Polyploidization of Murine Mesenchymal Cells Is Associated with Suppression of the Long Noncoding RNA H19 and Reduced Tumorigenicity

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Abstract

Mesenchymal stromal cells (MSC) are used extensively in clinical trials; however, the possibility that MSCs have a potential for malignant transformation was raised. We examined the genomic stability versus the tumor-forming capacity of multiple mouse MSCs. Murine MSCs have been shown to be less stable and more prone to malignant transformation than their human counterparts. A large series of independently isolated MSC populations exhibited low tumorigenic potential under syngeneic conditions, which increased in immunocompromised animals. Unexpectedly, higher ploidy correlated with reduced tumor-forming capacity. Furthermore, in both cultured MSCs and primary hepatocytes, polyploidization was associated with a dramatic decrease in the expression of the long noncoding RNA H19. Direct knockdown of H19 expression in diploid cells resulted in acquisition of polyploid cell traits. Moreover, artificial tetraploidization of diploid cancer cells led to a reduction of H19 levels, as well as to an attenuation of the tumorigenic potential. Polyploidy might therefore serve as a protective mechanism aimed at reducing malignant transformation through the involvement of the H19 regulatory long noncoding RNA. Cancer Res; 72(24); 6403–13. ©2012 AACR.

Introduction

Bone marrow stromal cells were first thought to serve as a tissue scaffold for hemopoietic cells (1). Further studies revealed their regulatory role in protecting hemopoietic stem cells from extinction through overdifferentiation (2). Eventually, these cells were designated as mesenchymal stromal/stem cells (MSC) to denote their capacity to differentiate into a spectrum of cell types (3). MSCs have potential cell therapy uses (3–5); however, the possible susceptibility of MSCs to malignant transformation puts their application in question. It appears that culturing of murine MSCs results in the accumulation of chromosomal aberrations, such as aneuploidy and polyploidy, which may lead to the acquisition of tumorigenic properties (6, 7). Although human MSCs were initially suspected of being prone to spontaneous malignant transformation in culture (8, 9), such claims were later dismissed (10–12). In fact, human MSCs generally undergo growth arrest after a few passages in culture, necessitating the use of mouse MSCs as a model. The possible tumorigenic potential of MSCs remains at the moment an open question.

Many tumor types contain cells with abnormal amounts of DNA, for example, aneuploid and polyploid cells (13–16). It is believed that polyploidy permits cells to acquire genetic defects, in a process termed chromosome instability (CIN; refs. 17–19). Such chromosomally unstable cells might eventually lead to tumor formation (20, 21). However, the fact that polyploid cells are also present in normal tissues, such as in the liver (22) and heart (23), suggests that polyploidy might have important physiologic roles. A recent study following polyploid liver cells in vivo showed that normal hepatocytes have properties similar to that of cancer cells, as they exhibit dynamic changes in their genome resulting in vast polyploidy and aneuploidy (24). It is suggested that such dramatic genomic abnormalities allows hepatocytes to adapt to the genotoxic stress present in the liver tissue.

In the current study, a collection of individually isolated mouse MSCs were examined for their ploidy and corresponding tumorigenic potential. Polyploid MSCs were found to be by far less tumorigenic than their diploid counterparts. We therefore aimed at unraveling the mechanism by which these polyploid cells maintain a nontumorigenic state. Genomic analysis implicated the noncoding RNA H19, a molecule shown to have both tumor-promoting (25, 26) and tumor-suppressing (27) activities, as the major molecular marker that distinguishes between diploid and polyploid MSCs and predicts their corresponding tumor-forming capacity.
Materials and Methods

Mice
The Weizmann Institutional Animal Care and Use Committee approved all animal experiments. C57Bl, BALB/c, SJL, and NOD.SCID mice were purchased from the Harlan Laboratories. NOD.SCID and NOD.SCIDβ2-microglobulin knockout mice were gifts of Prof. Tsvee Lapidot [Weizmann Institute of Science (WIS), Rehovot, Israel] and Dr. Nadir Askenazy (Schneider Hospital, Tikvah, Israel).

Cell culture
MSCs were grown in murine MesenCult Basal Media and supplement (Stem Cell Technologies), or in Dulbeccos’ Modified Eagles’ Media (Invitrogen) with 10% fetal calf serum (Biological industries), added with 60 μg/mL penicillin and 100 μg/mL streptomycin.

Derivation of MSC populations and clones
MSC populations were derived as previously described (28, 29). Briefly, cells were flushed from femurs and tibias using MSC medium and cultured until sufficient amounts of cells were obtained. For clonal isolation, MSCs at passages 5 to 7 were seeded in 96-well plates (Falcon) at a concentration of 0.2 cells per well and grown in MSC medium. Only wells containing single colonies were used for further experiments.

Cytogenetic analysis
Chromosome preparations and spectral karyotyping (SKY) analysis were acquired as previously described (30), and 25 to 75 mitoses were analyzed for each cell line.

DNA content estimation using flow cytometry
Cells were fixed with 70% ethanol/PBS, treated with 20 μL RNaseA 10 mg/mL (Sigma), and stained with 20 μL propidium iodide 2.5 mg/mL (Sigma). Labeled cells were analyzed using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems). Fresh splenocytes were used as control diploid cells.

In vivo tumorigenic potential estimation
Cells were injected subcutaneously (1 × 10⁶, 3 × 10⁶, or 5 × 10⁶ cells) or intravenously (0.5 × 10⁶ cells) into 12- to 14-week-old NOD.SCID, NOD.SCIDβ2, or syngeneic mice. Tumor formation was inspected for 6 months. The doubling time was calculated using the following formula:

\[ T_d = \log(2) \times \left( T_2 - T_1 \right)/ \log(V_2/V_1) \]

where \( T_d \) is doubling time; \( T_2 - T_1 \) is time between measurements; and \( V_1,2 \) the volume of the tumor at time points 1 and 2. Sections were prepared from sample tissues and tumor biopsies, stained with hematoxylin–eosin staining, and photographed using an Olympus IX71 equipped with a DP51 camera.

Real-time PCR
RNA was extracted using Tri-Reagent (MRC), and cDNA was prepared using M-MLV Reverse Transcriptase enzyme (Promega) according to the manufacturer’s protocols. All samples were treated with TURBO DNA-free Kit (Ambion). Real-time PCR was carried out using Platinum SYBR Green qPCR Super-Mix (Invitrogen) and processed using ABI 7300 (Applied Biosystems). Primers are listed in Supplementary Table S1. The cDNA of normal and immortalized primary human fibroblasts was provided by Prof. Varda Rotter (WIS).

Microarray analysis
Experiment was carried out using 100 ng of total RNA in a DNA Mouse Gene ST 1.0 microarray (Affymetrix) at the Genomic Technologies Unit (Biological Services, WIS). Analysis was conducted at the Bioinformatics Unit (Biological Services, WIS) using Partek Genomics Suite (Partek Inc.). Data were preprocessed and normalized using the Robust Multichip Average (RMA) algorithm. Gene enrichments were found using Ingenuity Systems Pathway Analysis, Gene Set Enrichment Analysis (GSEA), and MSigDB softwares. Microarray data are available at the Gene Expression Omnibus (GEO) website, accession number GSE39410.

Immunoblot analysis
The following antibodies were used: p53 (Santa Cruz), AuroraB (BD Biosciences), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Sigma), and c-Myc (Cell Signaling). Denitometry was conducted using ImageJ.

Isolation of hepatocytes with defined ploidy
Primary hepatocytes (from 10- to 14-day suckling mice and 8- to 10-weeks adult BALB/c mice) were isolated by 2-step perfusion (24). Cells were incubated with 15 μg/mL Hoechst 33342 (Invitrogen) and sorted using a SORP FACSAriaII cell sorter (Becton Dickinson).

Generation of tetraploid cells
Tetraploidization was conducted as previously reported (31). Briefly, cells were treated with 1 mmol/L hydroxyurea (Sigma) followed by 5 mmol/L sodium butyrate (Sigma). Control treatment included 24-hour incubation with fresh medium before sodium butyrate treatment.

H19 knockdown
Procedure was done according to the Amaxa (Lonza) manual using 2 μg H19 siRNA (Stealth RNAi siRNA, Invitrogen. Sequences: CUUUCUGUCACAUUGACCACACCUG or UCUAGAAAGCGACGAAUCUUCGUGAUUC). For control, 2 μg of Stealth RNAi siRNA Negative Control Med GC (Invitrogen) was used.

Seeding efficiency
A total of 500 cells were seeded in 100-mm plates (Falcon), incubated for 9 days, and stained with Giemsa/May-Grunwald (Sigma). Seeding efficiency was calculated by dividing the number of colonies counted with the number of cells seeded.

Doubling time
A total of 5,000 to 20,000 cells were seeded in 60-mm plates (Falcon), collected upon reaching approximately 50% confluence and counted. Doubling time was calculated using the following formula:

\[ T_d = (t_2 - t_1) \times \log(2)/\log(q_2/q_1) \]

where \( t_2 - t_1 \) is
$t_1$ equals the time of incubation, $q_1$ is the initial amount of cells, and $q_2$ is the final amount of the cells.

UV sensitivity
Cells were irradiated at UV-C (254 nm) using a low-pressure mercury lamp (TUV 15w G15T8, Philips). The number of cells 1 day after irradiation was divided by the number of cells in nonirradiated cultures to evaluate UV sensitivity.

Immunofluorescence
Cells on coverslips were fixed with ice-cold methanol, blocked and incubated with a γ-tubulin (Sigma) or AuroraB (Cell signaling) antibodies, followed by incubation with Cy3 secondary antibody (Jackson). Coverslips were mounted and counterstained using 10 μL of DAPI II (Vysis). Photographs were taken using a Zeiss Axio Imager Z1 microscope (Carl Zeiss).

Statistics
Significance of Kaplan–Meier comparisons was determined using log-rank test. All statistical analysis was done using SPSS (IBM) or Medcalc statistical software.

Results
Polyploid MSCs maintain a nontumorigenic state
We analyzed cytogenetically 4 independently isolated MSC populations and 1 clone, using SKY (Fig. 1A). The presence of structural chromosomal aberrations on single or paired homologous chromosomes in nondiploid cells was detected (Fig. 1A and Supplementary Table S2). This made it possible to follow the order of numerical and structural changes in chromosomes (Fig. 1B). Three of the cell populations studied had a near diploid–tetraploid chromosomal content (MSC7, 8, and 9). One population (MSC20) and one clone (Clone1) were near-tetraploid. The proportion of each subpopulation was examined by flow cytometry (Fig. 1C). The tetraploidy of MSC20 and Clone1 was stable during long-term culture. In contrast, MSC7, 8, and 9 were unstable; at different passages, pure diploid genotypes were observed and designated accordingly (MSC1, 2, and 3 correspondingly). To explore the extent of polyplody of MSCs in culture, we derived a total of 28 independent MSC populations, which showed variable ploidy (Fig. 1D). Most of these populations were polyploid or contained a polyploid subpopulation, and only a few MSCs remained diploid (6 of 28 populations). We also derived 47 clonal isolates from 3 of the diploid–tetraploid populations (MSC7, 8, and 9); none of them were found to be diploid, suggesting that the diploid cells tend to become polyploid. Under syngeneic conditions, most MSCs did not form tumors and therefore immunocompromised mice were used to more critically assess tumorigenicity (Fig. 2). The tetraploid cells, MSC20 and Clone1, were nontumorigenic at all passages. In contrast, MSC7, 8, and 9 were capable of tumor formation but showed limited tumorigenic potential, compared with their diploid counterparts MSC1, 2, and 3, respectively (Fig. 2A and Supplementary Table S3). Typically, MSC1 formed fibrosarcomas, whereas MSC2 and 3 formed fibroosteosarcomas (Fig. 2B). Surprisingly, the tetraploidy of MSCs predicted lower rather than higher tumorigenicity. To validate this observation, the tumorigenic potential of the various MSC populations and clones was evaluated and is summarized in Fig. 2C (The tumorigenicity of individual cells is shown in Supplementary Tables S4 and S5.) We found that the tumorigenicity of MSCs decreased significantly with increased ploidy. Injection of diploid MSCs resulted in the highest tumorigenic incidence, followed by mixed diploid–tetraploid MSCs (>3-fold reduction), pure tetraploid MSCs (>13-fold reduction), and finally over-tetraploid MSCs, which were completely devoid of tumor-forming capacity. Tumors derived from MSC7, 8, and 9 were analyzed cytogenetically (Supplementary Fig. S1). Apparently, all the tumors formed harbored cytogenetical aberrations identical to those first identified in the cultured cells before their introduction into animals. A tumor formed by MSC7 contained 2Xder(19)t(7;19), MSC8 tumor contained der(4)t(4;18), and MSC9 tumor 4 contained an additional chromosome 6 (+6). Some new aberrations have accumulated in vivo, however, most were nonclonal (detected in single cells) and overall were not common. Importantly, the tumors formed in vivo originated from the diploid rather than from the tetraploid subpopulations of MSC8 and 9. This is evident from the isochromosome 3 i(3) and der(17)t(3;17) found in all of the MSC8 and 9 (respectively) tumor diploid cells, which doubled in the tetraploid tumors. The fact that the MSC7 tumor only harbors 2 der(19)t(7;19) translocations, and not 4, also suggests that this tumor was formed from the MSC7 subpopulations with lower ploidy. These data indicate that the cells responsible for tumor formation in vivo were diploid and that the polyploid cells within the tumors emerged in situ.

Expression of the noncoding RNA H19 distinguishes between diploid and polyploid cultured MSCs and uncultured liver cells
To identify a possible gene expression profile, which might explain the difference in the tumorigenic potential among MSCs, a DNA microarray analysis comparing 3 diploid with 3 tetraploid cells was conducted (Supplementary Fig. S2). From a total of 136 differentially expressed genes ($P < 0.05$, fold change $> 2$), 40 were upregulated in diploid cells, and 96 were upregulated in tetraploid cells (Supplementary Fig. S2A). The top 10 expressed genes in diploid and tetraploid MSCs are shown in Supplementary Fig. S2B and S2C, respectively. H19, a paternally imprinted noncoding RNA molecule (32, 33), showed the highest increase in the diploid cells with a fold change of 35.9 and $P = 0.0018$ (Supplementary Fig. S2B). In addition, the function of major transcriptional regulators was different between diploid and tetraploid MSCs (Supplementary Fig. S2D). Apparently, c-Myc was more active and p53 was less active in diploid than in tetraploid MSCs (Supplementary Fig. S2E and S2F). Finally, many cell cycle–related genes were upregulated in diploid compared with tetraploid MSCs (Supplementary Fig. S2G).

Differences in H19 expression were also evident in nonmesenchymal primary mouse liver cells (Fig. 3). We confirmed previous data showing that tetraploid cells arise in the adult mouse liver (Fig. 3A, i), whereas in suckling mice, they are diploid (Fig. 3A, ii; ref. 34). H19 was significantly decreased in
tetraploid mesenchymal cells (283-fold change), as well as in adult mouse livers (4,157-fold change), compared with diploid mesenchymal cells and livers of suckling mice, respectively (Fig. 3B and C). As adult livers are composed of mixed diploid–polyploid cells, the 2 populations were separated by flow cytometry (Supplementary Fig. S3A and S3B). It was found that the diploid fraction expressed higher levels of H19 than the tetraploid fraction (142-fold change, Fig. 3D). The reduction in H19 expression in the adult mouse was specific to the liver, as no differences were detected in 2 other epithelial organs (lung and kidney) and neither in the spleen (Fig. 3E). Thus, polyploidy in both normal liver cells and in cultured MSCs is linked with markedly reduced H19 expression compared with diploid cells.

**H19 suppression mediates polyploid phenotypes**

To understand the role of H19, we compared diploid with tetraploid MSCs with respective high and low levels of H19 (Fig. 4A, i) and also conducted H19 knockdown in the diploid cells.
(Fig. 4A, ii). The doubling time (Fig. 4B, i and ii), seeding efficiency (Fig. 4C, i and ii), and UV sensitivity (Fig. 4D, i and ii) of the cells were analyzed. We found that tetraploid cells had a longer population doubling time (Fig. 4B, i) and lower seeding efficiency (Fig. 4C, i) than diploid cells. The sensitivity of tetraploid MSCs to UV irradiation was lower than that of diploid cells (Fig. 4D, i). Polyploidy was associated with uneven cell divisions and unstable centrosome numbers (17). The stability of the tetraploid MSC genotype in our experiments suggests differently. Examination of centrosome numbers of 2N, 2N + 4N, and 4N MSCs showed that most cells contain 1 to 2 centrosomes (Supplementary Fig. S4A). Nevertheless, 4N MSCs had a small increase in the proportion of cells with more than 2 centrosomes (8.2% compared with 4.6% to 4.8% in 2N and 2N + 4N MSCs). Importantly, no multipolar divisions were detected in 4N MSCs (160 mitotic cells inspected), as multiple centrosomes seem to cluster during mitosis (Supplementary Fig. S4B–S4D), possibly accounting for the stability of the polyploid state (35).

To examine the consequences of reduced H19 expression, we specifically knocked it down in diploid MSCs (Fig. 4A, ii). Apparently, H19 siRNA caused diploid cells to acquire properties resembling those of tetraploid cells; the doubling time of H19 siRNA–treated cells increased (Fig. 4B, ii), seeding efficiency decreased (Fig. 4C, ii), and UV sensitivity decreased (Fig. 4D, ii). Interestingly, H19 expression was elevated, dose dependently, by UV irradiation in tetraploid but not in diploid MSCs, which might explain the relative resistance of tetraploid...
cells to UV stress (Fig. 4E). However, H19 levels were increased in both diploid (Supplementary Fig. S5A) and tetraploid (Supplementary Fig. S5B) cells when reaching confluence. Most importantly, a shift toward polyploidy was detected after H19 knockdown in diploid cells (Fig. 5A and B), which similarly augmented the polyploidization effect of UV irradiation (Fig. 5C and D).

We then compared protein expression [Supplementary Fig. S6A (i and ii) and S6B (i and ii)], mitotic behavior [Supplementary Fig. S6C (i and ii)], and RNA expression [Supplementary Fig. S6D (i and ii)] in diploid, tetraploid, and H19 knocked-down MSCs. Protein analysis revealed that the levels of c-Myc were significantly higher in diploid MSCs [3-fold difference, Supplementary Fig. S6A (i) and S6B (i)]. This result is in line with the microarray data (Supplementary Fig. S2D and S2E) and with a previous report showing that c-Myc was able to induce H19 expression (36). The levels of p53 as well as AuroraB (regulator of chromosomal segregation) were relatively similar. Also, AuroraB localization was found normal in 20 mitoses from each cell were inspected, Supplementary Fig. S6C (ii)]. The RNA levels of c-Myc, p21, and p53 were not affected by H19 knockdown [Supplementary Fig. S6D (ii)]. Finally, the levels of c-Myc and p53 mRNAs were extremely heterogeneous among the different tumors formed by MSCs (Supplementary Fig. S7A and S7B). In contrast, H19 expression correlated well with the tumor-forming propensity of the cells.

**Tetraploidization of diploid cells reduces tumorigenic potential and lowers H19 expression**

To critically examine the implied connection between polyploidy associated with decreased H19 expression and tumorigenicity, we tested the effect of artificial tetraploidization (31) on diploid MSCs. The diploid MSC3, MSC4, and MSC5 successfully underwent polyploidization as evident from the tetraploid G2–M cells that formed (Fig. 6A and Supplementary Fig. S8A–S8D). Knockdown of H19 increased the efficiency of artificial tetraploidization, providing further evidence for its role in the control of cell ploidy (Fig. 6B).
Clonal isolates from 2 of the MSCs driven to tetraploidy showed that the proportion of 4N cells is about 25% (3 of 11 and 2 of 8 4N clones from MSC4 and MSC5, respectively), and that none of these cells had a tumorigenic potential. Artificial tetraploidization of MSC3 (MSC3-tet) also resulted in a significant reduction of the tumorigenicity compared with control-treated MSC3 (MSC3-control, Fig. 6C). The reduction in tumorigenicity of MSC3-tet was accompanied by a reduction in H19 expression (Fig. 6D). The artificial tetraploidization procedure resulted in a population with mixed ploidy, and the diploid cells remained at a significant amount, possibly masking the full extent of H19 reduction in the newly generated tetraploid cells. Therefore, artificial tetraploid clones and their diploid counterparts were isolated from the populations after

Figure 4. Suppression of H19 shifts diploid MSCs toward tetraploid-like phenotypes. A, H19 expression: in 2 diploid MSCs (3 and 5) and 2 tetraploid MSCs 25 and 22; i) after siRNA knockdown conducted on MSC5 (representative experiment; ii). Comparison of doubling time (B), seeding efficiency (C), and UV sensitivity (15 J/m²; D) between diploid (2N) and tetraploid (4N) MSCs (i, 3 experiments, post hoc Tukey analysis) and between control- and H19 siRNA–treated diploid MSCs (ii, 5 experiments, paired t test). Cells used had at least 70% knockdown. E, H19 expression after UV irradiation in MSC22 (4N) and MSC5 (2N). Experiment was carried out in triplicate and confirmed using additional 3 diploid and 3 tetraploid cells; representative data are shown (Student t test). Bars indicate mean ± SD. *, P ≤ 0.0001; **, P ≤ 0.005; ***, P < 0.05.
the artificial tetraploidization procedure. The tetraploid clones were found to express 6.5-fold less H19 than the diploid clones (Fig. 6E). Taken together, these data suggest that artificial tetraploidization of MSCs reduces tumorigenic potential in conjunction with suppression of H19 expression. In contrast, primary human fibroblasts immortalized using hTERT expressed significantly higher levels of H19 compared with control cells (Fig. 6F). This implies that H19 might also be involved in the transformation of human mesenchyme.

Discussion

Conflicting information related to MSC tumorigenicity has been reported (6–12, 37). Our study shows that tumor formation by mouse MSCs is a relatively rare event. Human cells are by far more stable genetically, and therefore their safety should be even greater (12). Nevertheless, tumorigenic capacity of human cells can be assessed in animal models that provide valuable information but underestimate cell capacities. Therefore, there is a need for alternative ways to assess the tumorigenicity of human cells. In this study, we focused on mouse MSCs to understand what might drive them toward malignant transformation. We found that tumorigenic mouse MSCs highly express H19. Furthermore, normal human fibroblasts transduced by TERT, a process that leads to their transformation, exhibit augmented expression of H19. The analysis of H19 could therefore serve as a marker for the selection of mesenchymal cells that are safe for clinical use. Furthermore, targeting of H19 in mesenchymal tumors may serve as means to eradicate sarcomas, as already suggested for non-mesenchymal cancers (38).

The inverse relationship between polyploidy and cancer reported here prompted experiments in which polyploidy was forced on diploid cells artificially. As a result, the cells became less cancerous than the control diploid population. This intriguing phenomenon substantiates our initial finding, that is, the higher tumorigenicity of diploid MSCs than that of polyploid ones. It is further implied that enforcement of polyploidy should be considered as means to attenuate tumor progression. Indeed, increased chromosomal content occasionally serves to decrease tumorigenic potential, as in Down syndrome (39, 40), or in neuroblastoma (41). Artificial tetraploidization also resulted in the suppression of H19 expression. Similarly, H19 knockdown led to increased polyploidization. Our study thus establishes a link between H19 expression and cell ploidy.
The majority of mouse MSCs in our study, as well as in other reports, are polyploid (42). In contrast, human MSCs maintain a rather stable chromosome number in culture. This is probably due to their very slow growth rate. Subsequently, the time frame of in vitro studies does not allow for accumulation of chromosomal changes in human MSCs in a way similar to that of mouse cells. From this point of view, our data have limited clinical relevance. However, it cannot be excluded, at this point, that the low incidence of polyploidization of human MSCs might predict higher propensity to transformation and cancer. Thus far, MSC transplantation did not result in their long-term tissue residence neither in mice nor in patients (43). If in the future better engraftment and long-term in vivo survival of human MSCs will be achieved, the possibility that such human diploid cells are potentially dangerous should be revisited.

It has unequivocally been shown that human cancers are often associated with aneuploidy (14). Chromosomal instability clearly contributes to cancer progression (19) and in this respect the fact that polyploid MSCs are rarely tumorigenic and exhibit properties in common with normal, rather than cancer cells (Figs. 2C and Fig. 4) is surprising. In an attempt to reconcile our findings with the vast literature on the role of CIN in cancer, we propose a modification of the current view: As culture conditions may impose stress on primary cells, it is hypothesized that polyploidization is one possible response, out of many (44–47), to environmental insults. We propose a putative function of polyploidy in counteracting malignancy under environmental stress, such as that imposed on the liver cell population in vivo or on cells in ex vivo culture (Fig. 7). Apparently, cells react to stress by elevating their H19 expression. When stress is excessive (Fig. 7A), diploid transformed cells and chromosomally unstable polyploid cells form, leading to malignancy (48, 49). Alternatively, some cells could avoid malignant transformation by undergoing polyploidization associated with repression of H19 expression (Fig. 7B). Such cells might be resistant to further stress as the numerical changes in chromosomal content possibly buffer structural changes. Indeed, the cytogenetic analysis in Fig. 1 supports this option: Nontumorigenic polyploid cells harbor chromosomal abnormalities in single chromosomes, in contrast to tumorigenic cells in which the polyploid subpopulation have doubled abnormalities in homologous chromosomes. This means that...
Figure 7. Some possible cell fates under stress. Cells under stress with increased H19 levels can undergo transformation and become cancerous cells, also through CN (A, red background). This study implies that, alternatively, tetraploidy accompanied by H19 suppression can maintain a noncancerous state (B, green background). Tetraploidization of cancerous diploid cells can attenuate their tumorigenic properties (Evasion) and reduce H19 expression. The in vivo occurrence of such an event remains to be examined.

the perturbation occurred before polyploidization. Such events may lead to the emergence of stable polyploid cells that are not tumorigenic as found in our experiments. The fact that we were able to attenuate the tumorigenicity of diploid cells by in vitro artificial tetraploidization suggests that doubling the amount of DNA, even at the cost of doubling possible existing genetic abnormalities, still has a positive effect. This might be explained by the associated reduction of H19 in the process. Thus, polyploidization might either have a cancer promoting or a cancer-preventing effect, as is the case in aneuploidy (50).

Further experiments should determine whether polyploidization occurring in vivo involves H19 or other unknown molecules and protects against transformation and subsequent tumorigenesis.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
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