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Review

Transcription factor ZBP-89 in homeostasis and disease

Sinju Sundaresan[1], Muyiwa Awoniyi[1,2] and Juanita L. Merchant[1,3]

[1]Division of Gastroenterology, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan; [2]University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; [3]Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan, USA.

ABSTRACT

Zinc-finger Binding Protein-89 (ZBP-89) is a Krüppel-type zinc-finger transcription factor encoded by the human *ZNF148* locus on 3q21 or *Zfp148* locus on mouse 16B3. It is a ubiquitous protein that typically binds to GC-rich DNA elements, also known to bind Sp1 family members. ZBP-89 functions as both a transcriptional activator and a transcriptional repressor depending on the cell context and gene targets. For example, its binding to the promoters of cyclin-dependent genes such as $p21^{waf1}$ activates gene expression, while binding to $p16^{INKa}$ suppresses gene expression. In addition, ZBP-89 can exert its effect through protein-protein interactions with p53 or ataxia telangiectasia mutated (ATM). The short-chain fatty acid butyrate induces ZBP-89 expression, suggesting the possibility of direct regulation by normal colonic flora. ZBP-89 induces apoptosis through its ability to repress anti-apoptotic genes *Bcl-xL* and *Mcl-1* while inducing the pro-apoptotic gene *Bak*. A consequence of its pleomorphic effects is its over-expression in a variety of cancers including those in the liver, stomach, breast, skin, pancreas, and lungs. In addition to its role in cell growth and transformation, ZBP-89 regulates a number of genes involved in inflammation and cooperates with NFκB. ZBP-89 regulates CD11b and *in vivo* studies demonstrate its essential role in myeloid cell activation. Moreover, ZBP-89 regulates myeloid cell progenitors. In the colon, conditional deletion of the *Zfp148* locus in mice has been shown to be essential in maintaining homeostatic levels of tryptophan hydroxylase 1 (*Tph1*) and anti-microbial peptides required for mucosal defense during infection by *Salmonella*. In summary, ZBP-89 plays a number of essential roles in various tissues during embryogenesis, homeostasis, inflammation and cancer.

KEYWORDS: ZBP-89, Krüppel-type transcription factor, apoptosis, cancer, immunity

1. Introduction

The Zinc-finger Binding Protein-89 (ZBP-89) is a ubiquitous transcription factor that can both activate and repress genes involved in both cell growth arrest and cell death [1-3]. The ZBP transcription factor family consists of four zinc-fingers in its N-terminal domain, which is in contrast to the Sp1 family, whose zinc-finger domain resides at the extreme C-terminus of the protein [4, 5]. However, ZBP-89 binds to the same GC-rich consensus DNA element as Sp1 and Sp3. Therefore, the sequence context and cellular background likely play significant roles in the overall regulation exerted by these zinc finger proteins. In addition to direct binding to the $p21^{Waf1}$ promoter [6, 7], ZBP-89 forms protein complexes with known tumor suppressor factors, such as p53 [1], p300 and ataxia telangiectasia mutated (ATM) [2, 8]. Based on these reports, there are increased efforts directed towards uncovering the role of ZBP-89 in homeostasis, mucosal repair and tumorigenesis. This review will summarize the recent developments of how

ZBP-89 contributes to the mechanisms underlying these varied cellular processes.

1.1. Structure of ZBP-89

ZBP-89 (also known as Zfp148, ZNF148, BFCOL1 and BERF-1) belongs to the Krüppel-type zinc-finger family of transcription factors that was originally cloned by screening an expression library using a GC-rich epidermal growth factor (EGF) response element (gERE) from the human gastrin gene (GAST) [4]. The chromosomal location designated *ZNF148* in humans and *Zfp148* in mice maps to human chromosome 3q21.2 and mouse chromosome 16, respectively. The full-length ZBP-89 protein consists of 794 residues (Figure 1) organized into 9 exons that form 5 distinct functional domains—an N-terminal acidic, a DNA binding, three basic and a C-terminal PEST (proline, glutamic acid, serine and threonine)-containing domains [9]. The four Krüppel-type zinc-fingers (Cys2-His2-type) reside within the N-terminus of the protein, which is in contrast to the Sp1 transcription factor family, whose zinc-finger DNA binding domain resides at the extreme C-terminus of the protein [10]. The two transcription factors typically bind to the same GC-rich element [4, 11] such that the configuration of their DNA binding domains could occupy the same DNA element. In addition to the zinc-finger domain, the N-terminal domain is quite acidic consisting of several glutamic-acid residues from amino acids 54-99. The three basic domains at amino acid residues 129-153, 313-335, and 470-485 flank the zinc-fingers while the serine-rich and PEST domains (at amino acids 569-596) are contained entirely within exon 9. This C-terminal domain contains a repressor since its deletion generates a functional protein from amino acids 1 to 450 that mediates transcriptional induction compared to the full length-protein [12, 13].

Another member of the ZBP family was cloned and designated ZBP-99 (also known as Zfp281,

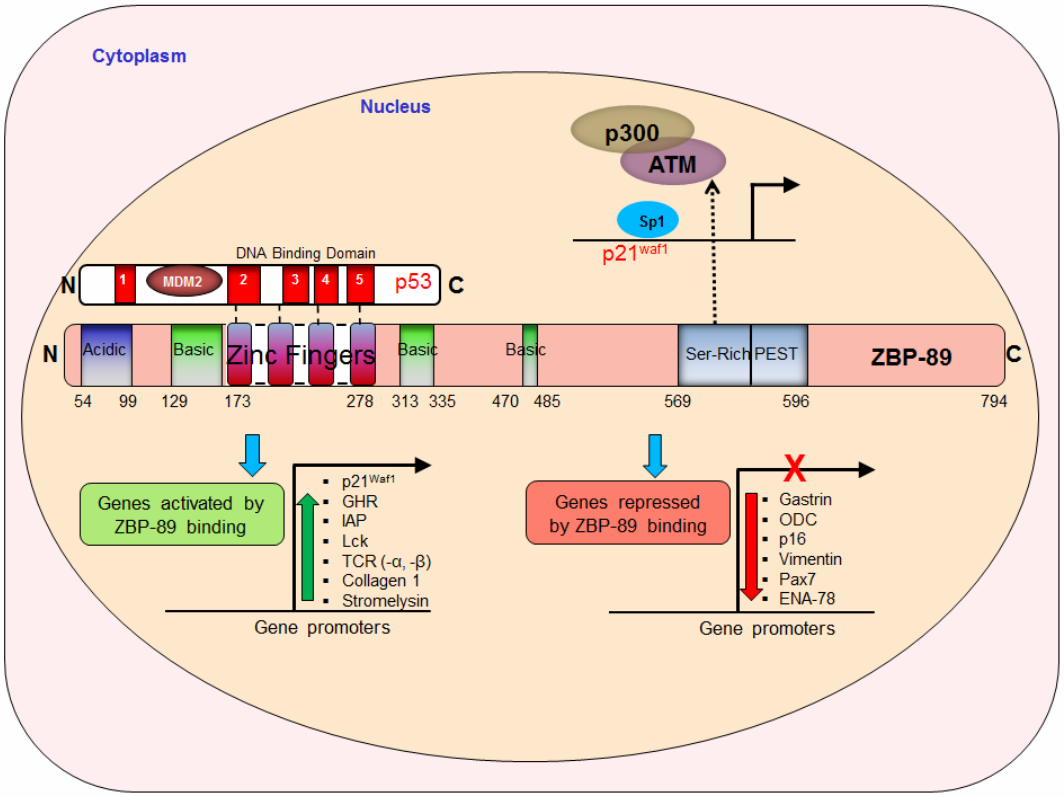

Figure 1. Schematic representation of structure and interactions of ZBP-89 with gene promoters, cell cycle regulators and mediators of apoptosis.

ZNF281) on the basis of homology to the zinc-finger domain, but has a molecular weight of 99 kDa [14] and resides on chromosome 1q32.1 [14, 15]. Murine ZBP-99 (Zfp281) is a key component of embryonic stem cells and is required for their pluripotency through direct transcriptional regulation of *Nanog* and physical interaction with Oct4, Sox2 and Nanog [16]. Although *Zfp281* is dispensable in the establishment and maintenance of embryonic stem cells, it is required for their ability to differentiate *in vitro* by functioning as a repressor of Nanog as well as a lineage specific gene *Gata6* [17]. Specifically, *Zfp148* recruits the NuRD repressor complex to the *Nanog* locus [18]. More recently, Hermeking and coworkers demonstrated that ZNF281 induces the intestinal stem cell markers LGR5 and CD133 [19]. In addition, the transcription factor was implicated in stem cell maintenance and epithelial to mesenchymal transition where it forms a complex with the transcription factor SNAIL [19, 20]. Sox4, another regulator of the stem cell niche, through its interaction with β-catenin/TCF [21] directly induces ZNF281 [22] and like ZBP-89, ZNF281 protein is phosphorylated by ATM and ataxia telangiectasia and Rad3-related (ATR) [23]. These studies raise the possibility that ZBP-89 (ZNF148) might also regulate stem cell function since its DNA binding domains are ~90% conserved. Nevertheless, their distinct N-terminal domains suggest different protein interacting partners. Lastly, *in silico* analysis has identified a third member of this zinc-finger family called *ZNF740* located on 12q13.13. However, roles and functions of ZNF740 have not been determined yet.

1.2. ZBP-89 in immune cells

Although ZBP-89 is a ubiquitously expressed protein, T-cells exhibit some of the highest levels of this transcription factor [24, 25], suggesting its strong role in cellular immunity. The initial report of the *ZNF148* locus in T-cells described a version of the ZBP-89 protein that was ultimately found to be truncated, since it consisted of only the first 459 amino acids instead of the full-length 794 residues. Since it was cloned from a T cell library and was found to regulate expression of the T cell α- and β-receptors, the novel clone was named hTβ [26]. In retrospect, hTβ is likely an alternative splice product of the full-length *ZNF148* locus that lacks exon 9. Since exon 9 contains the repressor domain and a PEST sequence, we presume that this truncated form functions as a transcriptional activator. pTα is typically expressed in pre-T cells in the thymus and extra-thymic T cell maturation sites but not in mature thymocytes, peripheral T cells, or other cell types, thereby serving as a marker for the T-cell progenitor. Reizes and Leder showed that *ZNF148* mRNA is expressed in the thymus at significantly higher levels than in any other organ tested, including the spleen, suggestive of a possible role in promoting T-lymphopoiesis [24].

Two other immune-related ZBP-89 gene targets include *CD11b* and the lymphocyte-specific protein-tyrosine kinase (lck) [25]. The integrin CD11b is a differentiation marker for the myelomonocytic lineage and is an important mediator of inflammation. The integrin plays a strong role in adhesion-dependent functions, which include migration, phagocytosis, degranulation, antibody-dependent cytotoxicity and release of proteolytic enzymes in response to inflammation. Using yeast one-hybrid screens, Park *et al*. showed that ZBP-89 binds to the *CD11b* promoter, acts as a transcriptional repressor and plays a role in the late stages of monocyte differentiation [27].

The lymphocyte-specific protein-tyrosine kinase (*lck*), $p56^{lck}$, is a member of the *src* kinase family, which plays a crucial role in signaling mediated through the T cell receptor (TCR) and pre-TCR complexes [28]. Transcription of the *lck* gene is regulated by two independent promoter elements: the proximal and distal promoters. The *lck* proximal promoter is positioned immediately adjacent to the first coding exon, and is active in the thymus, but is essentially silent in peripheral T cells, demonstrating that it is active only at an early developmental stage of T-lymphopoiesis [29]. The distal promoter is located far 5′-upstream of the proximal promoter and is active during all developmental stages of T-cell development [30, 31]. The transcriptional regulators of the proximal promoter play a critical role in the developmental program of hematopoietic cells within the T-lymphocyte lineage. ZBP-89 (also known as murine mtβ) is required for trans-activating the

proximal *lck* promoter [25]. This was supported by mutagenesis experiments demonstrating that ZBP-89 contributes to full activation of the *lck* proximal promoter. Mutating the ZBP-89 binding element or reducing ZBP-89 protein level significantly impaired the *lck* promoter activity [25].

Other gene targets include genes related to mesenchymal cell types such as type 1 collagen [12], stromelysin [32], and vimentin [33, 34]. Other tissue-specific genes regulated by ZBP-89 include intestinal alkaline phosphatase [13], and growth hormone receptor (GHR) [35]. Genes involved in cell growth include gastrin [4, 9, 11], ornithine decarboxylase (ODC) [14, 36], and the cell cycle-dependent kinase inhibitors p16 [37] and $p21^{Waf1}$ [6-8]. The Hox-related genes *SOX18* [38] and *Pax7* [39] have also been reported to be ZBP-89 gene targets. ZBP-89 has also been shown to promote differentiation in bone marrow [40, 41] and skeletal muscle [39, 42] as well as the germ cells [43]. As such, ZBP-89-mediated differentiation plays an important role in myogenesis, hematopoiesis, erythrogenesis and megakaryogenesis.

1.3. Interactions of ZBP-89 with genes

Like other Krüppel-type zinc-finger-containing transcription factors, ZBP-89 modulates genes involved in cell proliferation and differentiation. Several studies demonstrate that ZBP-89 arrests cell proliferation through its interactions with p53 [1, 44] and $p21^{waf1}$ proteins [6], (Figure 1). The tumor suppressor gene *p53* can function as an activator or inhibitor of multiple genes. ZBP-89 interacts with *p53* and prevents its ability to translocate from the nucleus to the cytoplasm where it is degraded by the proteasome [1]. The nuclear retention of p53 by ZBP-89 requires the amino terminal zinc-finger DNA binding domain and the DNA-binding and carboxy-terminal domains of p53 [1]. Over-expression of ZBP-89 results in p53 protein accumulation and growth arrest in human gastric adenocarcinoma (AGS) cells, effects that are abolished in p53-null cells [44-46], suggesting that the growth arrest function mediated by ZBP-89 is p53-dependent. A ZBP-89 splice variant called $ZBP\text{-}89^{\Delta N}$, lacking the amino terminal amino acids from 1-127 of the full-length protein ($ZBP\text{-}89^{FL}$), induces growth delay, reduced survival and increased susceptibility to dextran sodium sulfate (DSS) [47], suggesting that loss of the acidic trans-activating domain alone is sufficient to reduce normal mucosal repair.

p53 induces the expression of $p21^{WAF1}$, an inhibitor of the G1 to S-phase cyclin-dependent kinases (CDKs) 2, 3, 4 and 6 [48]. $p21^{WAF1}$ binds to cyclin/CDK complexes and controls cell cycle progression through both p53-dependent and independent mechanisms [49]. Moreover ZBP-89 directly and indirectly regulates $p21^{WAF1}$ gene expression through GC-rich elements and protein-protein interactions, respectively [50, 51]. Regulation of the $p21^{WAF1}$ promoter is mediated through proximal GC-rich elements that bind Sp1 and other zinc-finger factors like ZBP-89 [51]. Specifically, ZBP-89 binds to the proximal $p21^{WAF1}$ DNA elements at -245 to -215 upstream from the cap site where it recruits the co-activator p300 [52]. By contrast, ZBP-89 indirectly activates $p21^{WAF1}$ by blocking p53 translocation to the cytoplasm [1], where it normally would be degraded, effectively prolonging its half-life. Taken together, ZBP-89 induces $p21^{Waf1}$ gene expression by two mechanisms: direct ZBP-89 binding to the $p21^{Waf1}$ promoter in response to extracellular signals, e.g., Histone deacetylase (HDAC) inhibition by butyrate and trichostatinA (TSA), or by stabilizing p53 and its binding to the $p21^{WAF1}$ promoter.

Histone deacetylase inhibitors (HDACi) induce growth arrest, differentiation and apoptosis of colonic epithelial cells [53]. Butyrate, the naturally occurring HDAC inhibitor [54], is produced in the human colon by intestinal bacteria during the fermentation of dietary fiber. Butyrate maintains the healthy differentiated state of normal colonic epithelial cells and promotes differentiation of neoplastic cells [53]. Moreover, butyrate induces apoptosis in a number of cancer cell lines [55-57] including colon cancer lines by a mechanism involving $p21^{WAF1}$ activation [58]. ZBP-89 over-expression in HT-29 colon cell lines potentiates butyrate-mediated induction of endogenous $p21^{WAF1}$ [6]. HDACi treatment of colonic cells promotes the formation of an ATM/ZBP-89/p300 multi-molecular complex at the $p21^{WAF1}$ proximal promoter [50]. Reduction of ZBP-89 or ATM with small interfering RNAs blocked HDACi-induced $p21^{WAF1}$ expression. Moreover, ZBP-89 is

phosphorylated by ATM kinase at Ser202 and disruption of the ATM phosphorylation site abrogated the ability of ZBP-89 to potentiate butyrate-mediated induction of $p21^{Waf1}$ gene expression [8]. In addition, silencing of ZBP-89 expression blocked HDACi-induced phosphorylation of ATM at Ser1981 and p53 at Ser15 [50]. Thus, phosphorylated forms of both proteins seem to be required for $p21^{WAF1}$ expression. The activated form of ATM ($pATM^{Ser1981}$) was reduced in the colons of DSS-treated mice expressing the N-terminally truncated form of ZBP-89, demonstrating again that the amino-terminal domain is an essential protein-protein interacting region and apparently plays a crucial role in mucosal homeostasis [47, 50].

1.4. Role of ZBP-89 in apoptotic pathways

The process of apoptosis or programmed cell death is critical for the regulation of normal cell numbers and function by elimination of damaged and de-differentiated cells [59]. During neoplastic or malignant transformation, apoptosis becomes repressed, resulting in unopposed proliferation and the net accumulation of cells [60]. Double-strand DNA breakage from ionizing radiation causes ATM activation in p53-mediated apoptosis after phosphorylation at Ser15 if the DNA is not repaired [61]. If DNA is repaired during $p21^{Waf1}$-mediated cell cycle arrest, then the cell does not undergo apoptosis [61]. Like ionizing radiation, HDAC inhibitors (TSA and butyrate) activate ATM and induce $p53^{Ser15}$ [62]. However, unlike gamma irradiation, ATM-mediated $p53^{Ser15}$ phosphorylation induced by butyrate requires ZBP-89 and induces $p21^{Waf1}$ [50]. Since colonic butyrate levels contribute to colonocyte homeostasis, we speculate that the butyrate-dependent activation of ZBP-89 and ATM contributes to maintaining DNA fidelity in these rapidly cycling cells bathed in butyrate from bacterial fermentation. The functional consequences of the ZBP-89/p53 interaction appear to inhibit the p53-mediated apoptotic pathway since *Zfp148* deficiency sensitizes epithelial cells to the anti-proliferative effects of p53 [50]. If the ZBP-89/p53 interaction is viewed in the context of DNA damage, one might speculate that loss of *Zfp148* would exacerbate mucosal damage by favoring p53-mediated apoptosis in the gastrointestinal epithelium. Therefore it is likely that ZBP-89 plays a role in the DNA damage pathway. Double-stranded breaks (DSBs) in DNA recruit the DNA repair complex that includes ATM. Activated ATM phosphorylates protein targets including $p53^{Ser15}$. Moreover, reduced *Zfp148* levels in an *ex vivo* model prevents p53 phosphorylation at Ser15 [1].

Primordial germ cell proliferation is controlled by phosphorylation of $p53^{Ser15}$ that promotes its translocation to the nucleus. Previously, it was reported that $Zfp148^{+/-}$ embryonic stem cells from chimeric $Zfp148^{+/-}$ embryos showed impaired phosphorylation of p53 at Ser15 [43]. In addition, $Zfp148^{+/-}$ embryonic stem cells were resistant to cell death after serum starvation. Taken together, these studies suggest that reduced ZBP-89 protein levels can render some cells more susceptible to p53-mediated apoptosis, e.g., embryonic stem cells are more susceptible to unregulated growth if $p53^{Ser15}$ phosphorylation is blocked.

ZBP-89 can also induce apoptosis independently of p53. ZBP-89-mediated apoptosis was observed in human AGS and HCT-116 gastrointestinal cancer cells to function through Jun kinase activation [2]. Ectopic expression of ZBP-89 in this study was accompanied by activation of all three Mitogen Activated Protein kinase subfamilies, including JNK1/2, ERK1/2 and p38 MAP kinase, although only JNK kinase was required for ZBP-89-mediated apoptosis. Other studies reported that ectopic expression of ZBP-89 suppresses anti-apoptotic proteins Mcl-1 and Bcl-xL, although no effects on the pro-apoptotic Bcl-2 family was observed. Suppression of apoptosis by inducing anti-apoptotic proteins Mcl-1 and Bcl-xL is p53-independent [63-66]. In addition, pro-inflammatory cytokines induce molecular mechanisms that can trigger p53-independent apoptosis. For example, the pro-inflammatory cytokine interferon-gamma (IFNγ) induces phosphorylation of the Signal Transducers and Activators of Transcription (STAT) family of transcription factors, specifically STAT1. STAT1 triggers apoptosis by interacting with molecules involved in DNA repair and replication such as the tumor suppressor BRCA1 (Breast Cancer type 1 susceptibility protein) and MCM5 (Minichromosome maintenance complex component 5) [67, 68]. Effects of STAT1 are mediated by the C-terminal transactivation domain of STAT-1

induced *via* phosphorylation on either tyrosine-701 or serine-727. In addition, a G-rich element from +171 to +179 within the first intron of the STAT1 gene is critical for optimal STAT1 promoter activity. ZBP-89 binds directly to this STAT1 G-rich element along with Sp1 and Sp3 [69]. Reduction of ZBP-89 gene expression with siRNA attenuates both basal and IFNγ-induced STAT1 expression and subsequently diminishes expression of apoptotic markers, caspase-3 and poly ADP-ribose polymerase (PARP), which demonstrates that ZBP-89 is required for constitutive STAT1 expression [69].

Consistent with the interaction between ZBP-89 and p53, Lindahl and coworkers used a *Zfp148*-deficient mouse generated by gene trapping to demonstrate that mouse ZBP-89 contributes to macrophage proliferation [70]. The investigators found that a mouse haploinsufficient for *Zfp148* on an apolipoprotein E null background does not develop atherosclerosis. Using bone marrow transplantation of the *Zfp148* mutant cells, they discovered that mitigation of the atherosclerotic lesions was due to a hematopoietic cell. Moreover, loss of the *Zfp148* allele increased phosphorylation of p53 at Ser18 (analogous to Ser15 in human p53). Thus they concluded that loss of *Zfp148* increases p53 phosphorylation that subsequently reduces macrophage proliferation in atherosclerotic plaques. Correlating these results with prior studies performed in human epithelial cells, one might speculate that ZBP-89 modulates p53 activity either through its protein-protein interaction or through its ability to modulate ATM, the PI3 kinase that phosphorylates p53 at Ser15 (or Ser18 in the mouse) [1, 50]. The latter scenario would imply that ZBP-89 inhibits ATM activation.

2. ZBP-89 and cancer

Evidence that ZBP-89 modulates cell growth and cell death prompted us to consider its role in cancer. The following section reviews the role of ZBP-89 in both gastrointestinal and other extra-GI cancers, including breast, and lung adenocarcinomas.

2.1. ZBP-89 and gastric cancer

In contrast to its ability to inhibit gastrin gene expression, ZBP-89 was over-expressed in AGS, MKN-45 and Kato III gastric cancer cell lines [71]. Consistent with this observation, ZBP-89 expression was observed in gastric cancer by immunohistochemistry [71]. Given its elevated levels in pre-malignant states of gastric cancers it was then examined whether ZBP-89 played a role in the early stages of cancer development. Remington *et al*. reported that over-expression of ZBP-89 resulted in DNA inhibition and decreased proliferation of GH_4 and AGS cells through repression of the ornithine decarboxylase (ODC) promoter activity [72]. The ODC promoter contains multiple GC-rich DNA elements that bind Sp1 to activate transcription [36, 73, 74]. ODC gene expression closely correlates with cell proliferation. Thus, it is possible that over-expression of ZBP-89 successfully competes with Sp1 for binding to the ODC promoter eventually leading to its repression. By contrast, the BMRF1 gene of Epstein–Barr virus (EBV), which causes about 10% of gastric cancer [75], increases ZBP-89 binding to the gastrin promoter in AGS cells and subsequently promoter activation [76]. This was attributable to the fact that BMRF1 enhances the transcriptional activity of both ZBP-89 and Sp1. Induction of Sp1 through BMRF1 in AGS cells was at least two-fold higher than that of ZBP-89, resulting in net activation of the gastrin promoter. Nevertheless, the finding that viral genes increase ZBP-89 binding to the gastrin promoter where it inhibits transcription and downstream expression has potential therapeutic value especially in gastric cancer. However further studies directed towards understanding its role in EBV-initiated cancers are warranted.

2.2. ZBP-89 and colorectal cancer

Colorectal cancer (CRC) is one of the leading causes of cancer death worldwide [77]. Accumulating evidence suggests that ZBP-89 might be protective against colorectal cancer. In HCT-116 human colon adenocarcinoma cell line, ZBP-89 was found to potentiate p53-mediated cell death by chemotherapy [78]. Etoposide- and staurosporine-mediated cell arrest in the G1 phase of the cell cycle was significantly enhanced in the presence of ZBP-89 both in HCT116 wild-type (WT) and p53-null cells but not in HT29 cells, which contain the p53 mutant R273H. Similar results were obtained when five other p53 missense mutations (Y236C, G266R, V143A, R175A, and R249S) occurring in 21 sporadic colon cancer patients were tested. These results suggest that

p53 mutations (within the DNA binding domain) exert a dominant negative effect on ZBP-89; cells that accumulate WT p53 or are null for p53 are more susceptible to chemotherapy-mediated cell death and show elevated ZBP-89 levels [79, 80]. Several studies have indicated that ZBP-89 mRNA and protein levels are elevated in early stage colon cancer, but decrease as the cancer becomes more advanced. However the number of cancers examined has been small. Therefore, to better assess the tumor suppressive effects of ZBP-89 in mice, Law *et al*. developed transgenic mice overexpressing ZBP-89 in the intestine using *villin* promoter [81]. Increased activation of apoptotic indicator procaspase-3 and retinoblastoma protein cleavage were observed in $Zfp148^{TgVZ}$ mice, confirming prior observations that ZBP-89 over-expression induces apoptosis and cell cycle arrest *in vitro* [1, 6]. To evaluate the biological significance of ZBP-89 over-expression, $Zfp148^{TgVZ}$ mice were crossed to the tumor prone $Apc^{Min}/+$ mice, which develop multiple intestinal neoplasia due to a nonsense mutation in the murine homolog of adenomatous polyposis coli (*APC*) gene. A significant 50% reduction in the number of adenomas was observed in the small intestine of $Apc^{Min}/+$ mice when compared to $Apc^{Min}/+$ littermates expressing wild-type *Zfp148*. Although no significant difference was observed in the number and size of colon tumors, there was a trend toward a decrease in tumor size. This may be attributable to the fact that the *villin* promoter is less active in the colon than in the small intestine [82].

It is known that in addition to epigenetic mutations, inflammation *per se* as observed in patients with inflammatory bowel disease (IBD) increases the risk of developing colon cancer [83]. As discussed earlier in this review, butyrate produced upon fermentation of dietary fiber by colonic bacteria acts as an HDACi and promotes colonic epithelial homeostasis by a mechanism involving $p21^{Waf1}$ activation. Specifically, ZBP-89-dependent assembly of an ATM and p300 multimolecular complex at the $p21^{Waf1}$ promoter contributes to $p21^{Waf1}$ induction [6]. Mice homozygous for an isoform of ZBP-89 ($ZBP\text{-}89^{\Delta N/\Delta N}$), which expresses truncated ZBP-89 without the N-terminal p300-interaction domain (PID), showed no ATM-activated cells in areas where inflammation was present [50].

2.3. ZBP-89 and other cancers

Several lines of evidence demonstrate a role for ZBP-89 in other cancer types including breast cancer [84], melanoma [66], hepatocellular carcinoma (HCC) [44], pancreatic adenocarcinomas [85], and human lung cancer [37]. The Neuregulin-1 (*Nrg1*) gene encodes a group of multiple growth factors that play critical roles in breast development, and stimulation of the Nrg-1 pathway has been implicated in breast cancer [86, 87]. Over-expression of ZBP-89 using *pcDNA3-Flag-ZBP-89* expression vector in Neuro-2A cells led to a 30% decrease in Nrg-1 promoter control, an effect probably explained by competition with Sp1 for binding to GC-rich sites on the promoter [88]. In contrast, ZBP-89 was found to be elevated in 3 human breast cancer cell lines including MCF7, SKBR3, and ZR-75-1 [84]. Thus, while ZBP-89 expression has been observed in multiple cancer tissue types and cell lines, its overall effect on cancer growth has only been evaluated in the intestine. The general conclusion is that the wild-type protein promotes proliferation and tumor progression. The repressor effects on individual promoters might suggest repression of differentiation factors.

ZBP-89 protein can stabilize *p53* through direct interaction, leading to its retention in the nucleus [1]. In a subset of 33 HCC patients with recurrent intra and extrahepatic tumors, ZBP-89 co-localized with *p53* to the nucleus in about 63% (12 of 19) of cases, suggesting that ZBP-89 may play a role in the nuclear accumulation of *p53* protein in a subset of recurrent HCC [44]. The function of *p53* protein depends on whether it translocates to the nucleus. ZBP-89 is able to bind wild-type *p53* and p53 mutants with mutations in non-binding N-terminal domains that do not have nuclear localization signals (located within the carboxyl terminus). This is of clinical significance in certain patients with mutated p53. With accumulation of p53 protein in the nucleus, tumor cells retain their ability to undergo apoptosis and are thus more susceptible to radiotherapy and chemotherapy [89, 90]. Therefore, by co-localizing with p53 protein, the expression of ZBP-89 may

define a subgroup of recurrent HCCs that may benefit more from radiotherapy and chemotherapy.

3. ZBP-89 in tumor growth and metastasis

In addition to transcriptional regulation of genes in tumor development, ZBP-89 has been reported to regulate the expression of various molecules that are involved in tumor growth, invasion and metastasis. ZBP-89 inhibits the expression of ornithine decarboxylase (ODC) [14, 91] and vimentin [34]. ODC and vimentin expression correlates with tumor development, invasion and metastasis [92-95]. Inhibition of ODC in targeting non-melanoma skin cancer has been reported [96], while vimentin suppression has been reported to prevent prostate cancer migration and invasion [97]. Mechanisms responsible for the inhibition of vimentin by ZBP-89 include HDAC1 recruitment to the *vimentin* promoter [33], and competition with Sp1 that also binds vimentin to enhance transcription [34]. In contrast to restricting tumor progression, a positive role for ZBP-89 in metastasis has also been reported. ZBP-89 induces *matrix metallopeptidase 3* (*MMP-3*) promoter. MMP-3 is involved in tumor angiogenesis, invasion and metastasis, and activation by ZBP-89 suggests that it might play a role in tumor progression [98, 99].

4. Summary

Accumulating data demonstrates that ZBP-89 exerts protective effects against development and progression of a variety of cancers *via* its ability to affect multiple processes related to cell growth independent of the tumor suppressor gene *p53*. Cooperation between ZBP-89 and p53 appears to be especially important for the regulation of the cell cycle downstream of the DNA damage pathway and chromatin remodeling mediated by HDACs.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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