FHIT

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Synonyms

Fragile histidine triad gene

Historical Background

To date, approximately 80 common fragile sites (CFS) have been identified in the human genome (Ruiz-Herrera et al. 2006). Unlike rare fragile sites, which map to sites that are genetically altered in individuals, CFS occur at consistent genomic locations in particular cell types, which are defined by the paucity of replication initiation events at these loci (Letessier et al. 2011). DNA breaks at CFS, referred to as expression of the fragile site, can be induced *via* treatment with inhibitors of DNA replication, carcinogens, and environmental stresses (Lukusa and Fryns 2008). Several CFS colocalize with common deletions and translocations in tumors. Early efforts to find tumor suppressor genes encoded within fragile sites focused on *FRA3B* at chromosomal location 3p14.2, the most frequently expressed human CFS. Perturbations at 3p14.2 had been noted in many cancers and the site includes the t(3;8) translocation breakpoint associated with hereditary clear cell renal carcinoma (cRCC) (Cohen et al. 1979; Dreijerink et al. 2001). Positional cloning identified the fragile histidine triad gene, *FHIT*, as the gene that spans *FRA3B* (Ohta et al. 1996). In addition to losses of *FHIT* on the short arm of chromosome 3, many cRCC malignancies have been subject to gene losses including *RASSF1A* at 3p21.3 (Dreijerink et al. 2001) and *VHL* at 3p25 (Latif et al. 1993).

FHIT is a Tumor Suppressor Gene

Numerous cancer cell lines and primary tumors exhibit both homo- and hemizygous deletions within the *FHIT* gene. These deletions are concomitant with loss of mRNA expression in esophageal, stomach, colon, kidney, breast, and lung cancer cells. Immunohistochemistry of several types of primary tumors, including esophageal cancers, colon lesions, and cervical and lung cancers show frequent loss or absence of Fhit protein expression (Saldivar et al. 2010). This evidence notwithstanding, acceptance of *FHIT* as a tumor suppressor gene initially met with resistance, as loss of genetic material at a fragile site could be attributed as a consequence of genomic instability rather than a cause of cancer. However, accumulated evidence indicates that loss of Fhit is selected in carcinogenesis.

Mouse modeling and functional analysis has been used to show that *FHIT* mutations are drivers of malignancy. The murine *Fhit* locus shares key similarities with human *FHIT*: *Fhit* encompasses a fragile site, *Fra14A2*, and *Fhit* expression is altered in several murine cancer cell lines, suggesting that a mouse model of *Fhit* inactivation would be relevant to human disease (Glover et al. 1998). Mice heterozygous and homozygous at *Fhit* alleles have been established. These mice are fertile, live normal life spans, and do not present with gross defects, although an immune deficiency related to a low granulocyte count has been noted (Zanesi et al. 2001). *Fhit* heterozygous mice possess increased susceptibility to development of carcinogen-induced tumors. Oral administration of the carcinogen *N*-nitrosomethylbenzylamine (NMBA) produces tumors in 25% of a wild-type cohort of mice, while administration of an equivalent dose produces tumors in 100% of *Fhit* heterozygotes (Fong et al. 2000). *Fhit* heterozygous and *Fhit*-deficient mice are also more susceptible to spontaneous tumor development (Zanesi et al. 2001).

Gene therapy studies established that *Fhit* re-expression not only prevents development of cancer, but also contributes to tumor regression via apoptosis. Delivery of *Fhit* via adenovirus (Ad-*Fhit*) or adeno-associated virus (AAV-*Fhit*) significantly inhibits tumor development after NMBA treatment (Dumon et al. 2001). Immunohistochemical staining of tissue resected from sacrificed animals showed opposite Bax and Bcl2 staining in control versus Fhit-expressing animals, suggesting that failure to induce apoptosis contributed to tumor development in control animals while apoptosis due to delivery of Fhit in vector-treated animals contributed to tumor regression (Ishii et al. 2003).

Fhit Encodes a Dinucleoside Polyphosphate Hydrolase

Fhit and its homologs constitute one branch of histidine triad (HIT) proteins that have been strongly conserved throughout evolution. HIT proteins encode enzymes that bind and hydrolyze or phosphorylyze unusual nucleotide substrates. The HIT motif (His-Ø-His-Ø-His-Ø-Ø, in which Ø is a hydrophobic amino acid) forms part of the active site (Brenner 2002). Fhit protein and its orthologs are dinucleoside polyphosphate hydrolases, enzymes which bind and cleave a class of dinucleotides, typically containing two adenosines joined by 5′-5′ bridges of 3–5 phosphates. The favored substrate of Fhit is ApppA (Barnes et al. 1996) and the substrate typically used in vitro is GpppBODIPY (Draganescu et al. 2000). Hydrolysis of such compounds produces a nucleoside monophosphate product (e.g., AMP or GMP) plus the other nucleotide or analog (e.g., ADP or ppBODIPY).

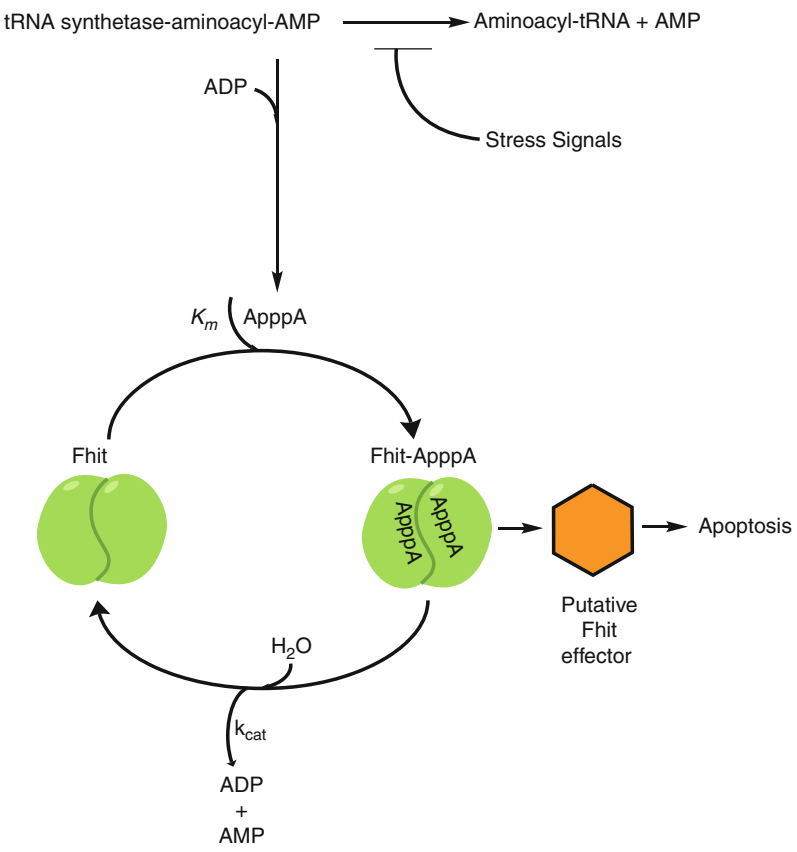
Fhit is a dimer, which contains two ApppA-binding sites per dimer. (Pace et al. 1998). ApppA molecules are by-products of reactions catalyzed by tRNA synthetases and are proposed to have several intracellular functions, including signaling stress responses (Campiglio et al. 2006). Interestingly, intracellular concentration of ApppA increases in mammalian cells exposed to the carcinogenic metal cadmium and the apoptosis-inducing topoisomerase poison etoposide (Fisher and McLennan 2008). Experiments were designed to determine whether the tumor-suppressing function of Fhit depends on ApppA-binding, hydrolysis, or both Appp-binding and hydrolysis. Re-expression of Fhit in Fhit-null cancer cells suppresses tumorigenicity in a manner that is largely insensitive to mutation of the active-site His (His96) to Asn (Siprashvili et al. 1997). This result suggested that the tumor suppressing function of Fhit might be independent of the ApppA substrate. However, a Fhit allele series was created to test the hypothesis that substrate binding but not hydrolysis is limiting for the pro-apoptotic activity of Fhit re-expression. Indeed, as mutations were created to reduce substrate binding, the pro-apoptotic activity of Fhit re-expression was reduced. In contrast, in mutations targeted to the active site His, which reduced catalytic activity by as much as 100,000-fold and reduced substrate binding by two-fold, only modest reductions in apoptotic activities were observed (Trapasso et al. 2003). As illustrated in Fig. 1, these data were interpreted to suggest that formation of a Fhit-ApppA substrate complex is limiting for interaction with a pro-aptoptic effector (Pace et al. 1998).

The Fhit Protein Interactome

Protein association studies have attempted to place Fhit into specific cellular pathways that might depend on diadenosine polyphosphates for signaling. Both wild-type Fhit and the catalytically inactive H96N mutant were shown to interact with tubulin in vitro and promote microtubule assembly in the presence of the microtubule binding proteins MAP2 and Tau (Chaudhuri et al. 1999). In addition, yeast two-hybrid data suggest that Fhit interacts with the SUMO conjugating enzyme, Ubc9 (Shi et al. 2000). Biochemical assays have confirmed an interaction in vitro with one report (Golebiowski et al. 2004) but not another (Shi et al. 2000) indicating ApppA-dependent protein binding. Another appealing model is that Fhit may bind and destablize Mdm2, an E3 ubiquitin ligase that marks p53 for degradation (Nishizaki et al. 2004).

Fhit and a member of the nitrilase superfamily are encoded as fusion proteins in worms and flies (Pekarsky et al. 1998), suggesting that Fhit and Nit1, the ortholog of the nitrilase-related domain may participate in the same pathway and associate as seen in the crystal structure of the worm NitFhit protein (Pace et al. 2000). The data suggest that like Fhit, Nit1 functions as a tumor suppressor. The *Nit1* knockout mouse shows increased susceptibility to NMBA-induced carcinogenesis and increased cell growth in culture (Semba et al. 2006). Moreover, loss of Fhit and loss of Nit1 are apparently additive for tumor suppression (Sun et al. 2009).

The Holy Grail in the molecular oncology of Fhit would be a set of protein interactions that provide a mechanism by which epithelial cells acquiring inactivating mutations in *FHIT* would obtain a survival advantage. The mechanism would also have to account for how Fhit re-expression induces apoptosis in a manner that depends on diadenosine polyphosphate binding. Though it is almost axiomatic that no unifying theory is accepted, there is one proposed mechanism notable for its far-reaching scope. According to this mechanism, Fhit transits to mitochondria *via* interaction with Hsp60 where it interacts



FHIT, Fig. 1 Stress is proposed to inhibit completion of the tRNA synthetase-catalyzed reaction, resulting in increased cytoplasmic ApppA. Fhit dimers, normally in the inactive state, now bind ApppA, forming pro-apoptotic Fhit-ApppA complexes. Fhit-ApppA complexes signal apoptosis *via* a putative Fhit effector. Cleavage of ApppA is proposed to terminate the pro-apoptotic state of Fhit

with and stabilizes ferredoxin reductase (Fdxr) (Trapasso et al. 2008). Fdxr transfers electrons from NADPH to cytochrome P450 via ferredoxin. Under stress, Fdxr levels increase, leading to depletion of NADPH, which is required to detoxify reactive oxygen species (ROS), thereby inducing ROS-mediated apoptosis. Consistent with the expectation that diadenosine polyphosphate binding is limiting for the tumor suppressing function of Fhit, the investigators have shown that mutations that reduce substrate binding also reduce mitochondrial localization, association with Hsp60 and Fdxr, and cellular responses to oxidative damage (Pichiorri et al. 2009).

Summary

Genetic analysis of Fhit has indicated that reintroduction of this protein induces apoptosis in cancer cells with *FHIT* deletions, which are a common cancer genotype. Challenges remain in defining the specific consequences of Fhit inactivation in the preneoplastic epithelial cells from which Fhit is typically lost and in defining the relationship between diadenosine polyphosphate signaling and cellular survival in cells, early in the process of carcinogenesis.

Cross-References

▶ p53

References

Barnes LD, Garrison PN, Siprashvili Z, Guranowski A, Robinson AK, Ingram SW, et al. Fhit, a putative tumor suppressor in humans, is a dinucleoside 5′,5‴-P1, P3-triphosphate hydrolase. Biochemistry. 1996;35(36):11529–35.

Brenner C. Hint, Fhit, and GalT: function, structure, evolution, and mechanism of three branches of the histidine triad superfamily of nucleotide hydrolases and transferases. Biochemistry. 2002;41(29):9003–14.

Campiglio M, Bianchi F, Andriani F, Sozzi G, Tagliabue E, Menard S, et al. Diadenosines as FHIT-ness instructors. J Cell Physiol. 2006;208(2):274–81.

Chaudhuri AR, Khan IA, Prasad V, Robinson AK, Luduena RF, Barnes LD. The tumor suppressor protein Fhit. A novel interaction with tubulin. J Biol Chem. 1999;274(34):24378–82.
Cohen AJ, Li FP, Berg S, Marchetto DJ, Tsai S, Jacobs SC, et al. Hereditary renal-cell carcinoma associated with a chromosomal translocation. N Engl J Med. 1979;301(11):592–5.
Draganescu A, Hodawadekar SC, Gee KR, Brenner C. Fhit-nucleotide specificity probed with novel fluorescent and fluorogenic substrates. J Biol Chem. 2000;275(7):4555–60.
Dreijerink K, Braga E, Kuzmin I, Geil L, Duh FM, Angeloni D, et al. The candidate tumor suppressor gene, RASSF1A, from human chromosome 3p21.3 is involved in kidney tumorigenesis. Proc Natl Acad Sci USA. 2001;98(13):7504–9.
Dumon KR, Ishii H, Fong LY, Zanesi N, Fidanza V, Mancini R, et al. FHIT gene therapy prevents tumor development in Fhit-deficient mice. Proc Natl Acad Sci USA. 2001;98(6):3346–51.
Fisher DI, McLennan AG. Correlation of intracellular diadenosine triphosphate (Ap3A) with apoptosis in Fhit-positive HEK293 cells. Cancer Lett. 2008;259(2):186–91.
Fong LY, Fidanza V, Zanesi N, Lock LF, Siracusa LD, Mancini R, et al. Muir-Torre-like syndrome in Fhit-deficient mice. Proc Natl Acad Sci USA. 2000;97(9):4742–7.
Glover TW, Hoge AW, Miller DE, Ascara-Wilke JE, Adam AN, Dagenais SL, et al. The murine Fhit gene is highly similar to its human orthologue and maps to a common fragile site region. Cancer Res. 1998;58(15):3409–14.
Golebiowski F, Szulc A, Szutowicz A, Pawelczyk T. Ubc9-induced inhibition of diadenosine triphosphate hydrolase activity of the putative tumor suppressor protein Fhit. Arch Biochem Biophys. 2004;428(2):160–4.
Ishii H, Zanesi N, Vecchione A, Trapasso F, Yendamuri S, Sarti M, et al. Regression of upper gastric cancer in mice by FHIT gene delivery. FASEB J. 2003;17(12):1768–70.
Latif F, Tory K, Gnarra J, Yao M, Duh FM, Orcutt ML, et al. Identification of the von Hippel-Lindau disease tumor suppressor gene. Science. 1993;260(5112):1317–20.
Letessier A, Millot GA, Koundrioukoff S, Lachages AM, Vogt N, Hansen RS, et al. Cell-type-specific replication initiation programs set fragility of the FRA3B fragile site. Nature. 2011;470(7332):120–3.
Lukusa T, Fryns JP. Human chromosome fragility. Biochim Biophys Acta. 2008;1779(1):3–16.
Nishizaki M, Sasaki J, Fang B, Atkinson EN, Minna JD, Roth JA, et al. Synergistic tumor suppression by coexpression of FHIT and p53 coincides with FHIT-mediated MDM2 inactivation and p53 stabilization in human non-small cell lung cancer cells. Cancer Res. 2004;64(16):5745–52.
Ohta M, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, et al. The FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t(3;8) breakpoint, is abnormal in digestive tract cancers. Cell. 1996;84(4):587–97.
Pace HC, Garrison PN, Robinson AK, Barnes LD, Draganescu A, Rosler A, et al. Genetic, biochemical, and crystallographic characterization of Fhit-substrate complexes as the active signaling form of Fhit. Proc Natl Acad Sci USA. 1998;95(10):5484–9.
Pace HC, Hodawadekar SC, Draganescu A, Huang J, Bieganowski P, Pekarsky Y, et al. Crystal structure of the worm NitFhit Rosetta stone protein reveals a Nit tetramer binding two Fhit dimers. Curr Biol. 2000;10(15):907–17.
Pekarsky Y, Campiglio M, Siprashvili Z, Druck T, Sedkov Y, Tillib S, et al. Nitrilase and Fhit homologs are encoded as fusion proteins in Drosophila melanogaster and Caenorhabditis elegans. Proc Natl Acad Sci USA. 1998;95(15):8744–9.
Pichiorri F, Okumura H, Nakamura T, Garrison PN, Gasparini P, Suh SS, et al. Correlation of fragile histidine triad (Fhit) protein structural features with effector interactions and biological functions. J Biol Chem. 2009;284(2):1040–9.
Ruiz-Herrera A, Castresana J, Robinson TJ. Is mammalian chromosomal evolution driven by regions of genome fragility? Genome Biol. 2006;7(12):R115.
Saldivar JC, Shibata H, Huebner K. Pathology and biology associated with the fragile FHIT gene and gene product. J Cell Biochem. 2010;109(5):858–65.
Semba S, Han SY, Qin HR, McCorkell KA, Iliopoulos D, Pekarsky Y, et al. Biological functions of mammalian Nit1, the counterpart of the invertebrate NitFhit Rosetta stone protein, a possible tumor suppressor. J Biol Chem. 2006;281(38):28244–53.
Shi Y, Zou M, Farid NR, Paterson MC. Association of FHIT (fragile histidine triad), a candidate tumour suppressor gene, with the ubiquitin-conjugating enzyme hUBC9. Biochem J. 2000;352(Pt 2):443–8.
Siprashvili Z, Sozzi G, Barnes LD, McCue P, Robinson AK, Eryomin V, et al. Replacement of Fhit in cancer cells suppresses tumorigenicity. Proc Natl Acad Sci USA. 1997;94(25):13771–6.
Sun J, Okumura H, Yearsley M, Frankel W, Fong LY, Druck T, et al. Nit1 and Fhit tumor suppressor activities are additive. J Cell Biochem. 2009;107(6):1097–106.
Trapasso F, Krakowiak A, Cesari R, Arkles J, Yendamuri S, Ishii H, et al. Designed FHIT alleles establish that Fhit-induced apoptosis in cancer cells is limited by substrate binding. Proc Natl Acad Sci USA. 2003;100(4):1592–7.
Trapasso F, Pichiorri F, Gaspari M, Palumbo T, Aqeilan RI, Gaudio E, et al. Fhit interaction with ferredoxin reductase triggers generation of reactive oxygen species and apoptosis of cancer cells. J Biol Chem. 2008;283(20):13736–44.
Zanesi N, Fidanza V, Fong LY, Mancini R, Druck T, Valtieri M, et al. The tumor spectrum in FHIT-deficient mice. Proc Natl Acad Sci USA. 2001;98(18):10250–5.

Fibulins

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Synonyms

BM-90; DANCE; EFEMP1; EFEMP2; EVEC; H411; Hemicentin; Him4; MBP1; S15; T16; TM14; UP50; UPH1