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Persistent Telomere Damage Induces Bypass of Mitosis and Tetraploidy

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SUMMARY

Tetraploidization has been proposed as an intermediate step toward aneuploidy in human cancer but a general mechanism for the induction of tetraploidy during tumorigenesis is lacking. We report that tetraploidization occurs in p53-deficient cells experiencing a prolonged DNA damage signal due to persistent telomere dysfunction. Live-cell imaging revealed that these cells have an extended G2 due to ATM/ATR- and Chk1/Chk2-mediated inhibition of Cdk1/CyclinB and eventually bypass mitosis. Despite their lack of mitosis, the cells showed APC/Cdh1-dependent degradation of the replication inhibitor geminin, followed by accumulation of Cdt1, which is required for origin licensing. Cells then entered a second S phase resulting in whole-genome reduplication and tetraploidy. Upon restoration of telomere protection, these tetraploid cells resumed cell division cycles and proliferated. These observations suggest a general mechanism for the induction of tetraploidization in the early stages of tumorigenesis when telomere dysfunction can result from excessive telomere shortening.

INTRODUCTION

Aneuploidy is a hallmark of solid human cancers. Diploid cells can acquire an aneuploid chromosome complement through repeated chromosome nondisjunction events (reviewed in Kops et al., 2005). However, as many solid tumors have subtetraploid karyotypes and supernumerary centrosomes, it is likely that the initial step toward aneuploidy is tetraploidization (reviewed in Storchova and Kuffer, 2008). Tetraploid cells, most likely because they can form multipolar spindles, have a high rate of chromosome missegregation, explaining how tumors attain subtetraploid karyotypes in which some chromosomes are present at four copies whereas other chromosomes have a lower copy number. Tetraploidization has been observed in the early stages of colon cancer (Danes, 1978; Levine et al., 1991), Barrett's esophagus (Galipeau et al., 1996; Rabinovitch et al., 1989), breast cancer (Dutrillaux et al., 1991), and cervical cancer (Olaharski et al., 2006). Three main mechanisms for tetraploidization in the context of human cancer have been proposed: cell fusion, a failure to complete mitosis (mitotic slippage), and a failure to complete cytokinesis (reviewed in Ganem et al., 2007).

Here, we show that tetraploidization can occur in response to the loss of telomere protection, which is thought to be a common event in human tumorigenesis (reviewed in Maser and DePinho, 2002). The telomeres of most human somatic cells undergo progressive telomere shortening due to the repression of telomerase. This process eventually limits cellular proliferation through the induction of apoptosis or senescence and is thought to represent a tumor-suppressor mechanism that can be subverted by the activation of telomerase (Kim et al., 1994; Bodnar et al., 1998). In agreement, extreme telomere shortening is observed in the early stages of tumorigenesis before telomerase is activated (Chin et al., 2004). Furthermore, most clinically relevant human tumors, including telomerase-positive tumors, have short telomeres that bear witness to the telomere shortening in their proliferative history (de Lange et al., 1990; Hastie et al., 1990). Thus, many human cancers might experience an episode of diminished chromosome-end protection during their development.

Telomeres that have become dysfunctional after extensive shortening activate the canonical DNA damage signaling pathways, mediated by the ATM and ATR kinases (d'Adda di Fagagna et al., 2003). At functional telomeres, ATM signaling is repressed by the shelterin component TRF2, whereas the single-stranded telomeric DNA-binding protein POT1 blocks the activation of the ATR kinase (Denchi and de Lange, 2007). In the current study, telomere dysfunction is experimentally induced in mouse embryo fibroblasts (MEFs) that contain floxed alleles of the two mouse POT1 genes, POT1a and POT1b (Hockemeyer et al., 2006). Depletion of POT1a/b induces an ATR kinase response that leads to accumulation of DNA damage factors at chromosome ends and activation of the effector kinases Chk1 and Chk2 (Denchi and de Lange, 2007). This DNA damage response is persistent because the repair of the damaged telomeres by NHEJ is repressed by TRF2, which remains associated with telomeres despite the removal of POT1a and -b (Hockemeyer et al., 2006).

When POT1a/b are deleted from MEFs that lack a functional p53 pathway, the cells undergo polyploidization, resulting in

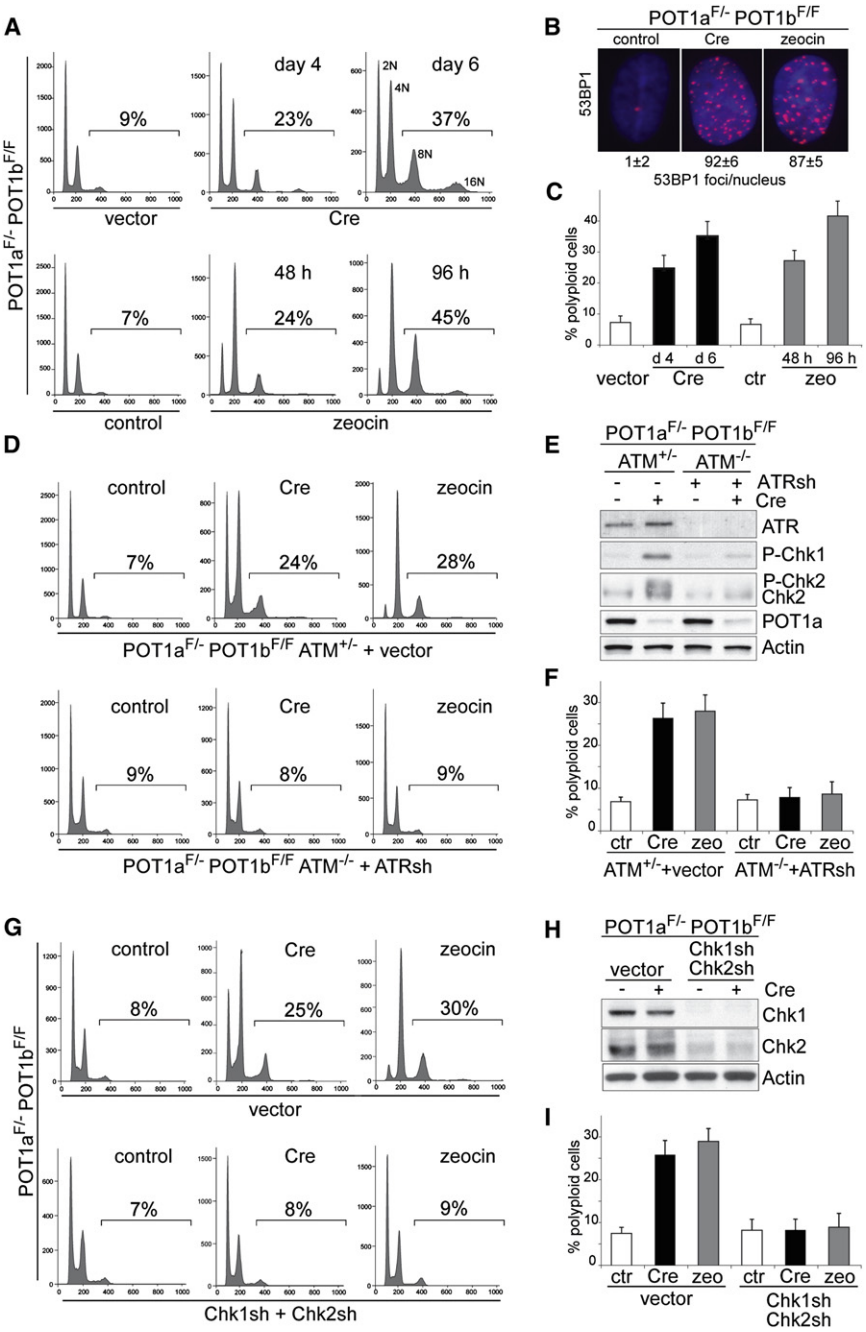

Figure 1. Polyploidy Induced by Persistent DNA Damage Signaling

(A) Polyploidization upon deletion of POT1a/b or continuous zeocin treatment. POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were treated with Cre, the vector control, zeocin, or left untreated and analyzed by FACS at the indicated time points The percentage of cells with DNA content > 4n is given. Representative FACS analyses are shown. See also related Figure S1A.

(B) 53BP1 foci in POT1a/b DKO cells and zeocin-treated cells. POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were treated with Cre, zeocin, or left untreated as in (A) and processed for IF for 53BP1 (red) (DNA stained with DAPI [blue]). Average 53BP1 foci/nucleus and SEMs are given (n > 50).

(C) Quantification of polyploidy induced by POT1a/b deletion or continuous zeocin. POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were treated and analyzed as in (A). The bars show the average values and SDs of three independent experiments.

(D–F) Diminished polyploidy after inhibition of ATM and ATR. POT1a$^{F/-}$POT1b$^{F/F}$ATM$^{-/-}$ and POT1a$^{F/-}$POT1b$^{F/F}$ATM$^{+/-}$ were treated with ATR shRNA or vector control. Polyploidy was measured as in (A). FACS profiles from a representative experiment (D) and quantification of the percentage of polyploid cells in three independent experiments with SDs (F) are shown. Immunoblotting showing ATR knockdown and phosphorylation of Chk1 and Chk2 in the indicated cells is shown in (E). See also related Figures S1C–S1E.

(G–I) Diminished polyploidy after impairment of Chk1 and Chk2. POT1a$^{F/-}$POT1b$^{F/F}$ MEFs were treated with Chk1 and Chk2 shRNAs (set 1) or vector control. Polyploidy was measured as in (D). FACS profiles from a representative experiment (G) and quantification of the percentage of polyploid cells in three independent experiments with SD (I) are shown. Immunoblotting showing Chk1 and Chk2 knockdown is in (H). See also related Figures S1F and S1G.

RESULTS

Polyploidization Induced by Persistent DNA Damage Signaling

The two mouse POT1 proteins were removed from telomeres through Cre-mediated gene deletion in POT1a$^{F/-}$POT1b$^{F/F}$MEFs, resulting in POT1a/b double knockout (DKO) cells. These and other cells used in this study are permissive for polyploidization because p53, which can block entry into S phase in tetraploid cells (Carder et al., 1993; Andreassen et al., 2001), is repressed by SV40 Large T antigen (SV40LT). As expected, POT1a/b DKO cells showed a DNA damage response and polyploidization characterized by FACS profiles with discrete 8n and 16n peaks (Figures 1A–1C and Figure S1A available online). The polyploid cell fraction (defined here as the fraction of cells with a DNA content >4n) increases from a basal level of 7% to 8% to 35% to 40% at day 6 after POT1a/b deletion 4n, 8n, and 16n DNA content (Hockemeyer et al., 2006). The FACS profile of these cultures shows discrete peaks suggesting that the whole genome is duplicated. Furthermore, the occasional metaphase spreads obtained from POT1a/b DKO cultures show diplo- and quadruplochromosomes indicating that these cells have undergone two or three rounds of DNA replication without resolution of the centromeric cohesin. Here, we document the consequences of telomere dysfunction in this setting and demonstrate that bypass of mitosis and endoreduplication are induced by the persistent DNA damage signal emanating from permanently damaged telomeres.