Immunohistochemical characterization of FHIT expression in normal human tissues

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Abstract: Background: Fragile histidine triad (FHIT) is a tumor suppressor gene that is commonly inactivated in human tumors. Interestingly, the normal pattern of FHIT expression is largely unknown. Aim: This study is aimed to characterize the expression of FHIT protein in normal human tissues. Materials and methods: A total of 119 normal human tissue specimens were analyzed for the FHIT expression using immunohistochemistry technique. The inclusion criteria included: normal/inflammatory tissue with no evidence of cellular atypia. Results: All studied specimens were stained positively with FHIT and showed either nuclear or cytoplasmic expression. Interestingly, the pattern of FHIT staining was similar among different specimens from each organ. FHIT is located predominantly in the nucleus, although cytoplasmic staining is also present in some cell types. Oral squamous epithelium, breast ductal epithelium, squamous and tubal metaplastic epithelium of the uterine cervix, esophageal squamous epithelium, salivary glands, and bronchial epithelia all strongly expressed the nuclear protein. In connective tissue, FHIT has shown strong cytoplasmic expression in histocytes including macrophages and dendritic cells, fibroblasts, and myofibroblasts. Conclusion: Documentation of the pattern of FHIT expression in normal tissues will contribute to our understanding of the normal function of this protein and to interpretation of potentially altered FHIT expression in human tumors.

Keywords: FHIT, immunohistochemistry, human, normal tissues

Introduction

The fragile histidine triad (FHIT) is a tumor suppressor gene and is located in FRA3B which is the most active common fragile site, where DNA damage leading to aberrant transcripts and translocations frequently occur [1-5]. Abnormal transcripts of FHIT have been detected in various types of cancer [6, 7]. Alteration of the FHIT gene through damage to the associated fragile region by environmental carcinogens contributes substantially to the human cancer burden [8, 9]. Overexpression of FHIT protein was found to inhibit tumorigenic activity and cause cell apoptosis in cancer cells [10]. FHIT protein is involved in apoptotic signal pathways, although the mechanisms of apoptosis induction have not been defined in detail. The FHIT gene can be inactivated by several mechanisms, including deletions, point mutations, methylation, loss of a whole chromo-
immunohistochemical analysis of FHIT expression in normal human tissues and compare FHIT expression with proliferation and differentiation within individual organs.

Materials and methods

The research project has been approved by the institutional research committee at Al-Farabi College for Dentistry and Nursing (IRB 14-06).

Tissue samples

A total of 119 formalin-fixed, paraffin-embedded specimens of normal adult human organs were obtained from surgical specimens. The studied tissues have included oral mucosa, lung, liver, spleen, kidney, uterus, ovary, breast, prostate, stomach, bowel, bladder, thyroid, salivary glands, hyaline cartilage, esophagus, skin, and tonsils. The inclusion criteria included: normal/inflammatory tissue with no evidence of cellular atypia. A standard hematoxylin–eosin histological examination was done to confirm the inclusion of each case. All slides were reviewed by at least by two pathologists independently (O.K. and A.Z.A.).

Immunohistochemical technique

FHIT immunohistochemical testing was performed according to the protocol that was developed earlier by Kujan et al. [12] using primary rabbit polyclonal anti-glutathione S-transferase-FHIT antibody (abcam, Cambridge, UK). Briefly, three paraffin-embedded, formalin-fixed sections from each studied organ were deparaffinized, rehydrated, and immersed in 0.5% H₂O₂–methanol for 5 min. Sections were microwaved in 0.01 M sodium citrate buffer (pH 6.0) for 15 min and blocked in 10% normal horse serum in 5% milk for 20 min at room temperature. The FHIT antibody was used at a 1:200 dilution in 1% bovine serum albumin–phosphate buffered saline (BSA–PBS) overnight at 4 °C. Sections were subsequently incubated for 1 h at room temperature with biotinylated horse anti-mouse IgG (1:1000 dilution; Dako Cytomation, Denmark) and then with the avidin–biotin complex (DAKO LSAB kit; Dako Cytomation, Glostrup, Denmark). Diaminobenzidine was used as a chromogen, followed by counterstaining with hematoxylin. For a negative control, the primary antibody was omitted. A previously known positive normal human salivary gland tissue was used a positive control.

Statistical analysis

For scoring, negative and low (no staining or immunoreactivity staining present in <10% of cells) staining scores and moderate (immunoreactivity in ≥10–50% of cells) and strong (immunoreactivity in >50% of cells) staining scores were combined as negative or positive, respectively, as described previously by Kujan et al. [12]. The software SPSS 22.0 was used to analyze all the statistical analyses. The statistical analysis includes the use of descriptive statistics, frequencies, or proportions.

Results

One hundred and nineteen samples meeting the inclusion criteria were enrolled in the study. The number and type of studied organs are shown in Table I. All studied specimens were stained positively with FHIT and showed either nuclear or cytoplasmic expression. Interestingly, the pattern of FHIT staining was similar among different specimens from each organ, regardless of fixation time. Specifically, Table II shows the distribution of FHIT expression in organs and cell types.

FHIT is located predominantly in the nucleus, although cytoplasmatic staining is also present in most cell types that express the protein. Strong nuclear FHIT expression occurs in epithelial and ductal cells such oral mucosa, skin, and salivary glands that are originated

| No. | Tissue type       | Number of examined specimens |
|-----|-------------------|------------------------------|
| 1   | Oral mucosa       | 10                           |
| 2   | Lung              | 7                            |
| 3   | Liver             | 5                            |
| 4   | Spleen            | 7                            |
| 5   | Kidney            | 5                            |
| 6   | Uterus            | 7                            |
| 7   | Ovary             | 7                            |
| 8   | Breast            | 7                            |
| 9   | Prostate          | 7                            |
| 10  | Stomach           | 7                            |
| 11  | Bowel             | 5                            |
| 12  | Bladder           | 5                            |
| 13  | Thyroid           | 7                            |
| 14  | Salivary gland (parotid) | 7 |
| 15  | Skin              | 10                           |
| 16  | Esophagus         | 5                            |
| 17  | Hyaline cartilage | 4                            |
| 18  | Tonsils           | 7                            |
| Total |                   | 119                          |
Table II  Expression of FHIT in organs and cell types

| Organ                       | Cell type                           | FHIT (nuclear) | FHIT (cytoplasmic) |
|-----------------------------|-------------------------------------|----------------|-------------------|
| Oral mucosa (squamous epithelia) | Basal and parabasal layer           | +++            | −                 |
|                             | Spinous layer                       | −              | −/+               |
| Lung                        | Alveoli (squamous epithelium)       | +++            | −                 |
|                             | Broncholiolos (columnar epithelium) | +++            | −                 |
| Liver                       | Hepatocytes                         | −              | ++                |
|                             | Kupffer cells/sinusoid macrophages  | ++             | −                 |
| Spleen                      | Red pulp (macrophages)              | −              | +++               |
|                             | White pulp (lymphocytes)            | −              | −                 |
| Kidney                      | Glomeruli                           | −              | +++               |
|                             | Tubular epithelium                  | ++             | −                 |
| Uterus                      | Cervix epithelium (basal and parabasal) | +++    | −                 |
|                             | Glands                              | ++             | −                 |
|                             | Endometrium proliferative           | −              | +                 |
|                             | Secretory                           | +              | ++                |
| Ovary                       | Tunica albuginea (squamous mesothelium) | −    | ++                |
|                             | Follicles (oocytes)                 | −              | +++               |
| Breast                      | Ducts                               | ++             | −                 |
|                             | Acini                               | ++             | −                 |
|                             | Stroma                              | +              | +++               |
| Prostate                    | Glands                              | ++             | −                 |
|                             | Stroma                              | −              | +++               |
| Stomach                     | Surface epithelium                  | +              | +++               |
|                             | Glands                              | +              | +++               |
|                             | Smooth muscles                      | −              | +++               |
| Bowel                       | Mucosa (epithelium)                 | +              | +++               |
|                             | Glands                              | +              | +++               |
| Bladder                     | Transitional epithelium             | ++             | ++                |
|                             | Smooth muscles                      | −              | +++               |
| Thyroid gland               | Follicles                           | +++            | −                 |
|                             | Stroma                              | −              | ++                |
| Salivary gland (parotid)    | Acini                               | +++            | −                 |
|                             | Ductal epithelium                   | +++            | −                 |
|                             | Stroma                              | −              | ++                |
| Skin                        | Squamous epithelium (basal and parabasal layers) | +++ | − |
|                             | Melanocytes                         | +++            | −                 |
|                             | Skin appendages                     | +              | +                 |
| Esophagus                   | Squamous epithelia (basal and parabasal layers) | +++ | − |
| Hyaline cartilage           | Lucanae (chondrocytes)              | +              | ++                |
| Tonsils                     | Surface of squamous epithelium      | +++            | −                 |
|                             | Crypt epithelium                    | +              | −                 |
|                             | Lymphocytes                         | −              | −                 |

+ = few positive cells; ++ = moderate number of moderate cells; +++ = many positive cells; − = no positive cells
from ectoderm (Fig. 1A–D). A remarkable pattern was observed in oral squamous epithelium where only basal and parabasal cells layers were expressed the FHIT protein. In contrast, surface epithelium and glands of organs originated from either mesoderm or endoderm such stomach, bowel, bladder, and endometrium proliferative have shown affinity for strong cytoplasmic expression. In connective tissue, FHIT has shown strong cytoplasmic expression in histocytes including macrophages and dendritic cells, fibroblasts, and myofibroblasts (Fig. 1E). These cells showed nuclear staining as well. We were un-

![Image](image_url)

**Fig. 1.** Immunohistochemical expression of FHIT is several human normal tissues. A) FHIT was detected in the basal and parabasal layer of oral squamous cell epithelium. Strong cytoplasmic expression in fibroblasts and myofibroblasts (×100). B) FHIT strongly expressed in the nucleus of oral basal cells epithelium (×400). C) FHIT expressed strongly in the ductal and acinar structures of breast tissue (×200). D) Strong nuclear expression in acinar and ductal structures of parotid salivary gland and strong cytoplasmic expression in fibroblasts (×200). E) Negative FHIT expression in lymphocytes and strong cytoplasmic FHIT expression in histocytes, phagocytes in spleen (×200)
able to identify lymphocytes, plasma cells, and neutrophils with FHIT protein expression. A cytoplasmic and nuclear localization of FHIT protein was evident in the chondrocytes (lacunae) of the hyaline cartilage.

Discussion

The FHIT gene and its protein product have been the focus of recent debate with regard to their role in tumorigenesis. FHIT gene located at human chromosome region 3p14.2 was identified and shown to be a large focus of recent debate with regard to their role in tumorigenesis. FHIT gene is shown to be about 1 Mb in size and encodes a 1.1-kb cDNA with 10 small exons. Furthermore, lack of detectable FHIT protein in both cancer cell lines and solid tumors was found correlated to be with FHIT gene deletions [8–11].

FHIT gene is considered as a tumor suppressor gene that could play an important role in major human carcinomas, because cancer cell-specific homozygous deletions within the FHIT gene and lack of expression of the protein product are hall markers of tumor suppressor gene [3, 4].

Several studies recommended using immunohistochemistry for evaluating the FHIT expression in a primary tumor because the proportion of cells exhibiting the protein, as well as their level of expression, can be determined [14, 15]. In addition to that, the FHIT gene is mostly inactivated by deletion rather than mutation [16–18]. Furthermore, FHIT hypermethylation is a more common event than deletion in studied carcinomas, in particular breast carcinomas. [19]. Subsequently, the aberrant FHIT transcripts in tumors and tumor-derived cell lines were associated with loss of FHIT protein expression [20]. Many published studies have used immunohistochemistry as the primary method to determine the levels of FHIT in other major types of cancer such as breast [21], cervix [22], and lung [8].

Little knowledge exists on exploring the intracellular localization and its function in the tumorigenesis. The first report was published by Golebiowski et al. [23] which demonstrated the distribution of FHIT protein and its intracellular localization in rat tissues and cells. The immunoblot analysis performed on the subcellular fraction of various rat tissues obtained by the differential and density-gradient centrifugation showed that FHIT protein was localized exclusively in the nucleus and the plasma membrane, supporting the hypothesis concerning FHIT as a signaling molecule [23], which was corresponded to the hypothesis that the enzyme–substrate complex from membrane to nucleus is the active form of FHIT.

Our study demonstrated that FHIT protein was located in the nucleus and cytoplasm; the latter could be in the plasma membranous system (endoplasmic reticulum, Golgi network, mitochondria, and transport vesicles), in similar findings to previous studies [23, 24].

Literally, prominent FHIT nuclear expression is seen in epithelia, including the oral, tonsil, esophageal, and skin squamous epithelium, in the cells of the basal layers. In addition, a nuclear staining of FHIT in all cases of normal salivary and breast parenchyma was also seen. However, an abundant FHIT cytoplasmic expression was observed in the surface epithelium and glands of organs originated from either mesoderm or endoderm such stomach, bowel, bladder, and endometrium proliferative.

Most interestingly, FHIT protein is always most prominently found in the nucleus of histiocytes including macrophage and dendritic cells within one observable microscopic background, and can strongly mean its nuclear signal role related to the function of histiocytes. Stable nuclear FHIT protein could be prominently found in the monocytes of the circulating blood cells, macrophages of the connective tissue, Kupffer cells of the liver, alveolar macrophages or dust cells of the lung, epithelioid cells under chronic inflammatory conditions, and dendritic cells of the lymph node. The neutrophil, another strongly migratory and phagocytic functional cell besides macrophage, did not express FHIT protein at all, either in nucleus or in cytoplasm

These discrepancies in FHIT expression pattern could be explained by the difference in the FHIT role in the cell. At the cellular level, FHIT has been shown to induce apoptosis and retard tumor cell proliferation in vitro and in vivo [25, 26]. Moreover, it has been established that the FHIT gene encodes a diadenosine polyphosphate (Ap3A) hydrolase [16, 19, 27]. Therefore, FHIT product is believed to have hydrolytic activity. FHIT catalyzes the Mg²⁺-dependent hydrolysis of Ap3A into ADP and AMP [28]. The reaction is thought to follow a two-step mechanism, in which a complex of Ap3A and Mg²⁺ reacts in the first step with His96 of the enzyme to form a covalent FHIT·AMP intermediate and release MgADP. In the second step, the intermediate FHIT·AMP undergoes hydrolysis to AMP and FHIT [27]. Another suggestion hypothesized by Zhao et al. was that the executive mode of the nuclear localization of FHIT protein is due to the binding with other effector molecules that have been reported, such as tubulin, Ubc9, Nic, Hint, CDK7, and so on [24].

A physical interaction between FHIT and human ubiquitin-conjugating enzyme 9 (hUBC9) has been found. It is well known that yeast UBC9 is involved in the regulation of M- and S-phase cyclins. These results suggest that FHIT may play an important role in cell cycle control through this interaction [29].
Zhao et al. also suggested that FHIT protein is highly stably expressed in the nucleus of both fixed or resident and free or elicited macrophages, suggesting that FHIT protein is not only a tumor suppressor protein but also a signal molecule related to immune function [24].

The function of FHIT nuclear localization in the histiocytes is not clear so far, but it may not be correlated with migration and phagocytosis as FHIT is not detected in the neutrophils and lymphocytes, and may not be associated necessarily with pro-apoptotic function because FHIT is highly expressed not only in quiescent but also in active histiocyte. In other words, the stable FHIT nuclear expression in histiocytes may be significantly associated with the robust tumor suppressor function, which could explain why the genuine histiocyte-derived tumor is extremely rare. The hypothesis suggested is that the function of FHIT nuclear localization in the monocyte derived cells to be one of signaling molecules for immune response is antigen-presenting and/or cytokine manufacturing [24]. However, the detailed mechanism between the nuclear localization and biological significance of FHIT in the histiocytes remains for larger studies to resolve.

The study has limitation due to the restricted selection of human body and organs that were examined and not to associate the current immunohistochemistry findings with the results from an immunoblot assay.

In summary, documentation of the pattern of FHIT expression in normal tissues will contribute to our understanding of the normal function of this protein and to interpretation of potentially altered FHIT expression in human tumors.

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