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Assignment[1] of the murine tumor susceptibility gene 101 (*tsg101*) and a processed *tsg101* pseudogene (*tsg101-ps1*) to mouse chromosome 7 band B5 and chromosome 15 band D1 by in situ hybridization

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[1] This is a more precise localization of *tsg101* previously assigned to chromosome 7 by PCR (Li et al., 1997). To our knowledge this is the first time *tsg101-ps1* has been mapped.

Rationale and significance

Tsg101 was discovered in mouse NIH 3T3 fibroblasts and the functional inactivation of this gene leads to cell transformation and tumors (Li and Cohen, 1996). The human TSG101 gene, which encodes a protein that is 94% similar to the mouse counterpart, has been mapped to chromosome 11p15.2→p15.1, a region that is associated with LOH in different types of tumors (Li et al., 1997). In the mouse, a *tsg101* sequence was detected on chromosome 7 using a PCR assay and a mapping panel of hybrid cell lines (Li et al., 1997). However, recent studies have shown that the mouse genome contains at least one processed pseudogene that is nearly identical to the *tsg101* cDNA sequence (Wagner et al., 1998). After analyzing both sequences, we found that the PCR assay used earlier to determine the chromosome location does not allow distinction between the pseudogene and the actual *tsg101* gene since the primer set amplifies a region within the last exon of *tsg101* that is identical to the pseudogene. Therefore, FISH analysis was performed to determine the chromosome location of both sequences independently.

Materials and methods

The isolation of BAC clones containing the mouse *tsg101* and *tsg101-ps* was described earlier (Wagner et al., 1998). The DNA was labeled with digoxigenin dUTP by nick translation and hybridized to metaphase chromosomes derived from mouse embryo fibroblasts. Specific hybridization signals were detected by incubating the hybridized slides with fluoresceinated antidigoxigenin antibodies followed by counterstaining with DAPI. To verify the location on specific chromosomes, the BAC clones were co-hybridized with P1 clones D7MIT259 and D15MIT13, respectively (Shi et al., 1997).

Probe names: *tsg101* and *tsg101-ps1*
Probe type: genomic DNA
Insert size: >150 kb
Vector: pBeloBAC 11
Proof of authenticity: DNA sequencing
Gene reference: GenBank accession nos. AF060868 for *tsg101*, and AF060867 for *tsg101-ps1*

Results

Mapping data
Location: *tsg101* on 7B5; *tsg101-ps1* on 15D1
Number of cells examined: 80 for both genes
Number of cells with specific signal: 76 exhibited specific labeling for *tsg101* (Fig. 1A) and 73 were positive for the processed pseudogene (Fig. 1D).
Most precise assignment: *tsg101* on 7B5; *tsg101-ps1* on 15D1

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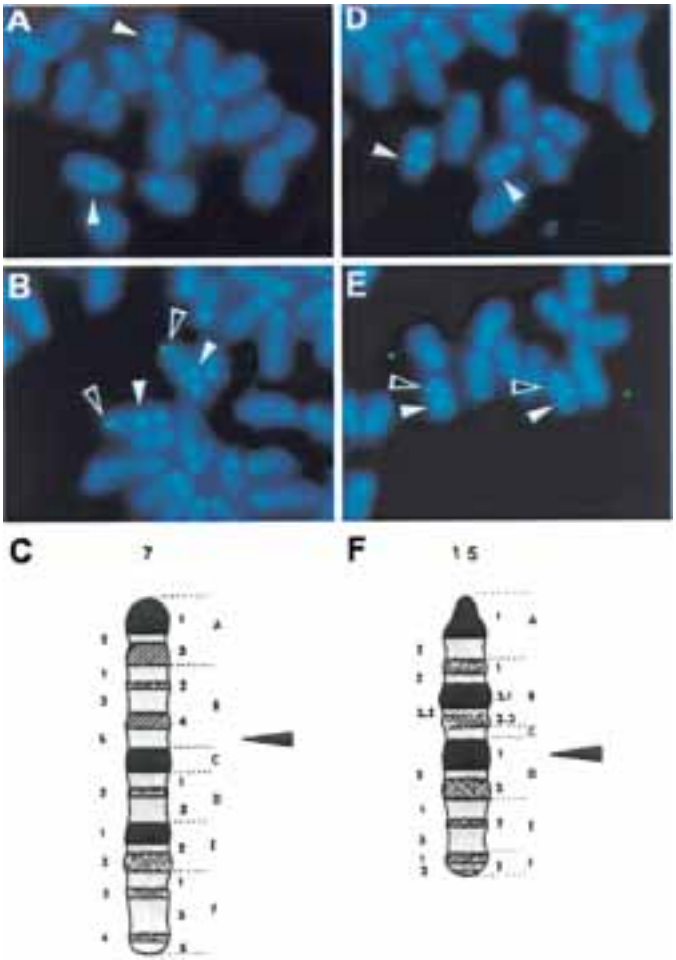

Fig. 1 . Chromosome location (solid arrowhead) of the mouse *tsg101* gene (**A**) and a processed *tsg101* pseudogene (**D**). The location of *tsg101* on chromosome 7 and *tsg101-ps1* on chromosome 15 was verified by co-hybridization with D7MIT259 (**B**) and D15MIT13 (**E**), respectively (open arrowheads). A minimum of 10 chromosomes were measured and the results indicated that *tsg101* maps to 7B5 (**C**) and *tsg101-ps1* localizes to 15D1 (**F**).

Location of background signals (sites with > 2 signals): none observed

The location of *tsg101* on chromosome 7 and *tsg101-ps1* on chromosome 15 was verified by co-hybridization with D7MIT259 (Fig. 1B) and D15MIT13 (Fig. 1E), respectively. Based on our mapping data, *tsg101* is located in the mouse within a cluster of genes syntenic to the human chromosome 11, band p15.1, such as *Kcnc1*, *Ldh1*, *Ldh3*, *Myod1*, *Tph*, and the *Saa* gene family (Mouse Genome Database, 1998). The *tsg101* processed pseudogene was localized on chromosome 15D1, but no gene has yet been mapped to this band. Interestingly, the *Myc* gene is located on 15D2–D3 (Boyle et al., 1992). Although *tsg101-ps1* is not expressed in normal mouse tissues (Wagner et al., 1998), it needs to be determined whether this sequence remains untranscribed in tumor models with a known amplification of the *Myc* locus.

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