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Consistent Effects of TSG101 Genetic Variability on Multiple Outcomes of Exposure to Human Immunodeficiency Virus Type 1†

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Tumor susceptibility gene 101 (TSG101) encodes a host cellular protein that is appropriated by human immunodeficiency virus type 1 (HIV-1) in the budding process of viral particles from infected cells. Variation in the coding or noncoding regions of the gene could potentially affect the degree of TSG101-mediated release of viral particles. While the coding regions of the gene were found to lack nonsynonymous variants, two polymorphic sites in the TSG101 5′ area were identified that were associated with the rate of AIDS progression among Caucasians. These single-nucleotide polymorphisms (SNPs), located at positions −183 and +181 relative to the translation start, specify three haplotypes termed A, B, and C, which occur at frequencies of 67%, 21%, and 12%, respectively. Haplotype C is associated with relatively rapid AIDS progression, while haplotype B is associated with slower disease progression. Both effects were dominant over the intermediate haplotype A. The haplotypes also demonstrated parallel effects on the rate of CD4 T-cell depletion and viral load increase over time, as well as a possible influence on HIV-1 infection. The data raise the hypothesis that noncoding variation in TSG101 affects the efficiency of TSG101-mediated release of viral particles from infected cells, thereby altering levels of plasma viral load and subsequent disease progression.

One fundamental feature of a successful virus is its ability to utilize the host cellular machinery in order to support its propagation. Understanding the interactions between host and viral proteins provides the opportunity to identify means for controlling outcomes of viral infection. Recent studies have disclosed a variety of cellular molecules that are exploited by human immunodeficiency virus type 1 (HIV-1) at different stages of its life cycle, one of which, the tumor suppressor gene 101 (TSG101) protein, is essential for budding of the virus from infected cells (10, 12, 21, 35).

TSG101 is an evolutionarily conserved gene located on human chromosome 11p15. It encodes a 46-kDa multidomain protein that contains an N-terminal ubiquitin-conjugating enzyme E2 variant (UEV) domain, a proline-rich domain, a coiled-coil region including a leucine zipper, and a C-terminal α-helical domain. The TSG101 protein has been detected in the nucleus and cytoplasm, and its localization is cell cycle dependent (38, 39).

A potential role for TSG101 as a tumor suppressor that was suggested in an early study of the gene (17) remains controversial (22, 34). Identification of molecules with which TSG101 interacts has suggested its involvement in transcriptional regulation (5, 13, 24, 32) and cell cycle control (6, 18, 27). Targeted deletion of tsg101 in mice results in early embryonic death due to a defect in cellular proliferation (29), and reports of cell cycle arrest and death in TSG101-deficient cells have further confirmed a critical role for TSG101 in cell survival (6, 16).

Many recent studies of TSG101 have focused on its role in endosomal trafficking. TSG101 and its yeast orthologue, Vps23, belong to the so-called “class E” proteins whose functions are essential for vacuolar protein sorting (1). TSG101/Vps23, along with two other proteins, Vps28 and Vps37, form a ~350-kDa complex named ESCRT-I (endosomal sorting complex required for transport) (14). ESCRT-I is involved in a series of protein-protein interactions that result in sorting of ubiquitylated proteins from early endosomes into multivesicular bodies (MVBs). During this process, early endosomes carrying protein cargo bud into MVBs, organelles that eventually fuse with lysosomes for subsequent protein degradation. This process entails direct binding of the TSG101/Vps23 UEV domain to ubiquitin, resulting in the delivery of ubiquitylated proteins to lysosomes for degradation.

† Supplemental material for this article may be found at http://jvi.asm.org.
proteins (that are destined for degradation) into MVBs (2, 12, 14, 20, 28, 33).

Some viruses have developed an ingenious mechanism for budding from cells that involves the host endosomal sorting process. This became evident after the discovery of the direct interaction between the UEV domain of TSG101 and a highly conserved motif in the p6 region of the HIV-1 Gag protein, Pro-Thr/Ser-Ala-Pro (PTAP), an interaction that was shown to be critical for the release of HIV-1 particles from the cellular membrane (10, 12, 21, 35). The HIV-1 PTAP motif belongs to a family of late (L) domains, so named for their late involvement in the viral life cycle. The viral L domains are also characterized by conserved PPXY and YXXL motifs, which, along with PTAP, interact with host proteins involved in the MVB pathway (reviewed in reference 9). The TSG101–HIV-1 relationship has been studied intensively by several laboratories, resulting in the identification of additional host proteins involved in the process of HIV-1 budding (30, 31, 36). The TSG101 molecule has also been shown to be involved in the budding of HIV-2 (25), Ebola virus (21), human T-cell leukemia virus type 1 (4), and bluetongue virus (37).

Given the central role of TSG101 in release of HIV-1 from infected cells, we hypothesized that genetic variations in TSG101 could potentially affect the functional activity of TSG101 protein in viral budding, thereby altering levels of circulating virus in the blood of infected individuals and the clinical course of AIDS. Here, we report two single-nucleotide polymorphism (SNP) variants, located at positions −183 and +181 relative to the translation start site, that associate with differences in viral load dynamics, in CD4 T-cell decline, and, correspondingly, with the rate of AIDS progression after infection. The −183 variant has been recently reported to be associated with faster CD4 decline in the Swiss HIV Cohort Study (SHCS) (3). Here, we demonstrate a more detailed analysis of the association between TSG101 variation and several outcomes of HIV-1 infection, which are consistent in the SHCS and a large sample of U.S. AIDS cohorts.

MATERIALS AND METHODS

Subjects. The study group included patients from five U.S. cohorts and a Swiss cohort: the AIDS Linked to the Intravenous Drug Experience Study, the Hemophilia Growth and Development Study, the Multicenter AIDS Cohort Study (MACS), the Multicenter Hemophilia Cohort Study, the San Francisco City Clinic Cohort Study (26), and the SHCS (http://www.shcs.ch). The seroconversion date was identified within a 2-year window. For over 90% of the 380 men included in the CD4 decline analysis, the seroconversion was known within a 1-year interval (median [interquartile range] = 0.51 [0.48 to 0.58] years).

RESULTS

Identification of TSG101 SNPs and haplotypes. An initial screening of the TSG101 coding region in 50 healthy Caucasian blood donors using the single-strand conformation polymorphism technique indicated the highly conserved nature of the gene, since no nonsynonymous nucleotide changes were identified. Sequencing of 79 randomly chosen Caucasian seroconverter patients (U.S. AIDS cohorts) was then performed in the 5′ area of the gene, which is likely to contain regulatory sequences. The sequenced fragment consisted of −2 kb around exon 1, where nine SNPs were identified (Fig. 1A). Analysis of the SNP genotypes revealed six haplotypes with estimated frequencies of >1% that could be defined by four haplotype-tagging SNPs (Fig. 1B) (positions −600 [rs3802966], −518 [rs1857909], −183 [rs2292179], and +181 [rs1395319] relative to the translation start site.

Analysis of TSG101 variants in the AIDS cohorts. The four TSG101 haplotype-tagging SNPs located in the 5′ area were typed in the U.S. AIDS cohorts. Haplotypes based on the four SNPs were estimated in a large population of 1,895 Caucasian individuals (Fig. 1C). The frequencies of the haplotypes in Caucasians differed only slightly from the initial estimation in 79 individuals (Fig. 1B).

The association of the individual SNPs and the five related haplotypes with disease progression was tested using the Cox proportional hazards model (7) and categorical analysis in which frequencies of genotypes were compared in people who developed AIDS during certain time periods. The Cox model did not reveal any significant effect for the four individual SNPs or for the five haplotypes (see Tables S1 and S2 in the supplemental material). However, we did observe differences in the frequencies of the −600G and −183C variants among individuals who developed AIDS before 7 years compared to those who developed AIDS later (see Table S3 in the supplemental material). In similar analyses, haplotype 2 conferred a susceptible effect (odds ratio [OR] = 1.6 to 2.5; P = 0.002 to 0.01), haplotypes 3 and 5 were protective (OR = 0.6 to 0.8, P = 0.007 to 0.2, and OR = 0.5 to 0.8, P = 0.06 to 0.5), while haplotype 4 was relatively neutral (OR = 0.8 to 1.1; P = 0.2 to 0.8) compared to a reference haplotype 1 (see Table S4 in the supplemental material). Of note, the −600G variant, which is in strong linkage disequilibrium (LD) with the −183T and +181C variants, specifies haplotype 3, and the protective effect...
of this haplotype corresponded to the effect of the individual SNP. Based on these data and the haplotype structures, we concluded that susceptibility and protection are associated with variation at positions −183 and +181. Three of the four possible haplotypes composed of these two SNPs were observed, indicating strong LD between the variants. The corresponding haplotypes, T-A, T-C, and C-C, were termed A, B, and C, respectively (Fig. 1D).

Haplotypes B and C are associated with different rates of AIDS progression. The frequencies of haplotypes A, B, and C among individuals who developed AIDS in one of six distinct time periods after seroconversion (≤3, 3 to 5, 5 to 7, 7 to 10, 10 to 12, and >12 years) were compared (data not shown). In this analysis, seroprevalent individuals were included in the last three groups depending on the date of an AIDS-defining outcome after their first HIV* visit. This analysis suggested that the effect of the TSG101 haplotypes on HIV disease is not gradual over time, an observation that was further elucidated by the absence of a significant effect of these variants on AIDS progression in survival analysis using the Cox model, where time is a continuous variable starting from seroconversion (see Table S5 in the supplemental material). Rather, differences in susceptible (C) and protective (B) haplotype frequencies appeared somewhat bimodal in that the frequency of the susceptible haplotype group was significantly greater among individuals who progressed to AIDS within 7 years after seroconversion relative to those who remained AIDS-free for at least 7 years (dominant model; OR = 1.72 to 2.30; P = 0.0001 to 0.002) (Table 1). Conversely, the protective haplotypes were observed significantly more frequently among those who remained AIDS-free for 7 years or longer after seroconversion compared to those who had progressed within 7 years (dominant model; OR = 0.56 to 0.64; P = 0.0005 to 0.01). Both the B and C haplotypes appeared to have dominant effects, since a codominant model did not fit the data as well as a dominant model. Haplotype A was relatively neutral (Table 1).

Protective, susceptible, and neutral TSG101 haplotype groups. Haplotypes B and C exhibited opposite dominant effects over the neutral haplotype A (Table 1), so it follows that the haplogenotypes A/B and B/B would both be protective, and A/C and C/C would both confer susceptibility in terms of AIDS progression before or after 7 years after seroconversion. We hypothesized that the dominant effects of haplotypes B and C would result in a neutral phenotype among B/C heterozygotes similar to the A/A haplotype. Thus, the three nonoverlapping groups based on genotypic data were tested for their effects on AIDS progression. As expected, the A/C-C/C grouping showed a strong susceptible effect (OR = 1.74 to 2.61; P = <0.0001 to 0.003), A/B-B/B associated with protection (OR =

![FIG. 1. SNPs and the corresponding haplotypes observed in the 5' area of TSG101. (A) Schematic map of the nine SNPs identified in the 5' area of TSG101. SNP positions are determined relative to the “A” nucleotide of the ATG start codon, which is shown within exon 1 (black box). (B) Haplotypes based on the nine SNPs. The haplotype frequencies were estimated based on sequencing data among 79 randomly chosen Caucasian seroconverters. Only haplotypes with frequencies >0.01 are listed. Alleles with minor frequencies are shown in boldface. F, frequency, CI, confidence interval. (C) Haplotypes based on the nine SNPs. The haplotype frequencies of Caucasian seroconverters. Only haplotypes with frequencies were estimated based on sequencing data among 79 randomly chosen Caucasian seroconverters. (D) Structure and frequencies of haplotypes A, B, and C estimated in 2,071 Caucasian individuals.

TABLE 1. Effects of TSG101 haplotypes on AIDS progression among Caucasians from the combined U.S. AIDS cohorts (dominant model)

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>AIDS outcome</th>
<th>No.* of fast progressors (F)</th>
<th>No.* of slow progressors (F)</th>
<th>OR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CD4 &lt; 200</td>
<td>218 (0.88)</td>
<td>637 (0.89)</td>
<td>0.94</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>AIDS-1993</td>
<td>245 (0.87)</td>
<td>775 (0.89)</td>
<td>0.91</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>AIDS-1987</td>
<td>132 (0.86)</td>
<td>995 (0.88)</td>
<td>1.06</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Death</td>
<td>92 (0.90)</td>
<td>1169 (0.88)</td>
<td>1.19</td>
<td>0.41</td>
</tr>
<tr>
<td>B</td>
<td>CD4 &lt; 200</td>
<td>74 (0.30)</td>
<td>286 (0.40)</td>
<td>0.64</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>AIDS-1993</td>
<td>82 (0.29)</td>
<td>353 (0.41)</td>
<td>0.60</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>AIDS-1987</td>
<td>44 (0.29)</td>
<td>451 (0.40)</td>
<td>0.60</td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td>Death</td>
<td>27 (0.26)</td>
<td>523 (0.39)</td>
<td>0.56</td>
<td>0.01</td>
</tr>
<tr>
<td>C</td>
<td>CD4 &lt; 200</td>
<td>77 (0.31)</td>
<td>145 (0.20)</td>
<td>1.78</td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td>AIDS-1993</td>
<td>89 (0.32)</td>
<td>184 (0.21)</td>
<td>1.72</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>AIDS-1987</td>
<td>52 (0.34)</td>
<td>251 (0.22)</td>
<td>1.78</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Death</td>
<td>40 (0.39)</td>
<td>291 (0.22)</td>
<td>2.30</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

* Numbers of individuals who had at least one of the corresponding haplotypes (dominant model).

† Seroconverter patients who progressed to an AIDS outcome in ≤7 years after HIV-1 infection.

‡ Patients who avoided an AIDS outcome for >7 years after HIV-1 infection (seroconverters and seroprevalent individuals).
0.51 to 0.61; \(P = 0.0001\) to 0.01), and the A/A-B/C grouping appeared neutral (Table 2). The protective and susceptible haplotype groups were further compared to the neutral group by the Cox proportional-hazards model (Fig. 2). The relative shapes of the curves in the Kaplan-Meier plots confirm that the effects of the TSG101 variants are not constant over time, but rather occur most obviously between about 4 and 12 years after seroconversion.

### TABLE 2. TSG101 haplogenotypes and AIDS progression among Caucasians from the combined U.S. AIDS cohorts

<table>
<thead>
<tr>
<th>Haplogenotype</th>
<th>AIDS outcome</th>
<th>No.(^a) of fast progressors(^b) (F)</th>
<th>No.(^a) of slow progressors(^c) (F)</th>
<th>OR</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral (A/A or B/C)</td>
<td>CD4 &lt; 200</td>
<td>133 (0.54)</td>
<td>357 (0.50)</td>
<td>1.17</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>AIDS-1993</td>
<td>147 (0.52)</td>
<td>426 (0.49)</td>
<td>1.14</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>AIDS-1987</td>
<td>72 (0.47)</td>
<td>560 (0.50)</td>
<td>0.89</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Death</td>
<td>47 (0.46)</td>
<td>668 (0.50)</td>
<td>0.85</td>
<td>0.47</td>
</tr>
<tr>
<td>Protective (A/B or B/B)</td>
<td>CD4 &lt; 200</td>
<td>56 (0.23)</td>
<td>251 (0.35)</td>
<td>0.54</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>AIDS-1993</td>
<td>64 (0.23)</td>
<td>307 (0.35)</td>
<td>0.54</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>AIDS-1987</td>
<td>37 (0.24)</td>
<td>384 (0.34)</td>
<td>0.61</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Death</td>
<td>21 (0.21)</td>
<td>447 (0.34)</td>
<td>0.51</td>
<td>0.006</td>
</tr>
<tr>
<td>Susceptible (A/C or C/C)</td>
<td>CD4 &lt; 200</td>
<td>59 (0.24)</td>
<td>109 (0.15)</td>
<td>1.74</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>AIDS-1993</td>
<td>71 (0.25)</td>
<td>137 (0.16)</td>
<td>1.80</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>AIDS-1987</td>
<td>45 (0.29)</td>
<td>183 (0.16)</td>
<td>2.13</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Death</td>
<td>34 (0.33)</td>
<td>214 (0.16)</td>
<td>2.61</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(a\) Numbers of individuals who had at least one of the corresponding haplotypes (dominant model).

\(b\) Seroconverter patients who progressed to an AIDS outcome in \(\leq 7\) years after HIV-1 infection.

\(c\) Patients who avoided an AIDS outcome for \(> 7\) years after HIV-1 infection (seroconverters and seroprevalent individuals).

**FIG. 2.** Kaplan-Meier plots for the three TSG101 haplotype groups. Four AIDS outcomes were analyzed using the Cox proportional-hazards model.
Individuals from different TSG101 haplotype groups exhibit different rates of CD4+ T-cell decline. The availability of longitudinal CD4 T-cell counts in two of our AIDS cohorts, the MACS and the SHCS (http://www.shcs.ch), provided the opportunity to determine whether the influence of TSG101 variants on AIDS progression might involve differential rates of CD4 T-cell decline. CD4 measurements over time were plotted, and the slopes of the fitted lines were compared between the three genotypic groups. For 380 MACS patients, CD4 T-cell decline was estimated from measurements obtained over a period of 13 years since seroconversion (Fig. 3A). An average of 12.9 CD4 measurements per individual, ranging from 2 to 32, was considered in the analysis. The SHCS cohort primarily consisted of seroprevalent individuals, so in this case, CD4 T-cell decline was measured over a period of 10 years starting at the point where CD4 counts fell in the range of 500 to 600 cells/mm³ (Fig. 3B). For 310 SHCS patients, an average of 11.5 CD4 T-cell count data points (ranging from 2 to 52) per individual were available for analysis. In both cohorts, the effects of the three genotypic groups on CD4 T-cell decline corresponded to the effects these genotypes had on AIDS progression, where the decline was progressively steeper in the following order: A/A-B/C (neutral group) > A/B-B/B (protective group) (Fig. 3). Differences in CD4 T-cell decline between the protective and susceptible groups were highly significant in both cohorts (P = <0.0001 to 0.0002).

Effects of the TSG101 haplotypes on viral load increase over time. Given the central role of TSG101 in HIV-1 budding and the known correlation between the viral load and the rate of AIDS progression (23), we tested whether the effect of TSG101 variation on CD4 T-cell decline and AIDS progression was also reflected in viral load changes over time. For this analysis, the increase in viral load over time among 373 MACS patients was measured, stratified by the three TSG101 genotypic groups (Fig. 4). An average of 9.6 measurements (ranging from 2 to 28 measurements) of viral load per patient over a period of 13 years since seroconversion were available for the analysis. No significant difference in HIV-1 RNA levels between the three genotypic groups was observed at the time of seroconversion. Strikingly, however, highly significant differences in log_{10} HIV-1 RNA slopes were observed between the protective and susceptible groups (P < 0.0001), strongly suggesting that variation in the TSG101 gene affects the HIV-1 viral load, potentially through the differential efficiency of TSG101 variants to mediate viral budding.

TSG101 genotypes and HIV-1 infection. The TSG101 genotypic groups were also tested for potential effects on HIV-1
infection by comparing the distributions of these genotypes in HIV+ patients with those in seronegative or high-risk exposed uninfected individuals. Although only marginally significant values ($P = 0.02$ to 0.08) were determined (Table 3), the effects of these genotypes on HIV-1 infection each paralleled their respective effects on the viral load, on CD4 T-cell decline, and on the rate of progression to AIDS.

**DISCUSSION**

The consistent protective (haplotype B) and susceptible (haplotype C) influences of TSG101 haplotypes on multiple outcomes after HIV-1 exposure, including longitudinal viral load levels, CD4+ T-cell decline, and subsequent disease progression, as well as a moderate effect on HIV-1 infection, support a physiological role for genetic variation near/within the TSG101 gene in HIV-1 pathogenesis. Whether the (−183, +181) haplotype variants have a direct effect on these outcomes remains in question. We did not find any nonsynonymous polymorphisms or 3’ untranslated region SNPs linked to the (−183, +181) variants that could explain the observed effects of these variants on HIV-1 disease. Further, the (−183, +181) haplogenotypes did not show any significant difference in the levels of TSG101 mRNA transcription in peripheral blood lymphocytes or purified CD4 cells as tested by real-time PCR, and no differences were observed in promoter activity using reporter constructs (data not shown). Likewise, no alternatively spliced forms of TSG101 mRNA associating with the genotypes were observed, negating splice variation as the underlying mechanism of the associations described. Although we were not able to detect an influence of the TSG101 haplotypes on gene transcription or mRNA splicing, we cannot rule out the possibility that the TSG101 SNPs may affect transcription/splicing under specific physiological conditions.

The protective and susceptible effects of TSG101 haplotypes on AIDS progression are not constant over time, as indicated by the Kaplan-Meier curves (Fig. 2). The relative shapes of the curves suggest that TSG101 variation has little or no effect during the early (≤4 years) and late (>12 years) stages of infection, but rather, only at an intermediate time period. Other host genetic, viral, or environmental factors that affect disease progression during the early and late stages of infection may override effects of TSG101. Alternatively, the TSG101 interaction with HIV and/or consequences of this interaction could be different during these two extreme time intervals compared with the intermediate period. However, the effects of TSG101 haplotypes occurring during the intermediate time interval were strong enough to be evident when the entire patient cohort was used in the categorical analysis, as well as the longitudinal analyses of CD4 T-cell decline and viral-load increase.

Recently, the (−183) variant, corresponding to haplotype C, was shown to associate with lower virus production ex vivo, a paradoxical finding given its association with faster CD4 T-cell decline (3) and susceptibility to AIDS reported here. Although this finding appears to contradict the genetic epidemiological findings presented here, the ex vivo assay may not be physiologically relevant to the described effect of TSG101 on AIDS progression given its time dependence as discussed above.

The HapMap genotype data (http://www.hapmap.org) suggest that TSG101 is located in a region of strong LD: the corresponding haplotype block defined according to Gabriel et al. (11) spans 118 kb. This block includes the entire TSG101 gene; its partial parologue, UEV-3 (15); and two additional gene fragments (the lactate dehydrogenase A-like 6A gene [GenBank accession no. NM144972] and a computationally predicted gene that may encode a protein similar to the mitochondrial carrier homolog 1 [XM497268]). Therefore, the (−183, +181) haplogenotypes may mark the true disease variant(s) through LD. In support of this, no effect on disease progression was detected in a smaller sample of African-American seroconverters (although longitudinal CD4 and viral-load data from these individuals were not available for analysis; also, the susceptible haplotype group was observed at a frequency of only 4%). However, given the requirement of TSG101 for HIV-1 budding in vitro, it seems likely that the effect described herein is due to variation in TSG101 and not to polymorphism in a neighboring locus. If so, this study represents the first genetic epidemiological evidence to support previous in vitro studies indicating a primary role for TSG101 in HIV-1 pathogenesis.

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**REFERENCES**

5. Burgdorf, S., P. Leister, and K. H. Scheidtmann. 2004. TSG101 interacts with apoptosis-antagonizing transcription factor and enhances androgen re-