PROSPECT
Journal of Cellular Biochemistry 109:858–865 (2010)

Journal of Cellular Biochemistry

Pathology and Biology Associated With the Fragile *FHIT* Gene and Gene Product

Joshua C. Saldivar,[1,2] Hidetaka Shibata,[2] and Kay Huebner[2]*

[1]*Integrated Biomedical Science Graduate Program, Ohio State University Medical Center, Columbus, Ohio*

[2]*Department of Molecular Virology, Immunology and Medical Genetics, Comprehensive Cancer Center, Ohio State University Medical Center, Biomedical Research Tower, Columbus, Ohio*

ABSTRACT

More than 12 years and >800 scientific publications after the discovery of the first gene at a chromosome fragile site, the *FHIT* gene at *FRA3B*, there are still questions to pursue concerning the selective advantage conferred to cells by loss of expression of *FHIT*, the most frequent target of allele deletion in precancerous lesions and cancers. These questions are considered in light of recent investigations of genetic and epigenetic alterations to the locus and in a retrospective consideration of biological roles of the Fhit protein discovered through functional studies. J. Cell. Biochem. 109: 858–865, 2010.

KEY WORDS: COMMON FRAGILE SITES; PROMOTER METHYLATION; DNA DAMAGE CHECKPOINT; *FHIT*; DNA REPAIR

Common fragile sites are large, unstable regions of the human genome that are highly sensitive to genotoxic stress and perturbation of replication [Durkin et al., 2008]. The molecular basis for susceptibility to genotoxic damage and instability is not well understood, in spite of numerous hypotheses and investigations [reviewed in Pichiorri et al., 2008a]. LeBeau and co-workers hypothesized that a unique epigenetic pattern might be involved and examined chromatin modification patterns at the six most highly recombinogenic human fragile sites and surrounding non-fragile regions [Jiang et al., 2009b]. Chromatin at most of the fragile sites exhibited less histone acetylation than surrounding non-fragile regions, and treatment with DNA demethylating agent or an inhibitor of histone deacetylation reduced chromosome breakage at the fragile loci. Chromatin at the *FRA3B*/*FHIT* locus, was more resistant to nuclease than flanking non-fragile sequences. The investigators concluded that histone hypoacetylation is characteristic of common fragile site chromatin, making it more compact, indicating a role for chromatin conformation in genomic instability at these fragile chromosome regions [Jiang et al., 2009b]. There is now general agreement concerning the extreme sensitivity of fragile regions to DNA replication stress, resulting in very frequent alterations to the more active fragile loci in cancers. Genes at the two most frequently activated human fragile sites, the *FHIT* and *WWOX* genes, are probably the most frequently altered genes in cancers, showing deletions, translocations, and promoter methylation in perhaps the majority of cancers. From detailed analyses of cancer-derived cell lines, many of the deletions are known to be identical in each cell of a derived cancer cell line, confirming clonal expansion, whether in the originating cancer or the derived cell line is not necessarily known. Are these regions so frequently deleted in cancers because they are most sensitive to DNA damaging agents, or does the deletion drive the expansion of the preneoplastic cell in which damage occurred, or do both mechanisms contribute?

Only further experimentation can conclusively answer these questions. Here we summarize recent results of genetic and epigenetic studies of the *FHIT* locus (see Fig. 1 for summary) that bear on the discussion, and review functional studies that examine the biological roles of Fhit protein that, when lost, could contribute to neoplastic expansion.

THE GENE AND EPIGENE

RECENT OBSERVATIONS OF ALTERATIONS AT THE *FHIT* LOCUS

Regions of recurrent genomic copy number alterations can harbor genes that drive initiation or progression of cancers. Evaluations of copy number changes have not usually considered whether a gene is always found in deleted or in amplified chromosome regions. A recent analysis did this across tumor samples by using high-resolution

Grant sponsor: NIH T32; Grant number: T32GM068412; Grant sponsor: National Cancer Institute; Grant numbers: CA132453, CA120516, CA115965.

*Correspondence to: Dr. Kay Huebner, PhD, Department of Molecular Virology, Immunology and Medical Genetics, Biomedical Research Tower, Rm 916, 460 W. 12th Ave., Columbus, OH 43210. E-mail: kay.huebner@osumc.edu

Received 30 November 2009; Accepted 1 December 2009 • DOI 10.1002/jcb.22481 • © 2010 Wiley-Liss, Inc.
Published online 15 January 2010 in Wiley InterScience (www.interscience.wiley.com).

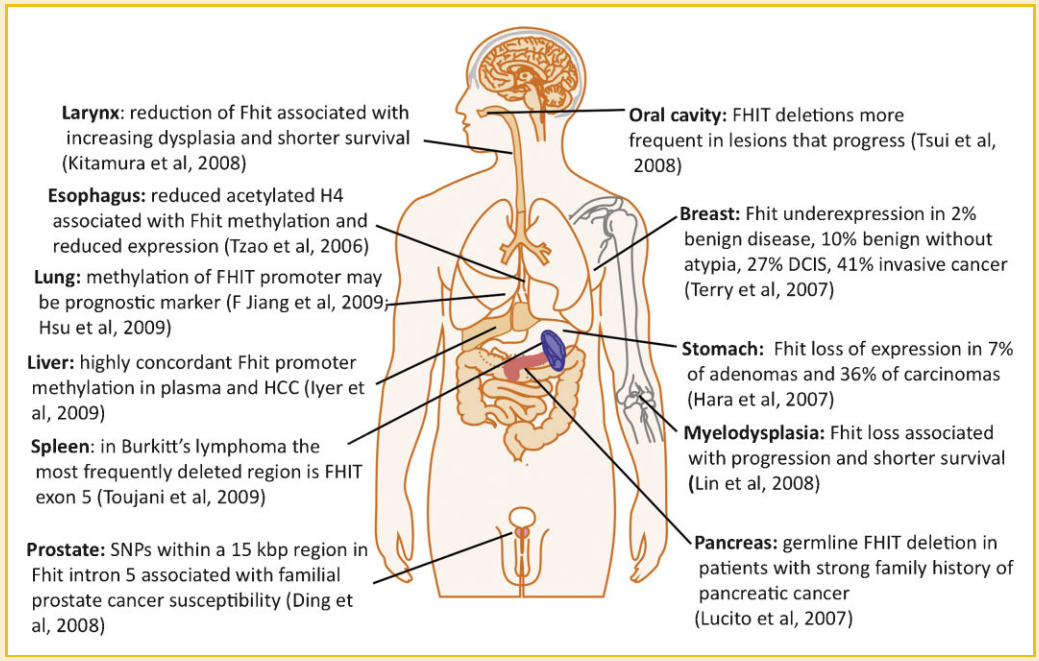

Fig. 1. Recent observations of genetic, epigenetic, and protein alterations in FHIT or Fhit in common human cancers. Summary of studies reporting loss or reduction of FHIT expression or alterations to FHIT alleles in cancers of many organs of males and females. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

comparative genomic hybridization microarrays. The results showed that ~30% of genomic clones were either "exclusively" deleted or amplified and could be considered non-random chromosomal events. Most of the known oncogenes or tumor suppressor genes, including *FHIT*, were in exclusively deleted regions, in lung adenocarcinoma. Authors concluded that joint analysis of deletions and amplifications highlights specific genomic areas with exclusively amplified or deleted recurrent copy number changes which are good candidates for harboring oncogenes or tumor suppressor genes [Broët et al., 2009].

Copy-number variants such as germ-line deletions and amplifications can also be associated with inherited genetic disorders, including familial cancer. Lucito et al. [2007] used representational oligonucleotide microarray analysis to characterize germ-line copy number variants in 60 cancer patients from 57 familial pancreatic cancer kindreds. A total of 56 unique genomic regions with copy-number variants not present in controls were identified, including 31 amplifications and 25 deletions. Two deleted regions were observed in two different patients, and one in three patients. The germ-line deletions included 81 known genes, including the *FHIT*, *PDZRN3*, and *ANKRD3* genes, and authors concluded that these deletions define potential candidate loci for familial pancreatic cancer genes [Lucito et al., 2007].

A candidate gene linkage approach on brother pairs affected with prostate cancer identified a prostate cancer susceptibility locus within *FHIT* intron 5. Subsequent association tests on 16 SNPs surrounding *D3S1234* in intron 5 in Americans of European descent revealed significant evidence of association for a single SNP within intron 5 of *FHIT*. In a more recent study, linkage and association of germline genetic variation in *FHIT* with prostate cancer were confirmed in a family-based sample of men with and without prostate cancer from Caucasian families, and in a community-based case–control sample of African American men with and without prostate cancer. The *FHIT* SNP, rs760317, was associated with prostate cancer in Caucasians and African American carriers [Levin et al., 2007]. Now in a very recent study, re-sequencing and genotyping within a 28.5 kb region surrounding this SNP has further delineated the association with prostate cancer risk to a 15 kb region. Multiple SNPs in sequences under evolutionary constraint within *FHIT* intron 5 defined haplotypes with an increased risk of prostate cancer in European-Americans. The results strongly support the involvement of the *FHIT* intronic region in an increased risk of prostate cancer [Ding et al., 2008].

FHIT ALTERATIONS THAT CONTRIBUTE TO DIAGNOSIS AND PROGNOSIS

Another approach to study selective inactivation involves detection of *FHIT* promoter methylation, exploration of these epigenetic changes in cancers and their usefulness in diagnostic and prognostic studies.

Methylation patterns can be useful biomarkers of cancer detection and risk assessment. The methylation status of six genes, including *FHIT*, was examined in plasma, tumor and normal lung tissues from patients with lung cancer and in plasma samples from cancer-free individuals. The concordance of methylation in tumor tissues and plasma samples was 86%, 87%, 80%, 75%, 76%, and 84% for the *BLU*, *CDH13*, *FHIT*, *CDKN2A*, *RARb*, and *RASSF1A* genes, respectively. The authors established that methylation of two or more of the markers met the criterion for an elevated risk

of cancer. Comparisons yielded a sensitivity of 73%, a specificity of 82%, and a concordance of 75% between the methylation patterns in tumor tissues and in corresponding plasma samples. The detection rate was relatively high in cigarette smokers with advanced squamous cell lung cancer. Authors concluded that multiple epigenetic markers in the plasma can be used for lung cancer detection [Hsu et al., 2007].

In another study, the various molecular alterations leading to the inactivation of *FHIT* gene function were investigated in lung cancer, and their use as biomarkers of risk for disease progression was validated. Fhit protein immunostaining was performed on tumor samples, and methylation status of the *FHIT* promoter was assessed in DNAs from tumor and normal lung samples. Loss of heterozygosity (LOH) at *FHIT* was detected in 62% of tumors, and strong association with complete loss of *FHIT* expression was observed when methylation and LOH were analyzed together. *FHIT* methylation in normal lung was associated with an increased risk of progressive disease. The results indicated that *FHIT* methylation in normal lung tissue could represent a prognostic marker for progressive disease [Verri et al., 2009].

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer mortality globally, and Iyer et al. [2009] hypothesized that aberrant DNA methylation could play a role in HCC development. They compared tumor methylation profiles for tumor suppressor genes *APC*, *FHIT*, *CDKN2A/B*, and *ECAD* in tumor tissues and plasma to test concordance between the two types of specimen. Concordance between the tissue and plasma results was highly statistically significant in all five genes and authors concluded that plasma DNA is reliable for determining methylation profile in liver cancer patients. Also, in a recent study of cervical carcinomas, a significant correlation was found between CpG site hypermethylation and low *FHIT* expression [Ki et al., 2008].

To examine the role of *FHIT* in myelodysplastic syndrome, Lin et al. [2008] examined the FHIT methylation status in patient samples. Methylation of the *FHIT* promoter was found in 47% of cases, and a significant difference was observed in frequencies of *FHIT* gene hypermethylation among patients with specific cytogenetically defined subgroups of cases. Patients with *FHIT* gene methylation in their dysplastic cells had significantly shorter survival times than those without *FHIT* methylation. The results suggested that aberrant methylation of the *FHIT* gene is involved in disease progression and is an adverse prognostic factor.

Global microarray expression analysis identified a number of differentially expressed genes in smoke-exposed bronchial epithelium and non-small cell lung cancers [Woenckhaus et al., 2008]. Immunohistochemical analysis of the differentially expressed gene products was then used to evaluate the prognostic relevance of these proteins in the lung cancers. Expression data were correlated with clinicopathologic features and clinical outcome. Significantly better overall survival was observed in adenocarcinomas compared with squamous cell cancers. Loss of *FHIT* expression was strongly associated with shorter overall survival in both histologic types of lung cancer, squamous cell cancers and adenocarcinomas. In adenocarcinomas, cytoplasmic expression of beta-catenin was associated with shorter survival; *MUC1* expression was associated with worse prognosis in patients with squamous cell cancers. The authors concluded that loss of Fhit protein expression and positivity for beta-catenin and MUC1 proteins are useful prognostic markers.

Computed tomography (CT) is used in diagnosis of lung cancer but has been limited by uncertain detection rates for early stage non-small cell lung cancer, especially central tumors. Genetic analysis of sputum has also been used in diagnosis, so efficacy of combining CT and genetic analysis of sputum for non-invasive diagnosis of stage in non-small cell lung cancer was evaluated [Jiang et al., 2009a]. Genomic copy number changes of *HYAL2*, *FHIT*, *CDKN2A*, and *SPA* genes were analyzed on a mini-chip in sputum from patients with stage I cancer and cancer-free controls. CT had higher sensitivity (85%) in detection of lung cancer compared with the mini-chip (70%), but there was no difference in specificity between the two tests (89% vs. 92%). CT showed higher sensitivity (93%) in identifying peripheral tumors; in detecting central tumors, CT had lower specificity (90%) than the mini-chip (98%), but higher sensitivity. Combining both tests offered higher sensitivity (91%) than a single test, with 92% sensitivity. Thus, the combined approach gave higher sensitivity, specificity, and accuracy for diagnosing central cancers than CT alone.

Bhutani et al. [2008] hypothesized that tobacco-induced molecular alterations in oral epithelium might be similar to changes in the lungs and could serve as surrogate tissue for assessing lung alterations. Methylation-specific PCR was used to analyze promoter methylation of the *CDKN2A* and *FHIT* genes in oral and bronchial brush specimens. At baseline, promoter methylation in bronchial tissue was detected in 23% of samples for *CDKN2A*, 17% for *FHIT*, and 35% for both; the percentages were comparable in oral tissue: 19% (*CDKN2A*), 15% (*FHIT*), and 31% (both). Data from both oral and bronchial tissues were available for 125 individuals, in whom the two sites correlated strongly with respect to alterations ($P < 0.0001$ for both *CDKN2A* and *FHIT*). At baseline, the mean bronchial methylation index was far higher in patients with oral tissue methylation (in either of the two genes) than in patients without oral tissue methylation. Similar correlations occurred at three months after intervention. Authors concluded that "the results support the potential of oral epithelium as a surrogate tissue for assessing tobacco-induced molecular damage in the lungs and have important implications for designing future lung cancer prevention trials and for research into the risk and early detection of lung cancer."

FHIT EXPRESSION CHANGES IN EARLY LESIONS

WWOX and *FHIT* genes are located in active fragile sites and reduction in expression of both has been associated with development of breast cancer. Wang et al. [2008] evaluated mRNA and protein expression for these gene products in breast tissue with normal histological appearances, atypical ductal hyperplasia, ductal carcinoma in situ, and invasive cancer. Compared with in situ and invasive cancer specimens, both normal and atypical hyperplasia specimens had greater rates of mRNA and protein expression; that is, expression of *FHIT* and *WWOX* decreased along with breast tissue progress from a normal histological appearance to atypical ductal hyperplasia, in situ cancer, and invasive cancer.

In immunohistochemical analyses of Fhit and PCNA expression in non-proliferative benign breast disease (BBD), proliferative BBD without atypia, proliferative BBD with atypia, carcinoma in situ or invasive carcinoma and of EGFR protein expression in a subset of these cases [Terry et al., 2007], Fhit underexpression was not detected in non-proliferative lesions, but occurred in 2% of proliferative BBD without atypia, 10% proliferative BBD with atypia, 27% of carcinoma in situ and 41% of invasive carcinoma, suggesting that it could be useful in assessing carcinoma in situ lesions (ductal, DCIS and lobular, LCIS) that are more likely to progress to malignancy. Also, microarray comparisons of DCIS and invasive carcinoma samples showed consistent downregulation of Fhit-related proteins, caspase 1 and BRCA1 in lesions under-expressing Fhit [Terry et al., 2007].

LOH at the *FHIT* locus is coincident with activation of DNA damage response checkpoint proteins [reviewed in Pichiorri et al., 2008a,b]; thus damage at fragile loci may trigger checkpoint activation. Cirombella et al. [2009] examined preneoplastic lesions adjacent to non-small cell lung carcinomas for alterations in expression of Fhit and activated checkpoint proteins and expression scores were analyzed for pair-wise associations and correlations among proteins and type of lesion. Hyperplastic and dysplastic lesions were positive for nuclear γH2AX expression; dysplastic lesions were negative for Fhit expression. Fhit positive lesions showed expression of most checkpoint proteins examined, while Fhit negative lesions showed absence of expression of Chk1 and phosphoChk1. The results showed that loss of expression of Fhit was significantly, directly correlated with absence of activated Chk1 in dysplasia and suggested a connection between loss of Fhit and modulation of checkpoint activity.

THE GENE PRODUCT

FHIT–SUBSTRATE COMPLEX AS THE ACTIVE SIGNALING MOLECULE

When the *FHIT* gene was identified and alterations observed in the locus in cancers, the conceptual Fhit amino acid sequence was found to be homologous to members of the histidine triad (HIT) family of proteins. Members of the HIT protein family each contain a His-X-His-X-His-XX sequence motif, that forms the core of the protein active site involved in nucleotide binding, hydrolysis or transfer [Brenner et al., 1999]. In addition to the Fhit branch, there are four other branches of the HIT protein family, including Hint, Aprataxin, GalT, and DcpS [Brenner, 2002; Kijas et al., 2006].

Alterations in expression of members of the Hint branch have also been associated with cancer [reviewed in Huber and Weiske, 2008]. *Hint1*$^{-/-}$ mice treated with *N*-nitrosomethylbenzylamine developed significantly more and larger squamous tumors than wild type mice [Su et al., 2003], as previously observed in *Fhit*$^{-/-}$ mice [reviewed in Pichiorri et al., 2008a,b]. Several other studies have been reported confirming a role for Hint1 in the suppression of cancer [Li et al., 2005; Wang et al., 2007]. There is now evidence that Hint2 may also play a role in tumorigenesis. Martin et al. [2006] have reported that HCC cells overexpressing Hint2 were suppressed for tumor formation when injected into nude mice compared to control cells. The authors further reported reduced expression of Hint2 in human primary HCC compared to adjacent normal liver tissue. Thus, it is likely that members of several HIT protein family branches, Hint1, Hint2, and Fhit have roles in tumor suppression.

As a member of the HIT family of nucleotide hydrolases, it was presumed that Fhit possessed enzymatic activity. Barnes et al. [1996] first reported Fhit to be a diadenosine 5′,5‴-P^1,P^3-triphosphate (Ap_3A) hydrolase. Fhit was also shown to hydrolyze Ap_4A, though Ap_3A was the preferred substrate. Site-directed mutagenesis of the central histidine of the triad demonstrated that this residue was critical for enzymatic activity of Fhit. Interestingly, diadenosine polyphosphates (Ap_nAs) are thought to be a novel class of signaling molecules, accumulating in response to cellular stress and affecting several cellular processes [Kisselev et al., 1998]. Recently, Ap_3A and Ap_4A were shown to induce proliferation of smooth muscle cells and this proliferation correlated with activation of the ERK1/2 MAP kinase pathway [Bobbert et al., 2008]. Fhit robustly lowers Ap_3A intracellular concentrations due to its hydrolase activity [Murphy et al., 2000], and might thus prevent Ap_3A mediated ERK1/2 activation and cell proliferation.

Early on, studies were conducted to determine if Fhit enzymatic activity was necessary for Fhit tumor suppressor function; for example, suppression of xenograft growth in nude mice after injection of cancer cells expressing exogenous Fhit was observed in the first tumor suppression experiment [cited in Trapasso et al., 2003]. Furthermore, the hydrolase "dead" Fhit mutant (mutated central histidine) also suppressed tumorigenicity in mice, suggesting that Fhit tumor suppression was not dependent on its enzymatic activity. Importantly, the hydrolase "dead" Fhit mutant (His-96 → Asn substitution) maintained affinity for Ap_3A and formed a Fhit–substrate complex [Pace et al., 1998]. This provided the first evidence that the Fhit–substrate complex was the active signaling molecule for tumor suppression.

It was further demonstrated that substrate-bound Fhit was the active signaling complex when Fhit mutants with reduced substrate binding and/or substrate hydrolysis were over-expressed in cancer cells and effect on induction of apoptosis was assessed, as a measure of suppressor activity. Importantly, induction of apoptosis was significantly reduced only in cancer cells expressing Fhit mutants with altered substrate binding. Fhit mutants that bound Ap_3A but were unable to hydrolyze the substrate still retained proapoptotic function [Trapasso et al., 2003]. Pace et al. [1998] discussed the possibility of a "Fhit effector" that binds the Fhit–substrate complex acting to slow hydrolysis of Ap_nA and sustain signaling. Soon after, Shi et al. [2000] reported a Fhit–Ubc9 interaction and Golebiowski et al. [2004] reported that Ubc9 interacted with Fhit and altered Fhit enzymatic kinetics resulting in delayed hydrolysis of Ap_3A and prolonged Fhit–Ap_3A signaling. However, we have not been able to confirm co-immunoprecipitation of Fhit by Ubc9 anitsera, or vice versa, even after protein cross-linking. From the Fhit mutant studies, it became clear that the Tyr 114 residue of Fhit was essential for the formation of Fhit–substrate complex and subsequent tumor suppressive signaling [Trapasso et al., 2003]. Interestingly, Tyr 114 is within a consensus sequence for Src kinase phosphorylation. Indeed, Src kinase was shown to phosphorylate Fhit at Tyr 114, and phosphorylation by Src kinase targeted Fhit for proteosomal degradation [Bianchi et al., 2006].

Recently, Fhit was shown to bind Hsp60, localize to mitochondria, interact with and stabilize Fdxr, leading to production of reactive oxygen species (ROS) under conditions of stress. These Fhit interactions resulted in cell cycle arrest and apoptosis in cancer cells [Trapasso et al., 2008]. Pichiorri et al. [2009] further demonstrated that Fhit localization to mitochondria, as well as Fhit interaction with Hsp60 and Fdxr, were limited by substrate binding; that is, Fhit mutants that did not bind substrate were defective in these functions. Collectively, these studies lead us to propose a model of the Fhit signaling molecular switch (Fig. 2). Upon addition of external stresses (peroxide, UV, etc.), intracellular Ap_nA concentrations increase, serving as a stress sensor. Inactive Fhit binds the Ap_nA substrate, activating the Fhit signaling pathway. Upon hydrolysis of Ap_nA to AMP and ADP, or possibly after Fhit phosphorylation at Tyr 114 and subsequent degradation [Bianchi et al., 2006], the Fhit pathway is switched off. Activated Fhit signaling in cancer cells induces cell cycle arrest and apoptosis.

Fhit deficient cancer cells show a mild response to production of ROS, crucial mediators of chemotherapy-induced cell death, confirming that Fhit deficiency could negatively influence treatment outcome. The *FDXR* gene is also a target gene of the p53 family. Overexpression of *FDXR* sensitized colon cancer cells to H_2O_2, 5-fluorouracil, and doxorubicin-induced cell death, indicating that Fdxr protein contributes to p53-mediated apoptosis through generation of oxidative stress in mitochondria. Thus, activated p53 induces apoptosis in response to cellular stresses in part through ROS, and simultaneously p53 increases transcription of the *FDXR* gene, which in turn enhances p53 function by increasing ROS induced apoptosis [Hwang et al., 2001]. Fhit also participates in this pathway, even in p53 deficient cells, by stabilizing Fdxr protein and enhancing ROS production.

Consistent with the studies by Trapasso et al. [2008] and Pichiorri et al. [2009], Rimessi et al. [2009] observed Fhit localization to the mitochondria. It was further demonstrated that Fhit sensitizes the low-affinity Ca^{2+} transporters of mitochondria, enhancing Ca^{2+} uptake into the organelle, both in intact and in permeabilized cells. Ca^{2+} accumulation in the mitochondria is believed to sensitize cells to apoptotic stimuli. Interestingly, increased Ca^{2+} uptake in Fhit overexpressing cells potentiated the effect of apoptotic agents. The authors concluded that Fhit regulates intramitochondrial calcium uptake and sensitizes cells to apoptotic signals.

GENOME INSTABILITY IN CANCER: SURVIVAL OF THE UN-FHIT

When *FHIT* was mapped to the *FRA3B* locus, it was presumed that the *FHIT* gene was highly susceptible to chromosomal alterations such as deletions and translocations, due to the frequency of DNA breaks associated with *FRA3B* upon genotoxin exposure. Indeed, chromosomal alterations at the *FHIT* locus and subsequent loss of Fhit expression are commonly observed in human cancer cells. Interestingly, and somewhat paradoxically, recent evidence suggests that Fhit functions to protect genomic integrity through involvement in the DNA damage response and DNA repair.

The cellular DNA damage response involves essential, complex signal pathways for the maintenance of genomic integrity. DNA-damaged cells must arrest the cell cycle and repair damaged DNA. Life and death decisions made by cells are dependent on the extent of damage and the completeness of repair. Cells unable to effectively repair damaged DNA trigger apoptosis, preventing inheritance of genetic mutations by daughter cells. Alterations to or interference with the DNA damage responses can result in loss of genomic integrity and initiation and progression of cancer.

FHIT-DEFICIENCY IS ASSOCIATED WITH RESISTANCE TO GENOTOXIC STRESS

The first indication that Fhit activity affected the DNA damage response was the finding that Fhit-deficient cancer and normal cells exhibited increased resistance to genotoxic stress-inducing agents,

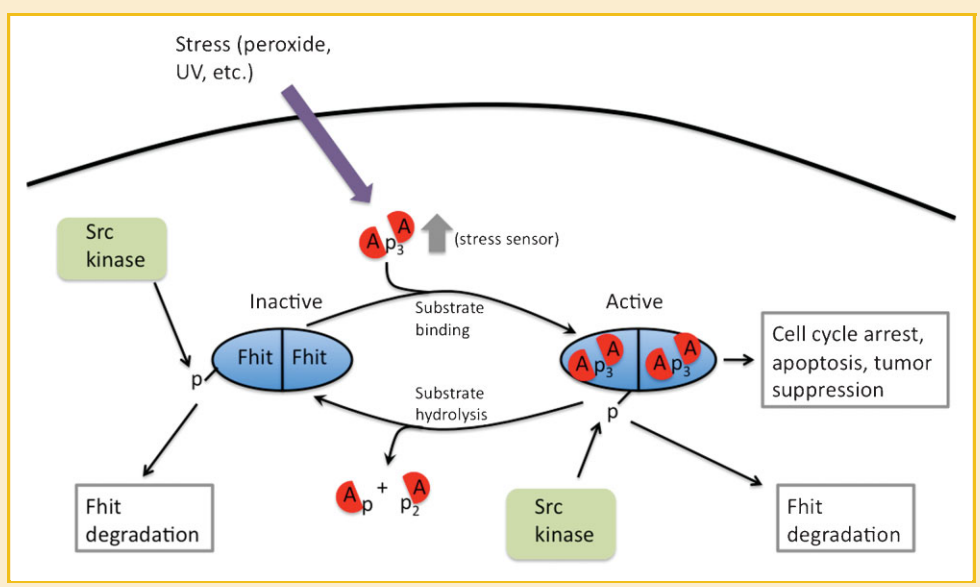

Fig. 2. Model of the Fhit–substrate complex as a molecular switch.

including mitomycin C and UVC. UVC surviving *Fhit*$^{-/-}$ cells appeared transformed and carried a >5-fold increase in mutations [reviewed in Pichiorri et al., 2008a,b].

More recently, Ishii et al. [2008] demonstrated that Fhit-deficient hematopoietic stem cells (HSC) survive hydroquinone exposure. In this study, HSCs were isolated from wild type and *Fhit*$^{-/-}$ mice, treated with hydroquinone, and hematopoietic colony formation was assessed to determine HSC susceptibility to hydroquinone. Interestingly, Fhit-negative HSCs were resistant to hydroquinone, as indicated by colony formation. Furthermore, Fhit-deficient bone marrow was shown to be resistant to hydroquinone in vivo, and Fhit-deficient HSCs retained potential for self-renewal and multipotent differentiation. Immunohistochemical staining of bone marrow tissue sections 120 days after transplantation of hydroquinone-exposed cells showed evidence of extensive apoptosis in wild type transplanted mice, while bone marrow of mice receiving *Fhit*$^{-/-}$ derived HSCs did not. Importantly, preneoplastic alterations were observed in tissues from *Fhit*$^{-/-}$ hydroquinone-exposed bone marrow-transplanted mice. In summary, Fhit-deficiency allows resistance to genotoxic stress and survival of cells carrying precancerous changes.

FHIT DEFICIENCY IS ASSOCIATED WITH ABERRANT CHECKPOINT RESPONSE

How Fhit participates in DNA damage response pathways to contribute to cell sensitivity to genotoxic stress has not been defined in detail; however, recent evidence suggests that Fhit modulates the G2/S checkpoint response. Hu et al. [2005a] reported an overactive Atr/Chk1 response and a stronger S/G2 checkpoint in *Fhit*$^{-/-}$ kidney epithelial cells, resulting in increased resistance to ionizing radiation and increased mutations in resistant cells. Fhit-deficiency is associated with increased resistance to genotoxic stress and DNA damage, and this may be explained in part by an overactive checkpoint in Fhit-deficient cells.

As a follow-up to the previous study, Hu et al. [2005b] observed an increase in homologous recombination repair (HRR) after Fhit knockdown. In contrast, Chk1 knockdown resulted in decreased HRR efficiency. Collectively, the results suggest that Fhit-deficiency leads to increased Chk1 activation, followed by increased HRR efficiency. Although HRR is regarded as an error-free repair pathway, we have observed an increase in both HRR and mutation frequency in Fhit-deficient hydroquinone-exposed cells, indicating that HRR is not always error-free [Ishii et al., 2008], as previously observed by Richardson et al. [2004].

In another study, it was shown that Fhit differentially modulated the DNA damage checkpoint of normal and cancer cells in response to UV exposure [Ishii et al., 2006]. In primary fibroblasts Fhit overexpression correlated with activation of Chk1, indicated by phosphorylated Chk1 (pChk1) at S317, with or without UV exposure. This presumably would allow DNA repair prior to mitosis, preserving genomic integrity. In contrast, esophageal cancer cells exhibited strong checkpoint activation upon UV exposure; however, overexpression of Fhit led to significant reduction of Chk1 and no detectable pChk1. This observation was confirmed in vivo. Consequently, mouse tumors with Fhit overexpression underwent apoptosis. Interestingly, experiments using Fhit mutants with very reduced substrate-binding demonstrated that Fhit modulation of the checkpoint response is dependent on Fhit binding of Ap_3A substrates [Pichiorri et al., 2009], providing further evidence that the Fhit–substrate complex is the active signaling molecule for the Fhit pathway.

PROSPECTS

THE GENE

It will be very important to understand more about the haplotypes within *FHIT* intron 5 that are associated with risk of prostate cancer. It will be especially interesting to determine if this region of intron 5 is involved in processing the ~1.7 megabase FHIT transcript or if this intron region may encode a functional non-coding RNA.

Secondly, *FHIT* and other genes encoded at fragile regions are frequently silenced by regulatory region CpG methylation. If this is not a mark of selection for inactivation then could it be a result of DNA damage? Are regulatory regions of damaged loci more likely to be methylated? And could this methylation be related to the unique chromatin properties at fragile sites, described by Jiang et al. [2009b]? Experiments could be devised to investigate these questions.

Finally, it should also be possible to directly investigate questions concerning the selection for absence of expression of fragile gene products that are possible tumor suppressors. For example, Fhit deficient mice are ~10-fold more susceptible than wild type mice to carcinogen induction of forestomach tumors. But the mouse *Fhit* locus with Fhit inactivated by deletion of exon 5 is still fragile; so perhaps by examining induced tumors from Fhit deficient mice versus wild type mice, it would be possible to determine if the wild type mice are more likely to exhibit deletions within the *Fhit* locus.

THE GENE PRODUCT

As summarized above, Fhit participates in a number of important signal pathways in response to endogenously or exogenously applied stressful agents, particularly those that cause DNA damage, whether to single or both DNA strands, and this includes responses to chemotherapeutic agents. Thus, detailed understanding of the mechanisms through which Fhit participates in stress responses, DNA damage response pathways and apoptotic pathways, is needed. The identification of Fhit-interacting proteins after cross-linking of overexpressed, tagged Fhit protein was a breakthrough in the sense that we know quite a lot now about how Fhit affects response to oxidative agents, through transport to the mitochondria, interaction with, and stabilization of Fdxr. Might this mechanism suggest that Fhit is involved in stabilization or destabilization of other important signaling proteins? It will be very important to determine if Fhit might affect stability of one or more proteins involved in checkpoint responses. It is also possible that Fhit, as a cytoplasmic protein, may be involved in other post-translational changes affecting checkpoints or apoptotic pathways. Post-translational changes are still difficult to examine on a global scale, particularly for low abundance proteins, although such studies may be feasible using the Fhit-deficient yeast strains developed by Charles Brenner.

A number of investigators in other laboratories have reported possible Fhit partner proteins in signal transduction, involving

interesting pathways that could contribute to tumor suppression, such as Mdm2 and βcat [reviewed in Huber and Weiske, 2008]; as mentioned in previous reviews, we have not detected direct interactions of Fhit with these proteins by co-immunoprecipitation. Nevertheless, further investigation of these possible Fhit-partner complexes and effects on signal pathways is warranted.

Currently, we are ''looking where the light is'' by examining expression differences in stressed Fhit positive and negative sister cells using microarray and RT-PCR array analyses, in order to identify pathways controlled, albeit indirectly, by Fhit.

ACKNOWLEDGMENTS

We thank Teresa Druck for preparation of figures, and our laboratory members for research contributions. We express regret to our colleagues whose original work is cited only through reviews, due to space limitations. Support for research in our laboratories is from grants from the National Cancer Institute, USPHS, funds from the Ohio State University Comprehensive Cancer Center. J.S. is funded by an NIH T32 training grant.

REFERENCES

Barnes LD, Garrison PN, Siprashvili Z, Guranowski A, Robinson AK, Ingram SW, Croce CM, Ohta M, Huebner K. 1996. Fhit, a putative tumor suppressor in humans, is a dinucleoside 5,5′-P1,P3-triphosphate hydrolase. Biochemistry 35:11529–11535.

Bhutani M, Pathak AK, Fan YH, Liu DD, Lee JJ, Tang H, Kurie JM, Morice RC, Kim ES, Hong WK, Mao L. 2008. Oral epithelium as a surrogate tissue for assessing smoking-induced molecular alterations in the lungs. Cancer Prev Res (Phila Pa) 1:39–44.

Bianchi F, Magnifico A, Olgiati C, Zanesi N, Pekarsky Y, Tagliabue E, Croce CM, Ménard S, Campiglio M. 2006. FHIT-proteasome degradation caused by mitogenic stimulation of the EGF receptor family in cancer cells. Proc Natl Acad Sci USA 103:18981–18986.

Bobbert P, Schlüter H, Schultheiss HP, Reusch HP. 2008. Diadenosine polyphosphates Ap3A and Ap4A, but not Ap5A or Ap6A, induce proliferation of vascular smooth muscle cells. Biochem Pharmacol 75:1966–1973.

Brenner C. 2002. Hint, Fhit, and GalT: Function, structure, evolution, and mechanism of three branches of the histidine triad superfamily of nucleotide hydrolases and transferases. Biochemistry 41:9003–9014.

Brenner C, Bieganowski P, Pace HC, Huebner K. 1999. The histidine triad superfamily of nucleotide-binding proteins. J Cell Physiol 181:179–187, Review.

Broët P, Tan P, Alifano M, Camilleri-Broët S, Richardson S. 2009. Finding exclusively deleted or amplified genomic areas in lung adenocarcinomas using a novel chromosomal pattern analysis. BMC Med Genomics 2:43.

Cirombella R, Montrone G, Stoppacciaro A, Giglio S, Volinia S, Graziano P, Huebner K, Vecchione A. 2009. Fhit loss in lung preneoplasia: Relation to DNA damage response checkpoint activation. Cancer Lett. DOI: 10.1016/j.canlet. 2009.10.017.

Ding Y, Larson G, Rivas G, Lundberg C, Geller L, Ouyang C, Weitzel J, Archambeau J, Slater J, Daly MB, Benson AB, Kirkwood JM, O'Dwyer PJ, Sutphen R, Stewart JA, Johnson D, Nordborg M, Krontiris TG. 2008. Strong signature of natural selection within an FHIT intron implicated in prostate cancer risk. PLoS One 3:e3533.

Durkin SG, Ragland RL, Arlt MF, Mulle JG, Warren ST, Glover TW. 2008. Replication stress induces tumor-like microdeletions in FHIT/FRA3B. Proc Natl Acad Sci USA 105:246–251.

Golebiowski F, Szulc A, Szutowicz A, Pawelczyk T. 2004. Ubc9-induced inhibition of diadenosine triphosphate hydrolase activity of the putative tumor suppressor protein Fhit. Arch Biochem Biophys 428:160–164.

Hara A, Yashima K, Yasugi A, Koda M, Kawaguchi K, Harada K, Andachi H, Shiota G, Ito H, Murawaki Y. 2007. Expression of Fhit, Mlh1, p16INK4A and E-cadherin in early gastric neoplasia: Correlation with histological grade and gastric phenotype. Oncol Rep 18:553–559.

Hsu HS, Chen TP, Hung CH, Wen CK, Lin RK, Lee HC, Wang YC. 2007. Characterization of a multiple epigenetic marker panel for lung cancer detection and risk assessment in plasma. Cancer 110:2019–2026.

Hu B, Han S, Wang X, Ottey M, Dicker A, Huebner K, Wang Y. 2005a. Involvement of the Fhit gene in the ionizing radiation-activated ATR/CHK1 pathway. J Cell Physiol 202:518–523.

Hu B, Wang H, Wang X, Lu HR, Huang C, Powell SN, Huebner K, Wang Y. 2005b. Fhit and CHK1 have opposing effects on homologous recombination repair. Cancer Res 65:8613–8616.

Huber O, Weiske J. 2008. β-catenin takes a HIT. Cell Cycle 7(10): 1326–1331.

Hwang PM, Bunz F, Yu J, Rago C, Chan TA, Murphy MP, Kelso GF, Smith RA, Kinzler KW, Vogelstein B. 2001. Ferredoxin reductase affects p53-dependent, 5-fluorouracil-induced apoptosis in colorectal cancer cells. Nat Med 7:1111–1117.

Ishii H, Mimori K, Inoue H, Inageta T, Ishikawa K, Semba S, Druck T, Trapasso F, Tani K, Vecchione A, Croce CM, Mori M, Huebner K. 2006. Fhit modulates the DNA damage checkpoint response. Cancer Res 66:11287–11292.

Ishii H, Mimori K, Ishikawa K, Okumura H, Pichiorri F, Druck T, Inoue H, Vecchione A, Saito T, Mori M, Huebner K. 2008. FHIT-deficient hematopoietic stem cells survive hydroquinone treatment long-term, carrying precancerous alterations. Cancer Res 68:3662–3670.

Iyer P, Zekri AR, Hung CW, Schiefelbein E, Ismail K, Hablas A, Seifeldin IA, Soliman AS. 2009. Concordance of DNA methylation pattern in plasma and tumor DNA of Egyptian hepatocellular carcinoma patients. Exp Mol Pathol (Epub ahead of print). DOI: 10.1016/j.yexmp.2009.09.012.

Jiang F, Todd NW, Qiu Q, Liu Z, Katz RL, Stass SA. 2009. Combined genetic analysis of sputum and computed tomography for noninvasive diagnosis of non-small-cell lung cancer. Lung Cancer 66:58–63.

Jiang Y, Lucas I, Young DJ, Davis EM, Karrison T, Rest JS, Le Beau MM. 2009. Common fragile sites are characterized by histone hypoacetylation. Hum Mol Genet 18:4501–4512.

Ki KD, Lee SK, Tong SY, Lee JM, Song DH, Chi SG. 2008. Role of 5′-CpG island hypermethylation of the FHIT gene in cervical carcinoma. J Gynecol Oncol 19:117–122. Published online 2008 June 20, 10.3802/jgo.2008.19.2.117.

Kijas AW, Harris JL, Harris JM, Lavin MF. 2006. Aprataxin forms a discrete branch in the HIT (histidine triad) superfamily of proteins with both DNA/RNA binding and nucleotide hydrolase activities. J Biol Chem 281:13939–13948.

Kisselev LL, Justesen J, Wolfson AD, Frolova LY. 1998. Diadenosine oligophosphates (Ap(n)A), a novel class of signalling molecules? FEBS Lett 427: 157–163, Review.

Kitamura K, Hayashi K, Kobayashi R, Ishii H, Matsubayashi J, Matsumoto T, Suzuki M. 2008. Clinicopathological significance of the fragile histidine triad transcription protein expression in laryngeal carcinogenesis. Oncol Rep 19:847–852.

Levin AM, Ray AM, Zuhlke KA, Douglas JA, Cooney KA. 2007. Association between germline variation in the FHIT gene and prostate cancer in Caucasians and African Americans. Cancer Epidemiol Biomarkers Prev 16:1294–1297.

Li H, Zhang Y, Su T, Santella RM, Weinstein IB. 2005. Hint1 is a haploinsufficient tumor suppressor in mice. Oncogene 25:713–721.

Lin J, Yao DM, Qian J, Wang YL, Han LX, Jiang YW, Fei X, Cen JN, Chen ZX. 2008. Methylation status of fragile histidine triad (FHIT) gene and its clinical

impact on prognosis of patients with myelodysplastic syndrome. Leuk Res 32:1541–1545.

Lucito R, Suresh S, Walter K, Pandey A, Lakshmi B, Krasnitz A, Sebat J, Wigler M, Klein AP, Brune K, Palmisano E, Maitra A, Goggins M, Hruban RH. 2007. Copy-number variants in patients with a strong family history of pancreatic cancer. Cancer Biol Ther 6:1592–1599.

Martin J, Magnino F, Schmidt K, Piguet AC, Lee JS, Semela D, St-Pierre MV, Ziemiecki A, Cassio D, Brenner C, Thorgeirsson SS, Dufour JF. 2006. Hint2, a mitochondrial apoptotic sensitizer down-regulated in hepatocellular carcinoma. Gastroenterology 130:2179–2188.

Murphy GA, Halliday D, McLennan AG. 2000. The Fhit tumor suppressor protein regulates the intracellular concentration of diadenosine triphosphate but not diadenosine tetraphosphate. Cancer Res 60:2342–2344.

Pace HC, Garrison PN, Robinson AK, Barnes LD, Draganescu A, Rosler A, Blackburn GM, Siprashvili Z, Croce CM, Huebner K, Brenner C. 1998. Genetic, biochemical and crystallographic definition of a substrate analog complex with the fragile histidine triad protein as the active signaling form of Fhit. Proc Natl Acad Sci USA 95:5484–5489.

Pichiorri F, Ishii H, Okumura H, Trapasso F, Wang Y, Huebner K. 2008a. Molecular parameters of genome instability: Roles of fragile genes at common fragile sites. J Cell Biochem 104:1525–1533.

Pichiorri F, Palumbo T, Suh S-S, Okumura H, Trapasso F, Ishii H, Huebner K, Croce CM. 2008b. The Fhit tumor suppressor: 'guardian of the preneoplastic genome'. Future Oncology, Fhit tumor suppressor: Guardian of the preneoplastic genome. Future Oncol 4:815–824.

Pichiorri F, Okumura H, Nakamura T, Garrison PN, Gasparini P, Suh S-S, Druck T, McCorkell KA, Barnes LD, Croce CM, Huebner K. 2009. Correlation of Fhit structural features with effector interactions and biological functions. J Biol Chem 284:1040–1049.

Richardson C, Stark JM, Ommundsen M, Jasin M. 2004. Rad51 overexpression promotes alternative doublestrand break repair pathways and genome instability. Oncogene 23:546–553.

Rimessi A, Marchi S, Fotino C, Romagnoli A, Huebner K, Croce CM, Pinton P, Rizzuto R. 2009. Intramitochondrial calcium regulation by the FHIT gene product sensitizes to apoptosis. Proc Natl Acad Sci USA 106:12753–12758.

Shi Y, Zou M, Farid NR, Paterson MC. 2000. Association of FHIT (fragile histidine triad), a candidate tumour suppressor gene, with the ubiquitin-conjugating enzyme hUBC9. Biochem J 352(Pt 2):443–448.

Su T, Suzui M, Wang L, Lin CS, Xing WQ, Weinstein IB. 2003. Deletion of histidine triad nucleotide-binding protein 1/PKC-interacting protein in mice enhances cell growth and carcinogenesis. Proc Natl Acad Sci USA 100:7824–7829.

Terry G, Ho L, Londesborough P, Duggan C, Hanby A, Cuzick J. 2007. The expression of FHIT, PCNA and EGFR in benign and malignant breast lesions. Br J Cancer 96:110–117.

Toujani S, Dessen P, Ithzar N, Danglot G, Richon C, Vassetzky Y, Robert T, Lazar V, Bosq J, Da Costa L, Pérot C, Ribrag V, Patte C, Wiels J, Bernheim A. 2009. High resolution genome-wide analysis of chromosomal alterations in Burkitt's lymphoma. PLoS One 4:e7089.

Trapasso F, Krakowiak A, Cesari R, Arkles J, Yendamuri S, Ishii H, Vecchione A, Kuroki T, Bieganowski P, Pace HC, Huebner K, Croce CM, Brenner C. 2003. Designed FHIT alleles established that Fhit-induced apoptosis in cancer cells is limited by substrate-binding. Proc Natl Acad Sci USA 100:1592–1597.

Trapasso F, Pichiorri F, Gasparo M, Palumbo T, Aqeilan RI, Gaudio E, Okumura H, Iuliano R, Okumura H, Di Leva G, Fabbri M, Birk DE, Raso C, Green-Church K, Spagnoli LG, Venuta S, Huebner K, Croce CM. 2008. Fhit interaction with ferredoxin reductase triggers generation of reactive oxygen species and apoptosis. J Biol Chem 283:13736–13744.

Tsui IF, Rosin MP, Zhang L, Ng RT, Lam WL. 2008. Multiple aberrations of chromosome 3p detected in oral premalignant lesions. Cancer Prev Res (Phila Pa) 1:424–429.

Verri C, Roz L, Conte D, Liloglou T, Livio A, Vesin A, Fabbri A, Andriani F, Brambilla C, Tavecchio L, Calarco G, Calabrò E, Mancini A, Tosi D, Bossi P, Field JK, Brambilla E, Sozzi G, EUELC Consortium. 2009. Fragile histidine triad gene inactivation in lung cancer: The European Early Lung Cancer project. Am J Respir Crit Care Med 179:396–401.

Wang L, Zhang Y, Li H, Xu Z, Santella RM, Weinstein IB. 2007. Hint1 inhibits growth and activator protein-1 activity in human colon cancer cells. Cancer Res 67:4700–4708.

Wang TT, Frezza EE, Ma R, Hu SY, Liu CZ, Zhang GY, Wachtel MS, Lü XM, Feng JB, Lü CX. 2008. Loss expression of active fragile sites genes associated with the severity of breast epithelial abnormalities. Chin Med J (Engl) 121: 1969–1974.

Woenckhaus M, Merk J, Stoehr R, Schaeper F, Gaumann A, Wiebe K, Hartmann A, Hofstaedter F, Dietmaier W. 2008. Prognostic value of FHIT, CTNNB1, and MUC1 expression in non-small cell lung cancer. Hum Pathol 39:126–136.