

1.36 ng mm<sup>-2</sup> and a  $k_d$  of 0.081 s<sup>-1</sup> (Fig. 4B). When the two proteins were mixed, a  $\Gamma_{eq}$  of 5.95 ng mm<sup>-2</sup> was reached. An apparent  $k_d$  > 0.0008 s<sup>-1</sup> was determined by fitting a mono-exponential function to the dissociation phase, which is 3 and 2 orders of magnitude lower as compared with the single proteins (fig. S7). Both SOPC and DOPS:SOPC bilayers showed no interaction with the control protein MBP (Fig. 4, A and B). Dissociation of bound CHMP2AΔC and CHMP3ΔC in Hepes buffered saline (HBS) containing 1 M NaCl revealed a  $k_d$  ≥ 1 s<sup>-1</sup>, which was much faster than dissociation in HBS alone. In contrast, the CHMP2AΔC-CHMP3ΔC polymer did not dissociate with a higher rate ( $k_d$  ≤ 0.00046 s<sup>-1</sup>) in the presence of 1 M NaCl (fig. S7), indicating resistance to change in ionic strength. Once assembled on membranes, CHMP2AΔC-CHMP3ΔC did not exchange with soluble or membrane-bound CHMP3ΔC (fig. S8). Thus, CHMP2A-3 complexes assembled on membranes in vitro in the absence of CHMP4-6 subcomplexes, even though yeast Snf7-Vps20 (CHMP4-6) complexes may recruit Vps2-Vps24 (CHMP2A-3) complexes to membranes in vivo (7).

To assess the influence of CHMP2AΔC-CHMP3ΔC tubes on membrane shapes, we used large unilamellar vesicles (LUVs) composed of DOPS:SOPC. LUV incubation with either CHMP2AΔC or CHMP3ΔC had no effect on their floatation in sucrose gradients (fig. S9, A and B), whereas preformed CHMP2AΔC-CHMP3ΔC tubes restricted LUV floatation to the middle of the gradient (fig. S9, C and D). Negative staining EM confirmed CHMP2AΔC-CHMP3ΔC tube membrane interaction via their outer surfaces (Fig. 4, C and D). However, no systematic remodeling of the LUV membranes was observed. Potential membrane remodeling by the CHMP copolymer or vice versa was further explored by assembling the polymer in the presence of LUVs. Although CHMP2AΔC-CHMP3ΔC assembly in the presence of SOPC LUVs had no effect on tube morphology (Fig. 4E), the presence of DOPS:SOPC LUVs produced shorter tubes (Fig. 4F), displaying loose helical coils (Fig. 4G) and cone-shaped tubes that appeared closed at the narrower end (Fig. 4H). Thus, this suggests a mechanism where lipid interaction affects CHMP polymerization.

Because modified VPS4 and CHMP3 exert dominant negative effects on HIV-1 budding (8, 9, 17, 25) and cytokinesis (6, 18), CHMP2A-CHMP3-VPS4 complexes may catalyze a common step such as membrane fission. The CHMP2A-CHMP3 polymer presents a membrane binding topology that is inverse to that of dynamin membrane complexes (26), which catalyze endocytotic vesicle abscission (27). ESCRT-III coupled to VPS4 may exert a similar role in budding processes directed away from the cytosol. Thus, we propose that a helical CHMP2A-CHMP3 polymer assembles on the inside of a membrane bud, which may induce membrane deformation, leading to constriction and eventually abscission when

coupled to VPS4 activity, the only energy-providing candidate in the pathway (2, 15) (fig. S10).

## References and Notes

1. R. L. Williams, S. Urbe, *Nat. Rev. Mol. Cell Biol.* **8**, 355 (2007).
2. S. Sakseena, J. Sun, T. Chu, S. D. Emr, *Trends Biochem. Sci.* **32**, 561 (2007).
3. J. H. Hurley, *Curr. Opin. Cell Biol.* **20**, 4 (2008).
4. P. D. Bieniasz, *Virology* **344**, 55 (2006).
5. J. G. Carlton, J. Martin-Serrano, *Science* **316**, 1908 (2007); published online 6 June 2007 (10.1126/science.1143422).
6. E. Morita *et al.*, *EMBO J.* **26**, 4215 (2007).
7. M. Babst, D. J. Katzmman, E. J. Estepa-Sabal, T. Meerloo, S. D. Emr, *Dev. Cell* **3**, 271 (2002).
8. U. K. von Schwedler *et al.*, *Cell* **114**, 701 (2003).
9. T. Muziol *et al.*, *Dev. Cell* **10**, 821 (2006).
10. A. Zamborlini *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 19140 (2006).
11. S. Lata *et al.*, *J. Mol. Biol.* **378**, 816 (2008).
12. Y. Lin, L. A. Kimpler, T. V. Naismith, J. M. Lauer, P. I. Hanson, *J. Biol. Chem.* **280**, 12799 (2005).
13. S. Shim, L. A. Kimpler, P. I. Hanson, *Traffic* **8**, 1068 (2007).
14. P. I. Hanson, R. Roth, Y. Lin, J. E. Heuser, *J. Cell Biol.* **180**, 389 (2008).
15. M. Babst, B. Wendland, E. J. Estepa, S. D. Emr, *EMBO J.* **17**, 2982 (1998).
16. N. Bishop, P. Woodman, *Mol. Biol. Cell* **11**, 227 (2000).
17. B. Strack, A. Calistri, E. Popova, H. Göttlinger, *Cell* **114**, 689 (2003).
18. J. D. Dukes, J. D. Richardson, R. Simmons, P. Whitley, *Biochem. J.* **411**, 233 (2008).
19. E. H. Egelman, *Ultramicroscopy* **85**, 225 (2000).
20. Materials and methods are available as supporting materials on Science Online.
21. M. D. Stuchell-Brereton *et al.*, *Nature* **449**, 740 (2007).
22. T. Obita *et al.*, *Nature* **449**, 735 (2007).
23. A. Scott *et al.*, *EMBO J.* **24**, 3658 (2005).
24. P. Whitley *et al.*, *J. Biol. Chem.* **278**, 38786 (2003).
25. J. Martin-Serrano, A. Yarovsky, D. Perez-Caballero, P. D. Bieniasz, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 12414 (2003).
26. S. M. Sweitzer, J. E. Hinshaw, *Cell* **93**, 1021 (1998).
27. K. Takei, P. S. McPherson, S. L. Schmid, P. De Camilli, *Nature* **374**, 186 (1995).
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## Supporting Online Material

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# A Neoplastic Gene Fusion Mimics Trans-Splicing of RNAs in Normal Human Cells

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Chromosomal rearrangements that create gene fusions are common features of human tumors. The prevailing view is that the resultant chimeric transcripts and proteins are abnormal, tumor-specific products that provide tumor cells with a growth and/or survival advantage. We show that normal endometrial stromal cells contain a specific chimeric RNA joining 5' exons of the *JAZF1* gene on chromosome 7p15 to 3' exons of the Polycomb group gene *JJAZ1/SUZ12* on chromosome 17q11 and that this RNA is translated into JAZF1-JJAZ1, a protein with anti-apoptotic activity. The *JAZF1-JJAZ1* RNA appears to arise from physiologically regulated trans-splicing between precursor messenger RNAs for *JAZF1* and *JJAZ1*. The chimeric RNA and protein are identical to those produced from a gene fusion found in human endometrial stromal tumors. These observations suggest that certain gene fusions may be pro-neoplastic owing to constitutive expression of chimeric gene products normally generated by trans-splicing of RNAs in developing tissues.

**R**ecurrent, specific gene fusions arising from chromosomal rearrangements are characteristic features of many neoplasms, especially those having hematopoietic and mesenchymal origins (1–6). In most fusions, recom-

bination occurs within introns that interrupt the coding sequences, giving rise to the expression of chimeric proteins (2). The prevailing view is that the chimeric proteins resulting from chromosomal rearrangements are entirely abnormal and have neoplastic effects leading to the growth and/or survival advantage of cells containing them.

An observation that seems at odds with this view is that chimeric mRNAs identical to those derived from fusion genes can often be detected in low abundance by reverse transcription–

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polymerase chain reaction (RT-PCR) of RNA from healthy tissues (7). The explanation generally offered for this finding is that specific chromosomal rearrangements occur within small numbers of cells in healthy tissues but that the chimeric proteins generated by them are alone insufficient to drive substantial clonal expansion.

We have previously described a gene fusion due to a (7;17)(p15;q21) chromosomal translocation found in about 50% of human endometrial stromal sarcomas (ESSs) (8, 9). The fusion joins the first three exons (from a total of five) in the gene *JAZF1* to the last 15 (from 16) in the Polycomb group gene *JJAZ1/SUZ12*. Expression of the chimeric *JAZF1-JJAZ1* protein in cultured human embryonic kidney (HEK) 293 cells confers resistance to apoptosis and, when accompanied by suppression of the unarranged *JJAZ1* allele, increased rates of proliferation (9).

We examined normal human endometrial tissues for possible chimeric *JAZF1-JJAZ1* RNA, beginning with endometrial stromal cell lines. RNA extracted from the immortalized, normal human endometrial stromal cell line (HESC) (10) was analyzed for the presence of *JAZF1-JJAZ1* chimeric RNA by RT-PCR with primers containing sense and antisense sequence flanking the site of joining between *JAZF1* and *JJAZ1* (11). A single amplification product generated by this reaction was identical in size to that amplified from human ESSs carrying a *JAZF1-JJAZ1* gene fusion due to the presence of a t(7;17)(p15;q21) (Fig. 1A). RT-PCR for the *JAZF1-JJAZ1* RNA in two additional, non-immortalized primary cell lines derived from the normal endometrial stroma of two other patients amplified similarly sized products. Nucleotide sequence analysis of the RT-PCR products from each cell line yielded the same sequence of nucleotides at the *JAZF1-JJAZ1* junction as was found in RNA of tumors with the gene fusion. RT-PCR for *JAZF1-JJAZ1* RNA failed to amplify products from the RNA extracted from a variety of other epithelial and mesenchymal cell lines, all of which contained *JAZF1* and *JJAZ1* RNA (fig. S1).

To investigate the specificity of the junction between *JAZF1* and *JJAZ1* RNA sequences in the HESC cell line, we carried out detailed RT-PCR studies on RNA from the HESC cell line using antisense primers for *JJAZ1* exon 3 sequence paired with six different sense primers for five exons of *JAZF1*. With primers for the first three exons of *JAZF1*, single products were obtained matching the sizes predicted for the joining of *JAZF1* exon 3 to *JJAZ1* exon 2 (Fig. 1B). Similar results were achieved when the sense primer for *JAZF1* exon 3 was paired with six different antisense primers distributed among the 16 exons of *JJAZ1*. These results are consistent with the *JAZF1-JJAZ1* RNA joined at exon 3 and exon 2 of the respective genes being the only abundant *JAZF1-JJAZ1* RNA in HESC cells.

To determine whether the *JAZF1-JJAZ1* RNA is translated into protein, we performed

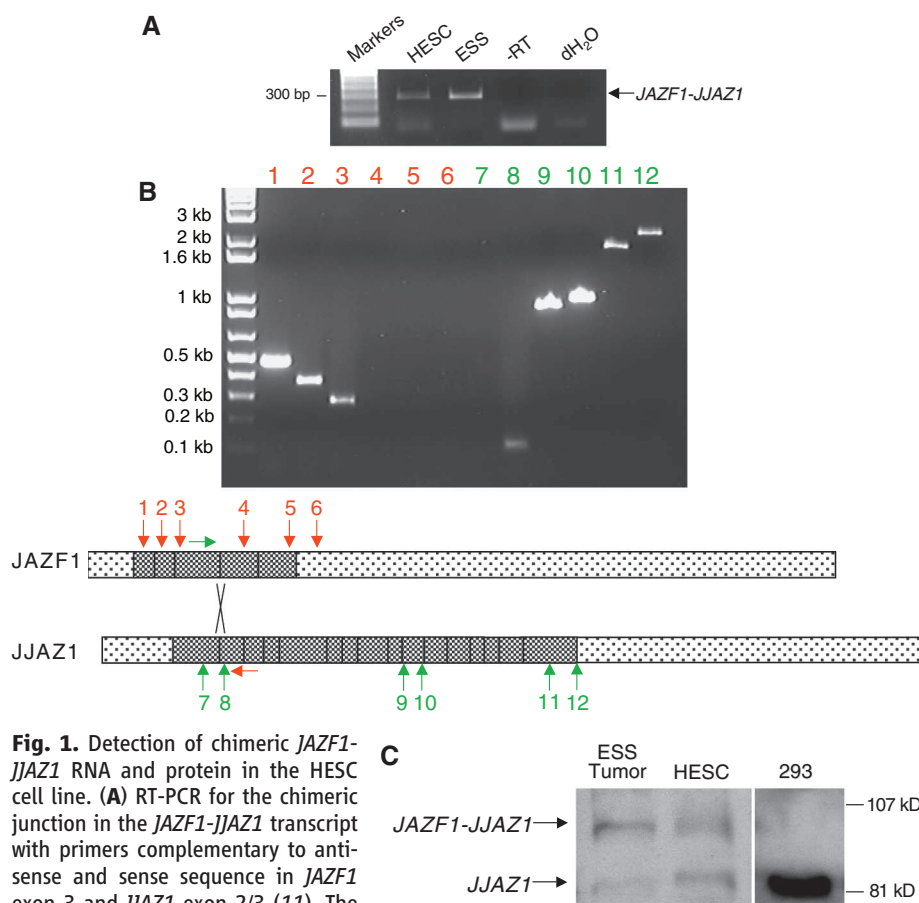
Western blot analysis with *JJAZ1*-specific antibody on protein extracts prepared from HESC cells. This analysis detected a protein identical in size to *JAZF1-JJAZ1* protein detected in ESSs (Fig. 1C and supporting online text).

The detection of *JAZF1-JJAZ1* RNA in endometrial stromal cell lines was duplicated by RT-PCR analysis of RNA extracted from formalin-fixed, paraffin-embedded tissues from normal human uteri. *JAZF1-JJAZ1* RNA was detected primarily in endometrium from late secretory and early proliferative phases of the menstrual cycle (Fig. 2A). No *JAZF1-JJAZ1* RNA sequences were amplified from normal myometrium at any phase of the cycle (fig. S2).

Because of the general association of the *JAZF1-JJAZ1* RNA with endometrium from particular phases of the menstrual cycle, we investigated the effects of steroid hormones on the production of the chimeric transcript in HESC

cells. Low concentrations of progesterone seemed to slightly increase amounts of the *JAZF1-JJAZ1* RNA seen in the absence of added hormone, whereas both estrogen and, at higher concentrations, progesterone suppressed detection of the chimeric RNA (Fig. 2B). These findings are consistent with the results of analyses on endometrial tissue, showing that *JAZF1-JJAZ1* RNA is present predominantly at the beginning and end of the menstrual cycle, when hormone concentrations are low.

Because normal endometrium is subjected to hypoxia and undergoes apoptosis during the late secretory phase of the menstrual cycle, we investigated whether hypoxia can induce production of *JAZF1-JJAZ1* RNA in HESC cells by treatment with desferrioximine (DFO), which simulates hypoxic conditions. HESC cells treated with 250  $\mu$ M DFO for 8 hours showed increased amounts of *JAZF1-JJAZ1* RNA (Fig. 2C). Another,



nonimmortalized normal endometrial stromal cell line, HESC-597, also showed up-regulation of *JAZF1-JJAZ1* RNA when treated with DFO. Cultures of cells derived from tissue other than endometrial stroma showed no detectable chimeric RNA with DFO treatment. Analysis of the RNA in DFO-treated HESC cells by a nuclease protection assay (fig. S3) indicated that somewhat less chimeric RNA was produced in these cells than in tumor cells containing the *JAZF1-JJAZ1* fusion, consistent with relative amounts of 10 to 35% detected by quantitative RT-PCR. The nuclease protection assay also confirmed that detection of chimeric RNA was not a methodologic artifact associated with RT-PCR. Quantitative RT-PCR revealed that treatment of HESC cells with DFO did not appreciably change the amount of either *JAZF1* or *JJAZ1* RNA (fig. S4), suggesting that DFO raises the levels of *JAZF1-JJAZ1* RNA by a mechanism independent of increased transcription of the two genes.

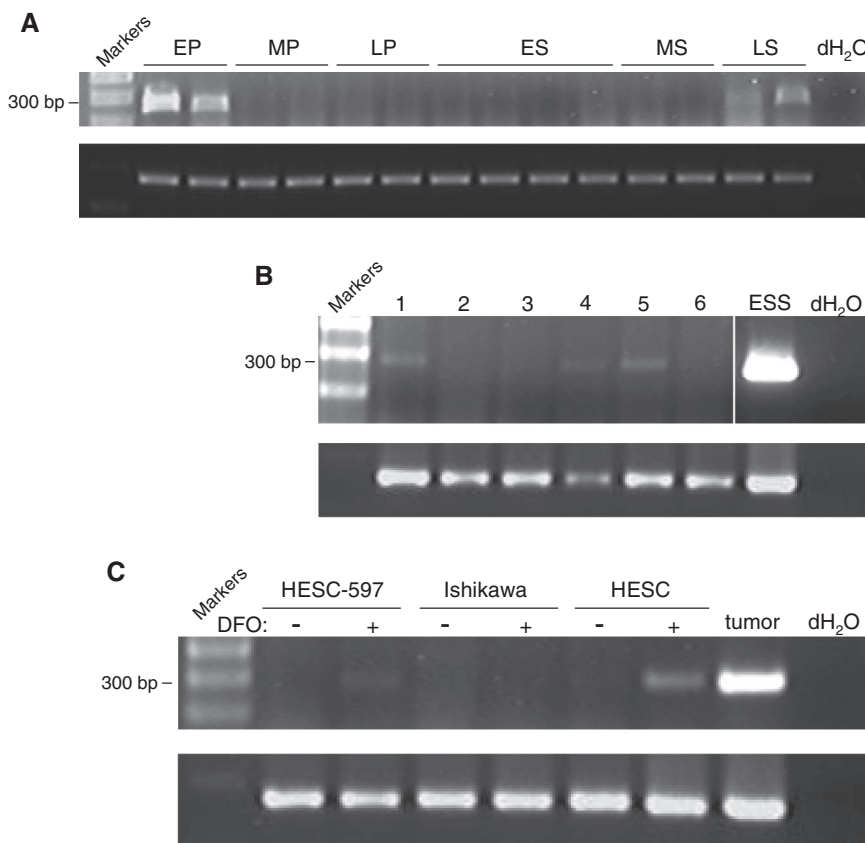
To investigate whether *JAZF1-JJAZ1* RNA is produced from a t(7;17)(p15;q21) in HESC cells, we first showed that this cell line, which had not intentionally been cloned, had in fact originated from a single cell immortalized in culture (fig. S5). We then performed Southern blot analyses of HESC DNA by using probes that had previously detected t(7;17)(p15;q21) rearrangements in ESSs. No rearranged bands were detected. Cytogenetic analysis of numerous metaphase spreads from HESC cells revealed no abnormalities in chromosomes 7, 17, or any other chromosome (Fig. 3A). Similarly, analysis by fluorescence in situ hybridization (FISH) with pairs of bacterial artificial chromosome (BAC) probes for DNA flanking on either side the chromosome 7p15 breakpoint and separately the 17q21 breakpoint detected no breakage in these regions of the genome (fig. S5). Additionally, a probe consisting of a yeast artificial chromosome (YAC) that contains DNA spanning the 7p15 breakpoint

showed no splitting of the fluorescent signal (Fig. 3B). Finally, no superimposition of signals was observed when probes for chromosomes 7p15 and 17q21 were used together in FISH studies.

To investigate the possibility that (7; 17)(p15;q21) translocations or their equivalents arose in cells in culture at some point after immortalization, we subcloned HESC cells by limiting dilution. Thirty-seven subclones derived on average from half a cell per culture were tested for the production of *JAZF1-JJAZ1* RNA. RT-PCR of RNA from these subclones detected *JAZF1-JJAZ1* RNA in all clones examined, and for most subclones, the amount of *JAZF1-JJAZ1* RNA increased when DFO was added to the culture (Fig. 3C). Furthermore, analyses of all clones examined were negative for rearrangements at the chromosome 7p15 site by FISH with flanking probes (Fig. 3D). Ten of these subclones were also tested for rearrangements at the 17q12 site by FISH, and none of these showed abnormalities.

Given the evidence against DNA recombination in HESC cells and the precise joining of sequences at exon boundaries in *JAZF1-JJAZ1* RNA, we reasoned that the mechanism most likely responsible for production of this RNA is trans-splicing of pre-mRNAs for the *JAZF1* and *JJAZ1* genes. To test this hypothesis, we prepared in vitro splicing extracts from the nuclei of HESC cells. Samples of this extract were mixed with samples of a nuclear extract from a primary rhesus fibroblast cell line RF (12). RT-PCR of *JJAZ1* intron 1 RNA sequence revealed that unspliced pre-mRNA was present in the HESC and RF nuclear extracts (fig. S7). Nucleotide sequence analysis of exon 3 in the *JAZF1* gene of RF cells showed two single base-pair sequence differences from the human *JAZF1* gene that permitted both selective RT-PCR of any RNA containing either rhesus or human exon 3 and the ability to distinguish between the products amplified from these RNAs. With selective primers and conditions, amplification of RNA after incubation of mixed extracts yielded products in which RF *JAZF1* exon 3 was joined to exon 2 of *JJAZ1* (Fig. 4B). Sequence analysis of the RT-PCR products confirmed that the amplified *JAZF1-JJAZ1* sequences contained exon 3 of RF *JAZF1* (Fig. 4C). The amount of product generally increased when the HESC extract was prepared from cells cultured with DFO, although the extent of increase varied considerably among experiments. No product was obtained from extracts of HESC cells or RF cells alone, or when adenosine 5'-triphosphate (ATP), an obligate cofactor for splicing, was omitted from the splicing reaction. Similar results were obtained with extracts from the nonimmortalized endometrial stromal cell line, HESC-597 (Fig. 4D).

To study the mechanism further and to rule out the possibility of polymerase switching during transcription, we carried out the in vitro trans-splicing assay with HESC nuclear extract mixed with purified RF RNA. The amount of trans-spliced product detected was similar to that in the

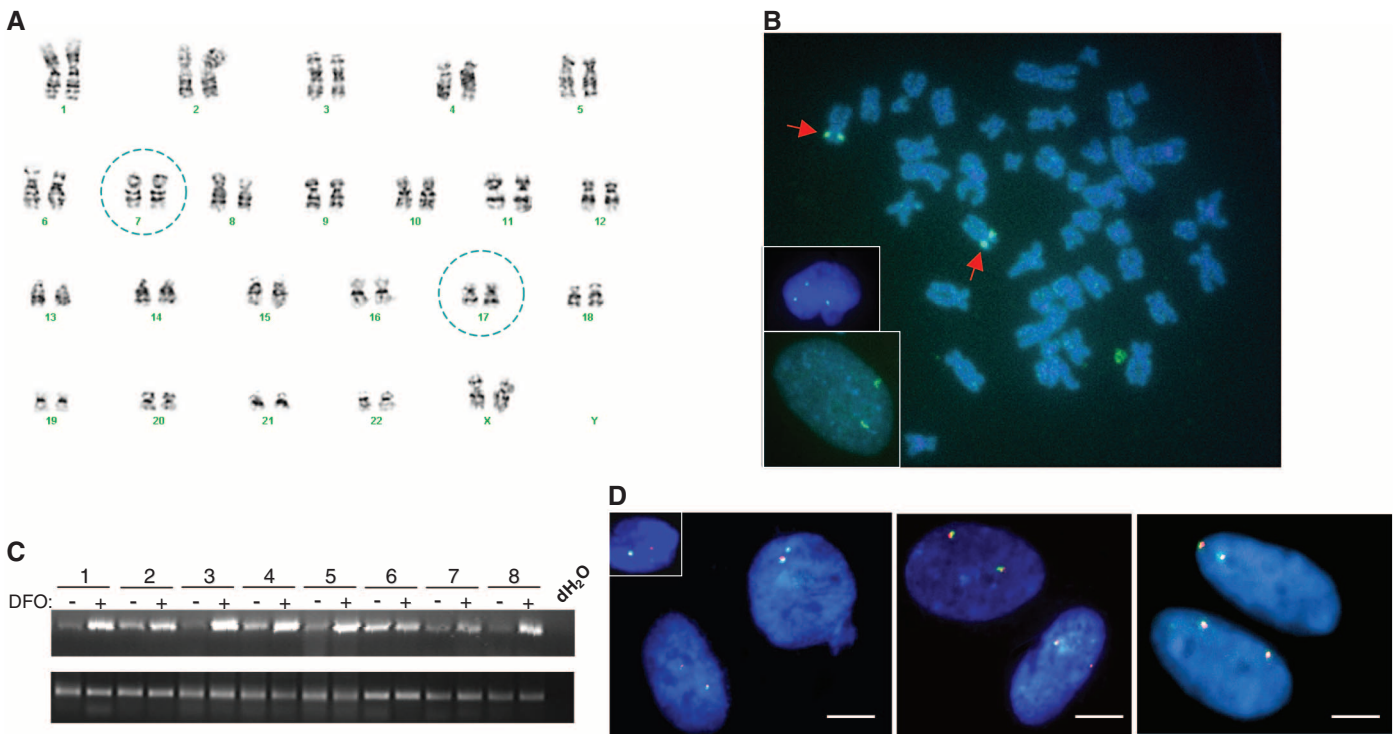


**Fig. 2.** Detection of *JAZF1-JJAZ1* RNA in endometrial tissues and effects of hormones and hypoxia on amounts of *JAZF1-JJAZ1* RNA in cultured cells. Analyses were performed by RT-PCR, as in Fig. 1A. Unlabeled panels show results of RT-PCR for β-actin RNA as a control for input RNA. (A) Detection of *JAZF1-JJAZ1* RNA in total RNA extracted from endometrial samples representing various phases of the menstrual cycle: EP, early proliferative; MP, mid-proliferative; LP, late proliferative; ES, early secretory; MS, mid-secretory; LS, late secretory. At least two separate uteri were tested for each phase. (B) Effect of hormone treatment on *JAZF1-JJAZ1* RNA in HESC cells. After 2 days of serum starvation, medium containing serum and no drug (lane 1), 17 β-estradiol at 5 × 10<sup>-8</sup> M (lane 2), progesterone at 1 × 10<sup>-6</sup> M, 1 × 10<sup>-7</sup> M, or 1 × 10<sup>-8</sup> M (lanes 3 to 5), or 17 β-estradiol at 5 × 10<sup>-8</sup> M plus progesterone at 1 × 10<sup>-7</sup> M (lane 6) was added to the cells for 24 hours. (C) Effect of DFO treatment on *JAZF1-JJAZ1* RNA in HESC and HESC-597 cells. No chimeric transcript could be detected in Ishikawa cells, an endometrial carcinoma line.



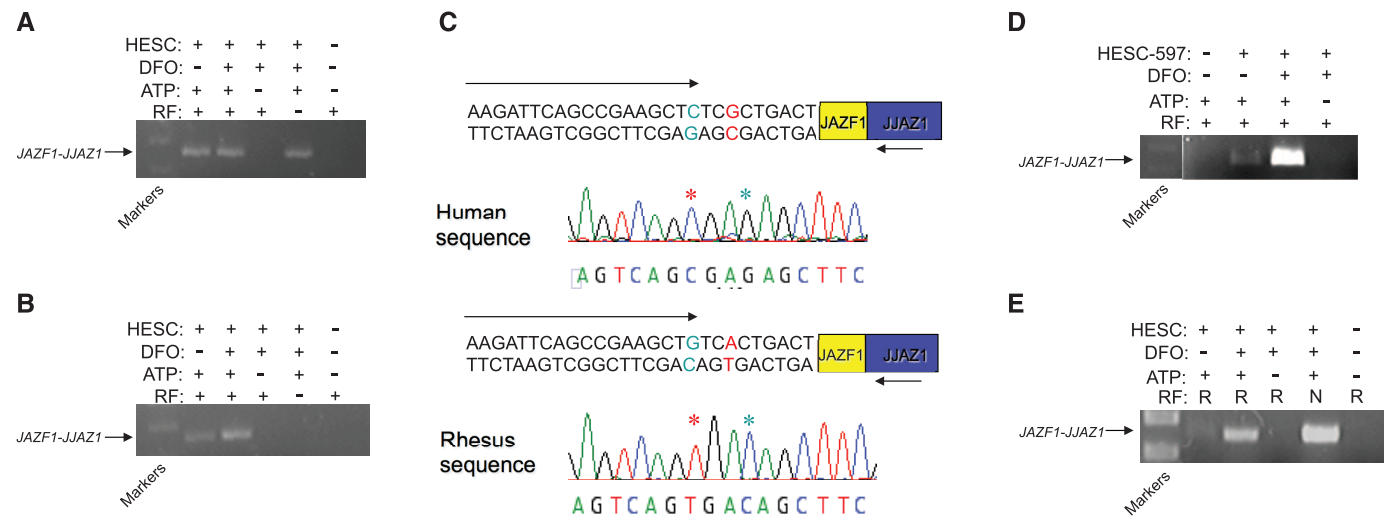
assay performed with a mixture of HESC and RF nuclear extracts (Fig. 4E). Elimination of all PCR-detectable traces of DNA from the RF RNA preparation by treatment with deoxyribo-nuclease I did not affect the production of the trans-spliced product (figs. S7 and S8).

The data presented here are consistent with trans-splicing of the pre-mRNAs transcribed from the *JAZF1* and *JJAZ1* genes in normal



**Fig. 3.** Absence of the t(7;17)(p15;q21) in HESC cells. **(A)** Cytogenetic analysis of HESC cells. Normal chromosomes 7 and 17 are circled. **(B)** FISH analysis of HESC cells with a YAC probe containing DNA spanning the *JAZF1* locus. Arrows point to the intact YAC signal in metaphase chromosomes. (Lower inset) A representative interphase HESC nucleus; (upper inset) an ESS control showing splitting of the probe signal. **(C)** RT-PCR

analysis for *JAZF1-JJAZ1* RNA in representative subclones of the HESC cell line with and without DFO treatment. **(D)** FISH analysis of three HESC subclones with two BAC probes, labeled red or green and each containing DNA sequences that flank the *JAZF1* locus on one side or the other. The juxtaposition of red and green signals indicates no separation of these sequences. (Inset) Separation of signals in an ESS control. Bars, ~10 μm.



**Fig. 4.** In vitro trans-splicing reactions. **(A)** RT-PCR analysis for *JAZF1-JJAZ1* RNA with a human-specific primer. RT-PCR products from chimeric RNA were amplified only when ATP was supplied, and the amount of product increased when the HESC nuclear extracts were prepared from cells pretreated with DFO. **(B)** RT-PCR analysis for *JAZF1-JJAZ1* RNA with a rhesus-specific primer. No band was detected in HESC or RF extracts alone, but products were observed when the two kinds of nuclear extracts were mixed. The amount of product increased when the HESC extract was prepared from the cells pretreated with DFO. **(C)** Sequence analysis of the RT-PCR products

amplified with antisense human- or rhesus-specific primers. Aqua-colored bases and stars indicate the interspecies sequence differences included in the species-specific primers; red bases and stars indicate the species-specific sequence differences detected in the products. **(D)** RT-PCR analysis for *JAZF1-JJAZ1* RNA in nuclear extracts of the HESC-597 and RF cells with rhesus-specific primers. Mixed extracts produced detectable signal in the presence of ATP. **(E)** RT-PCR analysis for *JAZF1-JJAZ1* RNA in HESC nuclear extracts mixed with purified RF RNA by means of amplification with the rhesus-specific primer. R, RNA; N, nuclear extract.

endometrial stromal cells and tissues to yield chimeric products identical to those produced by a recurrent gene fusion in endometrial stromal tumors. Trans-splicing of noncoding, leader exons to separately transcribed pre-mRNAs is common in certain lower eukaryotes, such as protozoa and nematodes (13–16). However, in vertebrates, only a few examples of trans-splicing have been described (17–25), and most of these involve splicing between pre-mRNAs of the same gene to generate mRNAs with duplicated exons (17–20). For these reasons, trans-splicing in vertebrates has sometimes been regarded as a nonfunctional by-product of a somewhat sloppy splicing system (26). This conclusion seems inapplicable to *JAZF1-JJAZ1* RNA because the *JAZF1-JJAZ1* fusion gene is a recurrent finding in a high fraction of endometrial stromal tissues, and fusion genes associated with chromosomal translocations in cancer have repeatedly been shown to contribute to the neoplastic phenotype of the tumors containing them (27, 28). Additionally, the expression of the *JAZF1-JJAZ1* coding sequences in cultured cells has demonstrated effects on cell survival and proliferation (9). Whether *JAZF1-JJAZ1* protein in tissues provides protection from hypoxia, to which endometrium is subjected during the late secretory phase and possibly the early proliferative phase of the menstrual cycle, remains to be determined.

The mechanisms involved in the trans-splicing of RNAs and the regulation of this process are unclear. Juxtaposition of the loci encoding the RNAs that participate in trans-splicing would not seem essential because *in vitro* splicing in nuclear extracts of RNAs at physiological concentrations was found to be efficient. These results also indicate that cotranscriptional splicing is not an absolute requirement for trans-splicing. Whether *in vivo* trans-splicing of RNA transcribed from loci that participate in chromosomal rearrangements predisposes DNA at those sites to recombination with or without prior intranuclear colocalization of the loci will require further investigation.

In view of the regulated trans-splicing between *JAZF1* and *JJAZ1* pre-mRNAs in normal endometrium, the t(7;17)(p15;q21) found in ESSs might be considered a mutation that leads to constitutive production of the *JAZF1-JJAZ1* mRNA and its protein product. This relation is similar to that seen in other oncogenic mutations associated with tumor development, namely, that mutations lead to overproduction or irreversible activation of gene products rather than to creation of “new” genes, as the fusion genes resulting from many chromosomal translocations and other DNA rearrangements have generally been thought to be.

If RNA products of fusion genes other than *JAZF1-JJAZ1* also mimic normal products resulting from trans-splicing of pre-mRNAs, it would explain the ability to frequently amplify from healthy tissues chimeric RNAs associated with chromosomal rearrangements in tumors. Considering the large number of recurrent gene

fusions found in tumors, it would further suggest that trans-spliced RNAs may be relatively common in normal cells and tissues (supporting online text). At a minimum, the finding of the trans-spliced *JAZF1-JJAZ1* RNA in normal cells implies a risk to inferring the presence of chromosomal rearrangements in tissue specimens for the diagnosis and detection of cancer, especially in the context of minimal disease. Additionally, it is possible that drugs designed to target chimeric proteins produced by neoplastic gene fusions may have toxicities due to inhibited function of similar proteins in normal cells.

#### References and Notes

1. M. A. Pierotti *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 1616 (1992).
2. F. Mitelman, B. Johansson, F. Mertens, *Nat. Rev. Cancer* **7**, 233 (2007).
3. O. Bernard *et al.*, *Oncogene* **6**, 1477 (1991).
4. P. J. Kourlas *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 2145 (2000).
5. A. Pardanani *et al.*, *Blood* **102**, 3093 (2003).
6. S. A. Tomlins *et al.*, *Science* **310**, 644 (2005).
7. S. Janz, M. Potter, C. S. Rabkin, *Genes Chromosomes Cancer* **36**, 211 (2003).
8. J. I. Koontz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 6348 (2001).
9. H. Li *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 20001 (2007).
10. G. Krikun *et al.*, *Endocrinology* **145**, 2291 (2004).
11. Materials and methods are available as supporting material on Science Online.
12. R. C. Desrosiers *et al.*, *J. Virol.* **71**, 9764 (1997).
13. L. Bonen, *FASEB J.* **7**, 40 (1993).
14. M. Krause, D. Hirsh, *Cell* **49**, 753 (1987).
15. N. Agabian, *Cell* **61**, 1157 (1990).
16. R. E. Sutton, J. C. Boothroyd, *Cell* **47**, 527 (1986).
17. C. Caudevilla *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 12185 (1998).
18. A. N. Akopian *et al.*, *FEBS Lett.* **445**, 177 (1999).
19. S. A. Frantz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 5400 (1999).
20. T. Takahara, S. I. Kanazu, S. Yanagisawa, H. Akanuma, *J. Biol. Chem.* **275**, 38067 (2000).
21. C. Finta, P. G. Zaphiropoulos, *J. Biol. Chem.* **277**, 5882 (2002).
22. G. Flouriot, H. Brand, B. Seraphin, F. Gannon, *J. Biol. Chem.* **277**, 26244 (2002).
23. C. Fitzgerald *et al.*, *J. Biol. Chem.* **281**, 38172 (2006).
24. Z. Jehan *et al.*, *Genome Res.* **17**, 433 (2007).
25. C. Zhang *et al.*, *DNA Cell Biol.* **22**, 303 (2003).
26. T. Maniatis, B. Tasic, *Nature* **418**, 236 (2002).
27. C. S. Huettner, P. Zhang, R. A. Van Etten, D. G. Tenen, *Nat. Genet.* **24**, 57 (2000).
28. A. T. Look, *Science* **278**, 1059 (1997).
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#### Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5894/1357/DC1

Materials and Methods

SOM Text

Figs. S1 to S8

References

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## Germline Allele-Specific Expression of *TGFBR1* Confers an Increased Risk of Colorectal Cancer

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Much of the genetic predisposition to colorectal cancer (CRC) in humans is unexplained. Studying a Caucasian-dominated population in the United States, we showed that germline allele-specific expression (ASE) of the gene encoding transforming growth factor- $\beta$  (TGF- $\beta$ ) type I receptor, *TGFBR1*, is a quantitative trait that occurs in 10 to 20% of CRC patients and 1 to 3% of controls. ASE results in reduced expression of the gene, is dominantly inherited, segregates in families, and occurs in sporadic CRC cases. Although subtle, the reduction in constitutive *TGFBR1* expression alters SMAD-mediated TGF- $\beta$  signaling. Two major *TGFBR1* haplotypes are predominant among ASE cases, which suggests ancestral mutations, but causative germline changes have not been identified. Conservative estimates suggest that ASE confers a substantially increased risk of CRC (odds ratio, 8.7; 95% confidence interval, 2.6 to 29.1), but these estimates require confirmation and will probably show ethnic differences.

The annual worldwide incidence of colorectal cancer (CRC) exceeds 1 million, being the second to fourth most common cancer in industrialized countries (1). Although diet and lifestyle are thought to have a strong impact on CRC risk, genes have a key role in the predisposition to this cancer. A positive family

history of CRC occurs in 20 to 30% of all probands. Highly penetrant autosomal dominant and recessive hereditary forms of CRC account for at most 5% of all CRC cases (2). Although additional high- and low-penetrance alleles have been proposed, much of the remaining predisposition to CRC remains unexplained (3).

## A Neoplastic Gene Fusion Mimics Trans-Splicing of RNAs in Normal Human Cells

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