Tumor Suppressor Genes: Does FHIT Fit?

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What is a tumor suppressor gene? From a functional point of view, it is a gene whose product can restrict the tumorigenic and metastatic processes at a certain step. From a genetic point of view, both alleles of a tumor suppressor gene are inactivated directly through deletion, inactivating mutations, or epigenetic modifications (e.g., methylation) in neoplastic cells but not in their normal counterparts.

The FHIT (fragile histidine triad) gene at chromosome 3p14 was initially identified by positional cloning in 1996 (1) and is considered a candidate tumor suppressor gene in multiple tumor types. Dinucleoside 5',5'-P1, P1-triphosphate (Ap1A) hydrolase activity is the only known function of pFHIT, and all four of the conserved histidines in the protein are required for full activity of the enzyme (2).

The discovery of the FHIT gene drew great attention in the cancer research community because loss of DNA sequences from chromosome 3p (including the 3p14 region) was recognized as one of the most frequent genetic and cytogenetic abnormalities present in a broad range of solid tumors. At least three tumor suppressor gene loci are suspected to be present on chromosome 3p. However, despite a rigorous search over the past two decades, only one tumor suppressor gene had been previously identified on chromosome 3, the VHL (von Hippel-Lindau disease) gene at 3p25, which was found to play a role in the tumorigenesis of renal cancer and hemangio-blastoma (3).

One could speculate that the aberrant transcripts may derive from subclones that occur randomly and are not associated directly with tumor initiation or progression. The presence of this fragile site may also account for the propensity to see deletions and no point mutations of the second allele. The fact remains that most tumor cell lines and primary tumors with FHIT abnormalities do not express—or express only low levels of—FHIT protein (6,8). One could speculate that the aberrant transcripts may inhibit protein translation or trigger protein degradation. Alternatively, another as yet unidentified gene within or close to FHIT may be the critical target while FHIT is an innocent bystander.

Finally, some investigators have noted that aberrant FHIT transcripts can be observed in many nontumor tissues (12,13) and that they also become more predominant in aging cells (14). These observations suggest that FHIT abnormalities are not tumor specific but represent the result of randomly occurring deletion events at a fragile site or reduced RNA-splicing fidelity in aging cells. However, loss of FHIT could be a very early clonal event in tumorigenesis. In fact, frequent loss of heterozygosity (LOH) at 3p14 is observed in oral premalignant lesions (15) and in normal-appearing bronchial epithelia of smokers (16). These findings support the notion that loss of the 3p14 region occurs early and frequently in tissues exposed to carcinogens. However, the direct role of the loss in tumorigenesis needs further investigation.

In this issue of the Journal, Muller et al. (17) report a study in which they have assessed LOH at FHIT, FHIT expression pat-
terns, and the presence of human papillomavirus (HPV) infection in cervical carcinoma cell lines, primary tumors, and normal epithelia. The authors confirmed previous findings that LOH in the 3p14 region, abnormal transcripts of FHIT, and infection with oncogenic HPV types are frequent in cervical cancer. Although the authors found an abnormal transcript of FHIT in normal cervical epithelium, it was distinct from the abnormal transcripts found in tumors (17), suggesting that different mechanisms may be involved in the genesis of these transcripts between normal cervical epithelium and cervical cancer. Muller et al. also found that infection with HPV 16, a high-risk HPV type, is associated with LOH in the FHIT region, supporting the importance of the 3p14.2 region in cervical carcinogenesis and again raising the question as to whether FHIT is the *bona fide* tumor suppressor in the region.

Further questions about the role of FHIT as a tumor suppressor gene are raised in another study by Otterson et al. (18), also in this issue of the Journal. The authors introduced a wild-type FHIT cDNA expression construct into HeLa cells, a cervical carcinoma cell line lacking endogenous pFHIT expression, to determine its ability to inhibit or reduce tumorigenicity. Although 58% (30 of 52) of human carcinoma cell lines that they studied—including HeLa—lack detectable pFHIT protein, expression of the cDNA construct did not change the rate of cell proliferation or alter tumorigenicity in animals (18). In contrast, a recent study by Siprashvili et al. (19) showed that transfection of FHIT DNA sequences into tumor cell lines from gastric, large-cell lung, nasopharyngeal, and renal cell cancers can significantly reduce the frequency of tumor formation and the size of tumors formed in nude mice. It is notable that the cell proliferation rate and the ability to form colonies in soft agar *in vitro* did not change after FHIT DNA transfection in both studies, clearly demonstrating that pFHIT does not play a role in the control of cell proliferation. These data argue against the hypothesis that elevated AP3A levels, resulting from a loss of control of cell proliferation. These data argue against the hypothesis that elevated AP3A hydrolase activity could also suppress tumorigenicity, suggesting that AP3A hydrolase activity is not essential for tumor suppression.

Now, the controversy is certain to intensify regarding the role of FHIT in tumorigenesis, in light of these contradictory results. One may argue that the apparent discrepancy may be the result of using different cell lines in the individual studies or of varying pFHIT expression levels in the independent experiments. It is clear that more studies are required to understand the biologic functions of the gene, especially pFHIT’s possible interactions with other proteins or nucleic acid elements. A search for possible cooperative effects of pFHIT with other cellular components could then be undertaken. Furthermore, it is crucial to understand the exact mechanisms underlying the generation of abnormal transcripts, which are frequently observed not only for FHIT but also for several other tumor-associated genes, such as TSG101 and mdm2 (20,21). If not caused by altered structure of the gene itself, aberrant transcripts may reflect reduced splicing fidelity, due to a defect in one or more components of the splicing machinery. It would not be surprising that this kind of defect could produce abnormal gene products, potentially acting as oncogenes or inhibitors of tumor suppressor genes. Finally, the long-awaited FHIT gene knockout experiments may shed light on this problem, especially if significantly more tumors can be observed in the gene-deficient animals.

Is FHIT a *bona fide* tumor suppressor or just an innocent bystander in a well-known fragile site? We can certainly classify a gene as a tumor suppressor when its inactivation patterns or functions fit the classical genetic and functional criteria for tumor suppressor genes. However, we should not prematurely reject a gene as a tumor suppressor when it displays atypical genetic and biologic patterns. We may need a bit of patience to fully understand the role of FHIT in human cancer.

References

Vitamins A and E: Further Clues for Prostate Cancer Prevention

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The initiation and progression of prostate cancer seems to involve a complex series of both endogenous and exogenous factors. While the frequency of latent prostate cancer is fairly evenly distributed among populations, the rate of transition from latent to progressive, clinically evident cancer shows considerable variation among populations (1). Migrants from countries with low rates of prostate cancer, moreover, tend to assume the higher rates of their new countries within a fairly short period of time, suggesting that environmental factors may play an important role in promoting clinically detectable cancer.

Known risk factors for prostate cancer include age, family history, and race. After age 50 years, both incidence and mortality rates from prostate cancer increase at a nearly exponential rate, more so than with any other major cancer. Younger age at onset of disease is also associated with a family history of prostate cancer: 18% of the patients who were less than 65 years of age at the onset of the disease have a positive family history compared with only 6% of older patients (2). The risk of developing prostate cancer also increases with the number of affected first-degree relatives and with a lower age of onset for those affected relatives (3). Several studies (3–5) suggest that a rare, highly penetrant dominant allele is responsible for this inherited form of prostate cancer, which may also account for some of the racial differences in disease patterns. Worldwide, African Americans have the highest incidence of clinically evident disease, at rates greater than 13 times those of men in China and Japan where rates are the lowest.

A variety of environmental and dietary elements have been proposed to promote prostate cancer, including dietary fat (1). Recently published research from the Health Professionals Follow-Up Study group shows that high intake of dietary and supplemental calcium also increases the risk of advanced prostate cancer by suppressing the formation of 1,25 OH2 in contrast to the association between high levels of vitamin D from 25 (OH)D. Laboratory and clinical studies suggest that this active form of vitamin D has an antitumor effect on prostate cancer (6).

The majority of research on dietary influence has focused on antioxidants. Free radicals are known to damage crucial cell components, such as DNA, proteins, enzymes, and membranes, and have been implicated in a wide spectrum of chronic diseases, including cancer. Vitamin A (retinol) and its active metabolites are essential for cell differentiation, visual function, physiologic growth, and reproduction, yet despite numerous studies, no consistent association with cancer risk has been established (7). Some studies show an inverse relationship between serum retinol and the risk of prostate cancer, while others indicate a positive association, particularly in men over the age of 70 years. Some of this discrepancy may be explained by the fact that vitamin A comes from both plant sources (carotenoids) and animal sources (vitamin A), so that a link with dietary fat may be a confounding variable.

There are more than 500 different carotenoids, and a considerable number of studies have examined their effect on cancer risk, often in conjunction with vitamin A or other antioxidants. Lycopene, a carotenoid that is not converted to vitamin A, is the most effective quencher of singlet oxygen of the major carotenoids and is the primary carotenoid in serum and various tissues, including the prostate gland. Several studies (8,9) have found an inverse association between prostate cancer and the intake of tomatoes and tomato-based products, the major dietary source of lycopene. β-Carotene, another important carotenoid, has also been studied extensively. Overall, data have suggested a protective effect, although several large studies have also shown no association (7).

Vitamin E also has the potential to decrease DNA damage and inhibit malignant transformation through its antioxidant

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