Review Article

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

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Received 14 September 2010; Accepted 23 November 2010

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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      | FHIT KO      | Radiation       | Increased multiorgan tumor involvement respect WT, but equal tumorigenesis | KO and WT mice were equally sensitive; FHIT**/- mice showed uterine preneoplasias          |
| WWOX 16q23.2     | Deletions translocations      | Wwox KO      | B(a)P (Benzo(a)pyrene) | KO and WT mice were equally sensitive; FHIT**/- mice showed uterine preneoplasias | KO and WT mice were equally sensitive; FHIT**/- mice showed uterine preneoplasias          |
|                  |                               |              | ENSO (Environmental cigarette smoke) | After exposure, FHIT loss was observed also in WT mice. WT and FHIT**/- mice did not show significant differences | After exposure, FHIT loss was observed also in WT mice. WT and FHIT**/- mice did not show significant differences |
|                  |                               |              | BBN (N-butyl-N-hydroxybutyl-nitrosamine) | 28% of FHIT**/- and 46% of FHIT**/- versus 8% of WT mice developed bladder invasive carcinomas | 28% of FHIT**/- and 46% of FHIT**/- versus 8% of WT mice developed bladder invasive carcinomas |
|                  |                               |              | BBN (N-butyl-N-hydroxybutyl-nitrosamine) | WT mice developed bladder invasive carcinomas | WT mice developed bladder invasive carcinomas |
| FHIT KO X Vhl KO |                               | Wwox KO      | NMBA (N-nitroso-methyl-benzylamine) | ~100% more gastric tumors than FHIT**/- alone | ~100% more gastric tumors than FHIT**/- alone |
| FHIT KO X Vhl KO |                               | Wwox KO      | DMN (Dimethyl-nitrosamine) | Lung adenocarcinomas in 44% | Lung adenocarcinomas in 44% |
| FHIT**/- X HER2/neu (mammary tumor promoter) |                               | Wwox KO      | ENSO (Environmental cigarette smoke) | Mammary tumors in 100% | Mammary tumors in 100% |
| FHIT KO X Nit KO |                               | Wwox KO      | NMBA (N-nitroso-methyl-benzylamine) | ~100% more gastric tumors than FHIT**/- alone | ~100% more gastric tumors than FHIT**/- alone |

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.
Table 1: Continued.

| Gene   | Human tumors                     | Animal model | Treatment                          | Induced phenotype                          | Spontaneous phenotype                  |
|--------|---------------------------------|--------------|------------------------------------|--------------------------------------------|----------------------------------------|
| PARK2  | Deletions, translocations        |              | Park2 KO                           | Motor and cognitive defects                 |                                        |
| 6q26   | Ovary, breast, esophagus, liver, colon, lung, renal, CNS, hematopoietic |              |                                    |                                            |                                        |
|        | Park2 X Apc                     |              |                                    | 4-fold increase in adenomas than control mice; all stages neoplastic lesions |                                        |
| CAV-1  | Deletions, translocations        |              | Cav-1 KO                           | Normal. Reduction of Cav-2 expression       |                                        |
| 7q31.2 | Downregulated in: ovarian, lung, mammary tumors Upregulated in: prostate, bladder, thyroid, esophageal carcinomas |              |                                    |                                            |                                        |
| Cav-1 KO | DMBA (7,12-dimethyl benzanthracene) | Development of epidermally derived tumors | Acceleration of the development of mammary lesions Decreased incidence in prostate tumors and loco-regional and distal metastasis |
| Cav-1 X MMTV-PyMT |                         |               |                                    |                                            |                                        |
| Cav-1 X Tramp |                         |               |                                    |                                            |                                        |
| TES    | Deletions, translocations        |              | Tes KO                             | Normal                                     |                                        |
| 7q31.2 | Head and Neck, gastric, breast, hematopoietic, prostate, CNS |              |                                    |                                            |                                        |
| Tes KO | NMBA(N-nitrosomethyl-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 x 1 or 0.1 Gy x 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 x 1 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 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 pointed 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, whilepreneoplastic 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 plastic lesions of the uterus were more frequent in FHIT−/− mice [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 genotoxic-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 polypl mutiplicity [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-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 preneoplastic 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.

Acknowledgments

The authors are grateful to Dr. Carlo M. Croce for helpful scientific contribution and Aaron Burch for technical assistance. N. Zanesi was a Kimmel Scholar. Some research described was supported by NIH grant no. CA115965 to K. Huebner and Grant support R01DK079217 to R. I. A.

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