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Gene Section

Review

NUDT6 (nudix (nucleoside diphosphate linked moiety X)-type motif 6)

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Identity

Other names: ASFGF2, bFGF, FGF-2, FGF-AS, FGF2AS, gfg, gfg-1
HGNC (Hugo): NUDT6
Location: 4q28.1
Note: NUDT6 is a novel nudix protein with unknown function.

DNA/RNA

Note
Human NUDT6 is located on chromosome 4 in the region of q28 on the reverse strand, opposite to FGF-2 gene locus. FGF-2 and NUDT6 genes overlap at 3' ends, and the mRNAs form a sense-antisense pair. The NUDT6 mRNA (referred to as FGF-AS) has been implicated in the regulation of FGF2 mRNA stability.

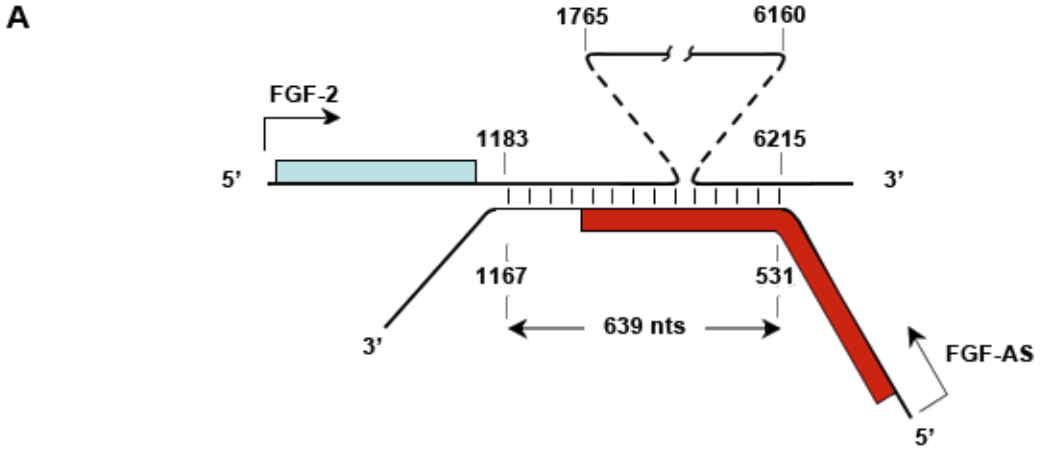

Figure A. The schematic representation of the overlap between human NUDT6 (FGF-AS) and FGF2 gene transcripts (colored boxes, coding region; connecting vertical lines, complementary regions between transcripts). Adapted from: MacFarlane LA, et al., 2010. Molecular Endocrinology 24.

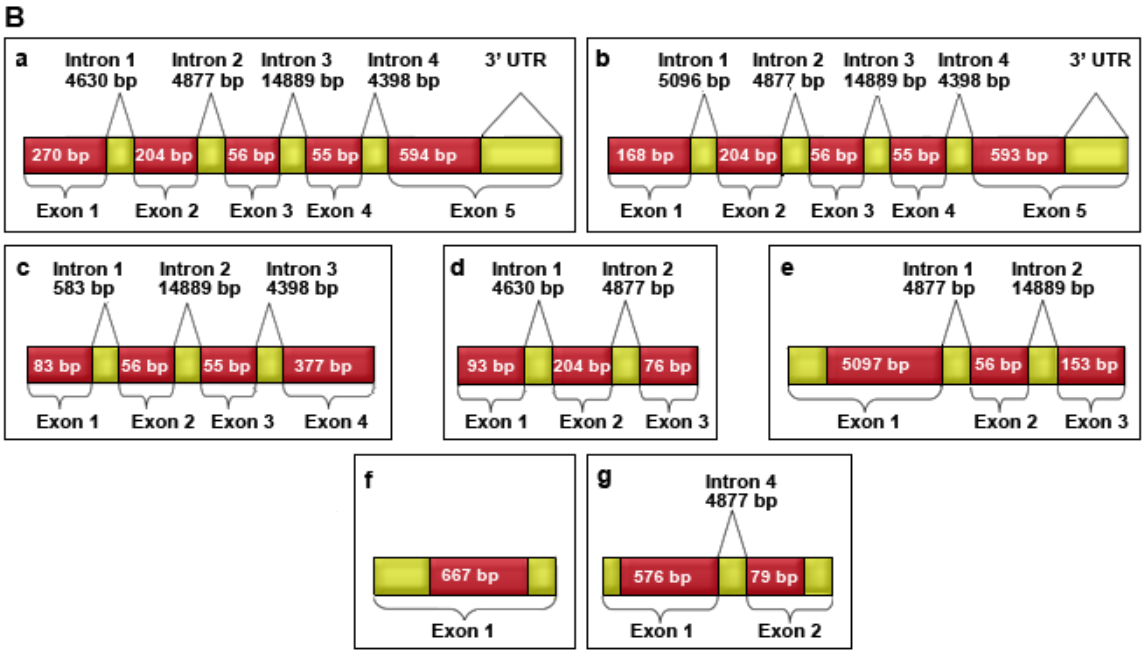

Figure B. The schematic representation of the human NUDT6 gene transcripts, variants a-g (red boxes, coding region; yellow boxes, untranslated region).

Description

The human NUDT6 gene is 34271 bp in length, composed of a 5'UTR, 16 exons, 6 introns and a 3'UTR. The 5' and 3'UTR contain a variety of regulatory elements that regulate NUDT6 expression. NUDT6 gene transcription is regulated by a core promoter mapping from -1871 to +181 (relative to the transcription start site +1, up-stream -), which is 44 kb downstream from the FGF-2 promoter, however the proximal -151/+181 region confers almost full transcription activity.

The promoter lacks a consensus TATA box or CCAAT element. The region between the first two exons contains two Sp1 transcription factor binding sites (-372/-58 relative to the first exon start site). The common upstream region (all subsequent positions relative to first exon start site) from these start sites contains a multitude of tissue specific transcription factor binding sites, which include lymphocyte specific factors Ets at -229 and -83, GATA at -662 and +56, Lyf-1 at -981; skeletal muscle consensus E-boxes at -901 and +30; cardiac factor Nkx-2.5 at -1501 and -582; liver and adipose C/EBP factor at -624; and testis specific factors SRY at -1740, -671, +163, +171 and Sox-5 at -1472, -632.

Two negative regulatory elements also reside in this shared upstream region, at -1871 and -1315. The NUDT6 3'UTR contains a singe AU-rich element (ARE) and seven AU-rich-like sequences which negatively regulate mRNA stability. A portion of the NUDT6 coding region and 3'UTR (+531/+1167) is fully complementary to the 3'UTR of FGF2 and interaction through this region leads to the formation of a sense-antisense pair.

Transcription

The primary transcript can be alternatively spliced to produce at least 7 splice variants, a-d. Full length variants a and b only differ in the use of an alternative first exon, designated 1A or 1B. The 3' ends of the variants share sequence similarity. Two transcriptional start sites have been identified, one 15 bp upstream of the 1A exon (designated +1) and the other 84 bp upstream of the 1B exon (+312). It is unclear whether another transcription start site specific for other variants are located further downstream.

NUDT6 transcripts are often designated FGF-AS (FGF antisense). The two longest transcripts, a and b, are classified as cis-antisense because they are transcribed from the same gene locus, on the opposite DNA strand and their 3'UTR is fully complementary to the 3'UTR of FGF-2 over two regions 583 bp and 56 bp in length.

Pseudogene

NA.

Protein

Note

Human NUDT6 encodes 3 novel nudix proteins with unknown function.

Description

NUDT6 splice variants a-c contain open reading frames (ORF) that predict isoforms of a novel nudix motif protein, originally designated GFG. The nudix box motif is defined by the consensus signature amino acid sequence

$GX_5EX_7REUXEEXGU$,

Schematic representing predicted NUDT6 isoforms encoded by alternate splice RNA transcripts (deep blue boxes, nudix motif; light blue box, MTSP-mitochondrial targeting signal peptide).

where X is any amino acid and U is a bulky hydrophobic amino acid, usually isoleucine, leucine or valine. To date, three different molecular weight isoforms have been identified in human, of 35, 28 and 17 kDa, which are presumably generated by alternative translation initiation. Isoforms are designated as a, b or c however, this does not necessarily indicate that the isoform was synthesized from the corresponding transcript variant. The 35 kDa isoform is synthesized from the full length FGF-ASa, by translation initiation from the in-frame AUG codon located in exon 1A. The origin of the 28 kDa isoform is unclear. It is suspected that it is synthesized from an in-frame CUG codon in exon 2 of either FGF-AS a or b.

However, it is possible that the 28 kDa product is a proteolytic fragment of the 35 kDa isoform. The 17 kDa isoform may arise from translation initiation at an in-frame CUG codon in exon 3 of FGF-ASb or AUG codon in the first exon of FGF-ASc. The NUDT6 isoforms are detected as stable homo- and hetero-dimers by western blotting, which can be disrupted by dithiothreitol (DTT) and boiling. Potential dimerization domains have been mapped to both the N-terminus and COOH-terminus of NUDT6.

Expression

NUDT6 is expressed in a tissue and developmental stage specific manner. RNA transcripts are detected in most human tissues including liver, thymus, spleen, peripheral blood leukocytes, heart skeletal muscle, testis, colon and kidney. However, which transcript variants are expressed appears to be tissue specific. The full length FGF-ASb is thought to be the predominant variant in most tissues however variant FGF-ASa is the major variant in normal hematopoietic tissues. Furthermore, some tissues co-express FGF-2 and the ratio between FGF-2 and FGF-AS transcripts varies with tissue and development stage. FGF-AS levels are relatively low in many embryonic tissues, with expression increasing dramatically in a tissue specific manner postnatally. FGF-2 and FGF-AS exhibit an inverse relationship in normal tissues, tumor cell lines, embryonic development and throughout cell cycle progression.

The level of NUDT6 expression and its ratio with FGF-2 expression is frequently altered in tumors. Normal pituitary expresses moderate levels of NUDT6 and no FGF-2 while pituitary tumors have reduced NUDT6 expression and high levels of FGF-2. The NUDT6/FGF-2 expression ratio decreases dramatically in tumors compared to normal tissue. Varying NUDT6/FGF-2 ratios have also been observed in esophageal adenocarcinomas. Additionally, transient increase in NUDT6 expression occurs in response to treatment with interleukin-2 and prolactin.

Localisation

NUDT6 can reside in the mitochondria, cytoplasm and nucleus, however its subcellular localization varies with isoform, cell type, disease state and extracellular stimulus. NUDT6a predominantly localizes to mitochondria whereas NUDT6b and NUDT6c primarily reside in the cytoplasm and nucleus. NUDT6 is only found in the cytoplasm of normal esophageal squamous epithelial cells whereas in normal lymphocytes it is exclusively nuclear. However, transformation of these cells results in the redistribution of NUDT6. Cells from esophageal adenocarcinoma tumors and lymph nodes of patient with immunoblastic lymphoma localize NUDT6 to the nucleus and cytoplasm.

Function

The RNA and protein products appear to have distinct biological functions. NUDT6 mRNA (FGF-AS) plays a role in FGF-2 regulation, proliferation, and cell survival. Additionally, the NUDT6 protein has been implicated in the control of hormone production in the pituitary, and possibly in the removal of potentially hazardous compounds and metabolites by virtue of its conserved nudix domain. However, it is not always clear whether a specific action is a result of the RNA or protein function and this is further complicated by multiple antisense splice variants and protein isoforms.

FGF-AS regulates FGF-2 transcript stability. Although the details of the mechanism involved are unclear, evidence suggest involvement of RNA interference and/or a dsRNA duplex formed between the 3'UTRs of FGF-AS and FGF-2. In addition to regulating FGF-2 abundance it has been suggested that it also controls FGF-2 isoform translation and localization. The regulatory role of FGF-AS over FGF-2 is thought to

account for observed effects on cell proliferation and survival.
NUDT6 protein is a nudix hydrolase which is a class of "house cleaning" enzymes capable of hydrolyzing a broad range of substrates, all defined as nucleoside diphosphates linked to some other moiety, that include nucleoside di- and triphosphates, dinucleoside and diphosphoinositol polyphosphates, nucleotide sugars and RNA caps. The substrate of human NUDT6 has yet to be elucidated and therefore its physiological function remains unknown. NUDT6 has observed effects on cell proliferation independent of those associated with FGF-AS. NUDT6 overexpression in human colorectal cancer cells increases proliferation. Perhaps NUDT6's effects on proliferation are dependent on expression level, isoform and/or cell type, as is the case with FGF-2. Furthermore, NUDT6 is involved in hormone production. GFG expression can increase levels of prolactin. However it is unclear if these effects are mediated through the same MAPK pathway utilized by FGF-2 to increase prolactin expression.
Additionally, NUDT6 alters the isoform ratio of growth hormone, by increasing synthesis of the 22 kDa isoform and not the 20 kDa.

Homology

NUDT6 contains a conserved nudix motif common to other members of the Nudix family of phosphohydrolases. The Nudix motif is GXXXXXEXXXXXXXREUXEEXGU where U is Isoleucine, Leucine, or Valine and X is any amino acid. NUDT6 is highly conserved among man, cow, mouse, worm, and fruit fly, and GFG homologs across species are more evolutionarily related to each other than to other nudix proteins from the same species.

Implicated in

Esophageal adenocarcinoma

Note
Esophageal adenocarcinoma refers to uncontrolled growth of glandular cells in the esophagus and the junction between the esophagus and the stomach.
Prognosis
Elevated expression of FGF-AS in FGF-2 expressing esophageal adenocarcinomas is associated with reduced tumor reoccurrence following surgical resection of tumors and increased survival rates, suggesting that it may be used as a prognostic indicator.
Oncogenesis
Esophageal adendocarcinoma tumors overexpressed FGF-AS and cytoplasmic GFG in comparison to normal match esophageal tissue. However, the reduced tumor reoccurrence and improved survival rates specifically correlated to FGF-AS levels, not GFG levels. Evidence suggests FGF-AS tumor suppressive role is a result of its post-transcription control over FGF-2.

Melanoma

Note
Melanoma is a malignant tumor of melanocytes, which are found primarily in the skin, however they can develop in melanocytes found in the eye and bowel. A characteristic of aggressive melanomas is their ability to form fluid-conducting vasculogenic-like networks.
Oncogenesis
A preliminary study investigating these 3D tubular networks within tumors identified NUDT6 as one of the many genes overexpressed in aggressive melanomas and speculated it is involved in promoting self-renewal and tumor cell plasticity in melanoma cancer networks. Additionally, they suggest FGF-AS could play a role in the development of the endothelia-lined vasculature networks in melanomas indirectly through its regulatory control over FGF-2 expression, which is associated with angiogenesis, proliferation and survival.

Colorectal cancer

Note
Colorectal cancer refers to uncontrolled growth of cells that line the colon, rectum and appendix, collectively the large intestine.
Oncogenesis
Induced overexpression of NUDT6 in a variety of human colorectal cells significantly increases cancer cell proliferation and their clonogenic capacity. NUDT6 is described as having tumor promoting functions in this cellular environment and it is suggested that it plays a role in colorectal cancer development and progression.

Endometriosis

Note
Endometriosis is a medical condition affecting the endometrium lining of the uterus. The endometrium is comprised of hormonally responsive cells that proliferate and secrete under the influence of estrogen and progesterone. Upon menstruation the endometrium lining is shed as a part of the menstrual flow. Endometriosis describes the presence of endometrial cells outside of the uterus, such as the ovaries, fallopian tubes, bladder and interstitial space in the abdominal cavity.
Patients with endometriosis lesions have reduced FGF-AS-b mRNA levels and elevated FGF-2 mRNA levels during the late proliferative phase of the menstrual cycle, compared to control patients. This increased FGF/FGF-AS ratio is thought to contribute to the development of endometriosis.

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