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Review Article

Common Fragile Site Tumor Suppressor Genes and Corresponding Mouse Models of Cancer

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Chromosomal common fragile sites (CFSs) are specific mammalian genomic regions that show an increased frequency of gaps and breaks when cells are exposed to replication stress *in vitro*. CFSs are also consistently involved in chromosomal abnormalities *in vivo* related to cancer. Interestingly, several CFSs contain one or more tumor suppressor genes whose structure and function are often affected by chromosomal fragility. The two most active fragile sites in the human genome are FRA3B and FRA16D where the tumor suppressor genes *FHIT* and *WWOX* are located, respectively. The best approach to study tumorigenic effects of altered tumor suppressors located at CFSs *in vivo* is to generate mouse models in which these genes are inactivated. This paper summarizes our present knowledge on mouse models of cancer generated by knocking out tumor suppressors of CFS.

1. Introduction

Fragile sites can be defined as heritable-specific loci on human chromosomes that exhibit nonrandom gaps or breaks when chromosomes are exposed to specific cell culture conditions [1]. Classification of fragile sites as rare or common depends on their expression frequency within the population [2]. Rare fragile sites are identifiable in less than 5% of the population, while common fragile sites are an intrinsic part of the regular structure of the chromosomes that are present in all individuals [1]. Fragile sites are often involved in deletions and translocations [3], in sister chromatid exchanges [4], in plasmid integration [5], and in intrachromosomal gene amplification [6]. Interestingly, some fragile sites, especially common sites, are involved in chromosomal tumor-related rearrangements, such as the deletions [7] and translocations [8] found in various tumors. The cytogenetic expression of common fragile sites is visible over wide chromosomal regions of mega-bases in size [1]. These sites seem therefore to represent regions of fragility, rather than specific loci [9]. The importance of common fragile sites in cancer is particularly relevant when one or more tumor suppressor genes are located within a specific region of fragility. For instance, *FRA3B*, the most active common fragile site on human metaphase chromosomes, maps to a region on chromosome 3p14.2 associated with deletions or translocation breakpoints in a vast number of human cancers, including lung [10], breast [11], esophagus [12], pancreas [13], and kidney [14]. The *FHIT* (Fragile HIstidine Triad) gene maps to the same chromosomal region of *FRA3B* [15]. It has been shown that this gene is frequently deleted [11, 15, 16] or involved in translocation breakpoints [15, 17] in a large number of tumor types. Other common fragile sites have been implicated in homozygous deletions or loss of heterozygosity (LOH) observed in various malignancies [1]: *FRA16D*, located on chromosome 16q23.2 and altered in breast [18], prostate [19], and hepatocellular carcinoma [20]; *FRA6E* on 6q26 [21], and *FRA7G* on 7q31.2 [22], both altered in ovarian cancer among others. The *WWOX* tumor suppressor gene, in the *FRA16D* region, the second most

Table 1: Synopsis of CFS tumor suppressor genes and their mouse models of cancer.

Gene	Human tumors	Animal model	Treatment	Induced phenotype	Spontaneous phenotype
FHIT* **3p14.2** Deletions translocations	Head and Neck, Lung, Larynx, Liver, Pancreas, Digestive Tract, Urinary Tract, Prostate, Spleen, Bone Marrow	FHIT KO	Radiation	Increased multiorgan tumor involvement respect WT, but equal tumorigenesis	
		FHIT KO	B(a)P (Benzo(a)pyrene)	KO and WT mice were equally sensitive; $FHIT^{+/-}$ mice showed uterine preneoplasias	
		FHIT KO	ECS (Environmental cigarette smoke)	After exposure, FHIT loss was observed also in WT mice. WT and $FHIT^{+/-}$ mice did not show significant differences	
		FHIT KO	BBN (N-butyl-N-hydroxybutyl) nitrosamine)	28% of $FHIT^{-/-}$ and 46% of $FHIT^{+/-}$ versus 8% of WT mice developed bladder invasive carcinomas	
		FHIT KO X Vhl KO			Lung adenocarcinomas in 44%
		FHIT KO X Vhl KO	DMN (Dimethyl-nitrosamine)	Lung adenocarcinomas in 100%	
		$FHIT^{+/-}$ X HER2/neu (mammary tumor promoter)			Mammary tumors in 100%
		FHIT KO X Nit KO	NMBA (N-nitroso-methyl-benzylamine)	~100% more gastric tumors than $FHIT^{-/-}$ alone	
WWOX **16q23.2** Deletions translocations	Lung, Digestive Tract, Liver, Pancreas, Breast, Ovarian, Prostate, CNS, Urinary Tract, Bone, Thyroid, Bone Marrow	Wwox KO			Postnatal lethality for $^{-/-}$ mice. Surviving mice showed metabolic disorders and developed femoral focal lesions resembling to osteosarcoma. $^{+/-}$ mice showed higher incidence of lung and mammary tumors than WT mice
			ENU (Ethyl-Nitroso-Urea)	80% of $^{+/-}$ mice developed lymphoblastic leukemia, lung, mammary, and liver tumors	
			NMBA (N-nitroso-methyl-benzylamine)	~100% of $^{+/-}$ mice with forestomach tumors	
		Wwox Hypomorphic strain (very low expression of Wwox)			Short lifespan. Females exhibited high incidence of lymphomas

Table 1: Continued.

Gene	Human tumors	Animal model	Treatment	Induced phenotype	Spontaneous phenotype
PARK2 **6q26** Deletions translocations	Ovary, breast, esophagus, liver, colon, lung, renal, CNS, hematopoietic	Park2 KO			Motor and cognitive defects
		Park2 X Apc			4-fold increase in adenomas than control mice; all stages neoplastic lesions
CAV-1 **7q31.2** Deletions translocations	Downregulated in: ovarian, lung, mammary tumors Upregulated in: prostate, bladder, thyroid, esophageal carcinomas	Cav-1 KO			Normal. Reduction of Cav-2 expression
		Cav-1 KO	DMBA (7,12-dimethyl benzanthracene)	Development of epidermally derived tumors	
		Cav-1 X MMTV-PyMT			Acceleration of the development of mammary lesions
		Cav-1 X Tramp			Decreased incidence in prostate tumors and loco-regional and distal metastasis
TES **7q31.2** Deletions translocations	Head and Neck, gastric, breast, hematopoietic, prostate, CNS	Tes KO			Normal
		Tes Ko	NMBA(N-nitroso-methyl-benzylamine)		High incidence of forestomach tumors

* FHIT findings include the period 2005–2010. Older data were previously reviewed.

active common fragile site in the human genome [23], has been cloned [24]. The involvement of *WWOX* in cancer has been reviewed recently by Del Mare et al. [25].

An important step in the functional characterization and validation of putative human tumor suppressor genes is the generation of recombinant mouse knockout models with both alleles of the gene of interest inactivated. Genes associated to well-characterized human CFSs are conserved in the mouse genome, but the level of fragility of CFSs may not be the same. In this paper we describe recombinant mouse strains carrying inactivated fragile site tumor suppressor genes, the fragile genes that have been most extensively examined for association with cancer development (Table 1).

2. FHIT

The role of *FHIT* as a tumor suppressor, its biochemistry, genetics, pathology, and biology, has been extensively reviewed since the discovery of the gene, 14 years ago (e.g., [26–28]). Previously, we summarized the insights that had emerged until 2004 into the genetics and biology of *FHIT*-deficient mice with particular focus on carcinogenesis and gene delivery studies [29]. New developments since 2004 concerning the *FHIT* gene and gene product are reviewed in this section.

Although the usefulness of a model like *FHIT*-deficient mouse rests mainly in the possibility to perform *in vivo* experiments, normal cells from different tissues with a defined *FHIT* genotype can also be isolated, cultured, and studied for specific purposes. For example, we established normal kidney cell lines from $FHIT^{+/-}$ and $FHIT^{+/+}$ mice that were then stressed and examined for differences in cell cycle kinetics and survival [30]. The same experiment was also performed with human *FHIT*-negative and -positive cancer cell clones. A larger fraction of *FHIT*-negative murine kidney cells survived treatment with mitomycin C or UVC light compared to *FHIT*-positive cells. Approximately 10-fold more colonies of mouse *FHIT*-deficient cells survived high UVC doses in clonogenic assays. Compared to wild type cells, similar results were also obtained with human cancer cells. After low UVC doses, the rate of DNA synthesis in mouse $FHIT^{-/-}$ cells decreased more rapidly and steeply than in $FHIT^{+/+}$ cells. UVC surviving $FHIT^{-/-}$ cells appeared transformed and exhibited more than 5-fold increase in mutation frequency. Such increased mutation burden

could explain the susceptibility of *FHIT*-deficient cells to malignant transformation *in vivo* [30].

An ionizing radiation study reported that FHIT could protect human cells from high doses of ionizing radiation-induced mutations at the *HPRT* locus [31], suggesting, once more, the potential protective effect of FHIT in DNA damage-induced carcinogenesis. However, it was still unclear whether FHIT could prevent high dose radiation-induced carcinogenesis or whether it plays any role in a low-dose environment. To investigate effects of multiexposure to low dose radiation at a high dose rate on tumorigenesis and whether FHIT plays a protective role in the process, Yu et al. [32] irradiated $FHIT^{+/+}$ and $FHIT^{-/-}$ mice with 1 Gy $\times$ 1 or 0.1 Gy $\times$ 10 exposures at a dose rate of 1 Gy/min, sacrificed the mice at 1.5 years after radiation and studied multiorgan tumorigenesis. The results showed that although the spontaneous tumorigenesis in these mice was relatively high, 1 Gy x1 exposure dramatically increased multiorgan tumor development and $FHIT^{-/-}$ mice showed more tumors than $FHIT^{+/+}$ animals. However, 0.1 Gy x 10 exposures did not increase tumorigenesis, and there was no significant difference between $FHIT^{+/+}$ and $FHIT^{-/-}$ mice. Thus, these results showed that FHIT could prevent high dose radiation-induced tumor development but has no effect in a low dose environment [32].

In the last five years, *FHIT*-deficient animals were used to produce mice deficient for multiple tumor suppressors or upregulated oncogene, and to discover effects due to the association of loss/reduction of FHIT and simultaneous deregulation of another cancer gene. Thus far, three articles have been published on this subject: two regarding the tumor suppressor genes *Vhl* and *Nit*, and one on the oncogene *HER2* (Figure 1).

Since alterations to the *FHIT* gene as well as other suppressor genes mapping to human chromosome 3 play an important role in development of lung and other cancers, we decided to determine if FHIT absence, in combination with deficiency of an additional tumor suppressor on human chromosome 3p, would affect the frequency of tumor induction. Thus, we examined the spontaneous and dimethylnitrosamine (DMNs-) induced tumor phenotype of $FHIT^{-/-}Vhl^{+/-}$ mice [33]. Whereas no spontaneous lung tumors were observed in $FHIT^{-/-}$ *or* $Vhl^{+/-}$ mice, 44% of $FHIT^{-/-}Vhl^{+/-}$ mice developed adenocarcinomas by two years of age. In addition, DMN induced lung tumors (adenomas and carcinomas) in 100% of $FHIT^{-/-}Vhl^{+/-}$ mice and adenomas in 40% of $FHIT^{-/-}$ mice by the age of 20 months. Thus, double deficiency in murine homologues of human 3p suppressor genes (Figure 1(a)) predisposes to spontaneous tumor formation and induced lung cancers recapitulating a pattern of lung cancer development similar to the human counterpart [33].

Manuela Campiglio's group had previously shown that FHIT protein levels could be regulated by FHIT proteasome degradation mediated by EGF-dependent activation of EGFR family members, including HER2, whose overexpression is linked to poor prognosis in breast cancer [34]. To test for a possible cooperation of the *FHIT* and *HER2* genes, these authors assessed tumor incidence in $FHIT^{+/-}$ mice

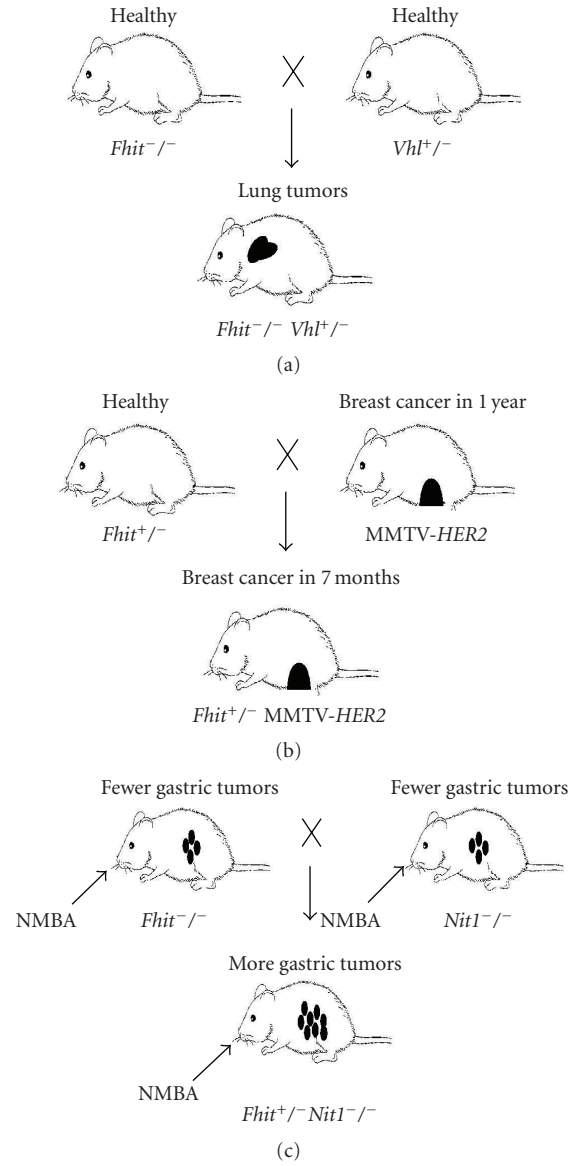

Figure 1: Cooperating effect of *FHIT* with *Vhl* (a), *HER2* (b), and *Nit1* (c) in mouse tumorigenesis.

crossed with animals carrying the *HER2/neu* proto-oncogene driven by the mouse mammary tumor virus promoter [35]. All *FHIT* heterozygous mice developed mammary tumors, whereas tumor incidence was reduced by 27% in *FHIT* wild type animals, which remained tumor-free at twenty months. These findings (Figure 1(b)) suggested a protective role for *FHIT* in *HER2*-driven mammary tumors and point to cooperation between FHIT loss and HER2 over-expression in breast carcinogenesis [35].

Mammalian *Nit1* is a member of a large gene family with some branches conserved from bacteria to mammals; interest in an association of *Nit1* alterations with cancer development in mammals began with the finding that in flies and worms Nit1 is fused to the N-terminus of the FHIT protein [36]. Conversely, mammalian Nit1 and FHIT

proteins are encoded by genes on different chromosomes. According to the "Rosetta stone" hypothesis, genes fused in one organism are likely to be involved in the same signal or metabolic pathways in organisms in which they map to separate locations [37]. To investigate roles for Nit1 in FHIT pathways, Semba et al. [38] generated Nit1 knockout mice and observed that these animals are more susceptible to tumor induction, like *FHIT*-deficient mice. Then the authors compared the tumor susceptibility of $FHIT^{-/-}$ mice with that of $FHIT^{-/-}Nit1^{-/-}$ double knockout animals after oral delivery of N-nitrosomethylbenzylamine (NMBA) and observed that double knockout mice developed significantly more spontaneous and NMBA-induced tumors than $FHIT^{-/-}$ mice [39]. When two distinct cancer genes are involved in the same pathway, the disruption of both their activities is not expected to lead to increased tumor susceptibility relative to the silencing of just one of the genes. Thus, results of the double knockout experiment (Figure 1(c)) suggested that *FHIT* and *Nit1* work, at least to some extent, in different pathways and their tumor suppressor activities are additive [39].

Silvio De Flora's group, in collaboration with us, was interested in studying the effect of *FHIT*-deficient mice after exposure to cigarette smoke or benzo[a]pyrene (B[a]P), a prototype of genotoxic and carcinogenic polycyclic aromatic hydrocarbons (PAHs) that are a fundamental component of cigarette smoke. In the first of these studies, wild type and $FHIT^{+/-}$ mice were treated with multiple doses of B[a]P by gavage [40]. All mice, irrespective of their FHIT status, were sensitive to induction of forestomach tumors, while preneoplastic lesions of the uterus were more frequent in $FHIT^{+/-}$ mice. The first generation of the cross between C57BL/6J and 129/SvJ strains (B6/129 F1) underwent spontaneous alopecia areata, an inflammatory skin disease, and hair bulb cell apoptosis. This phenotype was greatly accelerated by *FHIT* heterozygosity, suggesting that FHIT may play a role in the pathogenesis of alopecia areata [40]. In a separate report, D'Agostini et al. [41] exposed wild type and $FHIT^{+/-}$ rodents to environmental cigarette smoke (ECS). Evaluation of FHIT expression in the respiratory tract after 14 days of exposure to ECS revealed unequivocal evidence that FHIT is an early, critical target in smoke-related carcinogenesis [41], but heterozygosity for FHIT does not seem to confer an increased susceptibility of mice in terms of early biomarkers like apoptosis, cell proliferation, bulky DNA adducts in the lung, and various cytogenetical damages [42].

To determine if a nonfragile, cDNA version of a *FHIT* allele, expressed from a chromosomal region outside the fragile site, could reduce susceptibility of mice to carcinogen-induced tumorigenicity, we generated *FHIT* transgenic mice on a $FHIT^{+/+}$ and $FHIT^{+/-}$ background, treated them with NMBA, and assessed their tumor burden relative to wild type and $FHIT^{+/-}$ mice [43]. The tumor burden in NMBA-treated male transgenic mice was significantly reduced, suggesting that indeed a nonfragile FHIT protein could protect from tumorigenesis, while female transgenic animals were not protected. To determine if the difference in protection could be due to differences in epigenetic changes at the transgene locus in male versus female mice, we examined expression, hypermethylation and induced re-expression of *FHIT* transgenes in males and females or cells derived from them. The differences detected in the epigenome did not explain the differences in protection between the two sexes [43] but this should be reexamined with more sensitive epigenome sequencing methods now available.

FRA3B, the most active or most fragile of the human common fragile sites, is frequently altered in environmental carcinogen-associated cancers and in hematopoietic disorders [23, 44]. We reported that absence of FHIT in mouse hematopoietic cells exposed to hydroquinone, a genotoxic benzene metabolite, led to resistance to induction of cell death *in vitro* and escape from bone marrow suppression in transplanted mice [45]. Immunohistochemical analyses of transplanted hydroquinone-exposed, $FHIT^{-/-}$, and $FHIT^{+/+}$ bone marrow revealed absence of apoptosis and senescence markers in KO bone marrow. Accordingly, the long-term survival of hydroquinone-exposed FHIT-deficient bone marrow-transplanted mice allowed accumulation of inaccurately repaired DNA lesions and premalignant alterations in bone marrow-derived cells, suggesting that FHIT deficiency leads to unscheduled survival of genotoxin-exposed bone marrow cells, and increases the population of stem or precursor cells with damaged genomes and resultant accumulation of genomic alterations [45].

Since the *FHIT* gene is altered in human bladder cancer [46], Raffaele Baffa's laboratory published a report on a chemically induced urinary bladder cancer model in FHIT-deficient mice [47]. Recently, they used the same model to investigate the chemopreventive role of a COX-2 inhibitor, rofecoxib, in the development of bladder cancer in $FHIT^{+/+}$, $FHIT^{+/-}$, and $FHIT^{-/-}$ mice [48]. Though the COX-2 inhibitor decreased the incidence of neoplastic lesions in all three genotypes, the authors confirmed that FHIT-deficient mice are highly susceptible to N-butyl-N-(-4-hydroxybutyl)-nitrosamine (BBN), providing an *in vivo* model suitable for bladder cancer preclinical studies [48].

3. Wwox

Since *WWOX* is down-regulated in most human cancers and shows tumor suppressor function in different cell lines, *Wwox* knockout mice may be a useful tool to study the tumor suppressor activity of Wwox [25]. The mouse *Wwox* gene is similar to its human homologue, it spans the CFS Fra8E1 [49], and its expression induces apoptosis in murine cell lines [50]. Consequently, the murine *Wwox* gene is an appropriate model for studying the anti-tumor function of the human *WWOX* gene [25]. Thus, in 2007, we developed a mouse strain lacking Wwox expression [51]. The resulting single- and double-allelic targeted ablations exhibiting two completely different phenotypes.

Heterozygous mice developed normally and allowed to assess how the inactivation of a single *Wwox* allele spontaneously contributes to tumorigenesis [51]. Thus, after monitoring $Wwox^{+/+}$ and $Wwox^{+/-}$ littermates for two years, we observed that the incidence of tumor formation in $Wwox^{+/-}$ mice was significantly higher than in wild type animals and included lung and mammary tumors [51].

Noteworthy was the fact that in some tumors the second *Wwox* allele remained intact, suggesting haploinsufficiency for tumor suppression. To evaluate the role of WWOX in tumor progression, $Wwox^{+/+}$ and $Wwox^{+/-}$ animals were then treated with different chemical carcinogens and the incidence of tumor formation was examined. In one study, we utilized the chemical mutagen ethyl-nitroso-urea (ENU) and forty weeks after its administration, incidence of tumor formation in $Wwox^{+/-}$ mice was 80% compared to 40% in $Wwox^{+/+}$ animals. The tumor spectrum included leukemia and lung, mammary, and liver tumors [51]. In another study [52], wild type and $Wwox^{+/-}$ mice were treated with the gastric carcinogen NMBA. Fifteen weeks after its administration, almost 100% of $Wwox^{+/-}$mice had developed forestomach tumors ranging from adenomas to invasive carcinomas, whereas tumors were present only in 29% of $Wwox^{+/+}$ animals. Interestingly, $Wwox^{+/-}$ forestomachs showed moderately strong staining of Wwox protein in the near normal epithelium but weak and diffuse staining in the carcinoma areas of the same sections, thus confirming the haploinsufficient feature of Wwox [52]. Another report provided further support for the *in vivo* tumor suppressor activity of Wwox [53]. These authors produced a hypomorphic strain of mice with very low levels of Wwox protein. *Wwox* hypomorphic mice were viable but with a significantly shorter lifespan in comparison to wild type animals and females exhibited a higher incidence of spontaneous lymphomas [53].

$Wwox^{-/-}$ mice are born with no obvious malformations [51, 54]. Nevertheless, these mice exhibit a metabolic disorder characterized by hypoglycemia and hypocalcaemia and die at 3-4 weeks of age, showing growth delay, and an impaired ratio of organ/body mass in several tissues including spleen, thymus, and brain. Though smaller in size, they did not display abnormal behavior or impaired motor skills [54]. These and other features of the *Wwox* homozygous phenotype support multiple and unique roles of Wwox in a number of biological activities, like lipid metabolism and steroidogenesis [25], that are beyond the scope of this paper.

Although $Wwox^{-/-}$ mice died very prematurely, by the age of three weeks they develop focal lesions along the diaphysis of their femurs resembling early osteosarcomas [51]. Osteosarcoma derives from proliferation of undifferentiated osteoblasts. Intriguingly, we observed an impaired differentiation in $Wwox^{-/-}$ osteoblasts, suggesting a possible relationship between these two observations. Wwox, in fact, seems to be essential in regulating proliferation and maturation of osteoprogenitor cells during bone formation [54]. We observed an increase in Runx2 levels, the master transcription factor specific for osteoblast differentiation, in femur bones of $Wwox^{-/-}$ mice. In biochemical terms, we have demonstrated a physical interaction between Wwox and Runx2 using co-immunoprecipitation assays [54]. This association suppresses Runx2 transactivation function, therefore, since RUNX2 autoregulates its expression [55], we speculated that absence of Wwox stimulates Runx2 transactivation function and hence upregulates its expression. Further investigations are however necessary to gain more insights into the functional role of the Wwox-Runx2 interactions in osteosarcoma [25]. Recent results in human osteosarcoma specimens and cell lines confirmed that attenuation of Wwox is associated with increased tumorigenicity and aberrant expression of Runx2 [56] according to the predictions of *Wwox* mouse model.

Since $Wwox^{-/-}$ mice generated using conventional techniques die very early in life, conditional *Wwox* knockout mice were generated to study the WWOX function in both normal and cancer tissues [57]. This new model will greatly facilitate the functional analysis of Wwox in adult mice and will allow more refined investigations of neoplastic transformation in specific target tissues [57]. Other similar *Wwox* models are currently in construction as well.

4. Others

Parkin (*PARK2*) on *FRA6E* (6q26) is a widely expressed ubiquitin E3 ligase that is thought to target specific proteins for proteasomal degradation, and its mutations are responsible for autosomal recessive juvenile Parkinson disease [58, 59]. Moreover, diminished or total loss of Parkin expression has been observed in primary tumors and cell lines derived from ovarian, liver and lung carcinomas [60, 61], whereas its restored expression reduced *in vivo* tumorigenesis in nude mice [62]. Three *Park2* deficient mice have been generated, but only two models had an abnormal phenotype [63, 64]. Targeted exon3 null mice did not show any degeneration of dopaminergic neurons of substantia nigra, the neuropathological hallmark of Parkinson disease, but demonstrated motor and cognitive defects that resembled the very early symptoms of patients, prior to the development of overt clinical symptoms. Null mice did not develop any tumor or preneoplastic lesion, but interbreeding of *Park2* heterozygous mice with *Apc* (min) mice resulted in a dramatic acceleration of intestinal adenoma development and increased polyp multiplicity [65].

Deletions at chromosome 7q have been reported in a variety of human neoplasias: leukemia [66], breast [67], ovary [68], colon [69], prostate [70], gastric [71], head and neck [72], pancreatic [73], and renal cell carcinomas [74]. *FRA7G* (7q31.2) harbors two putative tumor suppressor genes: *CAVEOLIN-1 (CAV-1)* and *TESTIN (TES)*.

Caveolin proteins are expressed by caveolae, specialized invaginations of the plasma membrane that function to regulate signal transduction within the cell. *Cav-1* knockout mice showed a remarkable lack of caveolae in all nonmuscle tissues and are viable and fertile [75–78]. In mice, the absence of Cav-1 protein is associated to the reduction of Cav-2 expression, without any change at the transcription level.

Cav-1 null mice did not spontaneously develop tumors, although Capozza et al. [79] showed that the skin of $Cav\text{-}1^{-/-}$ mice is more susceptible to chemical carcinogenic (7,12-dimethylbenzanthracene, DMBA) treatment, resulting in the formation of epidermally derived tumors that are associated with cyclin D1 up-regulation and ERK1/2 hyperactivation. Young virgin *Cav-1* null mice developed a hyperplastic ductal epithelium [80] and complete loss of *Cav-1* accelerated the appearance of mammary dysplastic lesions in

polyoma middle T tumor prone transgenic mice (MMTV-PyMT) [81]. Loss of *caveolin-1* gene expression accelerates the development of dysplastic mammary lesions in tumor-prone transgenic mice. These findings were enforced by a study demonstrating that *CAV-1* haploinsufficiency is sufficient to induce the partial transformation of human breast epithelial cells [82].

However, *Cav-1* does not behave as a tumor suppressor in all cellular contexts. Genetic ablation of *Cav-1* in *TRAMP* mice decreased the incidence of prostate tumors at 28 weeks and reduced metastasis to regional lymph-nodes and distant organs [83]. An increased expression of *CAV-1* has been observed in metastatic lesions and metastasis-derived cell lines, as compared to primary tumors and primary tumor-derived cell lines respectively [84]. In summary, *CAV-1* is down-regulated in ovarian, lung and mammary carcinomas, as well as mesenchymal sarcoma, while it is up-regulated in prostate, bladder, esophageal and thyroid carcinomas, with some exceptions.

TESTIN (TES) is a cytoskeleton-associated protein that localizes along actin stress fibers, at cell-cell contact areas, and at focal adhesion plaques, where it directly interacts with Mena [85]. Loss of Tes expression has been observed in several tumor-derived cell lines [86] while its restored expression reduced *in vivo* tumorigenesis in nude mice [87]. Moreover, Tes overexpression enhanced cell spreading and decreased cell motility [88].

Tes knockout mice were viable and fertile, reproducing at mendelian ratio, and did not show an increased rate of tumor incidence compared to control littermates [89]. *Tes* null mice developed NMBA-induced gastric tumors after a zinc sufficient or deficient diet. Interestingly, *Tes* heterozygous and homozygous mice developed tumors, independently from the diet, at the same rate.

Recently, Ma et al. [90], showed that TES is down-regulated in primary gastric cancer tissues and its expression level correlates with prognosis: patients with a loss of expression of TES had a shorter life span than those with an expressing tumor. The same prognostic correlation has been also found for head and neck squamous carcinomas [91].

5. Conclusions

A number of knock-out and transgenic mouse models have been generated to study the *in vivo* functions of tumor suppressor genes mapping on CFSs: some of them presented a phenotype associated with pathophysiological abnormalities, whereas most of them, because of their embryonic lethality, showed developmental defects. In both instances, the absence of prezneoplastic or malignant proliferation in mouse models does not exclude their role in human cancer.

The future in this field of investigation probably rests in the creation of compound mice in which two or more engineered CFSs tumor suppressors are combined together to represent more closely human conditions in which multiple CFSs are often involved at the same time [92]. For example, since sequence fragility usually makes CFS particularly sensitive to environmental genotoxins, we can ask what might be the outcome of exposing a potential $FHIT^{-/-}Wwox^{+/-}$ mouse to such chemical insults when compared to single knockout animals. This approach could be even more informative in cases like $Tes^{-/-}$ mice, where a spontaneous cancer phenotype was not reported and the combination with other CFS models could reveal complex aspects of the malignant disease that no single CFS gene can cause alone.

Recently, it has been determined that, on average, fragile sites are denser than other genomic regions not only in protein coding genes but in microRNAs as well [93]. Since the number of microRNA engineered mouse models of cancer is increasing by the month [94, 95], it is likely that future modeling of human neoplasia will be greatly refined when CFS models include alterations of coding and non-coding genes alike.

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References

[1] T. Lukusa and J. P. Fryns, "Human chromosome fragility," *Biochimica et Biophysica Acta*, vol. 1779, no. 1, pp. 3–16, 2008.

[2] M. Schwartz, E. Zlotorynski, and B. Kerem, "The molecular basis of common and rare fragile sites," *Cancer Letters*, vol. 232, no. 1, pp. 13–26, 2006.

[3] T. W. Glover and C. K. Stein, "Chromosome breakage and recombination at fragile sites," *American Journal of Human Genetics*, vol. 43, no. 3, pp. 265–273, 1988.

[4] T. W. Glover and C. K. Stein, "Induction of sister chromatid exchanges at common fragile sites," *American Journal of Human Genetics*, vol. 41, no. 5, pp. 882–890, 1987.

[5] F. V. Rassool, T. W. McKeithan, M. E. Neilly, E. Van Melle, R. Espinosa, and M. M. Le Beau, "Preferential integration of marker DNA into the chromosomal fragile site at 3p14: an approach to cloning fragile sites," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 88, no. 15, pp. 6657–6661, 1991.

[6] A. Coquelle, E. Pipiras, F. Toledo, G. Buttin, and M. Debatisse, "Expression of fragile sites triggers intrachromosomal mammalian gene amplification and sets boundaries to early amplicons," *Cell*, vol. 89, no. 2, pp. 215–225, 1997.

[7] K. Mimori, T. Druck, H. Inoue et al., "Cancer-specific chromosome alterations in the constitutive fragile region FRA3B," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 13, pp. 7456–7461, 1999.

[8] C. M. Wilke, S. W. Guo, B. K. Hall et al., "Multicolor FISH mapping of YAC clones in 3p14 and identification of a YAC spanning both FRA3B and the t(3;8) associated with hereditary renal cell carcinoma," *Genomics*, vol. 22, no. 2, pp. 319–326, 1994.

[9] O. Handt, E. Baker, S. Dayan et al., "Analysis of replication timing at the FRA10B and FRA16B fragile site loci," *Chromosome Research*, vol. 8, no. 8, pp. 677–688, 2000.

[10] K. Hibi, T. Takahashi, K. Yamakawa et al., "Three distinct

regions involved in 3p deletion in human lung cancer," *Oncogene*, vol. 7, no. 3, pp. 445–449, 1992.

[11] M. Negrini, C. Monaco, I. Vorechovsky et al., "The FHIT gene at 3p14.2 is abnormal in breast carcinomas," *Cancer Research*, vol. 56, no. 14, pp. 3173–3179, 1996.

[12] D. Michael, D. G. Beer, C. W. Wilke, D. E. Miller, and T. W. Glover, "Frequent deletions of FHIT and FRA3B in Barrett's metaplasia and esophageal adenocarcinomas," *Oncogene*, vol. 15, no. 14, pp. 1653–1659, 1997.

[13] R. Shridhar, V. Shridhar, X. Wang et al., "Frequent breakpoints in the 3p14.2 fragile site, FRA3B, in pancreatic tumors," *Cancer Research*, vol. 56, no. 19, pp. 4347–4350, 1996.

[14] B. Zbar, H. Brauch, C. Talmadge, and M. Linehau, "Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma," *Nature*, vol. 327, no. 6124, pp. 721–724, 1987.

[15] M. Ohta, H. Inoue, M. G. Cotticelli 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*, vol. 84, no. 4, pp. 587–597, 1996.

[16] L. Virgilio, M. Shuster, S. M. Gollin et al., "FHIT gene alterations in head and neck squamous cell carcinomas," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 18, pp. 9770–9775, 1996.

[17] J. M. Fang, M. F. Arlt, A. C. Burgess, S. L. Dagenais, D. G. Beer, and T. W. Glover, "Translocation breakpoints in FHIT and FRA3B in both homologs of chromosome 3 in an esophageal adenocarcinoma," *Genes Chromosomes and Cancer*, vol. 30, no. 3, pp. 292–298, 2001.

[18] C. M. Aldaz, T. Chen, A. Sahin, J. Cunningham, and M. Bondy, "Comparative allelotype of in situ and invasive human breast cancer: high frequency of microsatellite instability in lobular breast carcinomas," *Cancer Research*, vol. 55, no. 18, pp. 3976–3981, 1995.

[19] B. S. Carter, C. M. Ewing, W. S. Ward et al., "Allelic loss of chromosomes 16q and 10q in human prostate cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 87, no. 22, pp. 8751–8755, 1990.

[20] N. Nishida, Y. Fukuda, H. Kokuryu et al., "Accumulation of allelic loss on arms of chromosomes 13q, 16q and 17p in the advanced stages of human hepatocellular carcinoma," *International Journal of Cancer*, vol. 51, no. 6, pp. 862–868, 1992.

[21] S. R. Denison, G. Callahan, N. A. Becker, L. A. Phillips, and D. I. Smith, "Characterization of FRA6E and its potential role in autosomal recessive juvenile parkinsonism and ovarian cancer," *Genes Chromosomes and Cancer*, vol. 38, no. 1, pp. 40–52, 2003.

[22] H. Huang, C. P. Reed, A. Mordi et al., "Frequent deletions within FRA7G at 7q31.2 in Invasive epithelial ovarian cancer," *Genes Chromosomes and Cancer*, vol. 24, no. 1, pp. 48–55, 1999.

[23] K. Huebner and C. M. Croce, "FRA3B and other common fragile sites: the weakest links," *Nature Reviews Cancer*, vol. 1, no. 3, pp. 214–221, 2001.

[24] A. K. Bednarek, K. J. Laflin, R. L. Daniel, Q. Liao, K. A. Hawkins, and C. M. Aldaz, "WWOX, a novel WW domain-containing protein mapping to human chromosome 16q23.3-24.1, a region frequently affected in breast cancer," *Cancer Research*, vol. 60, no. 8, pp. 2140–2145, 2000.

[25] S. Del Mare, Z. Salah, and R. I. Aqeilan, "WWOX: its genomics, partners, and functions," *Journal of Cellular Biochemistry*, vol. 108, no. 4, pp. 737–745, 2009.

[26] Y. Pekarsky, A. Palamarchuk, K. Huebner, and C. M. Croce, "FHIT as tumor suppressor: mechanisms and therapeutic opportunities," *Cancer Biology and Therapy*, vol. 1, no. 3, pp. 232–236, 2002.

[27] K. Huebner and C. M. Croce, "Cancer and the FRA3B/FHIT fragile locus: it's a HIT," *British Journal of Cancer*, vol. 88, no. 10, pp. 1501–1506, 2003.

[28] J. C. Saldivar, H. Shibata, and K. Huebner, "Pathology and biology associated with the fragile FHIT gene and gene product," *Journal of Cellular Biochemistry*, vol. 109, no. 5, pp. 858–865, 2010.

[29] N. Zanesi, Y. Pekarsky, and C. M. Croce, "A mouse model of the fragile gene FHIT: from carcinogenesis to gene therapy and cancer prevention," *Mutation Research*, vol. 591, no. 1-2, pp. 103–109, 2005.

[30] M. Ottey, S. Y. Han, T. Druck et al., "Fhit-deficient normal and cancer cells are mitomycin C and UVC resistant," *British Journal of Cancer*, vol. 91, no. 9, pp. 1669–1677, 2004.

[31] L. Lu, B. Hu, F. Yu, and Y. Wang, "Low dose radiation-induced adaptive response preventing HPRT mutation is Fhit independent," *International Journal of Radiation Biology*, vol. 85, no. 6, pp. 532–537, 2009.

[32] X. Yu, L. Lu, S. Wen, and Y. Wang, "The effects of Fhit on tumorigenesis after multi-exposure to low-dose radiation," *International Journal of Clinical and Experimental Medicine*, vol. 2, no. 4, pp. 348–353, 2009.

[33] N. Zanesi, R. Mancini, C. Sevignani et al., "Lung cancer susceptibility in Fhit-deficient mice is increased by Vhl haploinsufficiency," *Cancer Research*, vol. 65, no. 15, pp. 6576–6582, 2005.

[34] F. Bianchi, A. Magnifico, C. Olgiati et al., "FHIT-proteasome degradation caused by mitogenic stimulation of the EGF receptor family in cancer cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 50, pp. 18981–18986, 2006.

[35] F. Bianchi, E. Tagliabue, S. Ménard, and M. Campiglio, "Fhit expression protects against HER2-driven breast tumor development: unraveling the molecular interconnections," *Cell Cycle*, vol. 6, no. 6, pp. 643–646, 2007.

[36] Y. Pekarsky, M. Campiglio, Z. Siprashvili et al., "Nitrilase and Fhit homologs are encoded as fusion proteins in Drosophila melanogaster and Caenorhabditis elegans," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 15, pp. 8744–8749, 1998.

[37] E. M. Marcotte, M. Pellegrini, H. L. Ng, D. W. Rice, T. O. Yeates, and D. Eisenberg, "Detecting protein function and protein-protein interactions from genome sequences," *Science*, vol. 285, no. 5428, pp. 751–753, 1999.

[38] S. Semba, S. Y. Han, H. R. Qin et al., "Biological functions of mammalian Nit1, the counterpart of the invertebrate NitFhit Rosetta Stone protein, a possible tumor suppressor," *The Journal of Biological Chemistry*, vol. 281, no. 38, pp. 28244–28253, 2006.

[39] J. Sun, H. Okumura, M. Yearsley et al., "Nit1 and Fhit tumor suppressor activities are additive," *Journal of Cellular Biochemistry*, vol. 107, no. 6, pp. 1097–1106, 2009.

[40] R. Balansky, F. D'Agostini, G. Ganchev et al., "Influence of FHIT on benzo[a]pyrene-induced tumors and alopecia in mice: chemoprevention by budesonide and N-acetylcysteine," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 20, pp. 7823–7828, 2006.

[41] F. D'Agostini, A. Izzotti, R. Balansky, N. Zanesi, C. M. Croce, and S. De Flora, "Early loss of Fhit in the respiratory tract of rodents exposed to environmental cigarette smoke," *Cancer Research*, vol. 66, no. 7, pp. 3936–3941, 2006.

[42] S. De Flora, F. D'Agostini, A. Izzotti, N. Zanesi, C. M. Croce, and R. Balansky, "Molecular and cytogenetical alterations induced by environmental cigarette smoke in mice heterozygous for Fhit," *Cancer Research*, vol. 67, no. 3, pp. 1001–1006, 2007.

[43] K. A. McCorkell, R. Mancini, Z. Siprashvili et al., "Influence of a nonfragile FHIT transgene on murine tumor susceptibility," *Cytogenetic and Genome Research*, vol. 118, no. 2–4, pp. 196–203, 2007.

[44] M. F. Arlt, S. G. Durkin, R. L. Ragland, and T. W. Glover, "Common fragile sites as targets for chromosome rearrangements," *DNA Repair*, vol. 5, no. 9-10, pp. 1126–1135, 2006.

[45] H. Ishii, K. Mimori, K. Ishikawa et al., "Fhit-deficient hematopoietic stem cells survive hydroquinone exposure carrying precancerous changes," *Cancer Research*, vol. 68, no. 10, pp. 3662–3670, 2008.

[46] R. Baffa, L. G. Gomella, A. Vecchione et al., "Loss of FHIT expression in transitional cell carcinoma of the urinary bladder," *American Journal of Pathology*, vol. 156, no. 2, pp. 419–424, 2000.

[47] A. Vecchione, C. Sevignani, E. Giarnieri et al., "Inactivation of the FHIT gene favors bladder cancer development," *Clinical Cancer Research*, vol. 10, no. 22, pp. 7607–7612, 2004.

[48] D. D'Arca, J. LeNoir, B. Wildemore et al., "Prevention of urinary bladder cancer in the FHIT knock-out mouse with Rofecoxib, a Cox-2 inhibitor," *Urologic Oncology*, vol. 28, no. 2, pp. 189–194, 2010.

[49] K. A. Krummel, S. R. Denison, E. Calhoun, L. A. Phillips, and D. I. Smith, "The common fragile site FRA16D and its associated gene WWOX are highly conserved in the mouse at Fra8E1," *Genes Chromosomes and Cancer*, vol. 34, no. 2, pp. 154–167, 2002.

[50] N. S. Chang, N. Pratt, J. Heath et al., "Hyaluronidase induction of a WW domain-containing oxidoreductase that enhances tumor necrosis factor cytotoxicity," *The Journal of Biological Chemistry*, vol. 276, no. 5, pp. 3361–3370, 2001.

[51] R. I. Aqeilan, F. Trapasso, S. Hussain et al., "Targeted deletion of Wwox reveals a tumor suppressor function," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 10, pp. 3949–3954, 2007.

[52] R. I. Aqeilan, J. P. Hagan, H. A. Aqeilan, F. Pichiorri, L. Y. Y. Fong, and C. M. Croce, "Inactivation of the Wwox gene accelerates forestomach tumor progression in vivo," *Cancer Research*, vol. 67, no. 12, pp. 5606–5610, 2007.

[53] J. H. Ludes-Meyers, H. Kil, M. I. Nuñez et al., "Wwox hypomorphic mice display a higher incidence of B-cell lymphomas and develop testicular atrophy," *Genes Chromosomes and Cancer*, vol. 46, no. 12, pp. 1129–1136, 2007.

[54] R. I. Aqeilan, M. Q. Hassan, A. De Bruin et al., "The WWOX tumor suppressor is essential for postnatal survival and normal bone metabolism," *The Journal of Biological Chemistry*, vol. 283, no. 31, pp. 21629–21639, 2008.

[55] G. S. Stein, J. B. Lian, A. J. Van Wijnen et al., "Runx2 control of organization, assembly and activity of the regulatory machinery for skeletal gene expression," *Oncogene*, vol. 23, no. 24, pp. 4315–4329, 2004.

[56] K. C. Kurek, S. Del Mare, Z. Salah et al., "Frequent attenuation of the WWOX tumor suppressor in osteosarcoma is associated with increased tumorigenicity and aberrant RUNX2 expression," *Cancer Research*, vol. 70, no. 13, pp. 5577–5586, 2010.

[57] J. H. Ludes-Meyers, H. Kil, J. Parker-Thornburg, D. F. Kusewitt, M. T. Bedford, and C. M. Aldaz, "Generation and characterization of mice carrying a conditional allele of the Wwox tumor suppressor gene," *PLoS ONE*, vol. 4, no. 11, Article ID e7775, 2009.

[58] T. Kitada, S. Asakawa, N. Hattori et al., "Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism," *Nature*, vol. 392, no. 6676, pp. 605–608, 1998.

[59] Y. Imai, M. Soda, and R. Takahashi, "Parkin suppresses unfolded protein stress-induced cell death through its E3 ubiquitin-protein ligase activity," *The Journal of Biological Chemistry*, vol. 275, no. 46, pp. 35661–35664, 2000.

[60] F. Wang, S. Denison, J. P. Lai et al., "Parkin gene alterations in hepatocellular carcinoma," *Genes Chromosomes and Cancer*, vol. 40, no. 2, pp. 85–96, 2004.

[61] R. Cesari, E. S. Martin, G. A. Calin et al., "Parkin, a gene implicated in autosomal recessive juvenile parkinsonism, is a candidate tumor suppressor gene on chromosome 6q25-q27," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 10, pp. 5956–5961, 2003.

[62] M. C. Picchio, E. S. Martin, R. Cesari et al., "Alterations of the tumor suppressor gene Parkin in non-small cell lung cancer," *Clinical Cancer Research*, vol. 10, no. 8, pp. 2720–2724, 2004.

[63] M. S. Goldberg, S. M. Fleming, J. J. Palacino et al., "Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons," *The Journal of Biological Chemistry*, vol. 278, no. 44, pp. 43628–43635, 2003.

[64] J. M. Itier, P. Ibáñez, M. A. Mena et al., "Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse," *Human Molecular Genetics*, vol. 12, no. 18, pp. 2277–2291, 2003.

[65] G. Poulogiannis, R. E. McIntyre, M. Dimitriadi et al., "PARK2 deletions occur frequently in sporadic colorectal cancer and accelerate adenoma development in Apc mutant mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 34, pp. 15145–15150, 2010.

[66] B. Pedersen and J. Ellegaard, "A factor encoded by 7q31 suppresses expansion of the 7q- clone and delays cytogenetic progression," *Cancer Genetics and Cytogenetics*, vol. 78, no. 2, pp. 181–188, 1994.

[67] I. Bieche, M. H. Champeme, F. Matifas, K. Hacene, R. Callahan, and R. Lidereau, "Loss of heterozygosity on chromosome 7q and aggressive primary breast cancer," *The Lancet*, vol. 339, no. 8786, pp. 139–143, 1991.

[68] M. I. Edelson, S. W. Scherer, L. C. Tsui et al., "Identification of a 1300 kilobase deletion unit on chromosome 7q31.3 in invasive epithelial ovarian carcinomas," *Oncogene*, vol. 14, no. 24, pp. 2979–2984, 1997.

[69] J. C. Zenklusen, J. C. Thompson, A. J. P. Klein-Szanto, and C. J. Conti, "Frequent loss of heterozygosity in human primary squamous cell and colon carcinomas at 7q31.1: evidence for a broad range tumor suppressor gene," *Cancer Research*, vol. 55, no. 6, pp. 1347–1350, 1995.

[70] A. Latil, O. Cussenot, G. Fournier, J. C. Baron, and R. Lidereau, "Loss of heterozygosity at 7q31 is a frequent and early event in prostate cancer," *Clinical Cancer Research*, vol. 1, no. 11, pp. 1385–1389, 1995.

[71] S. Nishizuka, G. Tamura, M. Terashima, and R. Satodate, "Commonly deleted region on the long arm of chromosome 7 in differentiated adenocarcinoma of the stomach," *British Journal of Cancer*, vol. 76, no. 12, pp. 1567–1571, 1997.

[72] K. Matsuura, K. Shiga, J. Yokoyama, S. Saijo, T. Miyagi, and T. Takasaka, "Loss of heterozygosity of chromosome 9p21 and 7q31 is correlated with high incidence of recurrent tumor in head and neck squamous cell carcinoma," *Anticancer Research*, vol. 18, no. 1, pp. 453–458, 1998.

[73] A. Achille, M. O. Biasi, G. Zamboni et al., "Chromosome 7q allelic losses in pancreatic carcinoma," *Cancer Research*, vol. 56, no. 16, pp. 3808–3813, 1996.

[74] V. Shridhar, Q. C. Sun, O. J. Miller, G. P. Kalemkerian, J. Petros, and D. I. Smith, "Loss of heterozygosity on the long arm of human chromosome 7 in sporadic renal cell carcinomas," *Oncogene*, vol. 15, no. 22, pp. 2727–2733, 1997.

[75] M. Drab, P. Verkade, M. Elger et al., "Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice," *Science*, vol. 293, no. 5539, pp. 2449–2452, 2001.

[76] B. Razani, J. A. Engelman, X. B. Wang et al., "Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities," *The Journal of Biological Chemistry*, vol. 276, no. 41, pp. 38121–38138, 2001.

[77] Y. Y. Zhao, Y. Liu, R. V. Stan et al., "Defects in caveolin-1 cause dilated cardiomyopathy and pulmonary hypertension in knockout mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 17, pp. 11375–11380, 2002.

[78] G. Cao, G. Yang, T. L. Timme et al., "Disruption of the caveolin-1 gene impairs renal calcium reabsorption and leads to hypercalciuria and urolithiasis," *American Journal of Pathology*, vol. 162, no. 4, pp. 1241–1248, 2003.

[79] F. Capozza, T. M. Williams, W. Schubert et al., "Absence of caveolin-1 sensitizes mouse skin to carcinogen-induced epidermal hyperplasia and tumor formation," *American Journal of Pathology*, vol. 162, no. 6, pp. 2029–2039, 2003.

[80] H. Lee, D. S. Park, B. Razani, R. G. Russell, R. G. Pestell, and M. P. Lisanti, "Caveolin-1 mutations (P132L and null) and the pathogenesis of breast cancer: caveolin-1 (P132L) behaves in a dominant-negative manner and caveolin-1 (-/-) null mice show mammary epithelial cell hyperplasia," *American Journal of Pathology*, vol. 161, no. 4, pp. 1357–1369, 2002.

[81] T. M. Williams, M. W. C. Cheung, D. S. Park et al., "Loss of caveolin-1 gene expression accelerates the development of dysplastic mammary lesions in tumor-prone transgenic mice," *Molecular Biology of the Cell*, vol. 14, no. 3, pp. 1027–1042, 2003.

[82] W. Zou, L. McDaneld, and L. M. Smith, "Caveolin-1 haploinsufficiency leads to partial transformation of human breast epithelial cells," *Anticancer Research*, vol. 23, no. 6, pp. 4581–4586, 2003.

[83] T. M. Williams, G. S. Hassan, J. Li et al., "Caveolin-1 promotes tumor progression in an autochthonous mouse model of prostate cancer: genetic ablation of Cav-1 delays advanced prostate tumor development in tramp mice," *The Journal of Biological Chemistry*, vol. 280, no. 26, pp. 25134–25145, 2005.

[84] G. Yang, L. D. Truong, T. L. Timme et al., "Elevated expression of caveolin is associated with prostate and breast cancer," *Clinical Cancer Research*, vol. 4, no. 8, pp. 1873–1880, 1998.

[85] B. Boëda, D. C. Briggs, T. Higgins et al., "Tes, a specific Mena interacting partner, breaks the rules for EVH1 binding," *Molecular Cell*, vol. 28, no. 6, pp. 1071–1082, 2007.

[86] C. Tatarelli, A. Linnenbach, K. Mimori, and C. M. Croce, "Characterization of the human TESTIN gene localized in the FRA7G region at 7q31.2," *Genomics*, vol. 68, no. 1, pp. 1–12, 2000.

[87] M. Sarti, C. Sevignani, G. A. Calin et al., "Adenoviral transduction of TESTIN gene into breast and uterine cancer cell lines promotes apoptosis and tumor reduction in vivo," *Clinical Cancer Research*, vol. 11, no. 2, pp. 806–813, 2005.

[88] E. Griffith, A. S. Coutts, and D. M. Black, "Characterisation of chicken TES and its role in cell spreading and motility," *Cell Motility and the Cytoskeleton*, vol. 57, no. 3, pp. 133–142, 2004.

[89] A. Drusco, N. Zanesi, C. Roldo et al., "Knockout mice reveal a tumor suppressor function for Testin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 31, pp. 10947–10951, 2005.

[90] H. Ma, D. Weng, Y. Chen et al., "Extensive analysis of D7S486 in primary gastric cancer supports TESTIN as a candidate tumor suppressor gene," *Molecular Cancer*, vol. 9, article 190, 2010.

[91] E. Gunduz, M. Gunduz, L. Beder et al., "Downregulation of TESTIN and its association with cancer history and a tendency toward poor survival in head and neck squamous cell carcinoma," *Archives of Otolaryngology*, vol. 135, no. 3, pp. 254–260, 2009.

[92] E. Giarnieri, N. Zanesi, A. Bottoni et al., "Oncosuppressor proteins of fragile sites are reduced in cervical cancer," *Cancer Letters*, vol. 289, no. 1, pp. 40–45, 2010.

[93] A. Laganà, F. Russo, C. Sismeiro, R. Giugno, A. Pulvirenti, and A. Ferro, "Variability in the incidence of miRNAs and genes in fragile sites and the role of repeats and CpG islands in the distribution of genetic material," *PloS one*, vol. 5, no. 6, Article ID e11166, 2010.

[94] N. Zanesi, Y. Pekarsky, F. Trapasso, G. Calin, and C. Croce, "MicroRNAs in mouse models of lymphoid malignancies," *Journal of Nucleic Acids Investigation*, vol. 1, pp. 39–40, 2010.

[95] U. Santanam, N. Zanesi, A. Efanov et al., "Chronic lymphocytic leukemia modeled in mouse by targeted miR-29 expression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 27, pp. 12210–12215, 2010.