

## Gene Section

### Review

# ZBTB7A (zinc finger and BTB domain containing 7A)

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## Identity

**Other names:** DKFZp547O146; FBI-1; FBI1; LRF; MGC99631; ZBTB7; ZNF857A; pokemon

**HGNC (Hugo):** ZBTB7A

**Location:** 19p13.3

### Local order

- EEF2 (19pter-q12) eukaryotic translation elongation factor 2
- SNORD37 (19p13.3) small nucleolar RNA, C/D box 37
- PIAS4 (19p13.3) protein inhibitor of activated STAT, 4
- **ZBTB7A** (19p13.3) zinc finger and BTB domain containing 7A
- MAP2K2 (19q13.3) mitogen-activated protein kinase 2
- CREB3L3 (19p13.3) cAMP responsive element binding protein 3-like 3

## DNA/RNA

### Description

Size: 21601 bases.

Orientation: minus strand.

Cloning of the 5'-upstream region of ZBTB7A revealed: a) the absence of putative TATA and CCAAT sequences, b) the presence of two consensus sequences for the binding transcription factor Sp1, SpA (-641~-636-) and SpB (-972~-967), as well as consensus sequences for the AP-1, AP-2, PU.1, Hb, CBF-1, GATA-1 elements and p53-binding sites, c) the

presence of three regulatory elements, POS-D, NEG-U and NEG-D, with a key role in the regulation of the Pokemon gene.

The nucleotide sequence from -83 to -71 contains the positive element POS-D, which is necessary for the strong activity of the Pokemon promoter. The region from -558 to -541 contains the NEG-U element, involved in the negative regulation of the Pokemon promoter. The region from -128 to -108, namely NEG-D, is also necessary for the negative regulation of the Pokemon promoter. Neither NEG-U nor NEG-D element alone can negatively affect Pokemon expression. Pokemon promoter activity is greatly dependent on the interaction (cooperation) between NEG-U and NEG-D elements. This synergistic inhibition on Pokemon expression requires a specific length between the regulatory elements. Once this distance is reduced, the interaction between the NEG-U and NEG-D elements is almost abolished.

### Transcription

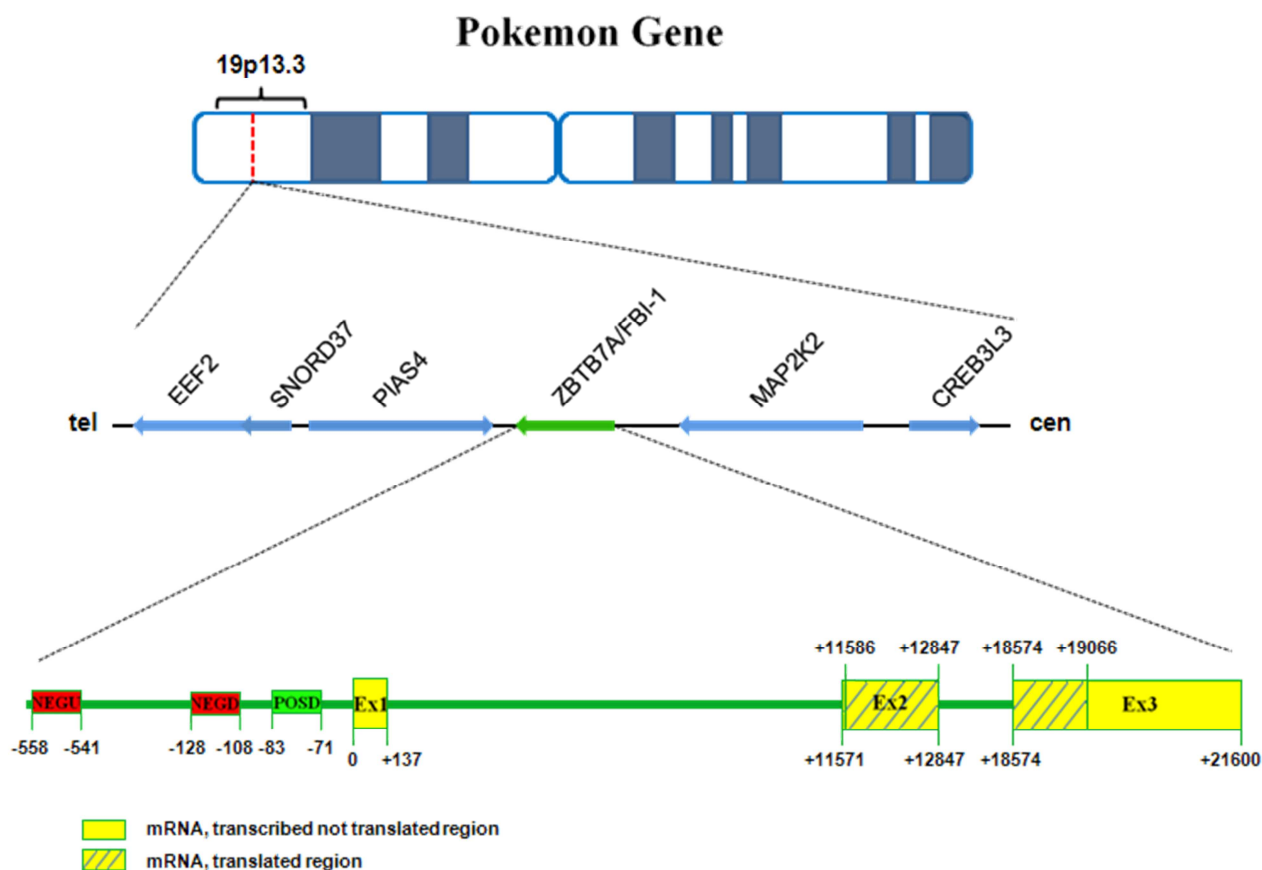
The gene is comprised of 3 exons, building a main transcript of 4456 bps.

#### Alternative splicing

Pokemon transcripts can be alternatively spliced, resulting in four mRNAs with different 5'-untranslated regions. The four alternative first exons are located at -11596, -10224, -9109 and -17 bp upstream of the translation start site of Pokemon, implying that Pokemon can putatively be regulated by four alternative promoters.

### Pseudogene

There are no known pseudogenes.



Schematic diagram depicting the structure and 19p13.3 chromosomal localization of the ZBTB7A/POKEMON gene.

## Protein

### Description

ZBTB7A protein is composed of 584 amino acids (a.a.) with a molecular weight of 86 kDa. It belongs to the POK (POZ and Krüppel) family of transcriptional repressors. Members of this family have a characteristic structure, containing a NH<sub>2</sub>-terminal POZ/BTB domain (poxvirus zinc finger/broad-complex tramtrack, bric-à-brac) and a COOH-terminal domain, consisting of C2H2 Krüppel-type zinc fingers, "surnamed" due to resemblance with the *Drosophila* segmentation protein Krüppel. Due to this structure, they are also known as POZ/ZF proteins. At the C-terminus a bipartite nuclear localization signal (NLS) is located.

POZ/BTB domain represents a 120 a.a. evolutionary conserved domain, present in more than 200 human proteins, which mediates protein-protein interactions. It is responsible for homodimerization as well as heterodimerization mediating the recruitment of corepressor complexes, such as nuclear co-repressor (N-CoR), silencing mediator of retinoic acid (SMRT), B-CoR (Bcl-6 co-repressor), mSin3A (SIN3 homolog A, transcription regulator) to the target genes. This also facilitates the binding of histone deacetylases within the target genes leading to its transcriptional repression. It also interacts with Sp1 zinc finger proteins.

C2H2 Krüppel-type zinc finger domain, found in more than 600 human proteins, represents a 25-30 a.a. domain, responsible for specific DNA recognition and binding. It is characterised by two conserved cysteine and histidine residue pairs that coordinate a single zinc atom.

### Expression

ZBTB7A is expressed in all proliferating cells. ZBTB7A is highly expressed in malignant tissues than in the corresponding benign or normal tissue (breast, lung, prostate).

### Localisation

ZBTB7A is constitutively nuclear, due to a Nuclear Localization Signal (NLS) located at the C-terminus (a.a. 498-502). Immunohistochemical (IHC) studies of ZBTB7A expression in NSCLCs (Non-small cell lung carcinomas) have shown a nuclear signal. In NSCLCs the immunostaining is prevalent in the cancerous nests, whereas few tumour-associated stromal cells and a small number of parabasal intermediate cells in the adjacent normal bronchial epithelium, are positively stained. ZBTB7A nuclear expression was also prevalent in malignant breast tissues and surrounding fibroblasts and lymphocytes, whereas stromal cells were weakly stained.



Schematic representation of human ZBTB7A protein structure. POZ domain: 24-131, ZF: 376-489, NLS: 489-503.

## Function

ZBTB7A has a variety of biological functions. ZBTB7A was originally identified as a factor binding to a specific sequence within the human immunodeficiency virus, type 1 promoter, and can physically interact with other POK family members such as BCL-6. Co-expression of ZBTB7A and BCL-6 in lymphoma, predicts the clinical outcome. ZBTB7A is also implicated in differentiation processes like osteoclastogenesis, chondrogenesis and adipogenesis. Furthermore, ZBTB7A knockdown in mice, results in embryonic lethality due to severe anaemia and impaired cellular differentiation.

ZBTB7A is a transcription factor which regulates the expression of many proteins, such as extracellular matrix collagen types I, II, IX, X and XI, fibronectin, elastin, human cartilage oligomeric matrix protein, p19ARF tumour suppressor and the c-fos and c-myc oncoproteins by binding to consensus sequence within their promoters. ZBTB7A preferable binding sites include either a single guanine-rich site or two half-sites, each with the consensus sequence G(A/G)GGG(T/C)(C/T)(T/C)(C/T), with a variety in spacing and orientation, supporting that ZBTB7A can bind to the DNA with great flexibility. In other cases, the interaction between Pokemon POZ domain and other transcription factors, such as Sp-1 or the p65 subunit of NF-kappaB, is necessary for its regulatory role.

ZBTB7A self-associates, as depicted in vivo and in vitro, via both its POZ domain and its zinc finger (ZF) domain. ZBTB7A can act as a transcriptional repressor or activator depending on the promoter context.

Finally, it plays a crucial role in cell tumorigenesis.

- Transcription regulator

1. ZBTB7A/FBI-1 + IST (HIV)

FBI-1 binds to IST, the HIV-1 inducer of short transcripts, and mediates its function. The first and second zinc fingers are required for efficient binding. FBI-1, via the zinc finger domain, interacts as well with Tat (transactivator of transcription), a viral transactivator protein, responsible for the synthesis of HIV-1 full-length transcripts. FBI-1 partially colocalizes with Tat and enhances its activity in a dose dependent manner, therefore modulating Tat-activated HIV-1 transcription.

2. FBI-1 + adipogenesis

FBI-1 acts as a dual regulator in adipogenesis. It promotes preadipocyte differentiation process and facilitates adipogenesis. On the other hand, it is implicated in termination of the mitotic clonal

expansion, in which growth-arrested preadipocytes re-enter the cell cycle and complete two rounds of cell division, followed by terminal differentiation phase.

3. FBI-1 + FASN (transcriptional activator)

Increased FBI-1 expression enhances FASN (fatty-acid synthase gene) expression, in stable cancer cells or immortalised stable cells. FASN expression appears to be low in almost all nonmalignant adult tissues, but is highly expressed in many carcinomas, where it is responsible for fatty acid synthesis. Furthermore, knockdown of FBI-1 expression leads to decreased FASN expression.

FBI-1 enhances FASN transcriptional activation through SREBP-1a (sterol-responsive element (SRE)-binding protein 1). FBI-1 and SREBP-1a form a complex and co-localize mainly in the nucleus. Specifically, FBI-1 through its ZFDBD-C-term domain (zinc finger DNA binding domain) interacts directly with the bHLH domain of SREBP-1. Sp1 binds to the proximal GC-box of the FASN promoter and acts as a major activator. It has been found that the regulatory elements GC-box and SRE/E-box of FASN promoter can be bound by all three, FBI-1, SREBP-1a and Sp1, transcription factors. This can lead to a binding competition between them.

4. FBI-1 + NF-kappaB (transcriptional activator)

FBI-1 POZ domain interacts directly with the RHD domain (Rel homology domain) of NF-kappaB p65 subunit.

FBI-1 enhances the transcription of NF-kappaB-responsive genes, like E-selectin, by increasing either the localization or stability of NF-kappaB in the nucleus. FBI-1 facilitates nuclear import and prolonged nuclear retention of the p65 subunit of NF-kappaB, either by inhibiting its export to the cytoplasm, or by increasing its stability. In addition, FBI-1 inhibits IkappaB (inhibitors of NF-kappaB) interaction with NF-kappaB, eventually enhancing NF-kappaB transcription in the nucleus.

5. FBI-1 + ADH5/FDH (transcriptional repressor)

FBI-1 POZ domain interacts with the C2H2-type zinc-finger domain of Sp1 (Specificity protein 1), thereby repressing the ADH5/FDH promoter activity.

6. ZBTB7A + ARF (transcriptional repressor)

ZBTB7A was characterized as a potential proto-oncogene that specifically represses transcription of p14ARF, a major tumour suppressor gene, (leading indirectly to p53 inactivation). ZBTB7A binds directly to p19ARF promoter (mouse homologue). ARF promoter contains several putative ZBTB7A-binding sites, rich in GC. However the one located 50 base

pairs upstream from the transcription start site is indispensable for p19ARF repression.

#### 7. FBI-1 + p21WAF1/CIP1 (transcriptional repressor)

FBI-1 represses transcriptional activation of P21CIP1, by the following ways.

Firstly, by inhibiting ARF and Hdm2 expression, and therefore p53, it indirectly affects p21WAF1/CIP1 expression. Secondly, as a GC-box-binding transcription factor, it competes with Sp1 (transcriptional activator) to bind to the GC-rich FRE/GC-box 3 element at the proximal promoter, critical for p21CIP1 transcription.

Furthermore FBI-1 competes with p53 for binding to the distal p53-binding elements of p21WAF1/CIP1 and represses its transcription.

Additionally, it interacts with corepressors such as NCoR (Nuclear receptor co-repressor), SMRT (silencing mediator for retinoid and thyroid receptors), and mSin3A and recruits HDAC complexes, leading to histone deacetylation at the proximal promoter, responsible for p21WAF1/CIP1 transcriptional repression.

#### 8. FBI-1 + CCS-3 + p21WAF1/CIP1

FBI-1 suppresses p21WAF1/CIP1 promoter activation by more than 70% by acting on both the proximal Sp1 binding GC-box 3 element and the distal p53 responsive regulatory elements. CCS-3, (cervical cancer suppressor -3), represents an isoform of eEF1A (eukaryotic translation elongation factor 1A), involved in various processes like embryogenesis, oncogenic transformation, senescence, cell proliferation, and cytoskeletal organization. CCS-3 enhances the FBI-1 mediated p21WAF1/CIP1-transcriptional repression, by another 30-50%. The above is accomplished because CCS-3 can interact with both co-repressors, (like SMRT and NCoR independently of FBI-1) and FBI-1 POZ domain. CCS-3 is able to recruit additional transcriptional co-repressor-HDAC complexes when bound to the FBI-1 POZ-domain, therefore enhancing FBI-1 transcriptional repression potential.

Furthermore, both FBI-1 and CCS-3 independently promote cell proliferation. Co-transfection of CCS-3 and FBI-1 results in a significant increase in cell population.

#### 9. LRF - COMP (transcriptional repressor)

LRF (mouse homolog of FBI-1) binds directly to the NRE element and specifically to a nine nucleotide (GAGGGTCCC) sequence - LRF binding element (LBE) in COMP (Cartilage oligomeric matrix protein) gene promoter. NRE represents a negative regulatory element, both necessary and sufficient to repress promoter activity. Therefore, it regulates COMP gene expression implicated in chondrogenic differentiation. Human COMP gene promoter also contains a typical consensus site (GGGGGCCTC, -1294 to -1302) for binding LRF/FBI-1, implying that FBI-1 may suppress COMP gene expression in human as LRF does in the mouse.

#### 10. FBI1 - AR (transcriptional repressor)

The androgen receptor (AR) is important for the proliferation and functioning of prostate cells.

FBI-1 physically interacts with AR and when overexpressed, it represses AR transcriptional activity in a ligand-dependent manner. More precisely, POZ domain of FBI1 is responsible for the interaction with the LBD domain (C-terminal ligand-binding domain) of AR. Furthermore, full-length FBI-1 is indispensable for the repression of AR transcriptional activity. On the contrary, FBI-1 suppression by siRNA enhanced AR transcriptional activity.

It is known that FBI-1 can interact with NCoR and SMRT, which are co-repressors of AR. (FBI-1 exhibited a much stronger interaction with SMRT compared to that with NCoR). It was shown that FBI-1 enhances the recruitment of NCoR and SMRT to endogenous ARE elements and through this mechanism it suppresses AR transcriptional activity. This notion is further supported by the fact that FBI-1 repressive effect on AR transcriptional activity, is inhibited upon endogenous NCoR and SMRT knockdown. Furthermore it was suggested that FBI-1 could recruit a co-repressor complex harbouring HDACs, resulting in repression of AR functions in the presence of R1881 (synthetic agonist of the androgen receptor). Last, it was shown that FBI-1 can play different roles in regulating androgen-dependent (LNCaP) and -independent (PC-3) prostate cancer cells. It inhibits LNCaP cell proliferation in the presence of R1881, whereas no difference in proliferation was observed in the absence of R1881. In contrast, knockdown of FBI-1 by siRNA led to a significant increase in LNCaP cell growth. Interestingly, either in the presence or absence of R1881, PC-3 cell proliferation was dramatically enhanced upon FBI-1 overexpression.

#### 11. Pokemon - survivin (transcriptional activator)

Tissue microarray technology has shown Pokemon overexpression in 86,8% of breast cancer tissue, but not in normal breast tissue. Furthermore Pokemon expression correlated positively with tumor size, lymph node metastasis and poor patient survival.

Survivin, a member of the inhibitor of apoptosis proteins (IAP), is implicated in cell carcinogenesis and tumor progression.

Survivin and Pokemon expression levels were found to be highly correlated in human breast cancer cells. Pokemon induces survivin expression by direct binding to the GC boxes in its promoter.

- Relation with ASF/SF2 through miRNAs

LRF is regulated by microRNA (miRNA) families.

In MEFs (Mouse Embryo Fibroblasts) it was shown that miR-28 and miR-505, which are negatively controlled by LRF, can affect ASF/SF2 (Alternative Splicing Factor/Splicing Factor 2) expression and therefore can induce apoptosis or cellular transformation. Deregulation of the LRF/miR-28/miR-505/ASF/SF2 axis affects the MEF senescence/apoptosis pathway.

- Relation with anticancer agents

Curcumin is a natural compound known for its antioxidant, anti-inflammatory and anticarcinogenic properties, since it can inhibit the proliferation and induce the apoptosis of cancer cells, such as gastric, colon, breast. Curcumin inhibits ZBTB7A transcriptional activation, by suppressing the Sp1 stimulatory effect on Pokemon promoter, as it inhibits Sp1 recruitment to the Pokemon promoter.

- Global mapping of ZBTB7A downstream target genes in HepG2 cells

ChIP-on-chip analysis performed in the human hepatocarcinoma cancer cell line HepG2, revealed 556 genes as potential direct targets of ZBTB7A. These genes are mainly divided in three categories: genes regulating metabolism, transcriptional regulators and cell signal transduction genes. Among the most ZBTB7A-targeted metabolic pathways are the aminosugar, arachidonic acid, tryptophan, pyrimidine and purine metabolic pathways. Moreover sucrose, vitamin B6, valine, leucine and isoleucine, folate, fatty acid and glycerolipid metabolism, are characterized as ZBTB7A regulatory targets. As far as signal transduction is concerned, ZBTB7A might play a regulatory role on the MAPK signalling pathway. Furthermore, four genes involved in axon guidance, a key stage in the formation of the neuronal network, are directly regulated by ZBTB7A expression in HepG2 cells. This pinpoints to a potential regulatory role for ZBTB7A in neural development.

- ZBTB7A and SUMOylation

ZBTB7A can be sumoylated as it contains an amino-acid sequence highly similar to the SUMO-1 conjugate consensus motif,  $\psi$ KXE ( $\psi$ : large hydrophobic residue, K: lysine to which SUMO-1 is conjugated, X: any amino acid, and E: glutamic acid). Sumoylation of several lysine residues of ZBTB7A is important in order to achieve full repressive activity.

## Homology

LRF (leukemia/lymphoma-related factor) is the mouse homologue of FBI-1. It co-immunoprecipitates and co-localizes with Bcl-6 and is involved in chondrogenesis and adipogenesis.

OCZF (osteoclast-derived zinc finger), the rat homologue of FBI-1, is a transcription repressor, involved in osteoclastogenesis. Mouse LRF and rat OCZF are 90 and 96% identical at the nucleotide and amino acid levels, respectively. Mouse LRF and human FBI-1 sequences are 89% identical at the amino acid level.

## Mutations

### Note

No known mutations.

## Implicated in

### Carcinogenesis

#### Note

Since ZBTB7A specifically represses transcription of p14ARF, a major tumor suppressor gene, it was characterized as a potential proto-oncogene, expected to play a significant role in carcinogenesis. ZBTB7A null MEFs (ZBTB7A<sup>-/-</sup>) had higher p19ARF protein levels than wild-type MEFs and resisted to proliferation and transformation upon oncogenic stimulation. The oncogenic nature of ZBTB7A was also examined in vivo in transgenic mice, which overexpressed ZBTB7A in T and B lymphoid lineage cells. These mice developed thymic lymphomas and tumour infiltration into bone marrow and poor survival compared to the wild-type ones.

ZBTB7A is overexpressed in diffuse large B cell lymphomas (DLBCL) and follicular lymphomas, as well as in anaplastic large cell lymphoma and angioimmunoblastic lymphoma, as far as T-cell malignancies are concerned.

ZBTB7A oncogenic role in human malignant gliomas was revealed with comparative genomic hybridization analysis (bioinformatics methods).

Tissue microarray analysis revealed ZBTB7A overexpression in colon, prostate, bladder, as well as in breast and lung carcinomas.

IHC and immunofluorescence analysis revealed a significantly higher expression of LRF protein in malignant breast specimens than in the corresponding benign or normal tissue, with a clear nuclear expression pattern. 40% and 15% of benign breast biopsy tissues expressed LRF mRNA transcripts and protein, respectively, probably because they contain premalignant cells and adipocytes.

Ductal hyperplasia showed weak LRF staining, whereas in invasive ductal carcinoma and invasive lobular carcinoma strong nuclear expression of LRF was observed.

In breast cancer tissues ZBTB7A expression levels were significantly correlated with histological grade, implying a potential role of ZBTB7A in breast cancer development, whereas were inversely correlated with the oestrogen receptor status. No correlation was confirmed between ZBTB7 overexpression and tumour size, stage, lymph node status, or PR status. However its overexpression predicted poorer overall survival and was associated with shorter recurrence-free survival.

In addition, ZBTB7A is expressed in the nuclei of human colon, renal, hepatocellular carcinomas, mesotheliomas and thymoma tumor cells.

IHC and immunoblotting analysis showed ZBTB7A overexpression in NSCLC specimens, compared to



adjacent normal tissue elements. Gene amplification proved to be a relatively frequent event leading to ZBTB7A overproduction in NSCLCs. Furthermore, a positive correlation between ZBTB7A expression and proliferation, as well as tumour size, was revealed. Survival analysis correlated ZBTB7A overexpression with poor prognosis. ZBTB7A expression is not associated with the NSCLC patients' sex, age, lymph node stage, tumor differentiation degree and histology. Furthermore IHC, immunoblotting and PCR analysis revealed significantly higher ZBTB7A (FBI-1) protein and mRNA expression levels in ovarian cancer samples and cell lines, compared to benign or borderline tumours. Gene amplification was proved to be, to a great proportion, the underlying mechanism of ZBTB7A overexpression in ovarian cancers. Upregulated ZBTB7A expression was found in metastatic foci and malignant ascites and was associated with advanced stage, poor overall survival and disease-free survival. Additionally, it was shown that ZBTB7A plays an important role in ovarian cancer cell migration, proliferation and invasiveness, through its direct binding to the promoter of MT1-MMP (membrane type 1-matrix metalloproteinase), which enhances MT1-MMP promoter activity. Moreover, ZBTB7A (LRF) is implicated in the pathogenesis of prostate cancer, since IHC, Western blot and PCR analyses revealed a significantly higher expression of LRF mRNA transcripts and protein in prostate cancer (PC) specimens than in benign prostate hyperplasia (BPH). A significant increase in LRF mRNA transcripts and protein levels was observed when normal human prostate cells (RWPE-1) as well as prostate cancer cells, (LNCaP androgen sensitive prostate cancer cells and PC-3 androgen-insensitive prostate cancer cells), were treated with EGF (epidermal growth factor). Recently, the development of a novel strategy for silencing Pokemon in vivo, with promising results, provided a marked anti-tumour activity in nude mice.

### **Cell growth and proliferation**

#### **Note**

ZBTB7A is implicated in cell growth regulation, as it transcriptionally represses Rb (Retinoblastoma), a gene involved in differentiation, cell cycle control, and tumor suppression. FBI-1, through its POZ domain, recruits HDACs to deacetylate histones H3 and H4 around the Rb proximal promoter region, resulting in Rb transcriptional repression. Additionally, FBI-1 binds to the 4 FREs (FBI-1 binding sites, GC-rich) on the Rb promoter and relatively strongly to the FRE2 element.

FBI-1 enhances cellular transformation as well. FBI-1 overexpression in HeLa cells resulted in formation of large foci and increased the number of HeLa cells in S phase. Moreover ZBTB7A is involved in cell proliferation in MEFs.

### **Differentiation**

#### **Note**

ZBTB7A is implicated in many differentiation processes including osteoclastogenesis, chondrogenesis and adipogenesis. ZBTB7A knockdown in mice resulted in embryonic lethality due to severe anaemia and impaired cellular differentiation. LRF was found to be essential for terminal fetal erythropoiesis. LRF is a downstream target of GATA1. GATA1 is a transcription factor significant for erythroblasts differentiation, maturation and protection from apoptosis. GATA1 binds directly to the two putative GATA-binding sites in ZBTB7A promoter region and activates its expression. Additionally, in the absence of LRF, both in embryos and adults, BIM a significant apoptosis inducer in hematopoietic cells was markedly upregulated. LRF directly binds to BIM proximal promoter region, which contains four potential LRF binding sites and represses its expression, during the late stages of erythroid differentiation. On the other hand, BIM loss rescues LRF knockout mice from fetal anemia.

From the above, it is resumed that the suppression of BIM-mediated apoptosis by LRF, which in turn is activated by GATA-1, is critical for effective erythropoiesis.

RANK and its ligand, RANKL (a member of the tumor necrosis factor - TNF superfamily), are critical molecules in osteoclastogenesis. RANKL also plays an important role in inflammatory bone loss. FBI-1/OCZF/LRF is highly expressed in osteoclasts and is an important regulator of RANKL-induced osteoclastogenesis. LRF siRNA inhibits osteoclast differentiation. Osteoclast formation is a crucial step in bone destruction in chronic arthritis. FBI-1/OCZF is highly expressed in osteoclasts in inflammatory arthritis, implying a role in inflammatory bone erosion. The above suggest that by enhancing osteoclastogenesis and survival of osteoclasts, FBI-1/OCZF/LRF might play important roles in bone destruction in vivo, including the bone loss and remodelling that occurs in chronic inflammatory arthropathies, such as rheumatoid arthritis.

### **Regulation of B lymphopoiesis**

#### **Note**

LRF (mouse homologue of ZBTB7A) regulates B lymphocytes development from lymphoid-restricted progenitors in mice, versus T lymphoid fate. Knockdown of LRF in HSCs (hematopoietic stem cells) and CLPs (common lymphoid progenitors), impaired B cell development and resulted in extrathymic T cell development in the bone marrow (BM). Furthermore, LRF blocks Notch signalling pathway in normal HSCs. This pathway is essential for T cell development. Notch deletion in mouse HSCs results in a decreased number of thymic T cells, whereas it enhances B cell development in the thymus.

Moreover, LRF regulates mature B cell lineage fate and humoral immune responses, through the obligatory formation of a dimer in B cells, important for its function. Furthermore, LRF inactivation in transformed B cells attenuated their growth rate. The above suggest the potential targeting of LRF dimers as a treatment of autoimmune diseases and B cell malignancies.

### **LRF bypasses RASV12-induced senescence**

#### **Note**

LRF enhances E2F-dependent transcription, with an unknown mechanism, which does not include Rb downregulation. LRF synergizes with RASV12 in inducing E2F. LRF bypasses RASV12-induced senescence by activating E2F-target genes, such as Cyclin E, Cyclin A and p107. Specifically, Cyclin E is shown to be indispensable, but not sufficient for LRF-mediated bypass of RASV12-induced senescence. The above mentioned bypass occurs independently of critical senescence inducers, such as p19ARF, p21CIP and p16INK4A.

### **LRF is post-transcriptionally controlled by MiR-20a: implications in senescence**

#### **Note**

MicroRNAs (miRNAs) are short 20-22 nucleotide RNA molecules, which act as post-transcriptional suppressors. They repress mRNA translation by base pairing to 3'UTR sequences.

Mir-20a, a member of miR-17-92 cluster, can interact directly with ZBTB7A 3'UTR. Upon miR-20a overexpression in MEFs, LRF protein is reduced by 40%. Conversely, miR-20a depletion resulted in increased LRF protein levels by 55%.

MiR-20a induces p19ARF expression to a smaller extent than siLRF. However, miR-20a is a more efficient senescence inducer than siLRF, implying the contribution of other factors to the senescence effect.

It has been shown that E2F1 expression is also under the miR-20a control in MEFs. When miR-20a is highly expressed there is a decrease in E2F1 protein levels, to a greater degree than that after siLRF. Conversely, miR-20a depletion slightly enhances E2F1 expression. However, E2F1 silencing by siE2F1 in MEFs is not enough to induce senescence, whereas miR-20a overexpression in LRF-null MEF, with the concomitant E2F1 downregulation, significantly enhances senescence. In addition, miR-20a and not siLRF, can also increase p16INK4A protein levels, a key player in senescence pathway, both in wild type and LRF-null MEFs.

The above data support the notion that miR-20a elicited cellular senescence results mainly from LRF downregulation and consequent p19ARF upregulation, with the contribution of E2F1 down-regulation and p16INK4A induction.

### **P-glycoprotein (Pgp) and transcriptional repression of Pokemon**

#### **Note**

Pgp is a 170 kD protein, encoded by the human MDR1 (ABCB1) gene and belongs to the ATP-binding cassette transporters family. Pgp and the other family members function as pumps to extrude anticancer drugs from cancer cells and are related to drug resistance phenotype. Pgp is often over-expressed in drug-resistant cancer cells. It has been found that Pgp represses the transcription of Pokemon, through decreasing Pokemon promoter activity. Furthermore, this Pgp regulated suppression of Pokemon is p53 dependent.

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