Gene Section
Review

FLI1 (Friend leukemia virus integration 1)

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Identity

Other names: EWSR2; SIC-1

HGNC (Hugo): FLI1

Location: 11q24.3

Note
NCBI mRNA Reference Sequence:
- NM_008026.4 (mus musculus)
- NM_002017.3 isoform 1 (homo sapiens)
- NM_001167681.1 isoform 2 (homo sapiens)

DNA/RNA

Description

Both the mouse and human fli-1 genes are approximately 120 kb, consist of 9 exons, and encode two protein isoforms, p51 (452 aa) and p48 (419 aa) (Sarrazin et al., 2000). Fli-1 is located on mouse chromosome 9 and human chromosome 11q24.1, a region of several abnormalities in human disease (Ben-David et al., 1990; Truong and Ben David, 2000). The fli-1 gene is located within 240 kb of the ets-1 locus, suggesting that these Ets transcription factors arose by gene duplication from a common ancestral gene (Ben-David et al., 1991). The first fli-1 intron is the largest at approximately 64 kb in length, and the last exon, 9, containing the Ets DNA binding domain, is the largest at approximately 2808 bp in length (Figure 1). The sequence of the 5'-untranslated region (UTR) is located within exon 1, while the sequence of the 3'-UTR is located within exon 9. At least two ATG translation initiation sites have been localized to nucleotide 342 (exon 1) and 441 (exon 2) of the human Fli-1 mRNA sequence (NM_002017.3),

responsible for the generation of the two protein isoforms, p51 and p48 (Sarrazin et al., 2000). The second isoform, p48, has a shorter N-terminus, contains a distinct 5'UTR, and lacks an in-frame portion of the 5' coding region, compared to isoform 1, p51. The sequences flanking the transcription initiation (CAP) sites show 94% conservation between the human and mouse isoforms. The fli-1 promoter contains a potential TATA box element, and multiple regulatory elements. These include GATA, EBS, GT-rich, GC-rich, AP-2, AP-3 and CTC elements, some of which are conserved between mouse and human. The fli-1 promoter also contains binding sites for Sp-1, c-Myc, GATA-1, Ets-2, Oct-3, TBP, PEA-3, EBP, ATF/CREB, and E2A-PBX1 (Barbeau et al., 1996; Barbeau et al., 1999; Dhulipala et al., 1998). The highly conserved 5' non-translated region of exon 1 is predicted to form a very stable hairpin structure, capable of post-transcriptional autoregulation (Barbeau et al., 1996).

Transcription

Transcription of the mouse fli-1 gene produces a full-length mRNA transcript of 3087 bp, and a processed length of 1359 bp. Transcription of the human fli-1 gene produces a full-length mRNA transcript of 3993 and 3941 bp, and a processed length of 1359 and 1260 bp, respectively.

Pseudogene

Although a pseudogene has not been identified for the fli-1 gene, comparison of the amino acid sequences of Fli-1 has revealed an 80% homology to the Ets-related protein Erg, localized adjacent to the ets-2 gene, on mouse chromosome 16 (Watson et al., 1992) and human chromosome 21q22.
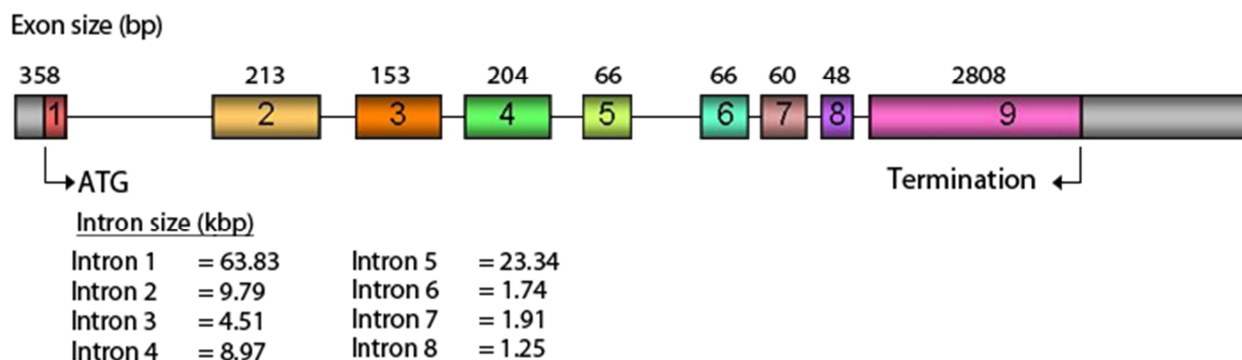
Figure 1: Genomic organization of human fli-1. The fli-1 gene is localized on human chromosome 11q23, contains nine exons extending over approximately 120 kb, with a processed mRNA transcript length of 1359 bp, and encodes two protein isoforms of 452 aa (p51) and 419 aa (p48).

Protein

Description

The fli-1 gene encodes two isoforms of 51 and 48 kDa, synthesized by alternative translation initiation sites, as mentioned above. Loss of function studies have provided evidence to suggest that both the p51 and p48 isoforms retain the same functional domains and activity (Melet et al., 1996). The functional domains located within the Fli-1 protein include the 5' Ets domain, and a Fli-1-specific region (FLS) referred to as the amino terminal transcriptional activation (ATA) domain, and a 3' Ets and carboxy terminal transcriptional activation (CTA) domain (Figure 2). The 5' Ets domain, sharing 82% sequence identity to Erg and 59-60% to Ets-1 and Ets-2, is located within amino acids 121-196. The FLS, which is absent in the Erg protein, is localized within amino acids 205-292. The 3' Ets domain, sharing 98% homology with Erg, is located within amino acids 277-360 and is responsible for sequence specific DNA-binding activity. The CTA domain, located within amino acids 402-452, is also involved in transcriptional activation and protein-protein interaction. Both the 5' and 3' Ets domain contain sequences of helix 1-loop-helix 2 (H-L-H) secondary structures that are also present in Erg (Rao et al., 1993), while the FLS and CTA domains contain sequences which resemble turn-loop-turn (T-L-T) secondary structures. The structures of the ATA and CTA domains contribute to the transcriptional activity of Fli-1. It has been suggested that the CTA region may serve simultaneously as a transcriptional activator and repressor (Rao et al., 1993). Recently, mice engineered to lack the CTA domain of Fli-1 by homologous recombination were shown to express negligible to low levels of the mutant of Fli-1 mRNA and protein (Spyropoulos et al., 2000). Furthermore, the recombinant Fli-1 protein lacking the CTA domain displayed only 50-60% of the transcriptional activity of wild- type Fli-1, providing further evidence of the CTA domain's involvement in transcriptional activation. The reduced levels of mutant Fli-1 in these mice suggest that the CTA domain also functions to autoregulate Fli-1 expression. NMR spectroscopy analyses have shown that the 3' Ets domain of Fli-1 consists of three alpha-helices and a four stranded beta-sheet that resembles the structures of the class of helix-turn-helix DNA-binding proteins found in the catabolite activator protein of Escherichia coli, as well as those of several eukaryotic DNA binding proteins including H5, HNF-3/forkhead, and the heat shock transcription factor (Liang et al., 1994a; Liang et al., 1994b). A comparison of the Fli-1 3' Ets domain to other structures has suggested that this 3' Ets domain uses a new variation of the winged helix-turn-helix motif for binding to DNA (Liang et al., 1994b).

Fli-1 binds to DNA in a sequence-specific manner, and it has been determined that the optimal DNA binding sequence for Fli-1 is ACCGGAAG/aT/c (Solomon and Kaldis, 1998; Mao et al., 1994; Cui et al., 2009). The bases flanking the core GGA Ets DNA-binding motif synergistically contribute to binding specificity among different Ets transcription factors. Gene promoters containing these Ets sequences have been shown to be transcriptionally regulated by Fli-1, including bcl-2 (Lesault et al., 2002), MDM2 (Truong et al., 2005), gpIX, gpIIb (Bastian et al., 1999), mpl (Deveaux et al., 1996), and recently SHIP-1 (Lakhanpal et al., 2010).

Phosphorylation of both Fli-1 protein isoforms has been predominately detected on serine residues. This phosphorylation is modulated by the concentration of intracellular calcium, similar to Ets-1 and Ets-2, and dephosphorylation is controlled, at least in part, by the phosphatase PP2A (Zhang and Watson, 2005). Post-translational modification by phosphorylation effects Fli-1 DNA binding, protein-protein interaction and transcriptional activation, thereby contributing to the control of gene function (Zhang and Watson, 2005; Asano and Trojanowska, 2009).

Expression

Fli-1 is highly expressed in all hematopoietic tissues and endothelial cells, and at a lower level in the lungs, heart and ovaries (Ben-David et al., 1991; Melet et al., 1996; Hewett et al., 2001; Pusztaszeri et al., 2006). The

ubiquitous expression of Fli-1 in all endothelial cells (Hewett et al., 2001) suggests a role for Fli-1 in endothelial cell fate and angiogenesis. It has been suggested that Fli-1 is the first dependable nuclear marker of endothelial differentiation (Rossi et al., 2004), is essential for embryonic vascular development (Spyropoulos et al., 2000), and acts as a master regulator establishing the blood and endothelial programmes in the early embryo (Gaikwad et al., 2007; Liu et al., 2008). Moreover immunohistochemical analysis has revealed that Fli-1 expression is a valuable tool in the diagnosis of benign and malignant vascular tumors.

Localisation

Similar to other Ets proteins, Fli-1 is a nuclear transcription factor and is generally localized within the nucleus, although Fli-1 protein has also been detected in the cytoplasm of specific cell types (Cui et al., 2009; Pusztaszeri et al., 2006).

Function

Fli-1 plays an important role in erythropoiesis. The constitutive activation of fli-1 in erythroblasts leads to a dramatic shift in the Epo/Epo-R signal transduction pathway, blocking erythroid differentiation, activating the Ras pathway, and resulting in massive Epo-independent proliferation of erythroblasts (Tamir et al., 1999; Zochodne et al., 2000). These results suggest that Fli-1 overexpression in erythroblasts alters their responsiveness to Epo and triggers abnormal proliferation by switching the signaling event(s) associated with terminal differentiation to proliferation (Zochodne et al., 2000). The constitutive suppression of Fli-1, mediated through RNA interference or dominant negative protein expression has revealed an essential role for continuous Fli-1 overexpression in the maintenance and survival of the malignant phenotype in both murine and human erythroleukemia (Cui et al., 2009).

Homology

Fli-1 is a member of the Ets transcription factor gene family. It is most related to Erg, located on mouse chromosome 16 and human chromosome 21. Similar to Fli-1, Erg is also activated through chromosomal translocation in human cancer. In prostate cancer, TMPRSS2 generates a fusion with ETV1, ETV4 and ETV5, and Erg share 98% homology within the Ets DNA binding domain. The fli-1 gene is conserved in human, mouse, chimpanzee, dog, cow, rat, chicken and zebrafish.

Mutations

Note

Fli-1 aberrant regulation is often associated with malignant transformation. Fli-1 was first identified as a target of proviral integration in F-MuLV-induced erythroleukemia (Ben-David et al., 1990). In addition to Friend erythroleukemia, integration at the fli-1 locus also occurs in leukemias induced by the 10A1 (Ott et al., 1994), Graffi (Denicourt et al., 1999), and Cas-Br-E viruses (Bergeron et al., 1991). While Fli-1 activation is associated with viral integration in mice, it is also associated with chromosomal abnormalities in humans. In Ewing's sarcoma and primitive neuroectodermal tumors a chromosomal translocation results in a chimeric EWS/Fli-1 fusion protein, containing the 5' region of EWS and the 3' ETS region of Fli-1 (Delattre et al., 1992). This oncoprotein acts as an aberrant transcriptional activator with strong transforming capabilities. In addition, Fli-1 has been implicated in human leukemias, such as Acute Myelogenous Leukemia (AML), involving loss or fusion of the tel gene (Kwiatkowski et al., 1998). Binding of wildtype Tel, an ETS transcription factor, to Fli-1 inhibits Fli-1's regulatory function. Therefore loss of the tel gene or generation of the Tel-AML fusion protein by chromosomal translocation eliminates the normal regulation of Fli-1 leading to an increase in Fli-1 activity. Tissue microarray and immunohistochemistry has also revealed the expression of Fli-1 in a wide variety of benign and malignant tumors, such as capillary hemangioma, neuroblastoma, small cell lung carcinoma, glioblastoma, medullar breast carcinoma, non-Hodgkin's lympoma, angiosarcoma, Kaposi's sarcoma and lymphoblastic lymphomas (Mhawech-Fauceglia et al., 2006).
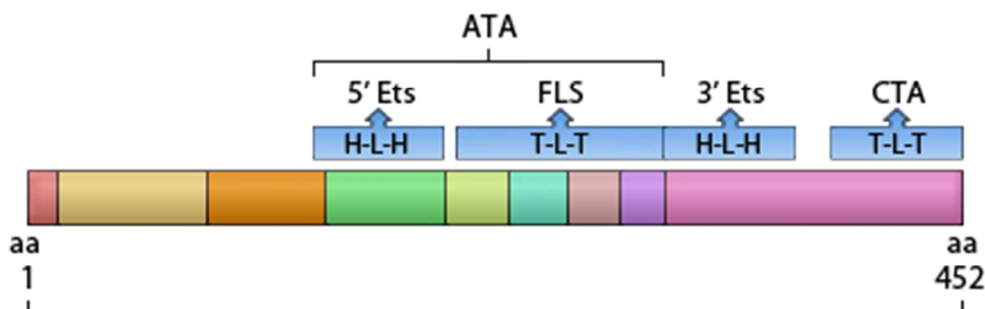

Figure 2: Fli-1 functional domains. Both human and murine Fli-1 proteins consist of 452 amino acids (aa) which contain the following domains: ATA: amino-terminal transcriptional activation domain, FLS: Fli-1 specific domain, CTA: carboxy-terminal transcriptional activation domain, H-L-H: helix-loop-helix structure, and T-L-T: turn-loop-turn structure.

Implicated in

Ewing's sarcoma and primitive neuroectodermal tumors

Note

Ewing's sarcoma is a malignant bone tumor affecting children. Such tumors usually develop during puberty, however there are few symptoms. Primitive neuroectodermal tumors are a rare group of tumors originating in cells from the primitive neural crest usually found in children under 10 years of age.

Prognosis

Since the EWS gene is fused to the fli-1 gene in the majority of Ewing's sarcomas, primers spaning these genes are used to amplify the junction for genetic diagnosis (Downing et al., 1995).

Oncogenesis

Expression of the EWS/Fli-1 fusion gene in the majority of Ewing's sarcomas is shown to be critical for cancer induction. Downregulation of this fusion oncoprotein using RNA interference inhibits cell growth and promotes apoptosis (Dohjima et al., 2003).

Jacobsen or Paris-Trousseau syndrome

Note

A relatively infrequent congenital disorder in which the fli-1 gene is commonly deleted. Clinical abnormalities include growth and mental retardation, cardiac defects, dysmorphogenesis of the digits and face, pancytopenia, and thrombocytopenia (Krishnamurti et al., 2001; Favier et al., 2003; Wenger et al., 2006).

Prognosis

Chromosomal deletions result in Fli-1 deficiency.

Systemic lupus erythematosus (SLE)

Note

A chronic autoimmune disease with variable symptoms, commonly affecting the skin, joints, kidneys, heart and lungs.

Prognosis

SLE patients display Fli-1 overexpression in peripheral blood leukocytes (Mayor et al., 2000). Interestingly, Fli-1 overexpression also occurs in the MRL/lpr murine lupus model, and a 50% reduction of Fli-1 levels markedly prolongs survival and significantly reduces renal disease in these mice (Mayor et al., 2000).

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