



Published in final edited form as:

J Cell Biochem. 2015 October ; 116(10): 2146–2154. doi:10.1002/jcb.25203.

Role and Regulation of Myeloid Zinc Finger Protein 1 in Cancer

Taka Eguchi^{1,*}, Thomas Prince^{3,*}, Barbara Wegiel², Stuart K Calderwood¹

¹Department of Radiation Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA02115

²Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA02115

³Urologic Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892

Abstract

Myeloid zinc finger 1 (MZF1) belongs to the SCAN-Zinc Finger (SCAN-ZF) transcription factor family that has recently been implicated in a number of types of cancer. Although the initial studies concentrated on the role of MZF1 in myeloid differentiation and leukemia, the factor now appears to be involved in the etiology of major solid tumors such as lung, cervical, breast and colorectal cancer. Here we discuss the regulation of MZF1 that mediated its recruitment and activation in cancer, concentrating on posttranslational modification by phosphorylation and sumoylation, formation of promyelocytic leukemia nuclear bodies and its association with co-activators and co-repressors.

(1) Introduction- MZF1 in cancer

Myeloid zinc finger 1 (MZF1 aka MZF1A, MZF1B, ZSCAN6, ZNF42 and ZFP98) is a SCAN-Zinc Finger (SCAN-ZF) transcription factor family member and has been implicated in a number of cancers and cellular malignancies (Hromas, Morris et al. 1995, Gaboli, Kotsi et al. 2001, Reymann and Borlak 2008, Mudduluru, Vajkoczy et al. 2010, Rafn, Nielsen et al. 2012, Deng, Wang et al. 2013). We will refer here to the gene or protein as MZF1 (Fig 2). Zinc finger proteins have in common the possession of small structural motifs, often occurring in clusters that can coordinate divalent cations such as Zn⁺⁺, forming finger-like structures that can bind to, most notably the major groove in DNA and permitting sequence-specific binding. MZF1 was first suspected to be involved in malignancy in studies on hematopoietic development along the myeloid lineage. MZF1 appeared to function as a gene repressor in this context, repressing the CD34 or *c-myb* promoter activity essential for hematopoietic differentiation (Perrotti, Melotti et al. 1995). Such a block towards differentiation suggested the emergence of a leukemic phenotype (Perrotti, Melotti et al. 1995). Neoplastic cell transformation by MZF1 was indeed demonstrated using the NIH3T3

Correspondence should be addressed to Stuart K Calderwood, 3 Blackfan Circle, Boston MA 02115, scaldew@bidmc.harvard.edu.

*Equal Contributors

&Conflict of interest.

The authors declare no conflict of interest.

transformation model, and forced expression of MZF1 led to emergence of cells that grew rapidly *in vivo* and initiated tumors in athymic mice (Hromas, Davis et al. 1996). Subsequent studies showed that immortalized myeloid cells were protected from IL-3 withdrawal-mediated apoptosis by MZF1 (Hromas 1996). MZF1 activity again resulted in cells that formed tumors in congenic mice. However, there was some ambivalence in the role ascribed to MZF1 in hematopoietic cancers. Gaboli *et al* showed that *mzf1*^{-/-} mice underwent a large increase in autonomous proliferation of hemopoietic progenitors and suggested that the *mzf1* gene might function as a suppressor of blood cell cancers (Gaboli, Kotsi et al. 2001). The reasons for discrepancies were not obvious although they could be connected to the findings that the *mzf1* gene can give rise to at least two transcripts that may have contrasting properties in the cell (see below).

The development of other types of solid tumor cancers have subsequently been associated with MZF1. Analysis of data from the TCGA website indicated significant amplification of the *MZF1* gene in human breast, uterine, lung and bladder cancers as well as increased levels of mutation in colorectal cancer (Fig.1). By contrast, in Glioma, a significant number of cancers showed *MZF1* deletion (Fig. 1). In addition, analysis of mRNA levels in a series of human cancers indicated large increases in MZF1 mRNA in myeloma and cutaneous melanoma compared to normal tissues (Oncomine: <https://www.oncomine.org>)

Likewise MZF1 appeared to play roles in multiple cancers as indicated by studies in cellular and animal models. In experiments in SK-Hep1 HCC tissue culture cells *in vitro*, inhibition of MZF1 expression using antisense technology led to reduced growth in hepatocellular carcinoma (Hsieh, Wu et al. 2007). This finding might not have been predicted from study of human liver tumors in which evidence of MZF1 amplification or mutation was low (Fig. 1). In addition, MZF1 was shown to mediate migration, invasion and *in vivo* metastasis in colorectal cancer models *in vitro* (RKO and SW480 cells) and cervical cancer cells (Mudduluru, Vajkoczy et al. 2010). In these studies the gene for the receptor tyrosine kinase AXL was shown to be activated by MZF1 at the transcriptional level, and knockdown of MZF1 by RNA interference reduced migration and invasion. AXL activity has been associated with a metastatic phenotype (Asiedu, Beauchamp-Perez et al. 2014). Moreover, MZF1 and AXL levels were positively correlated and significantly higher in resected colorectal tumors compared to normal tissues (Mudduluru, Vajkoczy et al. 2010). In three-dimensional breast cancer model derived from primary human breast cancer, MZF1 was found to bind to the enhancer element and drive the expression of the *cathepsin B (CTSB)* gene, a key molecule required for tumor invasion (Rafn, Nielsen et al. 2012) Rafn et al were able to define a signaling cascade that was initiated by ERBB2 activation through transforming growth factor receptor RII (TGFβRII) binding and propagated by the kinases, CDC42BP, ERK2, PAK4 and PKCA. This study gave the first convincing indication of an activating signaling pathway that might be located upstream of human MZF1 in cancer signaling (see below). A role for the TGFβRII ligand, TGF-1β in MZF1 function was likewise indicated by studies showing that TGF-β1 released by MDA_MB-231 mammary cancer cells could cause mesenchymal stem cells to differentiate into cancer associated fibroblasts (CAF) in a process dependent on MZF1 (Weber, Kothari et al. 2014). TGF factors classically regulate transcription through a series of Smad factors that can translocate to the nucleus and modulate gene expression (Derynck and Zhang 2003). TGF-1β can play

paradoxical roles in cancer, in some contexts inhibiting tumor growth although in others triggering cell invasion and local immune suppression (Massague 2008). Effects on CAF may play a significant role in such TGF- β functions. In lung adenocarcinoma another mechanism for MZF1 activation has been described (Tsai, Wu et al. 2014). MZF1 activity was triggered by the loss of Liver Kinase B1 (LKB1) leading to elevated levels of MYC, increased migration and invasion of CL-1 lung adenocarcinoma cells. Loss of LKB1 was shown to be associated with inactivation of the tumor suppressor TP53 and enhanced expression of the lung proto-oncogene protein NK2 homeobox 1 (NKX2-1) (Tsai, Wu et al. 2014). MZF1 was also reported to be activated downstream of NKX2 in human papillomavirus driven cancer. MZF1 led to upregulation of FOXM1 expression, again promoting migration, invasion and stemness that may be related to increase in Wnt / β -catenin signaling (Chen, Cheng et al. 2014). Another cancer relevant transcriptional target of MZF1 may be the protooncogene CDC37, which is required for oncogenic kinase maintenance and plays a role in the emergence of prostate carcinoma (Huang, Grammatikakis et al. 1998, Gray, Prince et al. 2008). Overexpression of CDC37 drives proliferation, survival and AR activity in prostate cancer, and thus induction of CDC37Cdc37 by MZF1 may have a critical role in development of prostate cancer (Calderwood 2015).

A recurrent theme in a number of the studies is a role for MYC in association with MZF1, and MYC is one of the factors that can bind the *MZF1* gene promoter (Suppl. Table 1). MYC appears to be regulated upstream and downstream of MZF1 suggesting a positive feedback loop. It may be significant that MZF1 target CDC37 can synergize with MYC in prostate carcinogenesis in transgenic mouse models (Stepanova, Finegold et al. 2000, Sironen, Karjalainen et al. 2002).

Thus changes in expression, mutation and regulation of the *MZF1* gene have been implicated in a wide spectrum of cellular malignancies. One common theme running through many of the studies was the finding of MZF1-dependent transcriptional changes associated with malignant cell migration and invasiveness. These effects of MZF1 could be mediated through repression of differentiation factors such as CD34 and c-myb or the *trans*-activation of genes encoding effector proteins such as AXL and cathepsin B. Interestingly, the effects of MZF-1 are dependent on cancer type and thus we speculate a specific genetic milieu influence activity of MZF1. Such interactions require further elucidation as might be critical for understanding cancer development as well as design of new therapeutic strategies.

As a whole, these effects of MZF1 appear to be mediated by complex transcriptional networks that require such factors as MYC, FOXM1 and NKX2-1. Consequently, elucidating the function and interactions of MZF1 within this network will require further analysis.

(2) MZF1 as a member of the SCAN-Zinc Finger transcription factor family

MZF1 belongs to the SCAN-ZF domain-pair gene cluster and is a component of the larger ZF, 600-member transcription factor family expressed only in vertebrates. The human genome has been shown to contain nearly seventy SCAN-ZF factors, including MZF1 itself

(Edelstein and Collins 2005). SCAN-ZF family members possess a varied number of Cys²H₂ zinc finger motifs that bind distinct DNA motifs, giving sequence specific recognition of target genes. In addition a small number of SCAN domain-only (SCAND) factors have been discovered that lack the DNA binding ZF domains and are currently thought to function as regulators of the intact SCAN-ZF factors that may be significant in cancer (Sander, Stringer et al. 2003, Edelstein and Collins 2005). As a promoter/enhancer-binding-type transcription factor, MZF1 functions as both a *trans* activator and *trans*-repressor, suggesting that its relative oncogenic activity may be an aggregate of increases and decreases in gene expression (Edelstein and Collins 2005).

The MZF1 activating and repressing kinase phosphorylation signals, inter-molecular interactions and transcriptional gene targets are described herein, organized in Table 1 and modeled in Figure 2.

(3) The *MZF1* gene

Understanding the organization of the *MZF1* gene and its regulation may give us a window through which to observe its involvement in malignancy. The human *MZF1* gene (NCBI Gene ID: 7593) is located on the end of chromosome 19q with its 5' end and promoter oriented towards the telomere, suggesting that telomere shortening in aging cells could affect MZF1 biology. *MZF1* encodes two unique isoforms produced by alternative splicing. A third isoform record of alternative transcription initiation was recently found to be an artifact and deleted from NCBI, however the isoform was maintained in the mouse record, thus suggesting a difference in species. The ability of the *MZF1* gene to encode multiple transcripts, likely with differing properties may play a significant role in its complex influences on tumorigenesis, as will be discussed below. The larger *MZF1* isoform 1 (NM_03422.2) encodes a longer protein known as both MZF1A and MZF1B producing a 734 aa protein product (NP_003413.2). This factor contains the N-terminal SCAN domain followed by a regulatory linker connected to a C-terminal DNA binding domain containing 13 Cys²H₂-type zinc finger (ZF) motifs. Studies in murine MZF1 place the *trans*-activation domain (TAD) within this linker region (Murai, Murakami et al. 1997). A shorter MZF1 isoform 2 (NM_01267033.1) encodes a 290 aa protein product (NP_001253962.1) comprising of the SCAN domain, part of the regulatory linker and a unique C-terminal motif. Interestingly this unique C-terminal motif lacks the phosphorylation sites predicted to reside in the corresponding region on MZF isoform 1 as well as the C-terminal ZF DNA binding domains, suggesting unique functions for both MZF1 isoform 2 and the phosphorylation of MZF isoform 1 (Figure 2) (Peterson and Morris 2000).

The full repertoire of mechanisms of regulation of *MZF1* gene expression have yet to be rigorously characterized. However global analysis of the human genome and proteome could provide some insights into the biology of MZF1. Comprehensive ChIP-Seq profiling of 161 transcription factors indicated that the *MZF1* gene promoter contained a single RNA polymerase 2 (POLR2A) binding site spanning the transcriptional start site (Hudson and Snyder 2006) (Euskirchen, Rozowsky et al. 2007). Within this POLR2A footprint 85 different transcription factors were shown to map to 111 binding sites, with 24 such factors binding to more than one site (Suppl Table 1). Notable transcription factors included MYC,

HSF1, TRIM28, STAT3, RELA, MAX and ZNF143. The observation that MYC bound to the *MZF1* gene promoter may be of significance in regulating expression of the *MZF1* gene in cancer (as discussed below). Protein profiling of normal human tissue indicated that the MZF1 protein was most highly expressed in the thyroid and brain (Uhlen, Fagerberg et al. 2015) (Uhlen, Bjorling et al. 2005) (www.proteinatlas.org).

(4) Regulation of MZF1 Function

The intracellular mechanisms of MZF1 regulation are largely uncharacterized but examination of the available data suggested a number of levels of regulation including posttranslational modifications, dynamic intra-nuclear localization, protein-protein association and binding to transcriptional co-factors.

(5) MZF1 entry into promyelocytic leukemia nuclear bodies (PML-NBs)

MZF1 has been shown to concentrate in PML-NBs (Bernardi and Pandolfi 2007, Heun 2007, Noll, Peterson et al. 2008). PML-NBs are nuclear structures ranging in size from 0.2–1.0 microns formed by the oligomerization of PML proteins along with, potentially over 160 other protein components (Van Damme, Laukens et al. 2010). PML-NBs have been reported to critically influence transcriptional activity and chromosomal structure through interaction with such other factors (Dellaire and Bazett-Jones 2004). However, it is still not clear how residence in PML-NB regulates the transcriptional activity of factors such as PML and, potentially MZF1.

MZF1 Regulation by SUMO modification

MZF1 was shown to co-associate with PML-NB protein PML, a member of the tripartite motif family and the principle coordinator of PML-NB assembly, within the nuclear bodies through its N-terminal region as determined using immunofluorescence analysis (Jensen, Shiels et al. 2001, Noll, Peterson et al. 2008). This region of MZF1 includes the SCAN domain, through which MZF1 also recruits other factors such as ZNF24 to PML-NBs (Noll, Peterson et al. 2008). Entry into PML-NBs appear to require the post-translational modification by SUMO (small ubiquitin-related modifier) (Heun 2007, Raman, Nayak et al. 2013).

SUMOylation of transcription factors has been associated with gene repression (Stielow, Kruger et al. 2010, Raman, Nayak et al. 2013). The SUMO modification allows recognition by SUMO interacting motifs (SIM) in associated proteins (Raman, Nayak et al. 2013). Inhibition of gene transcription after SUMOylation was shown to be due to recruitment of repressor proteins with SIM motifs, including Mi-2 containing complexes, SETDB1 and an LSD1-CoREST-HDAC1 complex (Stielow, Kruger et al. 2010, Tanaka and Saitoh 2010). SIM domains were found in each of the repressors. In addition SUMO/SIM interactions are critically involved in recruiting transcription factors to PML bodies (Noll, Peterson et al. 2008). Indeed PML itself contains a functional SIM and is modified by SUMOylation and these domains are involved in its role as a scaffold protein coordinating the assembly of PML-NBs (Cappadocia, Mascle et al. 2015). Recruitment of the repressor DAXX to the PML-NBs also requires a SIM domain (Chang, Naik et al. 2011).

Noll et al suggested that SUMOylation occurred within amino acids 37–128 of MZF1 (Noll, Peterson et al. 2008). Notably, this sequence contains only one lysine residue at K81. The site of the SUMO recognition site was not ascertained by this group although Noll et al suggested a sequence in the N-terminus-(aa 15–28) (Noll, Peterson et al. 2008). In addition, this region contains a consensus SIM site in the N terminus with a motif containing 4 adjacent hydrophobic amino acids- (hydrophobic amino acids at aa 86–90 (LLVL) (Figure 4). This site is embedded within a region of negative charge with a serine residue at aa79- again found in many SIMs. Recent studies by Cappadocia et al have shown phosphorylation within the SIM domains of PML and DAXX strongly enhances interactions with SUMO (Cappadocia, Mascle et al. 2015).

There is strong evidence to indicate that PML-NB may be involved in gene repression and indeed have been also associated with tumor suppression (Jensen, Shiels et al. 2001): In addition, the loss of PML-NBs was shown to play a key role in development of acute promyelocytic leukemia(Jensen, Shiels et al. 2001). The studies of Pandolfi *et al* showed that knockout of the MZF1 gene led to development of cancer strongly suggesting a potential cancer suppressive activity by MZF1 in this disease(Gaboli, Kotsi et al. 2001). MZF1 SUMOylation might thus play a key role in transcriptional function and it is tempting to speculate that the SUMOylated form may play key roles in gene repression, as with PML.

(6) Upstream activation of MZF1 by phosphorylation and SUMOylation

In accordance with the phospho-SIM studies of PML one might speculate that S79 of MZF1 could be phosphorylated when MZF1 enter the PML-NBs. S79 is in a region rich in negative charge and could thus be a potential substrate for CK2 that modifies S or T residues within regions of negatively charged amino acids (as is seen with PML). As mentioned above, MZF1 can be activated downstream of TGF β -receptor II-activated Erb-B2 in breast cancer through a signaling pathway involving the ERK2 kinase, although the phosphorylation site on MZF1 was not characterized (Rafn, Nielsen et al. 2012). Screening by mass spectrometry has identified 5 phosphorylated residues in MZF1, including at T210, S256, S274, S294 and S347 in the linker domain / *TAD* (*trans activation domain*), and T592 in the ZF domain (<http://www.phosphosite.org>) (Fig. 21). In addition a screen for CDK4 and CDK6 phosphorylation indicates MZF1 as a confirmed candidate for modification by both kinases (Anders, Ke et al. 2011). S256, S274 and S294 each resemble consensus cyclin dependent kinase sites on the basis of amino acid sequence and S274 could potentially be a substrate for the kinase ERK2. However, at this time, none of these phosphorylation sites have been characterized as activating or suppressing MZF1 in focused biology studies, although they might suggest a key regulatory role for the linker / *TAD region*. Studies on the murine Mzf1 aka Mzf2/Zfp121/Zfp98/Znf42 (NCBI Reference Sequence: NP_665818.2) encoded by the murine *mzf1* gene (NCBI gene ID: 109889), that shares considerable homology with homo sapiens MZF1, similarly containing 13 ZF motifs indicated multiple phosphorylation by MAP kinase family members such as ERK (Ogawa, Murayama et al. 2003). Phosphorylation took place in the Linker / *TAD domain* and led to inhibition of MZF1-mediated transcription. It is not clear to what degree these results can be extrapolated to human MZF1 although the considerable sequence similarity might suggest this possibility (Murai, Murakami et al. 1997). These phosphorylation sites in murine Mzf1 (S257, 275,

295) occur on similar residues to those found in human MZF1 isoform 1 at S256, 274, 294 (as mentioned above), suggesting possible functional significance for these sites in human MZF1. Phosphorylation does not inevitably enhance sumoylation of adjacent residues. For instance in the protein TRIM28/Kap1, a factor that associates with Kruppel family ZF proteins, phosphorylation on serine 824 by PIKK family kinases led to loss of Sumo from the N-terminus, inhibition of gene repressive activity and gain of *trans*-activating function (Bunch, Zheng et al. 2014) (Iyengar and Farnham 2011). Interestingly, both TRIM28 and PML belong to the *tripartate motif* family of factors and TRIM28 has been found to accumulate in Kap1 bodies, structures that have marked similarity to PML-NBs. (Briers, Crawford et al. 2009)

(7) Regulation of MZF1 by protein-protein interactions

As mentioned above, the SCAN box is a key protein interaction domain allowing SCAN-ZF proteins such as MZF1 to form dimers (Edelstein and Collins 2005). These interactions include formation of both MZF1 homodimers and heterodimers with other SCAN box-containing proteins (Sander, Stringer et al. 2003, Edelstein and Collins 2005, Noll, Peterson et al. 2008). The importance of dimerization for SCAN-ZF transcriptional activity was shown in studies on ZNF174 using GAL4 VP16 two hybrid experimental system. In these studies a ZNF SCAN-VP16 *trans*-activating fusion construct and a ZNF174 SCAN-GAL4 DNA binding fusion construct were shown to co-activate transcription from a GAL4 binding sites containing reporter construct, indicating that SCAN domain interacts / dimerizes with another SCAN domain in this system and synergistically activates transcription (Williams, Blacklow et al. 1999).

The presence of a highly conserved SCAN box in the SCAN-ZF family also suggested that a network of interactions between family members may occur through heterodimerization (Edelstein and Collins 2005). MZF1 for instance has been shown to interact with family members ZNF24, ZNF174 and ZNF202 in SCAN-SCAN box interactions (Noll, Peterson et al. 2008). Indeed family member ZNF24 was shown to accompany MZF1 into PML-NBs in a SCAN box dependent localization manner (Noll, Peterson et al. 2008). However, although the SCAN box is highly conserved, not all SCAN domains are able to form dimers indicating some limit to potential partnering between SCAN-ZF members (Edelstein and Collins 2005).

MZF1 can also interact with a SCAN box-containing protein deficient in ZF domains known as RAZ1 (SCAN-related protein associated with MZF1) or SCAND1 /RAZ108, which are also shown as SCAND1 variant 1 and variant 2 (Sander, Haas et al. 2000). The exact significance of this interaction is not clear although “zinc fingerless” SCAND1 might attenuate MZF1 signaling through diminished affinity for DNA targets or other interactions (Sander, Haas et al. 2000). Indeed ZF domains can interact with proteins as well as DNA (Edelstein and Collins 2005).

Transactivation by MZF1 would likely involve the recruitment of co-activating molecules although little information is available in this regard. However study of the murine Mzf1 paralog indicated its interaction with the SWI/SNF2-type DNA remodeling protein

mDomino (Ogawa, Ueda et al. 2003). mDomino was shown to bind the TAD of Mzf1 and activated transcription of a reporter construct (Ogawa, Ueda et al. 2003). In addition human MZF1 was shown to bind to LDOC1, a protein whose expression is reduced in pancreatic and gastric cancer. LDOC1 was shown to encode a leucine zipper domain and SH3 like proline rich domain and this association led to enhancement of apoptosis in Jurkat and K562 cells (Inoue, Takahashi et al. 2005).

MZF1 has also been associated with *trans*-repression of some genes. Takahashi et al showed that MZF1 could bind the co-repressor FHL3 (four and a half Lim domain protein-3) that interacts with cancer development regulators Smad 2, 3 and 4 (Takahashi, Matsumoto et al. 2005). Human Fc epsilon RI beta-chain expression was repressed by MZF1, through an element in the fourth intron and transcriptional repression by MZF1 required FHL3 as a cofactor (Takahashi, Matsumoto et al. 2005). Gene repression by factors such as PML and DAXX has been strongly associated with residence in PML-NBs suggesting the possibility that MZF1 found in such locations might be repressive to target genes.

(8) Conclusions

There is a growing evidence of a role for MZF1 in cancer and certainly its posttranslational modification will influence its role as a gene repressor or activator. Although the initial studies of MZF1 in malignant progression concentrated on its role in promyelocytic leukemia, the factor now appears to be involved in the etiology of a number of major solid tumor types, such as lung, cervical, breast and colorectal cancer (Tsai, Hwang et al. 2012) (Mudduluru, Vajkoczy et al. 2010, Rafn, Nielsen et al. 2012, Tsai, Wu et al. 2014). These properties may at least partially reflect increases in *MZF1* gene copy number and rate of mutation in many cancers (Fig. 1).

A theme common to many of the studies reviewed here was the finding of MZF1-dependent transcriptional changes in malignant cell migration and invasiveness (Reymann and Borlak 2008, Mudduluru, Vajkoczy et al. 2010, Rafn, Nielsen et al. 2012). These effects of MZF1 at least in promyelocytic leukemia could be mediated through repression of differentiation factors such as CD34 and c-myb or the *trans*-activation of genes encoding effector proteins such as AXL and cathepsin B (Perrotti, Melotti et al. 1995, Mudduluru, Vajkoczy et al. 2010, Rafn, Nielsen et al. 2012). It is interesting to note that a number of studies place MZF1 downstream of TGF- β , a factor known to be important in invasion, migration and the EMT phenotype (Massague 2008). MZF1 activation by TGF- β , may involve one or more of the phosphorylation sites characterized in MZF1 (Driver, Weber et al. 2015) (Fig. 3).

Although much information has accrued regarding mechanisms of transcriptional regulation by MZF1, major questions remain unanswered. These include the nature of PTMs that regulate function and their coupling to upstream signaling. MZF1 is modified by phosphorylation and SUMOylation as mentioned above, although the significance of these processes is still emerging. Phosphorylation of MZF1 appears to be required for responses to the activating effects of TGF- β stimulation (Rafn, Nielsen et al. 2012, Driver, Weber et al. 2015). Likewise the role of SUMOylation and entry of MZF1 into PML-NBs may play a significant role in regulation of gene expression in the malignant cell (Noll, Peterson et al.

2008). Indeed the SUMOylation sites and SUMO-interaction motifs (SIMs) are predicted in human and mouse MZF1 (Fig. 4). SUMOylation of transcription factors is usually associated with the recruitment of co-repressors, and this process may thus mediate some of the repressive processes orchestrated by MZF1 in differentiation and cancer (Stielow, Kruger et al. 2010, Tanaka and Saitoh 2010).

Other questions related to MZF1 and its role in cancers that may require study include: (1) the nature of associated transcription factors including dimerization partners containing the SCAN domain and their role in *trans* activation. SCAN domain partners may either synergize with MZF1 or, in the case of the zinc fingerless protein SCAND1 may inhibit the factor. This may be a highly significant mode of inhibition as SCAND1 levels are high in normal prostate and may inhibit unscheduled MZF1 activity (*GeneAtlas U133A gcrma*). In addition MZF1 may interact with transcription factors from other families outside the SCAN-ZF cluster. Of particular significance is MYC, a protein that may function both upstream and downstream of MZF1 (Tsai, Wu et al. 2014) (Suppl. Table 1). (2) Recruitment of co-activators and co-repressors by MZF1. Murine Mzf1 is known to interact with the chromatin remodeling factor mDomino, a protein that can function to insert the non-canonical histone H2AZ into chromatin (Ogawa, Ueda et al. 2003, Fujii, Ueda et al. 2010). Elevated levels of H2AZ are associated with rapid transcription (Redon, Pilch et al. 2002). Sumoylated MZF1 might be expected to interact with the SIM domains of gene repressors as described above, although this connection has not been formally proven. Future studies will address cell signaling in cancer and association of activators and repressors with MZF1. (3) Global interactions of MZF1 with the human genome. Determining the global interactions of MZF1 with the genomes of normal and malignant cells is clearly an important next step in the project of assessing the importance of this factor and its transcriptional targets in cancer.

Answers to these questions may permit us to consider strategies to targeting this intriguing factor in cancer or assessing MZF1 as a biomarker for cancer diagnosis and prognosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH research grants RO-1CA047407, R01CA119045 and RO-1CA094397

References

- Anders L, Ke N, Hydbring P, Choi YJ, Widlund HR, Chick JM, Zhai H, Vidal M, Gygi SP, Braun P and Sicinski P (2011). "A systematic screen for CDK4/6 substrates links FOXM1 phosphorylation to senescence suppression in cancer cells." *Cancer Cell* 20(5): 620–634. [PubMed: 22094256]
- Asiedu MK, Beauchamp-Perez FD, Ingle JN, Behrens MD, Radisky DC and Knutson KL (2014). "AXL induces epithelial-to-mesenchymal transition and regulates the function of breast cancer stem cells." *Oncogene* 33(10): 1316–1324. [PubMed: 23474758]
- Bernardi R and Pandolfi PP (2007). "Structure, dynamics and functions of promyelocytic leukaemia nuclear bodies." *Nat Rev Mol Cell Biol* 8(12): 1006–1016. [PubMed: 17928811]
- Bettermann K, Benesch M, Weis S and Haybaeck J (2012). "SUMOylation in carcinogenesis." *Cancer Lett* 316(2): 113–125. [PubMed: 22138131]

- Briers S, Crawford C, Bickmore WA and Sutherland HG (2009). "KRAB zinc-finger proteins localise to novel KAP1-containing foci that are adjacent to PML nuclear bodies." *J Cell Sci* 122(Pt 7): 937–946. [PubMed: 19258395]
- Bunch H, Zheng X, Burkholder A, Dillon ST, Motola S, Birrane G, Ebmeier CC, Levine S, Fargo D, Hu G, Taatjes DJ and Calderwood SK (2014). "TRIM28 regulates RNA polymerase II promoter-proximal pausing and pause release." *Nat Struct Mol Biol* 21(10): 876–883. [PubMed: 25173174]
- Calderwood SK (2015). "Cdc37 as a co-chaperone to Hsp90." *Subcell Biochem* 78: 103–112. [PubMed: 25487018]
- Cappadocia L, Mascle XH, Bourdeau V, Tremblay-Belzile S, Chaker-Margot M, Lussier-Price M, Wada J, Sakaguchi K, Aubry M, Ferbeyre G and Omichinski JG (2015). "Structural and Functional Characterization of the Phosphorylation-Dependent Interaction between PML and SUMO1." *Structure* 23(1): 126–138. [PubMed: 25497731]
- Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C and Schultz N (2012). "The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data." *Cancer Discov* 2(5): 401–404. [PubMed: 22588877]
- Chang CC, Naik MT, Huang YS, Jeng JC, Liao PH, Kuo HY, Ho CC, Hsieh YL, Lin CH, Huang NJ, Naik NM, Kung CC, Lin SY, Chen RH, Chang KS, Huang TH and Shih HM (2011). "Structural and functional roles of Daxx SIM phosphorylation in SUMO paralog-selective binding and apoptosis modulation." *Mol Cell* 42(1): 62–74. [PubMed: 21474068]
- Chen PM, Cheng YW, Wang YC, Wu TC, Chen CY and Lee H (2014). "Up-regulation of FOXM1 by E6 oncoprotein through the MZF1/NKX2–1 axis is required for human papillomavirus-associated tumorigenesis." *Neoplasia* 16(11): 961–971. [PubMed: 25425970]
- Dellaire G and Bazett-Jones DP (2004). "PML nuclear bodies: dynamic sensors of DNA damage and cellular stress." *Bioessays* 26(9): 963–977. [PubMed: 15351967]
- Deng Y, Wang J, Wang G, Jin Y, Luo X, Xia X, Gong J and Hu J (2013). "p55PIK transcriptionally activated by MZF1 promotes colorectal cancer cell proliferation." *Biomed Res Int* 2013: 868131. [PubMed: 23509792]
- Derynck R and Zhang YE (2003). "Smad-dependent and Smad-independent pathways in TGF-beta family signalling." *Nature* 425(6958): 577–584. [PubMed: 14534577]
- Driver J, Weber CE, Callaci JJ, Kothari AN, Zapf MA, Roper PM, Borys D, Franzen CA, Gupta GN, Wai PY, Zhang J, Denning MF, Kuo PC and Mi Z (2015). "Alcohol Inhibits Osteopontin Dependent Transforming Growth Factor-beta1 Expression in Human Mesenchymal Stem Cells." *J Biol Chem*.
- Edelstein LC and Collins T (2005). "The SCAN domain family of zinc finger transcription factors." *Gene* 359: 1–17. [PubMed: 16139965]
- Euskirchen GM, Rozowsky JS, Wei CL, Lee WH, Zhang ZD, Hartman S, Emanuelsson O, Stolc V, Weissman S, Gerstein MB, Ruan Y and Snyder M (2007). "Mapping of transcription factor binding regions in mammalian cells by ChIP: comparison of array- and sequencing-based technologies." *Genome Res* 17(6): 898–909. [PubMed: 17568005]
- Fujii T, Ueda T, Nagata S and Fukunaga R (2010). "Essential role of p400/mDomino chromatin-remodeling ATPase in bone marrow hematopoiesis and cell-cycle progression." *J Biol Chem* 285(39): 30214–30223. [PubMed: 20610385]
- Gaboli M, Kotsi PA, Gurrieri C, Cattoretti G, Ronchetti S, Cordon-Cardo C, Broxmeyer HE, Hromas R and Pandolfi PP (2001). "Mzf1 controls cell proliferation and tumorigenesis." *Genes Dev* 15(13): 1625–1630. [PubMed: 11445537]
- Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C and Schultz N (2013). "Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal." *Sci Signal* 6(269): 2004088.
- Gray PJ Jr., Prince T, Cheng J, Stevenson MA and Calderwood SK (2008). "Targeting the oncogene and kinome chaperone CDC37." *Nat Rev Cancer* 8(7): 491–495. [PubMed: 18511936]
- Heun P (2007). "SUMO organization of the nucleus." *Curr Opin Cell Biol* 19(3): 350–355. [PubMed: 17467254]

- Hromas R, Boswell S, Shen RN, Burgess G, Davidson A, Cornetta K, Sutton J, Robertson K (1996). "Forced over-expression of the myeloid zinc finger gene MZF-1 inhibits apoptosis and promotes oncogenesis in interleukin-3-independent FDCEP.1 cells." *Leukemia* 10: 1049–1050. [PubMed: 8667641]
- Hromas R, Davis B, Rauscher FJ 3rd, Klemsz M, Tenen D, Hoffman S, Xu D and Morris JF (1996). "Hematopoietic transcriptional regulation by the myeloid zinc finger gene, MZF-1." *Curr Top Microbiol Immunol* 211: 159–164. [PubMed: 8585946]
- Hromas R, Morris J, Cornetta K, Berebitsky D, Davidson A, Sha M, Sledge G and Rauscher F 3rd (1995). "Aberrant expression of the myeloid zinc finger gene, MZF-1, is oncogenic." *Cancer Res* 55(16): 3610–3614. [PubMed: 7627970]
- Hsieh YH, Wu TT, Huang CY, Hsieh YS and Liu JY (2007). "Suppression of tumorigenicity of human hepatocellular carcinoma cells by antisense oligonucleotide MZF-1." *Chin J Physiol* 50(1): 9–15. [PubMed: 17593797]
- Huang L, Grammatikakis N and Toole BP (1998). "Organization of the chick CDC37 gene." *J Biol Chem* 273(6): 3598–3603. [PubMed: 9452487]
- Hudson ME and Snyder M (2006). "High-throughput methods of regulatory element discovery." *Biotechniques* 41(6): 673, 675, 677 passim. [PubMed: 17191608]
- Inoue M, Takahashi K, Niide O, Shibata M, Fukuzawa M and Ra C (2005). "LDOC1, a novel MZF-1-interacting protein, induces apoptosis." *FEBS Lett* 579(3): 604–608. [PubMed: 15670815]
- Iyengar S and Farnham PJ (2011). "KAP1 protein: an enigmatic master regulator of the genome." *J Biol Chem* 286(30): 26267–26276. [PubMed: 21652716]
- Jensen K, Shiels C and Freemont PS (2001). "PML protein isoforms and the RBCC/TRIM motif." *Oncogene* 20(49): 7223–7233. [PubMed: 11704850]
- Massague J (2008). "A very private TGF-beta receptor embrace." *Mol Cell* 29(2): 149–150. [PubMed: 18243107]
- Mudduluru G, Vajkoczy P and Allgayer H (2010). "Myeloid zinc finger 1 induces migration, invasion, and in vivo metastasis through Axl gene expression in solid cancer." *Mol Cancer Res* 8(2): 159–169. [PubMed: 20145042]
- Murai K, Murakami H and Nagata S (1997). "A novel form of the myeloid-specific zinc finger protein (MZF-2)." *Genes Cells* 2(9): 581–591. [PubMed: 9413999]
- Noll L, Peterson FC, Hayes PL, Volkman BF and Sander T (2008). "Heterodimer formation of the myeloid zinc finger 1 SCAN domain and association with promyelocytic leukemia nuclear bodies." *Leuk Res* 32(10): 1582–1592. [PubMed: 18472161]
- Ogawa H, Murayama A, Nagata S and Fukunaga R (2003). "Regulation of myeloid zinc finger protein 2A transactivation activity through phosphorylation by mitogen-activated protein kinases." *J Biol Chem* 278(5): 2921–2927. [PubMed: 12427756]
- Ogawa H, Ueda T, Aoyama T, Aronheim A, Nagata S and Fukunaga R (2003). "A SWI2/SNF2-type ATPase/helicase protein, mDomino, interacts with myeloid zinc finger protein 2A (MZF-2A) to regulate its transcriptional activity." *Genes Cells* 8(4): 325–339. [PubMed: 12653961]
- Perrotti D, Melotti P, Skorski T, Casella I, Peschle C and Calabretta B (1995). "Overexpression of the zinc finger protein MZF1 inhibits hematopoietic development from embryonic stem cells: correlation with negative regulation of CD34 and c-myc promoter activity." *Mol Cell Biol* 15(11): 6075–6087. [PubMed: 7565760]
- Peterson MJ and Morris JF (2000). "Human myeloid zinc finger gene MZF produces multiple transcripts and encodes a SCAN box protein." *Gene* 254(1–2): 105–118. [PubMed: 10974541]
- Rafn B, Nielsen CF, Andersen SH, Szyniarowski P, Corcelle-Termeau E, Valo E, Fehrenbacher N, Olsen CJ, Daugaard M, Egebjerg C, Bottzauw T, Kohonen P, Nylandsted J, Hautaniemi S, Moreira J, Jaattela M and Kallunki T (2012). "ErbB2-driven breast cancer cell invasion depends on a complex signaling network activating myeloid zinc finger-1-dependent cathepsin B expression." *Mol Cell* 45(6): 764–776. [PubMed: 22464443]
- Raman N, Nayak A and Muller S (2013). "The SUMO system: a master organizer of nuclear protein assemblies." *Chromosoma* 122(6): 475–485. [PubMed: 23917899]
- Redon C, Pilch D, Rogakou E, Sedelnikova O, Newrock K and Bonner W (2002). "Histone H2A variants H2AX and H2AZ." *Curr Opin Genet Dev* 12(2): 162–169. [PubMed: 11893489]

- Reymann S and Borlak J (2008). "Transcription profiling of lung adenocarcinomas of c-myc-transgenic mice: identification of the c-myc regulatory gene network." *BMC Syst Biol* 2: 46. [PubMed: 18498649]
- Sander TL, Haas AL, Peterson MJ and Morris JF (2000). "Identification of a novel SCAN box-related protein that interacts with MZF1B. The leucine-rich SCAN box mediates hetero- and homoprotein associations." *J Biol Chem* 275(17): 12857–12867. [PubMed: 10777584]
- Sander TL, Stringer KF, Maki JL, Szauter P, Stone JR and Collins T (2003). "The SCAN domain defines a large family of zinc finger transcription factors." *Gene* 310: 29–38. [PubMed: 12801630]
- Sironen RK, Karjalainen HM, Torronen K, Elo MA, Kaarniranta K, Takigawa M, Helminen HJ and Lammi MJ (2002). "High pressure effects on cellular expression profile and mRNA stability. A cDNA array analysis." *Biorheology* 39(1–2): 111–117. [PubMed: 12082273]
- Stepanova L, Finegold M, DeMayo F, Schmidt EV and Harper JW (2000). "The oncoprotein kinase chaperone CDC37 functions as an oncogene in mice and collaborates with both c-myc and cyclin D1 in transformation of multiple tissues." *Mol Cell Biol* 20(12): 4462–4473. [PubMed: 10825210]
- Stielow B, Kruger I, Diezko R, Finkernagel F, Gillemans N, Kong-a-San J, Philipsen S and Suske G (2010). "Epigenetic silencing of spermatocyte-specific and neuronal genes by SUMO modification of the transcription factor Sp3." *PLoS Genet* 6(11): e1001203. [PubMed: 21085687]
- Takahashi K, Matsumoto C and Ra C (2005). "FHL3 negatively regulates human high-affinity IgE receptor beta-chain gene expression by acting as a transcriptional co-repressor of MZF-1." *Biochem J* 386(Pt 1): 191–200. [PubMed: 15453830]
- Tanaka N and Saitoh H (2010). "A real-time SUMO-binding assay for the analysis of the SUMO-SIM protein interaction network." *Biosci Biotechnol Biochem* 74(6): 1302–1305. [PubMed: 20530889]
- Tsai LH, Wu JY, Cheng YW, Chen CY, Sheu GT, Wu TC and Lee H (2014). "The MZF1/c-MYC axis mediates lung adenocarcinoma progression caused by wild-type Ikb1 loss." *Oncogene*.
- Tsai SJ, Hwang JM, Hsieh SC, Ying TH and Hsieh YH (2012). "Overexpression of myeloid zinc finger 1 suppresses matrix metalloproteinase-2 expression and reduces invasiveness of SiHa human cervical cancer cells." *Biochem Biophys Res Commun* 425(2): 462–467. [PubMed: 22846578]
- Uhlen M, Bjorling E, Agaton C, Szgyarto CA, Amini B, Andersen E, Andersson AC, Angelidou P, Asplund A, Asplund C, Berglund L, Bergstrom K, Brumer H, Cerjan D, Ekstrom M, Elobeid A, Eriksson C, Fagerberg L, Falk R, Fall J, Forsberg M, Bjorklund MG, Gumbel K, Halimi A, Hallin I, Hamsten C, Hansson M, Hedhammar M, Hercules G, Kampf C, Larsson K, Lindskog M, Lodewyckx W, Lund J, Lundberg J, Magnusson K, Malm E, Nilsson P, Odling J, Oksvold P, Olsson I, Oster E, Ottosson J, Paavilainen L, Persson A, Rimini R, Rockberg J, Runeson M, Sivertsson A, Skollermo A, Steen J, Stenvall M, Sterky F, Stromberg S, Sundberg M, Tegel H, Tourle S, Wahlund E, Walden A, Wan J, Wernerus H, Westberg J, Wester K, Wrethagen U, Xu LL, Hober S and Ponten F (2005). "A human protein atlas for normal and cancer tissues based on antibody proteomics." *Mol Cell Proteomics* 4(12): 1920–1932. [PubMed: 16127175]
- Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szgyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J and Ponten F (2015). "Proteomics. Tissue-based map of the human proteome." *Science* 347(6220): 1260419. [PubMed: 25613900]
- Van Damme E, Laukens K, Dang TH and Van Ostade X (2010). "A manually curated network of the PML nuclear body interactome reveals an important role for PML-NBs in SUMOylation dynamics." *Int J Biol Sci* 6(1): 51–67. [PubMed: 20087442]
- Weber CE, Kothari AN, Wai PY, Li NY, Driver J, Zapf MA, Franzen CA, Gupta GN, Osipo C, Zlobin A, Syn WK, Zhang J, Kuo PC and Mi Z (2014). "Osteopontin mediates an MZF1-TGF-beta1-dependent transformation of mesenchymal stem cells into cancer-associated fibroblasts in breast cancer." *Oncogene*.
- Williams AJ, Blacklow SC and Collins T (1999). "The zinc finger-associated SCAN box is a conserved oligomerization domain." *Mol Cell Biol* 19(12): 8526–8535. [PubMed: 10567577]

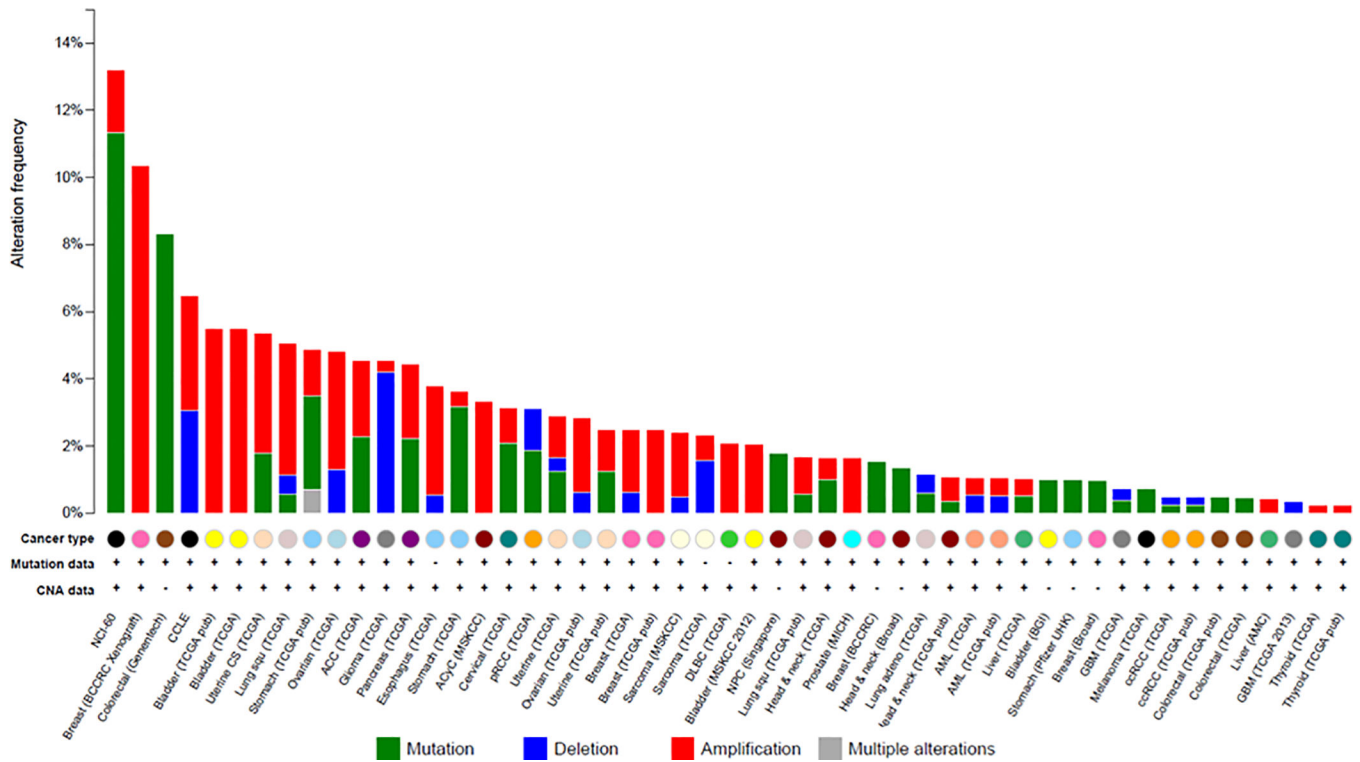
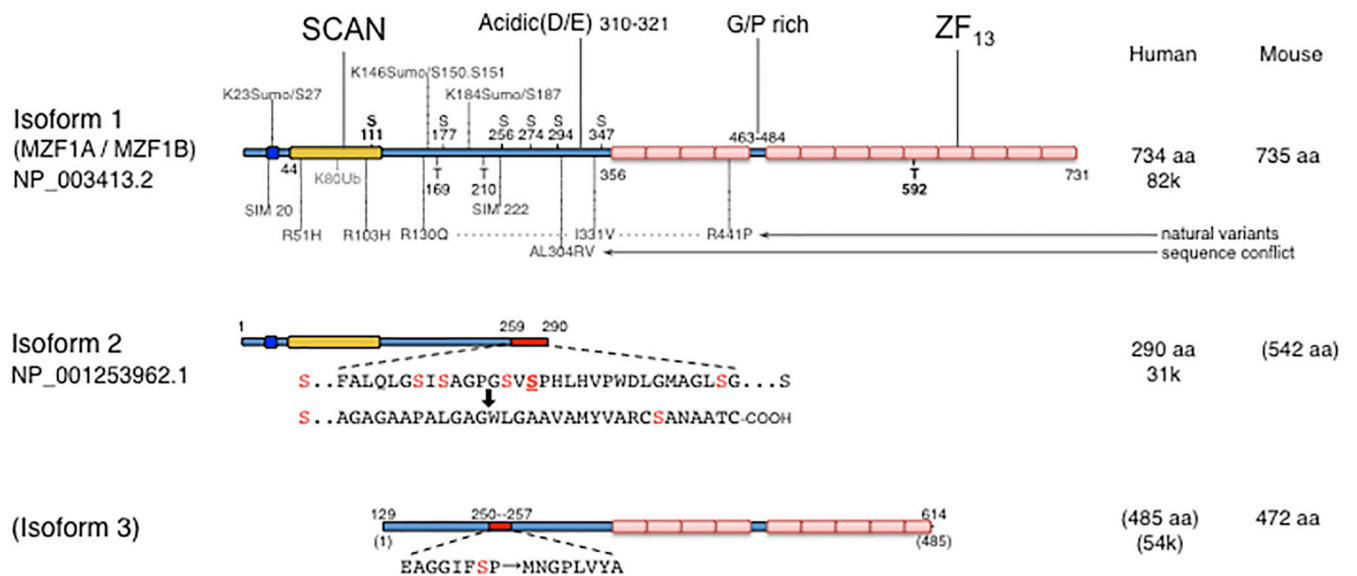


Figure 1. Frequency of MZF1 alterations across tumor types profiled by The Cancer Genome Atlas (TCGA).

Samples of each tumor type or cell line collection, listed along the x-axis, were analyzed for MZF1 gene copy number alterations (CNA) and open reading frame mutations. The percentage of alterations for each sample set is listed along the y-axis. The NCI-60 cancer cell line collection shows the largest percentage of alterations at 13.2% (7 out of 53 cases) for MZF1. This is followed by Breast Cancer Xenografts at 10.3% (3 out of 29 cases), Colorectal Adenocarcinoma at 8.3% (6 mutations out of 72 cases), Cancer Cell Line Encyclopedia at 6.5% (30 amplifications, 27 deletions out of 881 cases) and Bladder Cancer at 5.5% (7 out of 127 cases). All other tumor types are listed in descending order of percentage of MZF1 alteration. Interestingly MZF1 was deleted in 4.2% (12 out of 286 cases) of Brain Low Grade Gliomas analyzed. All samples were processed as a part of the U.S. national TCGA initiative to quantitatively characterize over 80 forms of cancer at the molecular level. Information was gathered and graphed by the cBioPortal site (Cerami, Gao et al. 2012, Gao, Aksoy et al. 2013).



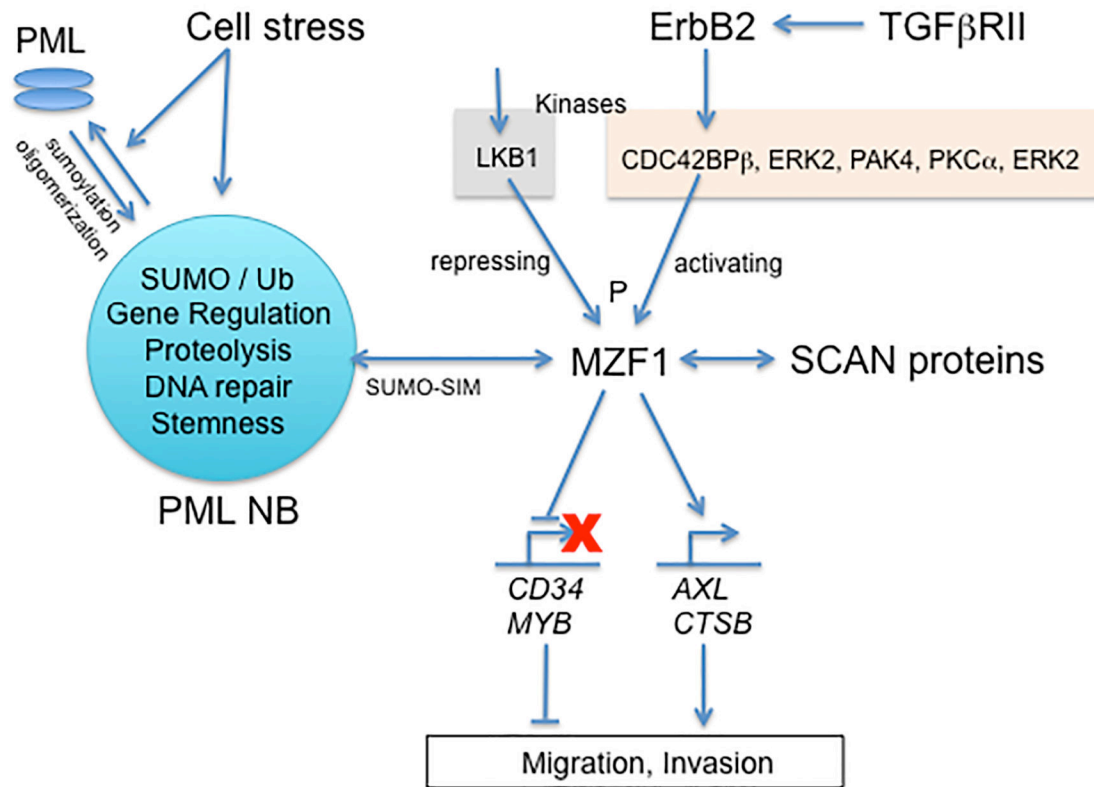


Figure 3. A model for regulation and roles of MZF1 in cancer.

LKB1 phosphorylates MZF1 for transcriptional repression of CD34 and MYB genes, which are inhibiting cancer cell migration and invasion (Perrotti, Melotti et al. 1995). In contrast, a battery of kinases was recently reported to be involved in MZF1 transcriptional activation of *AXL* and *CTSB*, both of which promote migration and invasion of cancer cells (Peterson and Morris 2000, Rafn, Nielsen et al. 2012). MZF1 can form homo- or hetero- dimers or oligomers with other SCAN-ZF or SCAN box proteins (Peterson and Morris 2000). MZF1 undergoes post-translational modifications (PTMs) including phosphorylation, sumoylation and presumably acetylation and ubiquitinylation (Noll, Peterson et al. 2008).

SUMO1 modification of MZF1 enables interaction with SUMO-interaction motif (SIM)-containing proteins such as PML (Noll, Peterson et al. 2008). Enzymes, transcription factors and other molecules stored in PML-NBs could potentially further give rise to further PTMs in MZF1 and modulate its function in gene regulation.

A (sumoylation motifs)

ψKxE	Consensus motif
ExKψ	Inverted motif (SUMO-2/3)
ψψψψKxE	Hydrophobic cluster motif (SUMO-2/3)
ψKxExxSP	PDSM
ψKxExxAcAcAc	NDSM

B (SIMs)

ψψxψAcAcAcAcAc	SIMa
ψψDLT	SIMb
AcAcAcAcAcψxψψ	SIMr (reversed)
ψ = V, I or L (hydrophobic aa)	
Ac = D, E or pS (negatively charged aa)	

C (sumoylation site prediction in MZF1)

species	position	alignment	score(P)	score(G)
h	K23	EGPVM V KLE D SEEE	0.93	58.988
m	K22	NEPAL V KLE D S DSD	0.93	
h	K184	PLGLQ V KEE S EVTE	0.93	44.651
m	K185	PLNLP M KEE T ELLG	0.8	
h	K146	QEVLS E KME P SSFQ	0.5	19.314
m	K147	KEVLS E KME P SSFQ	0.5	

D (SIM prediction in MZF1)

Position	Peptide	Score
20–24	PP E DEGP V M V K L E D S EEEG	58.988
222–226	E A Q R C G T V L D Q I F P H S K T G	57.35

Figure 4. Prediction of sumoylation and SUMO-interaction motifs (SIM) in MZF1.

(For references see (Bettermann, Benesch et al. 2012, Raman, Nayak et al. 2013, Cappadocia, Mascle et al. 2015)) (A) Sumoylation motifs in MZF1: PDSM, phospho-directed SUMO modification. NDSM, negatively charged amino acid dependent SUMO modification. SUMO-2/3 targets inverted motifs and hydrophobic cluster motifs. (B) different types of SIMs. Serine can be negatively charged upon phosphorylation. Ac, acidic amino acids. (C) Sumoylation site prediction in MZF1. Sites were predicted in *SUMOplot* (P) and *GPS-SUMO* (G) shown in scores. K and E are boxed. human MZF1 isoform 1 and mouse MZF1 isoform 1 (NP_665818.2) were input and aligned. (D) SIM prediction in MZF1. SIMs are predicted in *GPS-SUMO* setting a low threshold as cut off score = 55.31. Note that MZF1 aa 13–30 PPEDEGPVMVKLEDSEEE contains both a potential sumoylation site and a predicted SIM.

Table 1.

List of reports of MZF1 in various types of cancers, tissues and cell lineages.

Model/Type, Cell lineage	Roles, Targeting and Signals for MZF1	reference
Hematopoietic development or leukemia (Myeloid/hematopoietic lineage)	repression of CD34 and c-myb	Perrotti et al
Athymic mice, NIH3T3	Neoplastic Transformation	Hromas 1996
Tumor formation in congenic mice, Immortalized myeloid cells	Anti-apoptotic (MZF1 protected cells from IL-3 withdrawal-mediated apoptosis.)	ibid
Hepatocellular carcinoma	Growth (cell growth was inhibited by MZF1 antisense oligo.)	Hsieh
Colorectal cancer model. Compared with normal tissue	MZF1 mediated migration, invasion and in vitro metastasis. MZF1 targeted Axl, a receptor tyrosine kinase.	Mudduluru
Breast carcinoma	MZF1 bind an enhancer element in the cathepsin B gene, a key molecule in tumor invasion. TGF β -receptor-activated RTK Erb-B2 activates MZF1 through a pathway involving the kinases Cdc42-binding protein kinase, ERK2, p21 activated kinase 4 and PKC α .	Rafn et al
Lung carcinoma	MZF1 was activated by loss of Liver Kinase B1 (LKB1) and this led to elevated levels of MYC, increased migration and invasion. Loss of LKB1 was evidently associated with inactivation of tumor suppressing p53 and enhanced expression of the lung protooncogene protein NK2 homeobox 1 (NKX2-1).	Tsai
Human papillovirus driven caner	MZF1 was activated downstream of NKX2.	Chen
	MZF1 led to upregulation of FOXM1, promoting migration, invasion and stemness	