Deficiency of Splicing Factor 1 Suppresses the Occurrence of Testicular Germ Cell Tumors

Rui Zhu1, Jason Heaney2, Joseph H. Nadeau2, Sara Ali1, and Angabin Matin1

Abstract

Testicular germ cell tumors (TGCT) originate from germ cells. The 129-Ter and M19 (129.MOLF-Chr19 consomic) mouse strains have extremely high incidences of TGCTs. We found that the expression levels of SF1-encoded splicing factor 1 (SF1) can modulate the incidence of TGCTs. We generated mice with inactivated SF1. SF1 null mice (SF1−/−) died before birth. Mice with one intact allele of SF1 (SF1+/−) were viable but expressed reduced levels of SF1. When SF1-deficient mice (SF1+/−) were crossed to the 129-Ter and M19 strains, we observed decreased incidence of TGCTs in SF1+/−.Ter and SF1+/−.M19/+ mice compared with that in control cohorts. Therefore, SF1 deficiency protects against TGCT development in both strains. SF1 is expressed in the testes. We found that SF1 levels vary significantly in the testes of inbred strains such as 129 and MOLF, and as such SF1 is an oncogenic tumor-susceptibility factor from 129. Our results also highlight the complications involved in evaluating SF1 levels and TGCT incidences. When a large number of tumor-promoting factors are present in a strain, the protective effect of lower SF1 levels is masked. However, when the dosage of tumor-promoting factors is reduced, the protective effect of lower SF1 levels becomes apparent. SF1 is involved in splicing of specific pre-mRNAs in cells. Alternate splicing generates the complex proteosome in eukaryotic cells. Our data indicate that SF1 levels in mouse strains correlate with their incidences of TGCTs and implicate the importance of splicing mechanisms in germ cell tumorigenesis. Cancer Res; 70(18); OF1–9. ©2010 AACR.

Introduction

Testicular germ cell tumors (TGCT) are the most common malignancy in young men. These tumors originate from germ cells at different stages of development (1, 2). Genetic factors, such as ethnicity and family history, and environmental factors contribute to TGCT development (3, 4). Evidence indicates that a combination of multiple genetic factors contribute to susceptibility to TGCT development (5–8). Individually, each of these factors contributes with relatively modest effects toward tumor development. It has been a challenge to identify the factors that cause TGCTs particularly because the tumors initiate in utero although the disease may become evident decades after birth.

In mice, TGCTs occur predominantly on the 129 strain background. About 10% of 129 males develop spontaneous TGCTs (9). The genetic factors from the 129 strain that support TGCT development have not been identified. However, a number of gene defects have been experimentally shown to increase (10–14) or suppress the incidences of TGCT (15). The tumors in mice originate from primordial germ cells (PGC) and initiate development around embryonic day (E) 11.5 to E13.5. For reasons not well understood, some PGCs on the 129 strain background become transformed to embryonal carcinoma (EC) cells. EC cells proliferate rapidly in the embryonic gonads. Soon after birth, EC cells differentiate randomly into embryonic and adult cells that constitute the TGCTs in the testes. TGCTs in mice resemble the pediatric TGCTs of humans (16).

Two 129-derived mouse strains, 129-Ter and M19, have extremely high rates of spontaneous TGCT development (Supplementary Fig. S1). The Ter defect is due to inactivation of the function of the RNA-binding protein dead end 1 (Dnd1; ref. 11). Dnd1 is essential for PGC viability (11, 17). Loss of Dnd1 results in progressive death of germ cells contributed to some extent by BAX-mediated apoptosis (18). This results in sterility in all Ter mice. However, 129 strain mice with inactivated Dnd1 (129-Ter mice) develop TGCTs in addition to being sterile due to germ cell loss (19, 20). Thus, some PGCs of the 129-Ter strain escape death to transform into EC cells, and EC cells subsequently differentiate to form large tumors in the testes.

A second mouse strain with high incidence of spontaneous germ cell tumors is the consomic, 129.MOLF-Chr19, mouse strain (also referred to as M19, chromosome substitution strain or CSS; ref. 21). The M19 strain differs from 129 only because chromosome (Chr) 19 of the MOLF strain replaces that of 129 (Supplementary Fig. S1). The M19 strain does not carry the Ter (inactivation of Dnd1) defect. Multiple TGCT susceptible loci have been mapped to Chr19 of the M19 strain. These
loci either independently or interact epistatically to contribute to testicular tumor development (22). Unlike in the 129-Ter strain, the TGCT-causing genes in M19 do not cause germ cell death. Thus, both normal and transformed germ cells are present in the M19 strain, and M19 males can be fertile despite having testicular tumors.

We identified splicing factor 1 (Sf1) as a TGCT candidate gene from the M19 strain (23). Here, we report the role of Sf1 in TGCT development. Interestingly, our results indicate that Sf1 expression levels influence the incidence of germ cell tumor development.

Sf1 [also known as splicing factor 1, mammalian branch point-binding protein (mBBP), zinc finger gene in MEN1 locus (ZFM1), or zinc finger protein 162 (ZNF162)] participates in the early spliceosome assembly step during pre-mRNA splicing (24, 25). Sf1 is involved in the assembly of the earliest spliceosome complex (E′ complex) committed to the splicing pathway (26, 27). Splice site recognition requires cross talk between multiple proteins that are involved in forming complexes that commit the pre-mRNA to splicing. Sf1 interacts cooperatively with U2 small nuclear ribonucleoprotein auxiliary factor (U2AF65), and these proteins bind to the branch point site and polypyrimidine tract in the intron of pre-mRNAs, respectively (28–30). Sf1 is essential for viability of cells in culture. Sf1 is not required for general splicing mechanisms in germ cell tumor development. Therefore, our studies implicate Sf1 and splicing mechanisms in germ cell tumor development.

Materials and Methods

Mouse strains

The 129.MOLF-Chr19 (21), 129 (129S1/SvImJ; J:R002448, The Jackson Laboratory), and 129-Ter (11) strains have been described.

Generation of Sf1 knockout mice

The gene trap 129/Ola strain embryonic stem (ES) cell clone, XD130, was purchased from BayGenomics (University of California, Davis). Trap vector insertion was validated by BayGenomics to contain gene trap cassette (Supplementary Fig. S2). Trap vector insertion was validated by Southern blotting and PCR determined that XD130 harbored tandem insertion of two copies of the gene trap in the first intron of Sf1 and 261 bp downstream of exon 1 (Supplementary Fig. S2). This disrupts expression of normal germline transmission of the gene trap, β-geo allele, were crossed to 129 mice.

Germline transmission was screened by PCR genotyping for the targeted allele. Primers for genotyping were gtf1F, gtf4R, v1F, and v1R (Supplementary Fig. S2). gtf1F/gt4R identify the wild-type allele. All animals were genotyped using the three sets of primers. Mice genotyped to be positive for both v1F/v1R and gtf1F/gtf4R were selected to maintain the Sf1 gene trap line. Inactivation of Sf1 by the β-geo encoding gene trap was further verified by Southern blotting and by sequencing of the genomic sequences flanking the vector and the vector insertion site.

Southern blotting

Genomic DNA extracted from the spleen of 129,Sf1+/− and +/+ mice, of G1 and G2 generations, were digested with BglII or EcoRV and used for Southern blotting. Blots were hybridized with a 32P-labeled 626-bp PstI/XbaI fragment derived from the β-geo cassette (Supplementary Fig. S2). A 10.4-kb BglII fragment or a 8.9-kb EcoRV fragment is expected when one copy of trap vector is inserted in the genome. However, 8.6 and 10.4 kb BgII and 8.6 and 8.9 kb EcoRV fragments are expected when two copies of the trap vector are tandemly inserted into the Sf1 gene.

X-gal staining and in situ hybridization

X-gal staining and in situ hybridization was performed as described (33).

Immunohistochemistry and Western blotting

Immunohistochemistry and Western blotting for detection of Sf1 used anti-Sf1 antibody (SC-21157, Santa Cruz Biotechnology). Additional antibodies included β-actin (A3516 Sigma), anti–SSEA1-Alexa Fluor 647 (SC-21702, Santa Cruz Biotechnology), and Sox9 (AB5535, Chemicon).

Real-time PCR and quantitative real-time PCR

Reverse-transcriptase-PCR (RT-PCR) and quantitative real-time PCR (qRT-PCR) were carried out as described (23). The primers used for qRT-PCR were as follows:

\[
\text{Sf1: 5′-ttctgctgctttgc-3′ and 5′-gttcatctccacc-3′; Gapdh: 5′-ttgtctctgtagtcaca-3′ and 5′-accagagagctgtgaa-3′.}
\]

The primers used for RT-PCR amplification were as follows:

\[
\text{Sf1: 5′-cagacgctccccatgccatc-3′ and 5′-gtgctgtctggaac-3′; Oct4: 5′-ggagagggcagcaaatga-3′ and 5′-tccacgcttgca-3′; Dmd1: 5′-gcgtctggtagaagctctc-3′ and 5′-gcgtctgctactaccgtg-3′; Sox9: 5′-ggagaggcagcaacacta-3′ and 5′-atcggggttgctctc-3′; HPRT: 5′-gtggagcga-3′.}
\]

Results

Sf1 is essential during embryonic development

We used a gene trap, ES cell clone XD130, to generate Sf1+/− mice. Southern blotting and PCR determined that XD130 harbored tandem insertion of two copies of the gene trap in the first intron of Sf1 and 261 bp downstream of exon 1 (Supplementary Fig. S2). This disrupts expression of normal
Instead, β-geo (fusion of LacZ encoding β-galactosidase and neo resistance gene) is fused with the first 10 amino acids of Sf1 and expressed from the targeted allele.

We intercrossed heterozygous Sf1+−/− (or Sf1β-geo/+−) mice but did not obtain any homozygous null mice, Sf1−−/− (Sf1β-geo/β-geo), at weaning or at E11.5 (Supplementary Table S1). This result is similar to that reported for genetically modified Sf1 mice made by Shitashige and colleagues (32). Thus, Sf1 is critical during embryonic development.

Next, we examined the levels of Sf1 transcripts in Sf1+−/− mice using qRT-PCR. Total RNA from postnatal day 1 (PN1) testes was used for qRT-PCR. We found that Sf1 mRNA levels were significantly lower (2- to 4-fold lower) in the testes of Sf1+−/− mice compared with that in wild-type mice (Fig. 1). SF1 protein levels had also decreased in PN1 testes of Sf1+−/− mice as determined by immunoblotting using anti-SF1 antibodies (Fig. 1B and C).

Sf1 expression in the testes

We examined the expression pattern of Sf1 in mouse testes. First, we examined PN1 testes for LacZ activity from the endogenous Sf1 promoter in Sf1+−/− (Sf1β-geo/+−) mice. X-gal staining of Sf1+−/− mice revealed that the germ cells were positive (Supplementary Fig. S2) but the supporting cells of the seminiferous tubules were not positive for LacZ activity.

We sought to verify if the X-gal expression pattern in the testes of Sf1+−/− mice was a true reflection of endogenous SF1 expression. We therefore performed in situ hybridization using antisense Sf1 probes as well as immunohistochemistry using anti-SF1 antibody on PN1 testes from normal, wild-type (+/+) mice. In contrast to the LacZ expression pattern, both in situ hybridization (Supplementary Fig. S2) and immunostaining indicated (Fig. 2) that SF1 is present in both the germ cells and Sertoli cells. Immunostaining indicated that SF1 was present in the nucleus as would be expected for a splicing factor.

Because we obtained contradictory data regarding LacZ expression as opposed to in situ hybridization and immunostaining, we used RT-PCR to determine whether Sf1 transcripts are indeed expressed in both the germ and supporting cells. Therefore, we compared Sf1 expression in the testes of Ter and wild-type mice. Mice homozygous for the Ter defect, and on a C57BL/6J background (B6-Ter/Ter), completely lack germ cells (11, 19). RT-PCR indicated that Sf1 was present in the testes of B6-Ter/Ter mice (Fig. 2C). This indicates that Sf1 transcripts are indeed present in the supporting cells of the testes. RT-PCR also indicated the lack of expression of germ cell markers (Dnd1 and Oct4) and the presence of Sertoli cell markers (Sox9) in the testes of the Ter strain (18).

To determine why LacZ activity was absent in somatic cells, we examined the lacZ transcript (Sf1-geo fusion transcript) in Sf1+−/−;Ter/Ter testes. Sf1+−/−;Ter/Ter mice also lack germ cells (strain described below). However, RT-PCR

![Figure 1. Reduction of Sf1 mRNA and protein levels in Sf1+−/− mice. A, qRT-PCR performed on total RNA from PN1 testis of wild-type and Sf1+−/− mice. Sf1 expression is normalized against Gapdh expression in the testes. Error bars, SD derived from two independent experiments. B, SF1 protein levels in PN1 testes of wild-type and Sf1+−/− mice, determined by immunoblotting using anti-SF1 antibodies as shown in C. Protein levels are normalized against GAPDH expression in the testes. Error bars, SD derived from four independent experiments. C, a representative immunoblot using anti-SF1 antibody for PN1 testis of wild-type and Sf1+−/− mice. The blots were reprobed with anti-GAPDH as controls.](https://www.aacrjournals.org/can耶/figs/OF3.jpg)
system is reported to be less sensitive than RT-PCR or antibody staining (34).

Taking together the results of the above studies, we conclude that SF1 is expressed in both germ and Sertoli cells of the testes. However, in our Sf1+/− (Sf1+/geo+/−) mice, for unknown reasons, lacZ activity is strong only in the germ cells.

Deficiency of SF1 reduces TGCT incidence

Next, we examined the effect of SF1 deficiency on germ cell tumorigenesis. We were unable to assess TGCT incidence in Sf1−/− adult mice because of their embryonic lethality. We therefore crossed Sf1−/− mice to mouse strains with inherent genetic predisposition to TGCT. Males of the Ter strain develop TGCTs at an exceptionally high rate. Ninety-four percent of male mice homozygous for 129-Ter (Ter/Ter) develop tumor in at least one testis (19). In cases where testes of 129-Ter mice are tumor free, it remains small in size because of the lack of germ cells.

We crossed Sf1+/− with Ter/+ mice and examined tumor incidence in the double-mutant progeny (crosses are illustrated in Supplementary Fig. S3). We found that 40% of Sf1+/−;Ter/Ter male mice have testicular tumors (Table 1). The tumor incidence of the control cohort of siblings of the Ter/Ter genotype was 77%. Thus, tumor incidence had decreased in Sf1+/−;Ter/Ter mice, and this change was statistically significant (P = 0.0174). The incidence of bilateral testicular tumors had also decreased in Sf1+/−;Ter/Ter mice. Forty percent Sf1+/−;Ter/Ter compared with 71% of Ter/Ter tumor-bearing mice developed bilateral tumors. However, there was a corresponding increase in the number of Sf1+/−;Ter/Ter males with sterile, bilateral small testes (60%) compared with that in Ter/Ter males (23%). Mice of Ter/Ter genotypes with unilateral tumors had contralateral small testes. Thus, neither Ter/Ter nor Sf1+/−;Ter/Ter cohorts had normal testes.

Next, we examined SF1 levels in Sf1+/−;Ter/Ter testes. qRT-PCR indicated that SF1 transcript levels were lower in Sf1+/−;Ter/Ter compared with that in Ter/Ter testes (Supplementary Fig. S4). In addition, SF1 protein levels were also decreased in Sf1+/−;Ter/Ter testes (Fig. 3A).

The incidence of tumors in Ter/Ter mice in this study was 77% and not the reported incidence of 94% (19). This could

![Figure 2](image.png)

**Figure 2.** Expression of Sf1 in the testes. Immunostaining of PN1 testes from wild-type mice using anti-SF1 antibody at (A) low (bar, 200 μm) and (B) higher (bar, 20 μm) magnifications. C, RT-PCR for Sf1, Dnd1, Oct4, Sox9, and Hprt using total RNA from PN1 testes of wild-type (N) and B6-Ter/Ter (Ter) mice. PCR was performed on equal amounts of cDNA. +, SuperScript was added during cDNA preparation. −, control lanes; no SuperScript was added.

Table 1. TGCT incidence in Ter mice deficient for Sf1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. with tumors</th>
<th>Bilateral tumors</th>
<th>Unilateral tumors</th>
<th>Sterile testes</th>
<th>Normal testes</th>
<th>No. of males examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ter/Ter</td>
<td>17 (77%)</td>
<td>12 (71%)</td>
<td>5</td>
<td>5 (23%)</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Ter/Ter;Sf1+/−</td>
<td>10 (40%)</td>
<td>4 (40%)</td>
<td>6</td>
<td>15 (60%)</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Ter/+</td>
<td>23 (45%)</td>
<td>5</td>
<td>18</td>
<td>0</td>
<td>28</td>
<td>51</td>
</tr>
<tr>
<td>Ter/+; Sf1+/−</td>
<td>21 (40%)</td>
<td>4</td>
<td>17</td>
<td>0</td>
<td>32</td>
<td>53</td>
</tr>
<tr>
<td>+/+</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>+/+; Sf1+/−</td>
<td>1 (5%)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>21</td>
<td>22</td>
</tr>
</tbody>
</table>

NOTE: Ter/+;Sf1+/− mice were intercrossed with Ter/+ mice (illustrated in Supplementary Fig. S3). The progeny were of genotypes listed in the table. The progeny were genotyped, and the testis phenotypes were examined. The significance of the difference in tumor incidences between the Ter/Ter and Ter/Ter;Sf1+/− strains is P = 0.0174. Differences in the tumor incidences of Ter/+ compared with Ter/+;Sf1+/− and +/+ compared with Sf1+/− were not statistically significant.
be because of the smaller sample size that was collected for this study (22 Ter/Ter and 25 Sf1+/-;Ter/Ter males were examined; Table 1). An alternate possibility may be that 129 substrain differences influenced the tumor incidence. The Sf1 gene trap was on 129/Ola strain ES cells; founder mice were crossed to 129S1/SvImJ and Ter Sf1 substrain (129T1/Svgeo @Na; ref. 35). This genetic variability between 129 substrains could have influenced the tumor incidences in Ter/Ter mice in our crosses.

Overall, SF1 deficiency lowers the incidence of testicular tumors in Ter/Ter males, it does not restore germ cells in the testes of Ter/Ter males. This suggests that SF1 deficiency does not prevent germ cell death in Ter (because of the lack of DND1) but more likely affects germ cell transformation. Because SF1 mediates splicing of pre-mRNAs, it is likely that deficiency of SF1 causes reduced production of "oncogenic" spliced variants, which leads to attenuation of germ cell transformation processes and an overall decreased level of germ cell tumors. This also suggests that SF1 functions downstream to DND1. The identities of the pre-mRNAs that are targets of SF1 in germ cells are presently unknown.

In conclusion, the lack of one allele of Sf1 results in decreased expression of SF1 and is correlated with a significant reduction of TGCT incidence in Ter mice. Thus, Sf1 is haploinsufficient and Sf1 levels influence germ cell transformation.

**Deficiency of Sf1 reduces tumor incidence of M19 males**

To determine how Sf1 affects TGCT incidence of the M19 mouse strain, we crossed Sf1+/- to M19 mice and examined tumor incidences in the male progeny, Sf1+/-;M19/− and M19/+ males (Supplementary Fig. S3). These males carry one MOLF chromosome as signified by M19/+ and one allele of Sf1geo in Sf1+/-;M19/+. We found reduced TGCT incidence in Sf1+/-;M19/+ males (17%) compared with that in M19/+ males (27%; Table 2). This reduction in tumor incidence was modest but statistically significant at P < .04. The incidence of bilateral tumors also decreased in Sf1+/-;M19/+ (18% bilateral tumors) compared with that in M19/+ (31% bilateral tumors) males.

We found that Sf1 levels had decreased by ~50% in Sf1+/-;M19/+ compared with the M19/+ strain (Fig. 3A). This also correlated with lower Sf1 levels in the Sf1+/-;M19/+ compared with that in M19/+ gonads and lower TGCT incidence in Sf1+/-;M19/+ mice. Next, we compared the Sf1 mRNA levels in the PN1 testes of M19 to that of M19/+ and M19/+;Sf1+/- males. The level of Sf1 transcript was lower (by ~4-fold) in the M19 compared with that in the M19/+ gonads (Fig. 3B), M19/+ has one Chr19 from MOLF and one from 129 (Fig. 4), whereas M19 is homozygous with both Chr19 being MOLF derived.

Overall, genetic analysis using both the Ter and M19 strains indicates that lowering of Sf1 levels in the testes protects against TGCT development. This indicates a common mechanism of action of Sf1 in these two strains that develop TGCTs due to different genetic defects.

**Sf1 levels in the 129 and MOLF inbred mouse strains**

We initially identified Sf1 from the M19 strain, and a comparative microarray screen found a 2- to 8-fold reduced
expression of Sf1 in M19 compared with 129 (23). Because M19 males have higher TGCT incidence compared with 129 (21), we expected that decreasing Sf1 levels would further increase TGCT incidence in Sf1+/−\;Ter or Sf1+/−\;M19 mice. Surprisingly, the results from our genetic data were opposite to that expected. We have verified that Sf1 levels are indeed reduced in Sf1+/−\;Sf1+/−\;Ter and Sf1+/−\;M19/+ mouse gonads. This raised the question as to why Sf1 levels are low in the M19 strain, which has high tumor incidence. One explanation could be because Chr19 of the M19 strain is derived from and identical to that of the MOLF strain (Fig. 4; ref. 21). As Sf1 is located on Chr19, it is possible that the expression level of Sf1 in M19 is inherited from MOLF.

Therefore, we examined Sf1 expression of the MOLF inbred mouse strain. qRT-PCR indicated that Sf1 levels in the PN1 testes of MOLF are indeed similar to that in M19 (Fig. 3C), and Sf1 levels in both MOLF and M19 are significantly lower than in the 129 strain. Therefore, MOLF has inherently low Sf1 expression compared with 129. Also notable is the fact that, unlike 129, the MOLF strain does not develop spontaneous TGCTs.

Thus, Sf1 expression levels in M19 are similar to that in the MOLF strain, although the testicular tumor frequency of M19 is extremely high and that of MOLF is extremely low.

Taking together all the above observations, we conclude that the high testicular tumor incidence of the M19 strain likely occurs despite the lower Sf1 levels. Tumor development in the M19 strain is due to multiple TGCT susceptibility loci from Chr19 (22, 23). The presence of multiple TGCT-promoting genes likely overrides the protective effect of lower Sf1 levels in M19. Thus, the M19 strain is an exception to the rule that lower Sf1 correlates with lower TGCT incidence.

However, when we reduce the dosage of the TGCT-causing genes from that in M19 (homozygous for MOLF Chr19) to M19/+ (heterozygous for MOLF Chr19), the tumor incidence in M19/+ decreases to 26% (Fig. 4). Sf1 levels are higher in M19/+ (Fig. 4). Only when we further lower the Sf1 levels, as in the Sf1+/−\;M19/+ strain, did both Sf1 level and tumor incidence decrease (Fig. 4). Thus, the protective effect of lower Sf1 levels becomes apparent when a single MOLF Chr19 is present as in Sf1+/−\;M19/+ mice.

### Table 2. TGCT incidence in M19 mice deficient for Sf1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. with tumors</th>
<th>Bilateral tumors</th>
<th>Unilateral tumors</th>
<th>Normal testes</th>
<th>Total no. of males examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>M19/+</td>
<td>36 (27%)</td>
<td>11 (31%)</td>
<td>25</td>
<td>95</td>
<td>131</td>
</tr>
<tr>
<td>M19/+;Sf1+/-</td>
<td>22 (17%)</td>
<td>4 (18%)</td>
<td>18</td>
<td>111</td>
<td>133</td>
</tr>
</tbody>
</table>

NOTE: M19 (or M19/M19) was crossed to Sf1+/− mice (Supplementary Fig. S3). The progeny were genotyped to determine the M19/+ and M19/+;Sf1+/- males. The testis phenotypes of all males were examined. The significance of the difference in tumor incidences between the strains is P = 0.0375.
Discussion

Our results show that SF1 levels modulate the incidence of TGCT in two different mouse models of TGCTs. Haploinsufficiency of SF1 correlated with decreases in tumor incidences in Sf1+/-:Ter/Ter and Sf1+/-:M19/+ mice. This argues for a common role of SF1 in germ cell transformation in both strains, considering that different genetic defects are responsible for germ cell tumorigenesis in the two strains. Reduction of SF1 levels likely reduces germ cell transformation rates and results in an overall reduced number of testicular tumors. Another possibility is that lower SF1 levels in germ cells may result in reduced viability of germ cells. Thus, fewer germ cells survive to transform. However, the PN1 testes of Sf1+/- mice did not seem to have fewer germ cells compared with wild-type mice, and Sf1+/- mice are fertile.

SF1 levels in both MOLF and M19 strains were found to be significantly lower than in the 129 strain. Thus, different mouse inbred strains inherently express varying SF1 levels in their tissues, and the SF1 levels could likely influence the tumorigenic potential of cells from these strains. Because the 129 strain has higher SF1 levels and is permissive for TGCT development, we propose that SF1 is an oncogenic genetic susceptibility factor from 129 that promotes TGCT development.

Interestingly, SF1 levels are observed to inherently vary in tissues of different inbred strains (National Center for Biotechnology Information Geo Profiles). This inherent variation in SF1 levels could likely influence normal and disease phenotypes in mice.

We note that no significant tumor incidences have been observed for the MOLF inbred strain, which has inherently low SF1 levels. Of the reported studies, TGCT development is found in the M19 (129.MOLF Chr19) consomic strain (21), and suppression of mammary tumorigenesis has been reported in FVB/N-Tg (MMTV-PyMT) and MOLF F1 hybrid mice (36).

The difference in SF1 levels between the 129 and MOLF strains may be due to a number of factors such as differences in the sequence and activity of the promoters between strains, differences in the nature of alternate spliced SF1 variants, or single nucleotide polymorphisms (SNP) in the transcripts that affect stability.

Contrary to other observations, we found that SF1 levels are low in M19; however, TGCT incidence is high in this strain. In this case, it seems that the protective effect of low SF1 levels does not overcome the effects of multiple tumor-promoting loci present in M19. However, when we reduce the dosage of tumor-promoting loci in M19 to M19/+, then we clearly observe the protective effect of lower SF1 levels in M19/+;SF1+/-, TGCT incidences correlate with SF1 levels in M19/+ and M19/+;SF1+/- mice. Thus, the protective effect of SF1 deficiency can be overshadowed by the presence of multiple tumor-promoting loci in the genome.

An earlier study reported a gene trap–inactivated SF1 mouse line in which the gene trap was inserted in the promoter region of SF1 (37). Treatment of SF1+/- mice with an organotropic carcinogen resulted in higher number of colon tumors (32). This indicated that lower SF1 levels are oncogenic, and this is contradictory to our data. One explanation for this discrepancy could be that SF1 functions as an oncogene or tumor suppressor depending on the cell type. In colon cells, SF1 may be responsible for generating a majority of splice variants with tumor-suppressor function, whereas in the testes, SF1 may generate mostly oncogenic splice variants. Thus, lowering of SF1 levels in the colon enhances tumorigenesis, whereas lowering of SF1 levels in the testes attenuates tumorigenesis. Another possibility is that application of DNA-damaging carcinogen likely induces additional oncogenic mutations in colon cells, and the effects of multiple oncogenes outweigh the protective effect of SF1 deficiency. A third possibility is that the difference in the SF1+/- mouse background in the two studies influences the differences in results.

SF1 has been shown to be critical for HeLa cell viability (31). We detected SF1 expression in developmentally important organs, such as the heart and brain (Supplementary Fig. S5). Presumably, there are cell type–specific pre-mRNA targets of SF1 that are critical for cell and embryonic viability.

In the testes, SF1 is found in both the germ cells and supporting cells of the seminiferous tubules. It is possible that SF1 function in the supporting Sertoli cells of the testes may also be important for germ cell tumorigenesis. Overall, alternative splicing events are most prevalent in the testis and brain (38), implying that splicing regulation is especially important in these tissues.

Transformation of cells frequently occurs in conjunction with dysregulation in alternative splicing (39–42). Because splicing factors usually regulate production of a variety of different mRNAs, changes in either the level or function of splicing factors can cause global changes in RNA levels and splicing variants. Specific splicing factors have been found to be overexpressed or downregulated in cancer tissues (42, 43). Studies on human testicular tumors have found novel testicular cancer–associated splice isoforms (44, 45), which could serve as potential diagnostic or prognostic markers.

Interestingly, in humans, SF1 has also been implicated in immune responses (46). SF1 interacts with the branchpoint sequence within the intron of the histocompatibility leukocyte antigen, HLA-DQB1. Naturally occurring and disease-associated branchpoint mutations in DQβ1 intron 3 show impaired SF1 binding and altered splicing, which influence their gene expression (46). A recent global analysis of the human transcriptosome revealed that many genes have subtle genetic changes, such as SNPs in splice site usage (47). Splicing differences due to SNPs were found to be frequent in human populations, affect disease-causing genes, and likely contribute to phenotypic diversity and susceptibility to complex diseases.

In another study, oncogenic alternate splice variants were generated when SF1 was transiently transfected into human colon cancer cell lines, and this correlated with cell transformation (37). This again indicates a proto-oncogenic capability of SF1 in human cells.
In summary, we provide genetic evidence showing that deficiency of SF1 suppresses testicular tumorigenesis. SF1, an RNA-binding protein (48, 49) necessary for spliceosome assembly of specific pre-mRNAs (24, 25), likely regulates alternative splicing of pre-mRNAs in the testes. Thus, higher SF1 levels are oncogenic in the testes. Our data lead us to propose that SF1 is a tumor-susceptibility factor for germ cell tumorigenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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Rui Zhu, Jason Heaney, Joseph H. Nadeau, et al.

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