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Importance and Presentation of Transcription Factor Fli-1 in Hematopoiesis and in Hematological and Other Malignancies

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Abstract: *Transcription factor Friend leukemia virus integration 1 (Fli-1) comes under 28 members in human Ets family of transcription factors. These members are involved in development of different tissues, proliferation, differentiation and apoptosis as well as in cancer progression. Avian erythroblastosis transforming retrovirus E26 (v-Ets), which produced leukemias in chicken, was initially detected in 1982. Its characteristic wing-turn-helix Ets DNA binding domain (DBD) was described in 1985. Fli-1 was first identified in 1990 as a proto-oncogene near a common site for retroviral integration in Friend virus-induced erythroleukemia. Fli-1 functions as both a transcriptional activator and repressor. The nuclear transcription factor Fli-1 plays an important role in hematopoiesis by coordinating the balance between proliferation and differentiation. Fli-1 is engaged in both erythroid and megakaryocytic development. Abnormal expressions of Fli-1 were detected in hematological malignancies such as acute myeloid leukemia and diffuse large B-cell lymphoma and also in other malignancies such as astrocytoma, melanoma, colon cancer, endometrial cancer, epithelial ovarian cancer, nasopharyngeal carcinoma, head and neck squamous cell cancer and is connected with progression of these diseases. Fli-1 negatively regulates the tumor suppressor p53 by directly binding to the promoter of MDM2 gene and stimulating of its transcription and up-regulating MDM2. Fli-1 acts as oncogene by promoting proliferation and inhibiting p53 function. Therefore, Fli-1 can be a potential prognostic biomarker and molecular target in hematological and other malignancies. Mithramycin, a DNA binding aureolic acid natural product targets oncogenic Fli-1. Cardenolides, calcium ionophores, topoisomerase I inhibitors and several other small compounds are also capable to inhibit Fli-1.*

Keywords: *ETS transcription factors, Fli-1, differentiation, proliferation, BET inhibitors, mithramycin*

1. INTRODUCTION

Friend murine leukemia virus (F-MuLV) – induced murine erythroleukemia is a very good model to study multi-step leukemogenesis, which means the multi-stage nature of cancer [1-3]. This model was used for identification of several oncogenes and tumor suppressors such as *Fli-1*, *Spi-1*/*PU.1*, *TP53*, *Fli-2*/p45 NFE2, and *Fli-3*/*miR-17-92* [4-16]. F-MuLV – induced erythroleukemia is characterized by proerythroblast expansion leading to anemia, splenomegaly, and eventually death within eight weeks of viral infection in susceptible strains of newborn mice. Integration of the provirus at the *Fli-1* locus drives enhancer-mediated Fli-1 over expression [4, 5, 13-15]. *Fli-1* viral integration induces expression of anti-apoptotic genes (*Bcl-2* /B-cell lymphoma 2/ and *Bcl-xL*) [17] and tumor suppressor genes (*TP53* and *p45 NFE2*) [6-8, 18, 19]. However, this *Fli-1* viral integration decreases *Rb* (retinobloastoma gene) expression [20] and changes *Epo* (erythropoietin gene) locus arrangement resulting in the constitutive activation of the Epo receptor signal transduction pathway and in growth and survival advantage *in vitro* and *in vivo* [21,22].

Fli-1 inhibits the apoptotic cell death program normally activated in erythroblasts with Epo deficiency. Fli-1 inhibits also the terminal differentiation program induced in these cells in response to Epo and induces their proliferation. An abnormally activated form of the receptor for Epo (EpoR) is not needed for effects of Fli-1 on erythroblast [12]. Erythroblasts expressing Fli-1 have enhanced survival which correlates with the increase of *Bcl2* expression. The rapid down regulation of cyclin D2 and D3 expression typical for Epo-induced differentiation is prevented by Fli-1. Transcription factor Fli-1 blocks also the down regulation of several other genes involved in cell cycle or cell proliferation control. These results show that over expression of Fli-1 causes the deregulation of the normal balance between differentiation and proliferation in primary erythroblasts [12]. Thus, the activation of Fli-1 expression in murine Friend erythroleukemia cells may provide a proliferative advantage to these cells and inhibit

terminal differentiation or cell death. The envelope glycoprotein gp55 is present in spleen focus-forming virus (SFFV) and binds and activates EpoR [23-26]. However, gp55 is not present in F-MuLV. Erythroid cells expressing gp55 proliferate in the absence of their normal regulator Epo.

While *Fli-1* is activated in about 90% of F-MuLV-induced erythroleukemias, small percentage of infected mice had retroviral insertion within *Fli-3* locus, leading to activation of a micro RNA (miRNA, miR) cluster labeled miR17-92 [27]. Both Fli-1 and miR17-92 are over expressed in various cancer cell types and activate similar signaling pathways associated with erythroid transformation [28-45].

Transcription factor Fli-1 is very important in megakaryocytic differentiation [46-58]. Fli-1 binds to and directly regulates the genes encoding several proteins that are essential for terminal megakaryocytic differentiation. Homozygous loss of functional *Fli-1* alleles in mice leads to embryonic lethality because of severe defects in fetal megakaryopoiesis and a high incidence of embryonic hemorrhaging. This is the result of impaired megakaryopoiesis as well as inefficient blood vessel formation. Thus, *Fli-1* functions as a master regulator of blood and endothelial development [59-61].

Moreover, transcription factor Fli-1 is a key component together with GATA-2 and SCL in the control of hematopoietic stem cell (HSC) specification [62]. Gata2, Fli1, and Scl (Stem cell leukemia)/Tal1 (T-cell acute lymphocytic leukemia protein 1) are expressed in embryonic day-11.5 dorsal aorta where HSCs originate and in fetal liver where they multiply. The three HSC enhancers in these tissues and in ES cell-derived hemangioblast equivalents are bound by each of these transcription factors. Pimkin et al. [57] mapped the genome-wide chromatin occupancy of four master hematopoietic transcription factors (GATA1, GATA2, TAL1, and FLI1) and three histone methylation marks (H3K4me1, H3K4me3, and H3K27me3) in lineage-committed erythroid and megakaryocytic cells. In addition, they defined global gene expression changes that accompany the development of these mature lineages from hematopoietic stem and progenitor cells (HSPCs). By correlating gene expression with transcription factor occupancy, they produced a global functional annotation of chromatin dynamics during erythro-megakaryopoiesis. Their findings provided new insights into GATA protein functions and reveal a robust, genome-wide mechanism of megakaryocytic lineage priming in multi potential hematopoietic progenitors.

Specification and differentiation of erythroid cells and megakaryocytes from the common megakaryocyte-erythroid progenitor (MEP) are governed by coordinated regulation of a precise balance of members of several transcription factors, adaptors and micro RNAs including GATA-binding transcription factors (GATA-1 and GATA-2), Ets factors (Fli-1 and GABPα /GA-binding protein α/), Krűppel-containing factors (KLF1/EKLF and Leukemia/lymphoma Related Factor /LRF/), basic helix-loop-helix factors (SCL), multiple adaptors (Friend of GATA-1 /FOG-1/, LIM domain-binding protein 1 /LDB1/ and) and micro RNAs (miR-150 and miR-451) [63-77]. Recent studies have also provided support for a model in which murine HSCs rapidly commit to unipotent megakaryocytic progenitors not derived from bipotent megakaryocytic and erythroid-committed progenitors [78].

A novel and simple technology to obtain platelets using trans differentiation of human bone marrow erythroblasts to mega karyocytes with over expression of the *Fli-1* and *Erg* genes has been recently described [79]. To generate *Fli-1* and *Erg* over expressing erythroblasts, the isolated erythroblasts were transduced with lentiviral vectors containing the full coding sequences of human *Fli-1* and *Erg* genes, respectively.

1.1. Friend Leukemia Integration-1 (Fli-1)

Fli-1 is located on mouse chromosome 9 and human chromosome 11q24, a region of frequent abnormalities in human disease [5, 80]. Both the murine and human homologs of the *Fli-1* gene are approximately 120 kb, consist of nine exons, end encode two protein isoforms, two protein isoforms, p51 (452 amino acid residues /aa/) and p48 (419 aa) [5, 81, 82]. The *Fli-1* gene is on the same chromosome as *Ets-1* gene located in the distance about 240 kb from *Ets-1* gene locus. Both genes arose probably by gene duplication from a common ancestral gene [5]. Two initiation codons of transation are localized to nucleotide 245 within exon 1 and 344 within exon 2 of the mouse Fli-1 mRNA sequence (NCBI Reference Sequence: NM_008026.5 [82]. These translation initiation codons are responsible for the synthesis of two Fli-1 protein

isoforms. Similar situation is in human Fli-1 mRNA where two ATG translation initiation sites have been localized to nucleotides 344 (exon 1) and 443 (exon 2) of the human Fli-1 mRNA sequence (NCBI Reference Sequence: NM_002017.4). These translation initiation codons are responsible for the synthesis of two Fli-1 protein isoforms p51 and p48 [82]. The second isoform, p48, has a shorter N-terminus and contains a distinct 5´ untranslated region (UTR) in comparison with isoform 1, p51.

Alternative exons 1 (1a and 1b) of murine and human *Fli-1* gene as the result of alternative splicing and translation of alternative Fli-1 mRNAs were also detected [83,84]. Exon 1b was not conserved in the mouse but was detected in every analyzed human cell, whereas exon 1a was present only in a part of analyzed human and murine cells. These results suggested that both mouse and human *Fli-1* gene expression might be under the control of several independent promoter regions. *Fli-1* gene expression was detected in lymphoid, myeloid and endothelial cells, spleen, thymus and at lower levels in lung, heart and ovaries [85]. *Fli-1* gene expression is potently down regulated during Epo-induced erythroid differentiation [20].

Several *cis*-regulatory elements within a 234 bp region (sequence from −271 to −37) that contibute to *Fli-1* gene promoter activity was found in a murine B cell line and T cell line [86, 87]. This region contains a GATA consensus site as well as two Ets binding sites. Indeed, introducing mutations within these Ets binding sites abolished the promoter activity. Transcription factor Spi-1/PU.1 binds to this region and positively regulates *Fli-1* gene expression on the transcription level [9].

Ets factors Elf1, Tel and Fli-1 bind *in vitro* to this region and increase endogenous *Fli-1* expression in a T cell line. These Ets transcription factors also stimulate *Fli-1* gene expression *in vivo* [86, 87]. This mechanism and polymorphic microsatellite of GA dinucleotide repeats located at the 5´ end of exon 1 have the important influence on *Fli-1* expression. The presence of a shorter microsatellite resulted in higher Fli1 promoter activity [88]. A significant association of this polymorphism was observed with systemic lupus erythematosus (SLE) of patients without nephritis [88].

The functional domains located within the Fli-1 protein include the 5´ Ets domain, and Fli-1-specific region (FLS), both together referred to as the amino-terminal transcriptional activation domain (ATA), and a 3´ Ets domain and carboxy-terminal transcriptional activation domain (CTA). The 5´ Ets domain is located within amino acids 121-196 and the FLS, which is absent in the Ets-related protein Erg with 81% homology to the Fli-1 protein, is localized within amino acids 205-292. The 3´ Ets domain was determined witin amino acids 277-360 and is important for sequence specific DNA-binding activity of Fli-1. The CTA domain, found within amino acids 402-452, is also involved in transcriptional activation and protein-protein interactions with other transcription factors.

Both, the 5´ and 3´ Ets domains contain sequences of helix-loop-helix (H-L-H) secondary structures, while the FLS and CTA domains contain sequences of turn-loop-turn (T-L-T) secondary structures. Transcriptional activity of Fli-1 is regulated by ATA and CTA domains. CTA domain may function also as transcriptional repressor [89]. Moussa et al. [90] generated mutant *Fli-1* mice that expressed a truncated Fli-1 protein lacking CTA region. Heterozygous mice with this defect in Fli-1 were viable, while homozygous mice with this truncated Fli-1 had reduced viability and reduced platelet number. Platelet aggregation and activation were also impaired. Homozygous mutants had the thrombocytopenia phenotype with extensive bleeding.

Both isoforms of Fli-1, p51 and p48, are phosphorylated primarily on serine residues in human T cells [91]. Phosphorylated Fli-1 has a short half-life (p51 isoform-105min and p48 isoform-80 min). In contrast, the phosphorylated Erg protein is much more stable with a half-life of 21 h. Fli-1 phosphorylation is dependent on the concentration of intracellular calcium. Protein phosphatase 2A is involved in Fli-1 dephosphorylation. Transforming growth factor beta (TGF-β) stimulated phosphorylation and acetylation cascade of Fli-1 in dermal fibroblasts [92]. In this case, Fli-1 phosphorylation was localized to threonine 312 located in the DNA binding domain of Fli-1. Protein kinase C delta (PKCö) caused this phosphorylation and was controlled by the c-Abl tyrosine kinase, which is required for nuclear localization of PKCö [93].

1.2. Fli-1 Target Genes

Fli-1 functions as both a transcriptional activator and repressor [17, 20, 48, 49, 59, 64, 70, 87, 90,

94-117]. A great part of results in this area was gained in F-MuLV – induced murine erythroleukemia model system as I described in Introduction. Fli-1 induced transcriptional up regulation of *Bcl-2* and *Mdm2* (the RING-E3 ubiquitin ligase) and blocks apoptosis. Mdm2 mediates deregulation of p53 by stabilizing Mdm2 via ubiquitination of p53 and its degradation in proteasomes. Therefore, direct upregulation of Mdm2 by Fli-1 [94] destabilizes the anti-apoptotic protein p53, which plays an important role in the initial transformation of erythroblasts by Friend virus [18] and accelerates tumor progression by inducing genomic instability. Fli-1 down regulates *EKLF/KLF1* and *GATA-1* genes expression [70, 95, 101]. Moreover, Fli-1 negatively regulates phosphatidyl-inositol polyphosphate 5-phosphatase (*Ship1*) gene expression. Down-regulation of Ship1 resulted in higher phosphorylation of AKT/PKB by PI3K and erythroid proliferation [96]. Fli-1 stimulated ribosomal gene expression and ribosome biogenesis in erythroleukemogenesis [97].

Fli-1 has emerged as a critical regulator of inflammatory mediators, including monocyte chemoattractant protein-1(MCP-1) [107,108], macrophage inflammatory protein-2 (CXCL2) [111], chemokine C-C motif ligand 5 (CCL5) / RANTES (Regulated on Activation, Normal T cell Expressed and Secreted [112], G-protein-coupled receptor 9 in the CXC chemokine receptor (CXCR3) [113], interleukin 6 (IL-6) [115], and endothelial protein C receptor (EPCR) [116]. Infiltration of T cells into the kidney is a typical feature of human and experimental lupus nephritis that contributes to renal injury. All these inflammatory mediators are promising therapeutic targets in lupus nephritis, where Fli-1 expression is elevated. The reducing the levels of Fli-1 in SLE and similar autoimmune kidney diseases may be protective against development of nephritis through down regulation of desribed inflammatory mediators.

The cytokine, granulocyte colony stimulating factor (G-CSF) regulates neutrophil precursor maturation and survival, and activates mature neutrophils. Fli-1 drives transcription from the G-CSF promoter and mutation of the Fli-1 DNA binding domain resulted in abolut 90% loss of transcriptional activity [114]. Acetylation of Fli-1 by histone acetyltransferases p300/CBP and p300/CBP associated factor (PCAF) significantly decreased Fli-1 specific activation of the G-CSF promoter [114]. On the other hand, G-CSF induces stabilization of Fli-1 during myeloid development [118].

Fli1 recruits HDAC1/p300 to the COL1A2 promoter and suppresses the expression of the COL1A2 gene by chromatin remodeling through histone deacetylation [117]. TGF-β-dependent phosphorylation of Fli1 at threonine 312 is a critical step regulating the remodeling of the Fli1 transcription repressor complex, leading to transcriptional activation of the COL1A2 gene [92].

Fli-1 DBD dimerization plays a role in transcriptional activation and repression by Fli-1 and its fusion proteins at promoters containing Fli-1 binding sites [119]. The homodimerization interface is helix-swapped and dominated by hydrophobic interactions, including those between two interlocking Phe 362 residues.

1.3. The Role of Fli-1 in Malignant Transformation

Fli-1 is over expressed in various cancer cell types [28-39]. A number of virally induced leukemias, including Friend virus-induced erythroleukemia, are associated with *Fli-1* over expression [12-15]. Fli-1 activates the Rho GTPase pathway and is associated with metastasis in breast cancer [120]. The high expression of *Fli-1* associated with adverse prognosis of endometrial cancer correlated with a high differentiation grade and mutated P53 expression [38]. The high expression of *Fli-1* connected with the progression of epithelial ovarian cancer correlated with advanced tumor stage, positive lymph nodal involvement, and poor overall survival (OS) and disease-free survival (DFS) [36]. Deregulation of *ETS1* and *Fli-1* c ontributes to the pathogenesis of diffuse large B-cell lymphoma [34]. DLBCL is the most common type of non-Hodgkin lymphoma. *ETS1 and Fli-1* sustained cell viability of a DLBCL cell line. Down regulation of ETS1 and Fli-1 through correspondent lentiviral shRNAs induced lower proliferation rate and decreased mRNA levels of target genes of ETS a Fli-1. High expression of FLI1 protein is an adverse prognostic factor in acute myeloid leukemia (AML) [33]. AML patients with high Fli-1 had lower complete remission CR) rate and shorter CR duration, resulting in a significantly shorter overall survival. However, high *Fli-1* expression is good prognostic marker in the human breast cell lines and in a small sample of human primary breast tumors [121]. Loss of Fli-1 has

similar effects as increased migration and invasion in the breast cancer cells and may promote tumor progression. Contrary to these results, another report showed that the expression of Fli-1 contributes to the progression of human breast cancer by up-regulation of the *bcl-2* gene and inhibition of apoptosis [30].

Gene fusion is another contribution of *Fli-1* to malignant transformation. *Fli-1* is the most frequently implicated in chromosomal translocation t(11;22)(q24;q12) [122-128]. Ewing sarcoma is defined by this chromosomal translocations that fuse *EWS* (*EWSR1*), located at 22q12, and a gene of the Ets family of transcription factors. In 85-90% of cases, the fusion gene is *EWS-Fli-1*, which encodes the N-terminal portion of EWS and the C-terminal portion of Fli-1, including the ETS DNA-binding domain. The resultant EWS-Fli-1 chimeric protein includes transactivation domain of EWS and DNA-binding domain of Fli-1. EWS-Fli-1 regulates the expression of a number of genes important for cancer progression and is the causative oncogene. EWS-Fli-1 affects the transcriptome, epigenome and proteome to reprogram cells in disease development. EWS-Fli-1 up regulates target genes such as *c-myc* [123], DNA-binding protein inhibitor 2 (*ID2*), cyclin D1 (*CCND1*), glioma-associated oncogene homolog 1 (*Gli1*) [127], Forkhead box family gene (*FOXM1*) [127], vascular endothelial growth factor A (*VEGFA*), matrix metallopeptidase 3 (*MMP-3*), nuclear receptor subfamily 0, group B, member 1 (*NR0B1*, alias *DAX-1*) [127] and histone methyl-transferase Enhancer of Zeste (*EZH2*), implicated in cell cycle, invasion and proliferation pathways. EWS-Fli-1 functions also as transcriptional repressor and inhibits the expression of some tumor suppressor genes such as $p21^{Cip1,\ Waf1}$, $p57^{Kip1}$, transforming growth factor beta receptor II (*TGF-βRII*), insulin-like growth factor binding protein 3 (*IGFBP3*) and lysine-6-oxidase (*LOX*) [127].

Direct pharmacological inhibition of EWS-Fli-1 to eradicate this cancer is unsuccessful but some new approaches downregulate important target genes. The bromodomain and extra-terminal domain (BET) inhibitors repressed these target genes but EWS-Fli-1 expression was not significantly affected [129-131]. BET proteins (bromodomain /BRD/ containing proteins BRD2, BRD3, BRD4, and the testis-specific BRDT) were found as an important class of epigenetic readers of histone acetylation involved in chromatin remodeling and transcriptional regulation. The human proteome contains 46 bromodomain-containing proteins with a total of 61 bromodomains with conserved structural features [132]. BET inhibitors led to inhibition of Ewing sarcoma cells proliferation and survival. Specific BET inhibitors such as dimethylisoxazole-based compound I-BET151 or triazolodiazepines-based compounds JQ1 and I-BET762 resulted in displacement of BRDs from chromatin and inhibition of transcription at key genes such as *BCL2*, *MYC*, and *CDK6* [129].

Preclinical data suggest that the histone lysine specific demethylase 1 (LSD1) inhibition [133] also disrupts the function of EWS-Fli-1.

Fli-1 is a novel Ets transcription factor involved in gene fusions in prostate cancer. *SLC45A3-Fli-1* fusion was described in prostate cancer [134]. *SLC45A3* exon 1 is fused with *Fli-1* exon 3. As the currently available prostate cancer cell lines do not harbor *Fli-1* overexpression, the development of a specific biological model of the *SLC45A3-Fli-1* fusion is needed to further clarify the oncogenic role *Fli-1* in prostate cancer.

1.4. Fli-1 Inhibitors

Transcription factors have been considered undrugable, but this concept has been recently changed. Several Fli-1 inhibitors are presently used to treat cancers. To identify small molecular weight compounds capable of inhibiting the transactivation ability of Fli-1, luciferase reporter assays were used [135]. The luciferase reporter assay is commonly used as a tool to study gene expression at the transcriptional level. It is widely used because it is convenient, relatively inexpensive, and gives quantitative measurements. A vector with the Fli-1 Ets DNA binding site cloned in front of a minimal promoter, immediately upstream of the luciferase reporter gene was utilized. The construct was co-transfected with the EWS/Fli-1 vector and control empty vector binto human embryonic kidney epithelial cell line 293T cells. Chemical library of about 4500 compounds was screened in this way [135]. Thirty compounds that down regulated EWS/Fli-1 luciferase activity by at least 50% in three independent experiments were found. The six functional drug groups were established from these 30 effective

compounds. Two compounds from the group of chemotherapeutic drugs, etoposide and dactinomycin (actinomycin D), are topoisomerase II inhibitors used to treat diverged cancer types including Ewing sarcoma [136-138]. These drugs kill tumor cells by inhibition of EWS/Fli-1 and by inducing DNA damage. Topoisomerases I and II are enzymes that control the changes in DNA structure by catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands during the cell cycle. Topoisomerase inhibitors block the ligation step of the cell cycle that leads to apoptosis and cell death. Dactinomycin intercalates between adjacent guanine-cytosine base pairs, blocking the transcription of DNA by RNA polymerase. It also causes single-strand DNA breaks, possibly via a free-radical intermediate or an interaction with topoisomerase II.

Most cardiac glycosides (e.g., digitoxin, digoxin, ouabain, and oleandrin) have been isolated from plants, including Digitalis purpurea, Digitalis lanata, Strophanthus gratus, and Nerium oleander. Some cardiac glycosides have also been found in amphibians and mammals, including digoxin, ouabain, bufalin, marinobufagenin, and telecinobufagin [139]. Ten compounds from this group inhibit EWS/Fli-1. Calcimycin (A23187) from the group of calcium ionophores is a rare divalent cation specific ionophore antibiotic that has many biochemical and pharmaceutical applications. Calcimycin inhibits binding of Fli-1 to DNA by regulation of PKCδ phosphorylation of Fli-1.

An aureolic acid natural product mithramycin (MTM, Mithracin®) was validated as a potent antagonists of the oncogenic transcription factor EWS-Fli-1 [140-142]. MTM (also known as plicamycin, trade name Mithracin) is member of tricyclic polyketides family and is produced by *Sreptomyces* plicatus. MTM binds in the minor groove of GC-rich DNA, thereby disrupting transcription of protooncogenes. MTM antagonizes EWS-Fli-1 without affecting its expression levels. MTM likely directly interferes with the EWS-Fli-1 function on DNA. Sub-micromolar concentrations MTMs stabilize Fli-1-DNAcomplex on GGAA repeats, which are critical for the oncogenic function of hybrid protein EWS-Fli-1 [141]. Formation of a ternary complex Fli-1-DNA-MTM on a single GGAA Fli-1/MTM binding site was also demonstrated by nuclear magnetic resonance. Phase I/II trial NCT 01610570 of mithramycin in children and adults with refractory extracranial solid tumors or Ewing sarcoma has been recently done.

1.5. Increased Level of Fli-1 Messenger Rna in Bone Marrow and Blood Mononuclear Cells of Mds Patients with 5q- Syndrome

Patients with the 5q- syndrome, a subtype of myelodysplastic syndrome (MDS) have macrocytic anemia and thrombocythemia with effective through dysplastic megakaryopoiesis. We found an increased level of Fli-1 mRNA in the mononuclear blood and bone marrow cells [55, 143]. We therefore proposed that in this MDS subtype, high Fli-1 preferentially induces differentiation of a common megakaryocytic and erythroid progenitor (MEP) into the megakaryocytic lineage and probably allows effective megakaryopoiesis leading to normal or even increased platelet numbers. Boultwood et al. [144] localized 40 genes to the commonly deleted region (CDR) of the long arm of chromosome 5. Some of the genes on the distal part of the CDR encode micro RNAs (miR-145 and miR-146a) [145]. Kumar et al. [146] identified *Fli-1* as a miR-145 target gene, which was confirmed in colon carcinoma [147] and Ewing sarcoma [148]. Our finding of a high Fli-1 mRNA level in 5q- syndrome was consistent with haploinsufficiency of this miR-145 in 5q- syndrome and stabilization of Fli-1 mRNA level and increased Fli-1 protein levels. Haploinsufficiency of miR-145 and miR-146a leads to increased expression of their target genes, *TIRAP* (Toll/interleukin-1 receptor /TIR/ homology domain-containing adaptor protein) and *TRAF6* (Tumor necrosis factor /TNF/ receptor associated factor 6), and consequently to an upregulation of *IL-6* expression [149]. IL-6 stimulates megakaryocyte differentiation and platelet production [150].

2. Conclusions and Perspectives

Transcription factor Fli-1 is a member of the Ets (E-twenty-six) family of winged helix-turn-helix transcription factors that bind a purine-rich consensus sequence GGA (A/T). Transcriptional activation of the *Fli-1* gene by either chromosomal translocation or proviral insertion leads to Ewing sarcoma in humans and erythroleukemia in mice, respectively. In Ewing sarcoma, the DNA binding (Ets domain) of *Fli-*

1 is translocated from chromosome 11 to the *EWS* locus on chromosome 22, forming a fusion protein with altered expression and transactivation properties. In mice infected with Friend murine leukemia virus, about 75% of the resulting erythroleukemias have insertional activation of the *Fli-1* gene. Thus, dysregulation of the *Fli-1* gene is a common factor in the progression of at least two distinct tumor types.

Transcription factor Fli-1 has important role in normal development and malignant transformation. Fli-1 is necessary for stem cell maintenance and differentiation, hematopoiesis, vasculogenesis and angiogenesis. Fli-1 is required for murine vascular and megakaryocytic development and is frequently hemizygously deleted in patients with thrombocytopenia (Jacobsen and Paris-Trousseau syndrome). The level of Fli-1 during megakaryopoiesis affects thrombopoiesis and platelet biology [151]. It is unknown how deregulated Fli-1 expression alters the balance between erythroid differentiation and proliferation. Fli-1 induces de-differentiation by reverting erythroid colony-forming (CFU-E) cells to erythroid burst-forming (BFU-E) cells in mouse erythroleukemia cells [152]. The nuclear transcription factor Fli-1 has been shown to increase cellular proliferation and tumorigenesis in many types of cancer [29-39, 85, 120, 153-156].

Transcription factors have long been counted as undruggable targets for therapeutics. Enhanced recognition of protein biochemistry as well as the need to have more targeted approaches to treat cancer has taken transcription factors approachable for therapy.It is possible to block protein-DNA or protein-protein interactions. Specific enantiomers, DNA-binding small molecular weight compounds (for exammple YK-4-279 and its individual enantiomers, etoposide, actinomycin D, calcium ionophores, mithramycin) are used or studied in pre-clinical trials. The main problem is that many these drugs are not specific for targeting Fli-1 or EWS-Fli-1 fusion proteins. The bromodomain and extra-terminal domain (BET) inhibitors repressed these target genes but EWS-Fli-1 expression was not significantly affected. BET proteins bind to the N-acetylated lysine residues on histone tails and induce an opened-chromatin structure to enhance transcription of several oncogenes (for example myc). Further progress in this area is required.

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