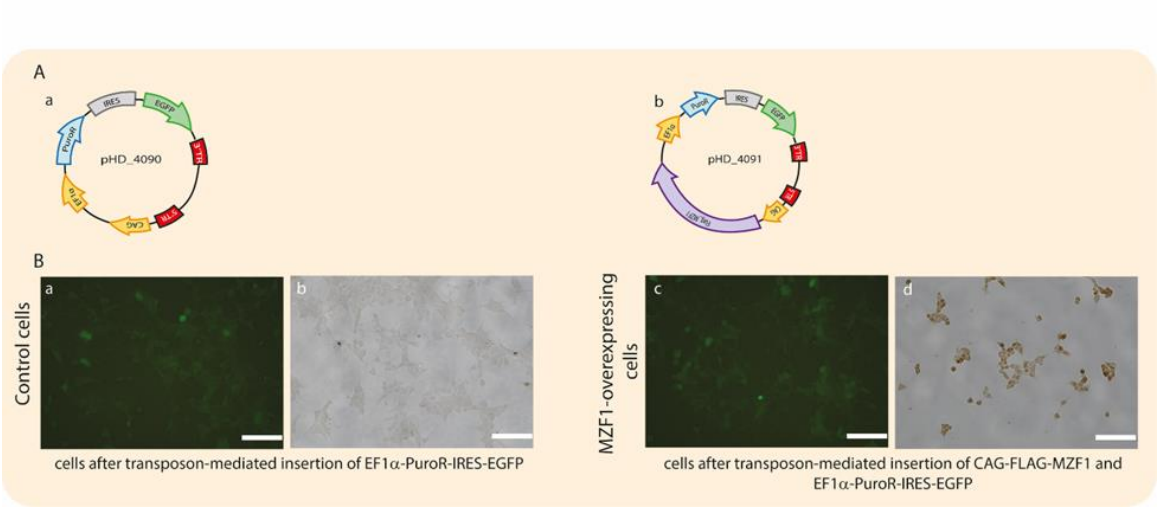

Figure 1. Generation of cells with stable overexpression of MZF1. A) The schematic maps of the pHD_4090 (a) and pHD_4091 (b) constructs. The pHD_4090 construct expresses only PuroR and EGFP under the control of EF1α. However, the pHD_4091 construct, in addition to PuroR and EGFP, encodes FLAG-MZF1 under the control of a CAG promoter. B) Fluorescent image of control cells (a) and cells overexpressing FLAG-MZF1 (c). Immunocytochemical confirmation of cells overexpressing FLAG-MZF1 is shown in d relative to the control cells (b). Scale bar: 100 µm

MZF1 overexpression did not affect the anchorage-independent proliferation of HEK293T cells

Using a soft agar colony formation assay, we also assessed the effects of MZF1 overexpression on the anchorage-independent proliferation, an indicator of cell stemness. After 21 days of culture in low-melting agarose, we did not find any statistical differences between the number (a median of 11.61 colonies in MZF1 overexpressing cells versus a median of 10.94 colonies in control cells per microscopic field) and size of colonies (a median of 210,370 squared pixels in MZF1- overexpressing cells versus a median of 241,004 squared pixels in control cells) in two groups of MZF1-overexpressing and control cells (Figure 3).

Discussion

In this study, our investigation on the effects of MZF1 stable overexpression did not reveal any significant changes in cell proliferation, cell viability, and its capability of anchorage-independent proliferation in HEK293T cells (Figs 1-3). Previous findings on the role of MZF1 in cancer cells can be categorized into three groups: those in which MZF1 functions as an oncogene and promotes proliferation and malignancy, the second group, in which MZF1 acts as a tumor suppressor and prohibits the initiation or development of cancerous states. The third group of reports indicates the lack of significant changes in proliferation and oncogenesis upon MZF1 overexpression (Table 1). The varying effects of MZF1 on cellular functions across different cell lines may stem from its influence on distinct target genes and signaling pathways specific to each cellular context (Figure 4). The first group of studies provides a link between MZF1 overexpression and tumor suppression. For instance, some of the MZF1 target genes are well-known tumor suppressors like *P53*. Although MZF1 overexpression does not change the mRNA or protein levels of p53, it can influence the p53 promoter activity and give rise to the enrichment of the P21 mRNA level as a downstream effector of p53. The SCAN domain of MZF1 physically interacts with the C-terminal basic domain of p53. Also, MZF1 binds the histone acetyltransferase p300/CBP-associated factor (PCAF), and by facilitating the p53 acetylation, suppresses breast cancer cell proliferation (Li et al., 2023) (Figure 4A). Moreover, in gastric cancer cells, the overexpression of MZF1 enhances SMAD4 transcript and protein levels. In this condition, AKT phosphorylation and β-catenin expression, as key regulatory molecules in migration, are down-regulated, and migration is suppressed (Lee et al., 2017a). Furthermore, MZF1, by activating the miR-328-3p expression, which is followed by down-regulation of CD4, repressed the malignant behavior of gastric cancer cells (Qi et al., 2022).

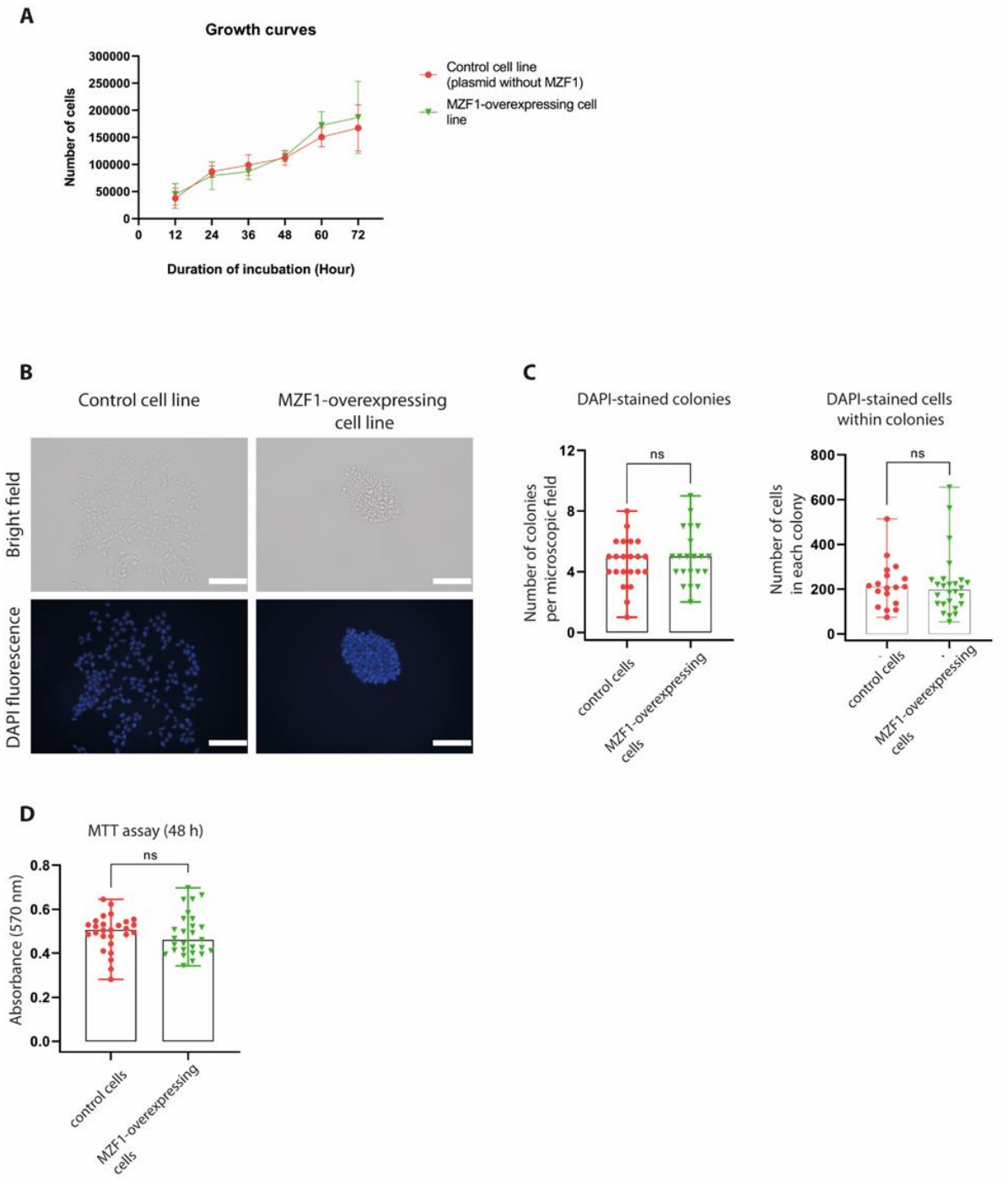

Figure 2. The stable overexpression of MZF1 did not affect the cell proliferation and cell viability of HEK293T cells A) The growth curves of the MZF1-overexpressing and control cells were assessed at various time points (12, 24, 36, 48, 60, and 72 hours). Error bars represent standard deviations obtained from the average of four biological replicates. B) Brightfield and DAPI-fluorescence images of representative colonies of two groups of MZF1-overexpressing and control cells during the colony formation assay. Scale bar: 100 µm C) The left panel shows the number of colonies in MZF1-overexpressing cells (n=21) and control cells (n=22). The right panel shows the number of cells in each colony of MZF1-overexpressing cells (n=26) and control cells (n=18). The data were collected from two independent experiments, were analyzed using the Mann-Whitney U-test, and were expressed as median and range. D) The cell viability assay of MZF1-overexpressing cells (n=27) compared with control cells (n=27), determined by MTT assay 48 h after seeding. Data was gathered from three biological replicates, statistically analyzed by the Mann-Whitney U-test, and reported as median with range.

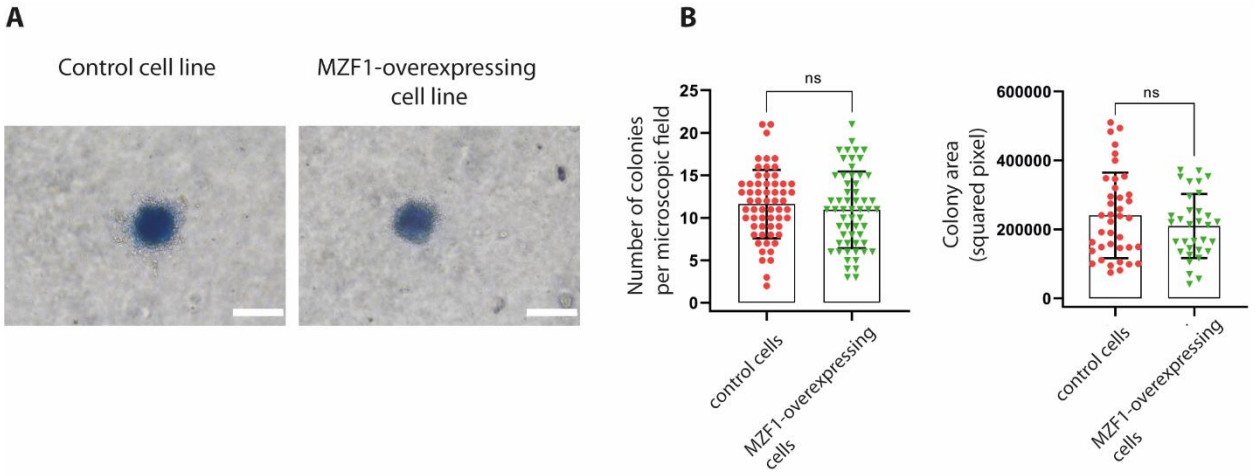

Figure 3. MZF1 overexpression did not affect the anchorage-independent proliferation of HEK293T cells. A) Representative colonies of the MZF1 overexpressing cells and control cells during soft agar assay. Scale bar: 100 µm. B) The left panel shows the number of colonies in MZF1-overexpressing (n=58) and control (n=60) cells, which were analyzed in each microscopic field of a soft agar assay. The right panel shows the colony area (in squared pixels) for the control (n=33) and MZF1-overexpressing cells (n=39). The colony areas were measured using ImageJ software and are represented by the mean ± SD. Statistical analysis between the two groups was carried out using the Welch correction test.

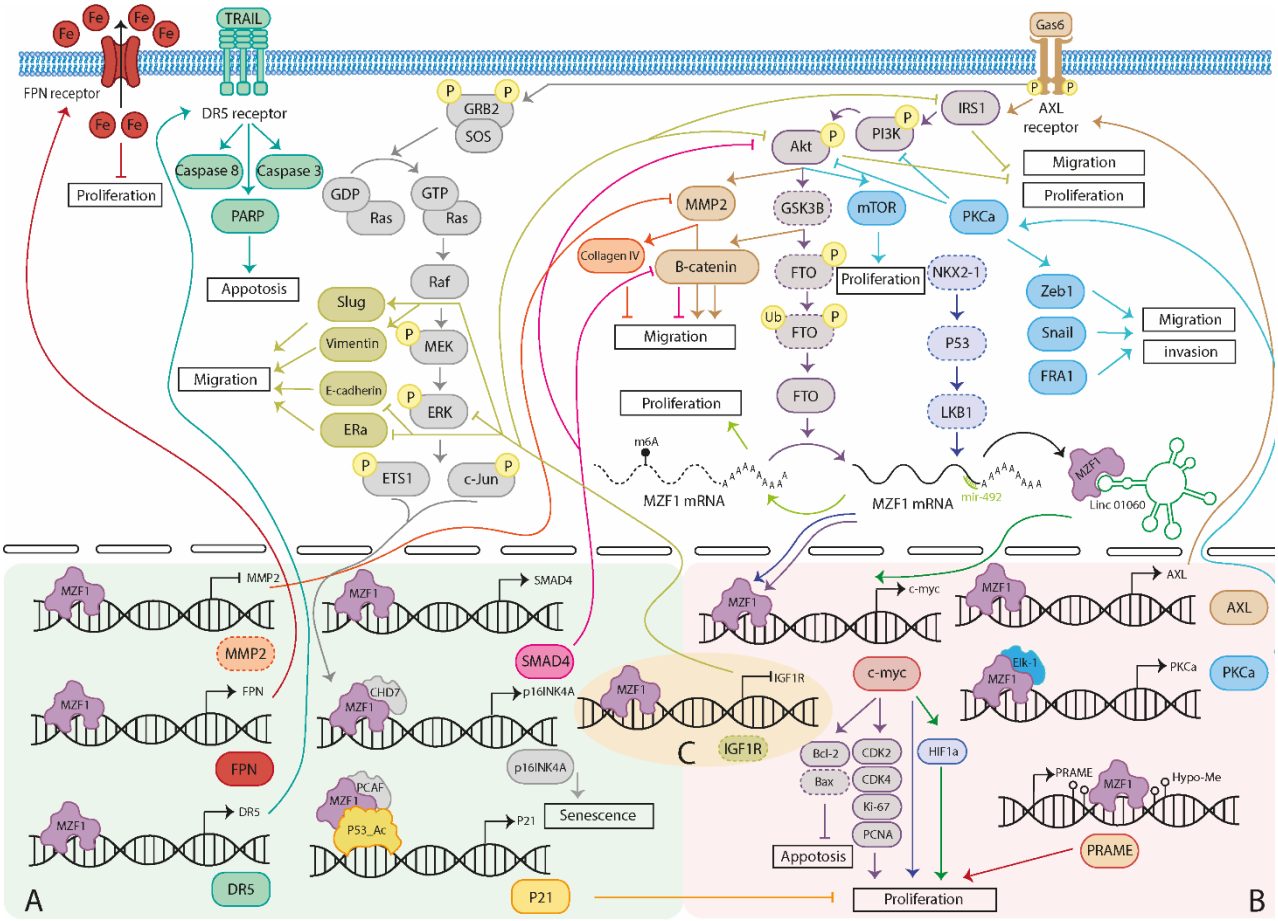

Figure 4. The schematic representation of the various regulatory roles of MZF1 in different cellular functions. (A) Tumor suppressor role, (B) oncogenic role, and (C) dual function of MZF1 in various cancers. Dotted outlines around a molecule indicate a reduction or absence of that molecule.

Table 1. The effects of MZF1 on various cellular functions in different cancer cells

Effect	Cell function/ Biological role	Cell line	Ref.
Functioning as a tumor suppressor (negative impact on cellular functions)	Proliferation	Breast cancer cells (MCF-7, ZR-75-30)	(Li et al., 2023, Tian et al., 2025)
		T-cell lymphoma (Karpas 299, SR-786, SUP-M2)	(Vishwamitra et al., 2015)
		Prostate cancer cells (PC3, DU145)	(Chen et al., 2015)
		Gastric cancer cells (HGC-27, AGS, SNU16)	(Qi et al., 2022, Tian et al., 2025)
		Lung carcinoma (A549)	(Li et al., 2024)
	Cell cycle	Breast cancer cells (MCF-7, ZR-75-30)	(Li et al., 2023)
	Cell viability	Breast cancer cells (MCF-7, ZR-75-30)	(Li et al., 2023)
		T-cell lymphoma (Karpas 299, SR-786, SUP-M2)	(Vishwamitra et al., 2015)
		Prostate cancer cells (PC3, DU145)	(Chen et al., 2015)
		Gastric cancer cells (HGC-27, AGS, SNU16)	(Qi et al., 2022)
	Stemness	T-cell lymphoma (Karpas 299, SR-786, SUP-M2)	(Vishwamitra et al., 2015)
		Gastric cancer cells (HGC-27, AGS, SNU16)	(Qi et al., 2022)
Functioning as an oncogene (positive impact on cellular functions)	Proliferation	Colorectal cancer cells (SW620)	(Zhang et al., 2021)
		Glioma cancer cells (PT#3, A172)	(Wang et al., 2022)
		Melanoma cancer cells (A375P)	(Lee et al., 2017b)
		liver adenocarcinoma (SK-Hep-1)	(Yue et al., 2015)
	Cell cycle (S-phase)	Colorectal cancer cells (SW620)	(Zhang et al., 2021)
	Stemness	Lung adenocarcinoma cells (CL1-0)	(Tsai et al., 2015)
No impact on cellular functions	Proliferation	Gastric cancer cells (MKN28, MKN74, MKN1, AGS)	(Lee et al., 2017a)
		Breast cancer cells (Hs578T, T47D, MDAMB-231)	(Li et al., 2023, Yue et al., 2019)
		Cervical cancer cell (SiHa)	(Tsai et al., 2012)
		HEK293T cell line	This study
	Cell cycle	Gastric cancer cells (MKN28, MKN74, MKN1, AGS)	(Lee et al., 2017a)
		Breast cancer cells (T47D)	(Li et al., 2023)
	Cell viability	Breast cancer cells (Hs578T)	(Yue et al., 2019)
		Cervical cancer cell (SiHa)	(Tsai et al., 2012)
		HEK293T cell line	This study
	Stemness	HEK293T cell line	This study

In line with this report, MZF1 directly binds to the matrix metalloproteinase 2 (MMP2; gelatinase-A), which can degrade type IV collagen, a pivotal component of the extracellular matrix. Hence, suppression of MMP2 by MZF1 decreases the invasion of SiHa human cervical cancer cells (Tsai et al., 2012) (Figure 4A). Also, MZF1 is positively regulated through direct binding of AP4 and c-Myb to its promoter. MZF-1 plays a suppressive role in prostate cancer by directly binding to the ferroportin (FPN) promoter. FPN is the only known iron exporter in mammalian cells that regulates the intracellular iron concentration. MZF1 increases FPN concentration, which is followed by elevated iron egress out of cells. Thus, the first outcome is the reduction of iron-related cellular activities, such as DNA replication and ATP synthesis, in prostate cancer cells relative to normal cells (Chen et al., 2015) (Figure 4A).

Another study demonstrated that sulindac sulfide increases the binding of the MZF1 transcription factor to the death receptor 5 (DR5) promoter, leading to its increased activity. DR5 is a receptor for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which sensitizes colon cancer cells to TRAIL-induced apoptosis, which is accompanied by cleavage of caspase-8, caspase-3, and PARP (Horinaka et al., 2014) (Figure 4A). Connected to these observations, the oncogenic Ras may increase the possibility of interaction between MZF1 and chromodomain helicase DNA-binding protein 7 (CHD7) by promoting the expression of both genes. Ras activates the Raf-1/MEK/ERK cascade, and ERK induces the phosphorylation of ETS1 and c-Jun, which bind to the MZF1 promoter. CHD7 is an ATP-dependent helicase without a DNA-binding domain. Hence, CHD7, through association with MZF1, can be recruited to the MZF1-binding sites on the p16INK4A promoter, leading to its expression and senescence. Based on these findings, in pancreatic adenocarcinoma (PAAD) cells with intact retinoblastoma protein (RB1) levels, the reduction of MZF1 is responsible for p16INK4A downregulation and the disruption of a senescence phenotype (Wu et al., 2022) (Figure 4A).

Normal lung epithelial cell lines exhibit higher expression levels of mitochondrial AMPK (mitoAMPK) compared to lung cancer cells. MZF1 is down-regulated by mitoAMPK. Then, Sirtuin 6 (SIRT6) expression increases as a result of less MZF1 binding to its promoter. SIRT6 reduces cancer state by inhibition of the expression of glycolysis-related genes (*SLC2A2*, *SLC2A4*, and *PKM2*) (Li et al., 2024).

The second group of studies provides evidence for the positive impact of MZF1 on tumorigenesis. For instance, MZF1 upregulates the expression of several cancer-driving genes or oncogenes, such as *c-Myc*, *AXL*, *PRAME*, *IGF1R*, and *PKCα*. The MZF1/c-Myc axis is responsible for promoting the carcinogenesis of colorectal, lung, and glioblastoma cells. In normal cells, the glycogen synthase kinase 3 beta (GSK3ß) kinase, a mediator of various signaling pathways, induces the phosphorylation and ubiquitination of fat mass and obesity-associated protein (FTO), which results in the degradation of FTO. However, in colorectal cancer cells, following the downregulation of GSK3ß, FTO becomes upregulated, which in turn removes the m6A mRNA modification of MZF1, its increased expression, and the increased expression of the proto-oncogene c-Myc. MZF1 overexpression causes an increase in the expression of CDK2, CDK4, Ki-67, PCNA, and Bcl-2, and suppresses the expression of Bax. Therefore, the MZF1/c-Myc axis promotes colorectal cancer cell proliferation by enhancing the proportion of S-phase cells and hampering cell apoptosis (Zhang et al., 2021) (Figure 4B).

In the lung adenocarcinoma cells, in the presence of inactivated Liver Kinase B1 (LKB1), as a result of direct MZF1 binding to the c-Myc promoter, the increased expression of c-Myc is followed by its oncogenic properties, including migration, invasion, and anchorage-independent growth (Tsai et al., 2015) (Figure 4B). In glioma cells, the A2 domain of MZF1 binds to the sense strand of Linc01060. This physical interaction, by protecting it from ubiquitin-mediated proteasomal degradation, stabilizes the MZF1 protein. Moreover, this interaction facilitates the translocation of MZF1 from the cytoplasm to the nucleus and enriches MZF1 at the c-Myc promoter. Thus, MZF1 exerts its oncogenic effect via the MZF1/c- Myc/HIF1α axis (Li et al., 2021) (Figure 4B). In colorectal cancer cells, the activation of tyrosine kinase AXL receptors evokes migration and invasion. MZF1, as part of its oncogenic role, interacts with the AXL promoter and induces AXL expression (Mudduluru et al., 2010) (Figure 4B).

Other studies suggest that the oncogenic role of MZF1 is exerted through promoting PRAME (Preferentially expressed Antigen in Melanoma) expression. PRAME is a cancer-testis antigen whose expression is restricted to the testis, with low levels detected in a few other tissues. PRAME is involved in various cancers. MZF1 directly interacts with the first intron of PRAME and, along with DNA demethylation, upregulates PRAME in melanoma cells, which in turn promotes cell proliferation (Lee

et al., 2017b). In agreement with the above reports, in hepatocellular carcinoma (HCC), cooperative interaction between the N-terminal region of MZF1 and the C-terminal region of Ets-like protein-1 (Elk-1) forms a complex that attaches to the promoter of protein kinase C alpha (PKCα) and drives its expression, leading to cell proliferation, migration, invasion, as well as tumorigenicity (Yue et al., 2015) (Figure 4B). In addition, MZF1 expression is related to the resistance to immunotherapy in HCC. Direct binding of MZF1 to the dependent kinase 4 (CDK4) promoter regulates CDK4 expression, which in turn modulates programmed death ligand 1 (PD-L1) ubiquitination. Also, CDK4, through phosphorylation of MZF1, makes a positive feedback loop in MZF1 regulation and PD-L1 ubiquitination. Therefore, using a CDK4 inhibitor can be used along with immunotherapy (anti-PD-L1 antibody), which can improve the immunotherapy effects by regulating the expression of PD-L1 (Kan et al., 2024). Besides, it has been shown that miR-492, which is overexpressed in hepatoblastoma, rectal cancer, and pancreatic adenocarcinoma, is also upregulated in prostate cancer and post-transcriptionally targets the 3' UTR of MZF1 mRNA. Therefore, miR-492, due to the decline of MZF1, promotes cell proliferation and cell viability (Chen et al., 2015).

The third group of studies indicates that MZF1 might simultaneously play opposite roles in tumorigenesis. MZF1, through direct interaction with the insulin-like growth factor-1 (IGF-1R) promoter, suppresses its transcriptional level. The inhibition of IGF-1R negatively influences the phosphorylation level of p38 mitogen-activated protein kinase (p38 MAPK), the protein levels of ERα, and the transcript levels of E-cadherin, while elevating the mesenchymal cell markers, such as Slug and Vimentin, maintains the mesenchymal phenotype and migration (Yue et al., 2019). On the other hand, the MZF1 physical association with the IGF-1R promoter reduces cell migration, cell viability, cell proliferation, and anchorage-independent colony formation in lymphoma cells. MZF1 overexpression exerts its inhibitory effects on the IGF-1R promoter not only by reduction of the mRNA and protein levels of IGF-1R but also by downregulation of the activated or phosphorylated forms of IGF-1R, as well as phosphorylation of downstream targets like pAKT and pIRS-1 (Vishwamitra et al., 2015) (Figure 4C).

In summary, we have demonstrated that the overexpression of MZF1 does not impact cell proliferation, viability, and anchorage-independent colony formation (stemness) in MZF1-overexpressing HEK293T cells.

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Conflict of Interests
The authors declare that they have no conflict of interest.

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