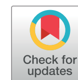

A p53-dependent response limits the viability of mammalian haploid cells

Teresa Olbrich[a], Cristina Mayor-Ruiz[a], Maria Vega-Sendino[a], Carmen Gomez[b], Sagrario Ortega[b], Sergio Ruiz[a,1], and Oscar Fernandez-Capetillo[a,c,1]

[a]Genomic Instability Group, Spanish National Cancer Research Center, Madrid 28029, Spain; [b]Transgenics Unit, Spanish National Cancer Research Center, Madrid 28029, Spain; and [c]Science for Life Laboratory, Division of Genome Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, S-171 21 Stockholm, Sweden

Edited by Stephen J. Elledge, Harvard Medical School, Boston, MA, and approved July 18, 2017 (received for review March 28, 2017)

The recent development of haploid cell lines has facilitated forward genetic screenings in mammalian cells. These lines include near-haploid human cell lines isolated from a patient with chronic myelogenous leukemia (KBM7 and HAP1), as well as haploid embryonic stem cells derived from several organisms. In all cases, haploidy was shown to be an unstable state, so that cultures of mammalian haploid cells rapidly become enriched in diploids. Here we show that the observed diploidization is due to a proliferative disadvantage of haploid cells compared with diploid cells. Accordingly, single-cell–sorted haploid mammalian cells maintain the haploid state for prolonged periods, owing to the absence of competing diploids. Although the duration of interphase is similar in haploid and diploid cells, haploid cells spend longer in mitosis, indicative of problems in chromosome segregation. In agreement with this, a substantial proportion of the haploids die at or shortly after the last mitosis through activation of a p53-dependent cytotoxic response. Finally, we show that p53 deletion stabilizes haploidy in human HAP1 cells and haploid mouse embryonic stem cells. We propose that, similar to aneuploidy or tetraploidy, haploidy triggers a p53-dependent response that limits the fitness of mammalian cells.

haploidy | embryonic stem cells | p53 | HAP1 | chromosome segregation

The main advantage of yeast as a model organism for genetic studies is the availability of haploid cells, so that the mutation of a single allele can suffice to reveal a phenotype. This approach has been of enormous importance for biomedical research in recent decades, as exemplified by the number of Nobel Prizes awarded to discoveries that used yeast as a model system, including the discovery of autophagy, telomeres, and the cell cycle (1). In any case, there are questions intrinsic to mammalian biology, such as stemness or differentiation, that are difficult to address using yeast as a model, and that could be answered by the availability of mammalian haploid cell lines.

The development of haploid animal cells started in the 1960s with cell lines derived from parthenogenetic frog embryos (2, 3). Although haploid cell cultures have been established from several invertebrates, including cockroaches and flies (4), similar attempts in vertebrates failed until recently. The isolation of a pseudohaploid human cell line from a patient with chronic myelogenous leukemia (KBM7) (5), along with the development of haploid embryonic stem (haES) cell cultures from zebrafish, mouse, rat, pig, monkey, and human cells (6–10), have greatly facilitated forward genetic screenings in mammalian cells. Examples of the potential of this approach include identification of the receptors used by the Ebola and Lassa viruses for their entry into the host cell (11, 12), and of all genes that are essential for mammalian cell viability (13, 14).

Whereas mutagenesis of the haploid genomes was originally done with retroviral or transposon gene traps, the subsequent development of CRISPR-Cas9 technologies has emerged as a powerful alternative (14). Moreover, genome-sequencing technologies also have enabled genetic screenings in haploid cells mutagenized by chemical agents (15). Although haploid mammalian cells are becoming a widely used model for screenings, their overall proliferation properties have been less well characterized. Here we present our data in this regard, which show that a p53-dependent response limits the fitness of mammalian haploid cells.

Results

Haploid HAP1 Human Cells Grow at Lower Rates than Diploids. As a model of mammalian haploid cells, we first used the near-haploid human HAP1 cell line, which was generated while trying to generate induced pluripotent stem cells from KBM7 cells (11) and has been widely used for forward genetic screenings (11, 16–18). While growing HAP1 cells, we noticed a rapid reduction in the percentage of haploid cells (Fig. 1*A*). This observation is consistent with previous studies using haploid cells that have linked this action to the "diploidization" of the haploids (19). To specifically analyze the growth properties of haploid HAP1 cells, haploid and diploid cell populations were FACS-sorted and grown independently. Clonogenic assays revealed that haploid HAP1 cells formed smaller colonies than their diploid counterparts (Fig. 1 *B* and *C*). In addition, reduced cell numbers were found in cultures initiated from the haploid fraction of HAP1 cells (Fig. 1*D*), indicative of reduced fitness of these cells.

The foregoing results suggest that the loss of haploid cells from HAP1 cultures could be a consequence to the better growth properties of the diploids, which would gradually overtake the culture. To directly address this possibility, FACS-sorted haploid and diploid fractions of HAP1 cells were infected with lentiviruses encoding for red fluorescent protein (RFP) and enhanced

Significance

Haploidy is a critical advantage for studies on gene function, because only one allele needs to be mutated to yield a phenotype. That is why yeast has led genetic screenings in recent decades. All mammalian cells except germ cells contain two or more sets of chromosomes. Recently, several mammalian haploid cell lines have been obtained; however, the haploid state is unstable, and cultures become progressively enriched in diploid cells. Here we reveal that chromosome segregation takes longer in mammalian haploid cells than in their diploid counterparts, and is followed by an increase in spontaneous cell death. By deleting p53, we can rescue the viability of haploid cells and thereby stabilize the haploid state in mammalian somatic cells.

Author contributions: S.R. and O.F.-C. designed research; T.O., C.M.-R., M.V.-S., C.G., S.O., and S.R. performed research; T.O., C.M.-R., and O.F.-C. analyzed data; and S.R. and O.F.-C. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. Email: sruizm@cnio.es or ofernandez@cnio.es.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1705133114/-/DCSupplemental.

CELL BIOLOGY

Downloaded from https://www.pnas.org by Centro Nacional de Investigaciones Oncologicas CNIO on February 9, 2024 from IP address 193.147.150.204.

Downloaded from https://www.pnas.org by Centro Nacional de Investigaciones Oncologicas CNIO on February 9, 2024 from IP address 193.147.150.204.

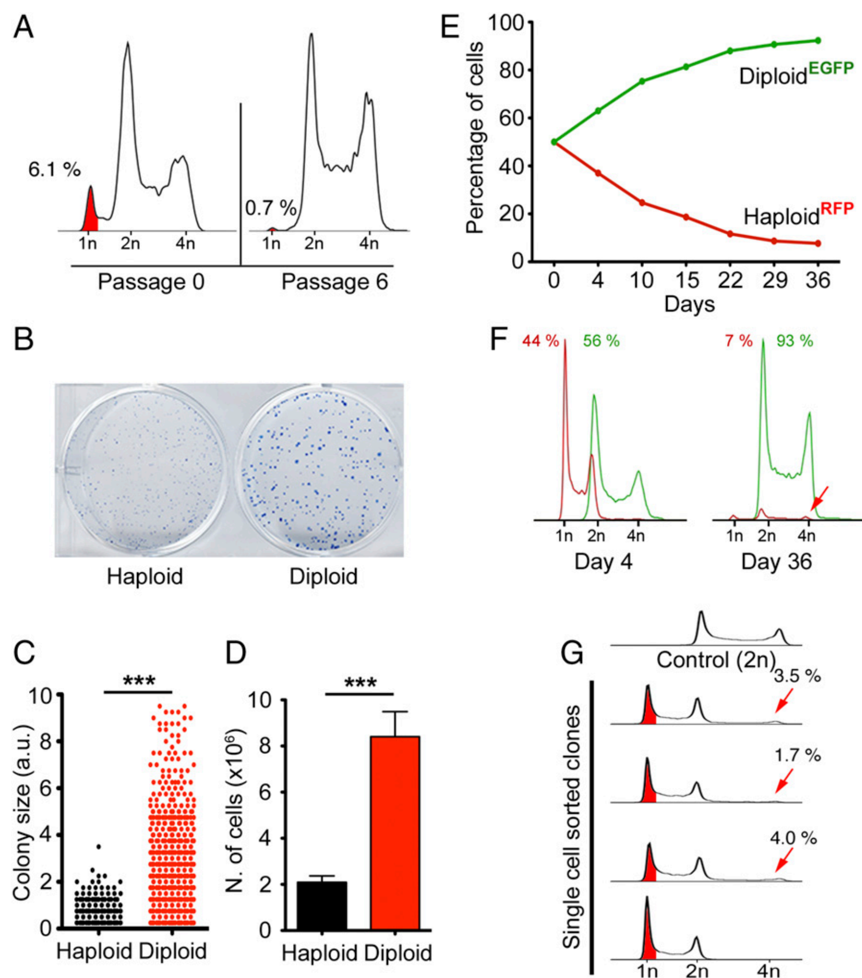

Fig. 1. Loss of haploid HAP1 cells is due to an overgrowth by diploids. (*A*) DNA content analysis from HAP1 cells illustrating (in red) the percentage of G1 haploids at passages 0 and 6. (*B*) Representative image of methylene blue-stained clonogenic cultures derived from haploid and diploid HAP1 cells at 5 d after plating. (*C*) Sizes of the colonies shown in *B* at 5 d after plating. Data are representative of two independent experiments each with three technical replicates. $***P < 0.001$. (*D*) Total cell numbers at 6 d after plating of 5,000 haploid and diploid HAP1 cells. Data are representative of two independent experiments each with three technical replicates. Error bars indicate SEM. $***P < 0.001$. (*E*) Percentages of RFP-positive haploid and EGFP-positive diploid HAP1 cells over the course of 36 d, in a culture that started with equal percentages of RFP and EGFP cells (50%). Two independent experiments were performed, and one representative experiment is shown. (*F*) DNA content analysis from the HAP1 cells shown in *E* at days 4 and 36 after the start of the experiment. The red arrow illustrates the 4n population found in RFP-positive cells. (*G*) DNA content analysis from single-cell–sorted haploid HAP1 clonal cell lines at 32 d after sorting. G1 haploid cells are shown in red. Control diploid cells are also shown. Red arrows indicate the percentage of 4n cells found in each case.

green fluorescent protein (EGFP), respectively, mixed together at a 1:1 ratio, and grown for 36 d. Serial FACS analyses of these cultures revealed a gradual increase in the percentage of diploid EGFP-positive HAP1 cells, so that only 7% of the cells were coming from the RFP-positive haploid fraction at day 36 (Fig. 1 *E* and *F*). Given that a competitive growth disadvantage was behind the loss of haploid cells in HAP1 cultures, we reasoned that cultures initiated from single-cell–sorted haploid HAP1 cells should remain haploid for longer. Accordingly, single-cell–sorted HAP1 lines remained haploid even after 1 mo in culture (Fig. 1*G*). Of note, a small percentage of diploids was observed on prolonged passaging in pool-sorted (Fig. 1*F*) and single-cell–sorted (Fig. 1*G*) haploid cultures. Nevertheless, although diploidization (i.e., the true conversion of a haploid cell into a diploid) may occur at a low frequency, our data demonstrate that the outgrowth by diploid cells is the main contributor to the progressive loss of haploid cells from HAP1 cultures.

p53 Limits the Growth of Human Haploid HAP1 Cells. We next investigated the possible factors limiting the growth properties of haploid HAP1 cells. Besides haploidy, other alterations in ploidy, such as aneuploidy and tetraploidy, are known to reduce the fitness of eukaryotic cells (20, 21), an effect that can be alleviated to some extent by the deletion of the tumor suppressor *p53* (22–25). Interestingly, p53 levels were increased in FACS-sorted haploid HAP1 cells compared with diploid HAP1 cells (Fig. 2*A*). Moreover, p53 was active in haploid HAP1 cells, as evidenced by a concomitant increase in the levels of the p53-target P21, which were abrogated by the expression of a *p53*-targeting shRNA (Fig. 2*A*). During the course of these experiments, we noticed that p53-depleted HAP1 cells exhibited a higher percentage of haploidy than control HAP1 cells (Fig. 2*B*), suggesting that indeed the increased levels of p53 could be partly responsible for the reduced growth properties of haploid HAP1 cells.

To further explore the role of p53 in HAP1 cells, FACS-sorted haploid HAP1 cultures were transfected with plasmids expressing the Cas9 nuclease as well as a *p53*-targeting short guide RNA (sgRNA). A total of 45 antibiotic-resistant clones were expanded, including 21 that still expressed p53 (wild type; WT) and 24 that had lost p53 expression (knockout; KO) (Fig. 2*C*). Whereas the proportions of isolated clones that were haploid and diploid were similar in the WT lines, 87.5% of the *p53* KO clones remained haploid on the first FACS analysis (Fig. 2*D*). Moreover, p53-deficient HAP1 cell lines consistently maintained a higher percentage of haploid cells than was seen in WT lines on continuous passaging (Fig. 2 *E* and *F*). Interestingly, CRISPR-mediated depletion of P21 did not increase the stability of haploid HAP1 cells (*SI Appendix*, Fig. S1), suggesting p53-dependent apoptosis rather than cell cycle arrest as the cause of the reduced fitness of mammalian haploids (see below).

p53 Deficiency Stabilizes Haploidy in Mouse Haploid Stem Cells. HAP1 cells derive from a cancer cell line, KBM7, that carries several mutations that could in principle have an influence on the observed phenotypes. To investigate the role of p53 in primary haploid cells, WT and $p53^{-}$ mouse haES cells were generated by

Downloaded from https://www.pnas.org by Centro Nacional de Investigaciones Oncologicas CNIO on February 9, 2024 from IP address 193.147.150.204.

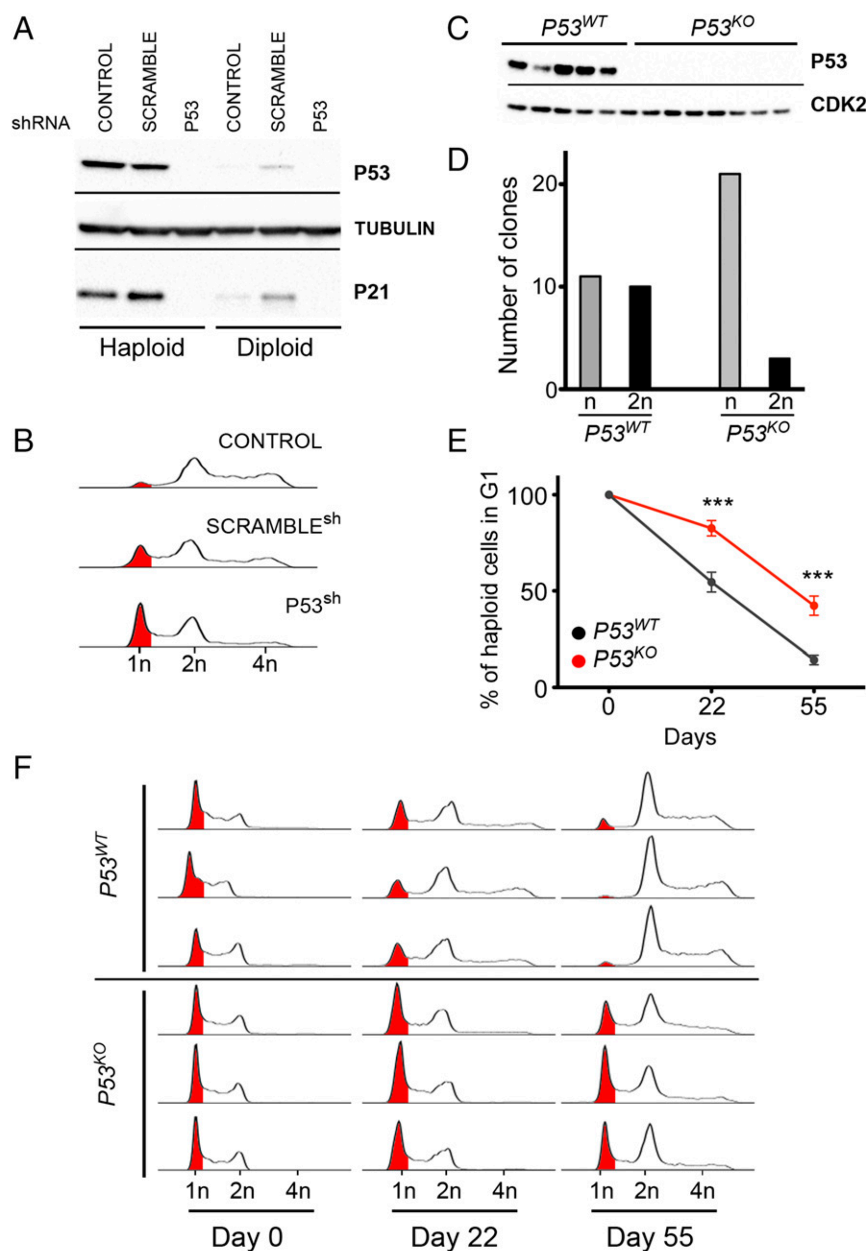

Fig. 2. Reduced p53 levels stabilize haploidy in HAP1 cells. (*A*) Western blot showing the expression levels of p53 and p21 in HAP1 cells infected with lentiviruses encoding for scramble shRNAs or p53-specific shRNAs and sorted into haploid or diploid pools. Control cells were infected with empty vectors. Tubulin expression was used as a loading control. (*B*) DNA content analysis of the cells shown in *A* at 43 d after infection. (*C*) Western blot illustrating the absence of p53 in HAP1 clonal cell lines after Cas9-dependent *p53*-specific gene editing. A representative number of the clones further used in *D* is shown as an example. CDK2 expression was used as a loading control. (*D*) Number of independently isolated WT (n = 21) and *p53*-deficient clones (n = 24) obtained after transfecting haploid HAP1 cells with a plasmid encoding for Cas9 and a *p53*-targeting sgRNA. Clones were analyzed for haploidy or diploidy by measuring DNA intensity by FACS. (*E*) Progression of the percentage of haploids cells in G1 in the WT (n = 11) and *p53*-deficient (n = 21) haploid clonal cell lines shown in *D*. Error bars indicate SEM. ***P < 0.001. (*F*) DNA content analysis from three representative haploid WT and *p53*-deficient HAP1 clonal cell lines shown in *E* at days 0, 22, and 55. G1 haploid cells are highlighted in red.

inducing parthenogenesis with strontium chloride (8, 9) on oocytes isolated from $p53^{+/-}$ female mice (Fig. 3 *A* and *B*). Consistent with previous reports, the generation of haES cells required repeated sorting for haploidy, owing to the rapid loss of haploid cells from haES cultures, which occurs at a much faster rate than on HAP1 cells. Accordingly, very few haploid cells were detectable in WT haES cultures at passage 5, the first passage at which sufficient numbers of cells could be obtained for FACS analyses (Fig. 3*C*). Remarkably, p53-deficient haES cultures showed significant numbers of haploid cells already at passage 5 (Fig. 3*C*). Moreover, the higher number of haploid cells in p53-deficient haES lines was accentuated by passage 15, after both WT and p53-deficient haES cultures had undergone two rounds of FACS sorting for haploidy (Fig. 3*D*).

As in the case of HAP1 cells, p53 levels were increased in haES cells compared with diploid ES cells (*SI Appendix*, Fig. S2*A*), and expression of a *p53*-targeting shRNA increased the percentage of haploid haES cells (*SI Appendix*, Fig. S2 *B* and *C*). Interestingly, restoration of p53 levels in these cultures by Cre-dependent excision of the shRNA sequence reduced the percentage of haploidy, indicating that p53-deficient haES cells do not permanently

Downloaded from https://www.pnas.org by Centro Nacional de Investigaciones Oncologicas CNIO on February 9, 2024 from IP address 193.147.150.204.

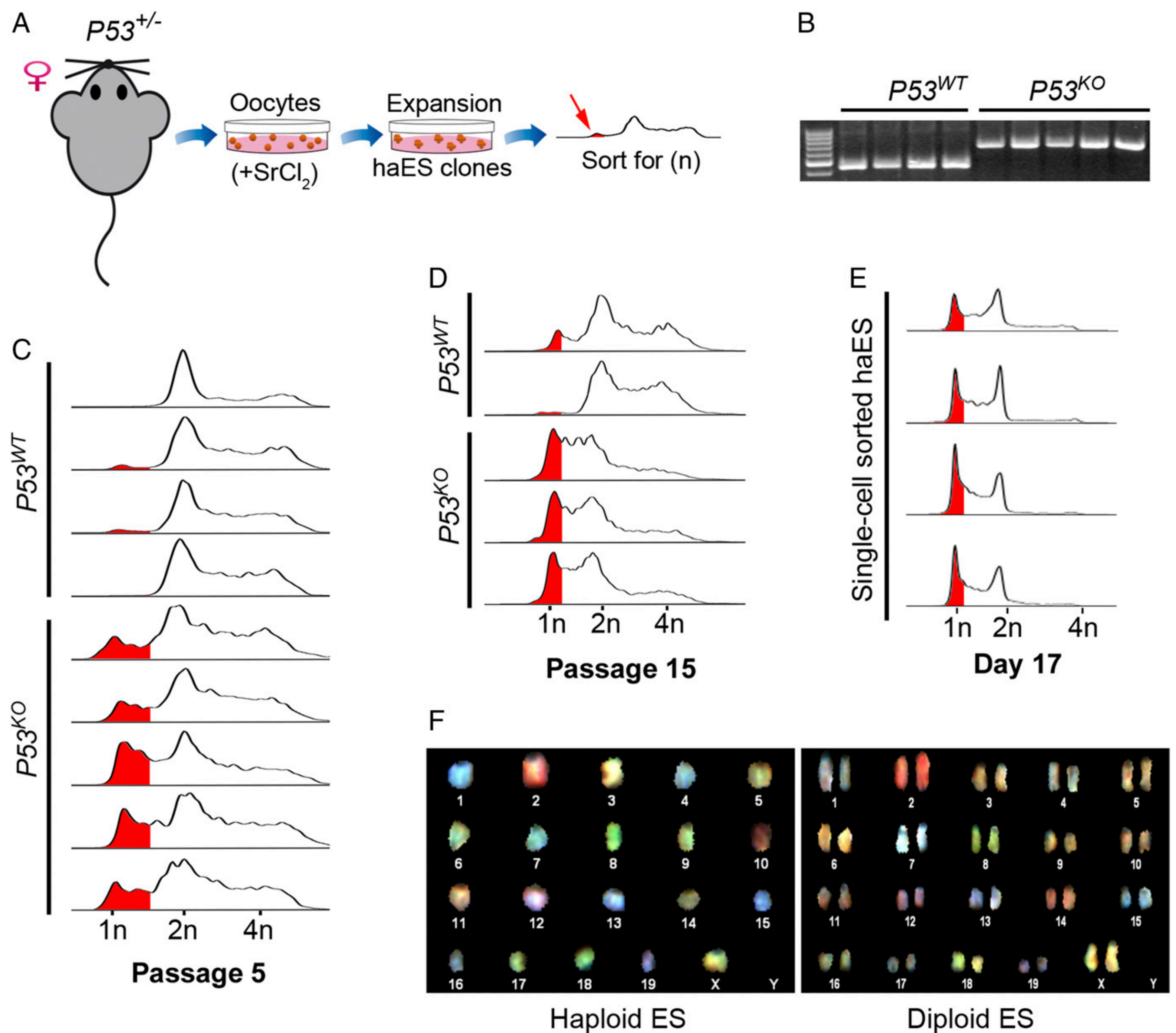

Fig. 3. *p53* deficiency facilitates the generation and maintenance of haploid mouse ES cells. (*A*) Schematic representation of the protocol used to generate haploid mouse ES cell lines from *p53*-heterozygous female mice. (*B*) Representative PCR analysis showing *p53* status in newly generated haploid ES cell lines. (*C* and *D*) DNA content analysis from representative haploid WT and *p53*-deficient ES cell lines determined at passage 5 (*C*) or 10 passages later, which also included two cell sorts for haploidy (*D*). G1 haploid cells are highlighted in red. (*E*) DNA content analysis from single-cell–sorted WT haploid ES clonal cell lines at day 17 after sorting. G1 haploid cells are highlighted in red. (*F*) SKY analysis of a representative single-cell–sorted haploid and diploid ES cell line.

"adapt" to the haploid state and remain sensitive to p53 activation (*SI Appendix*, Fig. S2 *B* and *C*). Finally, single-cell sorting also facilitated the maintenance of haploid cells in haES cultures (Fig. 3*E*), indicating that a p53-dependent reduced fitness of the haploids was again responsible for the observed reduction in the percentage of haploids in haES cells. Of note, single-cell–sorted haES lines were truly haploid, as determined by spectral karyotype (SKY) analysis (Fig. 3*F*). In summary, the reduced percentage of haES cells is due to an overgrowth of the cultures by diploid ES cells, which can be alleviated by either single-cell sorting or *p53* deletion.

Increased Cell Death Rate, Rather than Slower Cell Cycle Progression, Limits the Growth of Mouse Haploid Stem Cells. We next investigated the reasons behind the poor growth properties of haES cells. Because mouse ES cells lack a p53-dependent G1/S checkpoint (26, 27), the impaired growth of the haploids probably is not due to reduced S-phase entry. Accordingly, WT and p53-deficient ES and haES cells showed similar percentages of replicating cells, as measured by the incorporation of ethynyl deoxyuridine (EdU) (Fig. 4*A*). To determine the reasons behind the loss of haploid haES cells, we infected *p53* WT and KO single-cell–sorted haploid haES with lentiviruses expressing a fusion between EGFP and histone H2B (H2B-GFP), and monitored their growth by recording videos over 24 h (Fig. 4*B* and *SI Appendix*, Fig. S3). These analyses revealed several interesting findings. First, similar numbers of mitotic events were detected in all cultures, consistent with the fact that cell cycle progression was not grossly affected in haploid cells. In contrast, the loss of haploids was due to their death, with 56.36% of the WT haES cells dying over the course of 24 h. Second, *p53* deletion significantly, but not completely, rescued the death of haES cells, which can explain its effect in facilitating the

Downloaded from https://www.pnas.org by Centro Nacional de Investigaciones Oncologicas CNIO on February 9, 2024 from IP address 193.147.150.204.

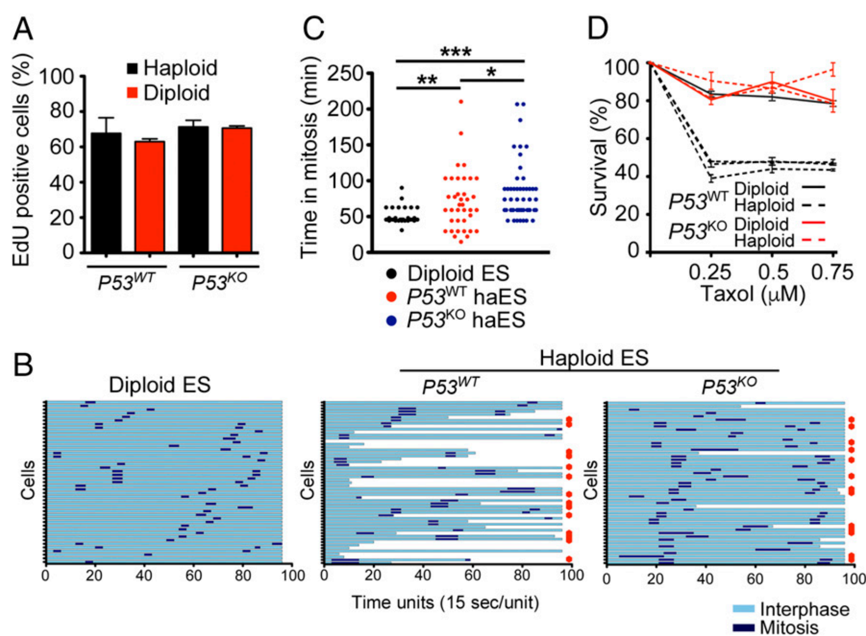

Fig. 4. p53-dependent death limits the expansion of mouse haploid ES cells. (*A*) Percentage of EdU-positive WT and p53-deficient cells observed after a 30-min EdU pulse. Numbers represent an average obtained from three independent single-cell–sorted haploid and diploid cell lines evaluated in duplicate. Error bars indicate SEM. (*B*) Schematic representation of the time spent in mitosis (dark blue) or interphase (light blue) in individual diploid and haploid single-cell–sorted ES cells. EGFP-H2B–expressing cells were imaged every 15 min for a total of 24 h. Time spent in mitosis was defined as the time between chromosome condensation and cytokinesis. Red dots indicate the presence of genomic abnormalities observed in these videos (including lagging chromosomes, micronuclei, or chromosomal bridges). At least 40 individual cells were analyzed per condition. Additional examples are shown in *SI Appendix*, Fig. S3. (*C*) Time spent in mitosis from the analyses shown in *B*. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. (*D*) Cell viability of haploid and diploid WT and *p53*-deficient single-cell–sorted ES cells at 24 h after incubation with various doses of taxol.

maintenance of haploid haES cells. Finally, the death of haploid cells frequently occurred at or shortly after mitosis, suggesting an association with problems occurring during this stage. Accordingly, the duration of mitosis was increased in haES cells compared with diploid ES cells (Fig. 4 *B* and *C*), and GFP-H2B images frequently revealed evidence of problems during segregation in haploid mitosis (Fig. 4*B* and *SI Appendix*, Fig. S3).

Chromosome Segregation Alterations in Mouse Haploid Stem Cells. The foregoing results suggest that the reduced viability of murine haES cells could be due to chromosome segregation problems, which ultimately trigger a p53-dependent cytotoxic response. In agreement with this idea, haES cells were more sensitive than diploid ES cells to the microtubule poison taxol in a p53-dependent manner (Fig. 4*D*). Recent work has shown that chromosome segregation problems can arise due to replication stress (RS), which leads to regions of the genome entering mitosis before they have been fully replicated (28). Although we cannot dismiss the possibility that discrete regions of the genome might be affected by RS in haES cells, we did not find an overall increase in RS or DNA damage markers, such as nuclear foci of 53BP1 or histone H2AX phosphorylation (*SI Appendix*, Fig. S4). Thus, the segregation deficiencies observed in haES cells should reflect bona fide mitotic problems. Given that animal oocytes lose their centrosomes during meiosis, which are provided by the sperm during fertilization, we considered the possibility that the segregation abnormalities could arise from a deficiency of centrosomes in haES cells. Surprisingly, however, immunofluorescence analyses of γ-tubulin revealed normal numbers of centrosomes in haES cells, implying the de novo formation of these structures in haploid stem cells (*SI Appendix*, Fig. S5).

Finally, we hypothesized that the different cell sizes and/or DNA contents of haES cells could increase the percentage of errors at the mitotic spindle, which is optimized to coordinate the segregation of a diploid genome. In agreement with this idea, immunofluorescence analyses of metaphase cells revealed smaller haploid metaphase cells along with a smaller mitotic spindle (*SI Appendix*, Fig. S6 *A–D*). However, although it was smaller, the mitotic spindle exhibited greater heterogeneity in its overall distribution in haES cells compared with diploid ES cells (*SI Appendix*, Fig. S6*A*). Surprisingly, even though haES cells have only one-half the DNA content of diploid ES cells, the distribution of their DNA at the metaphase plate was wider than that in diploid ES cells (*SI Appendix*, Fig. S6 *E* and *F*), consistent with the more heterogeneous distribution of the mitotic spindle (*SI Appendix*, Fig. S6*A*). In summary, these data indicate an intrinsic instability in the arrangement of DNA at the mitotic spindle in haploid ES cells.

Discussion

From the earliest attempts in insects and amphibians, a progressive reduction in the percentage of haploid cells has been noted in animal haploid cultures. Although this phenomenon has been considered a consequence of diploidization (19), here we have shown that it is due to reduced fitness and growth properties of haploid cells, which are outcompeted by the diploid cells existing in these cultures. At least in mammals, this reduced fitness is explained in part by the activation of a p53-dependent cytotoxic response, associated with difficulties arising during the segregation of haploid DNA content.

Based on our findings, we suggest two strategies to facilitate the maintenance of haploidy in human and mouse cell cultures: single-cell sorting and *p53* deletion. Of note, although *p53* deletion facilitates the maintenance of haploid HAP1 or mouse haES cells, it does so by enabling the survival of genomically unstable cells. Thus, we favor single-cell sorting as a simple procedure in mammalian haploid cell studies. Whereas mouse haES cells can contribute to mouse chimeras, the tissues that arise from these cells are composed of diploid ES cells (8). It is tempting to speculate that

strategies aimed at stabilizing the haploid state also might facilitate the generation of mammalian haploid tissue or even animals. In this regard, it is noteworthy that a recent report revealed increased levels of p53 and cell death in tetraploid mouse ES cells. Strikingly, *p53* deletion rescued the viability of tetraploid ES cells and enabled the generation of late-stage mouse tetraploid embryos (29).

Although our data on single-cell–sorted haES and HAP1 cells shows that diploidization (the conversion of a haploid into a diploid) does occur, it is an infrequent event. However, once it happens, diploid cells rapidly overtake the culture owing to their better growth properties. We propose that this phenomenon of diploidization is probably similar to the spontaneous tetraploidization observed in various primary mammalian cells, such as mouse embryonic fibroblasts. In support of this idea, diploidization has been proposed to arise as a result of mitotic nondisjunction (30), which is known to be the origin of tetraploidization (31). However, such segregation problems could be more frequent in haploid cells than in diploid cells, leading to p53-dependent apoptosis, because the spindle likely has evolved to deal optimally with a 2n karyotype. An alternative proposal is that mitosis could simply take more time in the haploids, but in the absence of mis-segregation. In this context, previous studies have shown that prolonged mitosis, even in the absence of segregation problems, leads to an USP28-, p53-, 53BP1-, and P21-dependent G1 arrest in the next interphase (32–35). However, our findings of no increase in 53BP1 foci in haploid cells, apoptosis rather than G1 arrest in haploid cultures, and no increase in the percentage of haploid HAP1 cells on USP28 or P21 deletion (*SI Appendix*, Fig. S1) argue against this idea. All things considered, we suggest that a general principle might underlie the reduction in fitness observed in eukaryotic cells with an altered ploidy (e.g., haploidy or tetraploidy), reflecting a higher frequency of mis-segregation events in cells with a DNA content differing from normal. Our findings should facilitate the use of animal haploid cells in scientific research, and thus open their use to a more laboratories and a wider range of technologies.

Methods

Complete details of the methods used in this study are provided in *SI Appendix, Materials and Methods*.

Derivation and Culture of Haploid Mouse ES Cells. Mouse haES cells were generated as described recently (8, 9) using oocytes from $p53^{+/-}$ female mice. The ES cells were cultured on feeder layers in N2B27-based medium plus LIF, PD0325901 (1 μM), and CHIR99021 (3 μM) and supplemented with 15% KO serum replacement (Invitrogen), 0.1 mM nonessential amino acids, and 0.35% BSA fraction V. DNA was extracted from the cell clones using standard procedures, and PCR was performed to evaluate *p53* status (primers: 1, 5′ CTA TCA GGA CAT AGC GTT GG 3′; 2, 5′ TAT ACT CAG AGC CGG CCT 3′; 5′ ACA GCG TGG TGG TAC CTT AT 3′). All mouse studies were conducted in accordance with the Guidelines for Humane Endpoints for Animals Used in Biomedical Research, under the supervision of the Ethics Committee for Animal Research of the Instituto de Salud Carlos III.

Live-Cell Imaging. To evaluate mitosis entry and duration, ES cells were infected with lentiviruses encoding the histone H2B-EGFP and seeded on an eight-well μ-Slide (Ibidi) pretreated with 0.1% gelatin. The next day, cells were imaged every 15 min for a total of 24 h in a Leica DMI 6000 B system. Mitotic duration was easily scored by chromatin condensation/decondensation. At least 40 cells were followed to evaluate the time spent in mitosis and interphase, as well as cell death, for each individual cell.

ACKNOWLEDGMENTS. We thank the members of the O.F.-C. laboratory and Monica Alvarez and Guillermo de Carcer for insightful comments, as well as the staff of the Transgenic Mice, Flow Cytometry, and Confocal Microscopy Units from the Spanish National Cancer Research Center for technical help. T.O. was supported by a doctoral fellowship from Boehringer Ingelheim Fonds, C.M.-R. was supported by a doctoral fellowship from La Caixa Foundation, and S.R. was supported by a Ramon y Cajal contract (RYC-2011-09242). This research was funded by Fundación Botín; Santander Bank, through its Santander Universities Global Division; the Spanish Ministry of Economy, Industry, and Competitiveness (Grants SAF2014-57791-REDC and SAF2014-59498-R, to O.F.; SAF-2013-44866-R, to S.O.; and SAF2013-49147-P and SAF2016-80874-P, to S.R.; cofinanced by the European Regional Development Fund of the European Union); Fundació La Marato de TV3; the Howard Hughes Medical Institute; and the European Research Council (Grant ERC-617840).

1. Hohmann S (2016) Nobel yeast research. *FEMS Yeast Res* 16:fow094.
2. Freed JJ, Mezger-Freed L (1970) Stable haploid cultured cell lines from frog embryos. *Proc Natl Acad Sci USA* 65:337–344.
3. Freed JJ (1962) Continuous cultivation of cells derived from haploid *Rana pipiens* embryos. *Exp Cell Res* 26:327–333.
4. Debec A (1978) Haploid cell cultures of *Drosophila melanogaster*. *Nature* 274: 255–256.
5. Andersson BS, et al. (1995) KBM-7, a human myeloid leukemia cell line with double Philadelphia chromosomes lacking normal c-ABL and BCR transcripts. *Leukemia* 9: 2100–2108.
6. Sagi I, et al. (2016) Derivation and differentiation of haploid human embryonic stem cells. *Nature* 532:107–111.
7. Yang H, et al. (2013) Generation of haploid embryonic stem cells from *Macaca fascicularis* monkey parthenotes. *Cell Res* 23:1187–1200.
8. Leeb M, Wutz A (2011) Derivation of haploid embryonic stem cells from mouse embryos. *Nature* 479:131–134.
9. Elling U, et al. (2011) Forward and reverse genetics through derivation of haploid mouse embryonic stem cells. *Cell Stem Cell* 9:563–574.
10. Yi M, Hong N, Hong Y (2009) Generation of medaka fish haploid embryonic stem cells. *Science* 326:430–433.
11. Carette JE, et al. (2011) Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. *Nature* 477:340–343.
12. Jae LT, et al. (2014) Virus entry. Lassa virus entry requires a trigger-induced receptor switch. *Science* 344:1506–1510.
13. Blomen VA, et al. (2015) Gene essentiality and synthetic lethality in haploid human cells. *Science* 350:1092–1096.
14. Wang T, et al. (2015) Identification and characterization of essential genes in the human genome. *Science* 350:1096–1101.
15. Forment JV, et al. (2017) Genome-wide genetic screening with chemically mutagenized haploid embryonic stem cells. *Nat Chem Biol* 13:12–14.
16. Papatheodorou P, et al. (2011) Lipolysis-stimulated lipoprotein receptor (LSR) is the host receptor for the binary toxin *Clostridium difficile* transferase (CDT). *Proc Natl Acad Sci USA* 108:16422–16427.
17. Jae LT, et al. (2013) Deciphering the glycosylome of dystroglycanopathies using haploid screens for lassa virus entry. *Science* 340:479–483.
18. Rong Y, et al. (2015) Genome-wide screening of genes required for glycosylphosphatidylinositol biosynthesis. *PLoS One* 10:e0138553.
19. Yilmaz A, Peretz M, Sagi I, Benvenisty N (2016) Haploid human embryonic stem cells: Half the genome, double the value. *Cell Stem Cell* 19:569–572.
20. Ganem NJ, Pellman D (2007) Limiting the proliferation of polyploid cells. *Cell* 131: 437–440.
21. Santaguida S, Amon A (2015) Short- and long-term effects of chromosome mis-segregation and aneuploidy. *Nat Rev Mol Cell Biol* 16:473–485.
22. Cross SM, et al. (1995) A p53-dependent mouse spindle checkpoint. *Science* 267: 1353–1356.
23. Fujiwara T, et al. (2005) Cytokinesis failure generating tetraploids promotes tumorigenesis in p53-null cells. *Nature* 437:1043–1047.
24. Li M, et al. (2010) The ATM-p53 pathway suppresses aneuploidy-induced tumorigenesis. *Proc Natl Acad Sci USA* 107:14188–14193.
25. Thompson SL, Compton DA (2010) Proliferation of aneuploid human cells is limited by a p53-dependent mechanism. *J Cell Biol* 188:369–381.
26. Aladjem MI, et al. (1998) ES cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage. *Curr Biol* 8: 145–155.
27. Hong Y, Stambrook PJ (2004) Restoration of an absent G1 arrest and protection from apoptosis in embryonic stem cells after ionizing radiation. *Proc Natl Acad Sci USA* 101: 14443–14448.
28. Mankouri HW, Huttner D, Hickson ID (2013) How unfinished business from S-phase affects mitosis and beyond. *EMBO J* 32:2661–2671.
29. Horii T, et al. (2015) p53 suppresses tetraploid development in mice. *Sci Rep* 5:8907.
30. Bürckstümmer T, et al. (2013) A reversible gene trap collection empowers haploid genetics in human cells. *Nat Methods* 10:965–971.
31. Shi Q, King RW (2005) Chromosome nondisjunction yields tetraploid rather than aneuploid cells in human cell lines. *Nature* 437:1038–1042.
32. Uetake Y, Sluder G (2010) Prolonged prometaphase blocks daughter cell proliferation despite normal completion of mitosis. *Curr Biol* 20:1666–1671.
33. Fong CS, et al. (2016) 53BP1 and USP28 mediate p53-dependent cell cycle arrest in response to centrosome loss and prolonged mitosis. *eLife* 5:5.
34. Lambrus BG, et al. (2016) A USP28-53BP1-p53-p21 signaling axis arrests growth after centrosome loss or prolonged mitosis. *J Cell Biol* 214:143–153.
35. Meitinger F, et al. (2016) 53BP1 and USP28 mediate p53 activation and G1 arrest after centrosome loss or extended mitotic duration. *J Cell Biol* 214:155–166.

Downloaded from https://www.pnas.org by Centro Nacional de Investigaciones Oncologicas CNIO on February 9, 2024 from IP address 193.147.150.204.