Hyperploidy of embryonic fibroblasts derived from Parp-1 knockout mouse

By Tadashige Nozaki,*, Hisako Fujihara,* Nozomi Kamada,*** Otoya Ueda,***
Tsuyoshi Takato,** Hitoshi Nakagama, Takashi Sugimura, M. J. A.,**
Hiroshi Suzuki,*** and Mitsuko Masutani*†

(Contributed by Takashi Sugimura, M. J. A., June 12, 2001)

Abstract: Various studies show that poly(ADP-ribose) polymerase-1 (Parp-1) is involved in genomic stability. We previously established Parp-1 knockout (Parp-1−−) mice by disrupting Parp-1 exon 1. Parp-1−− and Parp-1+− embryonic fibroblasts were isolated and they became immortalized spontaneously after two months of culture. Parp-1−− embryonic fibroblasts showed extensive hyperploidy compared to Parp-1+− counterparts, and most of the cells contained a single nucleus. These results suggest that disruption of Parp-1 could result in the perturbation of chromosomal ploidy control through endoreduplication.

Key words: Parp; ploidy; endoreduplication; embryonic fibroblast; genomic stability.

Introduction. Poly(ADP-ribosylation) reactions are catalyzed by poly(ADP-ribose) polymerase family proteins, including Parp-1, tankyrase, VPARP, PARP-2, PARP-3 using NAD as a substrate. Parp-1 is present in nuclei and activated by binding to DNA strand breaks. Recently, Parp-1 was also demonstrated to localize to the centrosome as well as chromosomes at cell division phase and interphase. Gene-disruption studies in mice showed that Parp-1 is involved in DNA repair, cell-death induction and the maintenance of genomic stability. In this study, we describe that Parp-1 deficiency in the mouse embryonic fibroblasts induced a substantial increase in the population of cells with extensive hyperploidy, which might be caused by endoreduplication, a process of repeated DNA replication without undergoing subsequent mitosis.

Materials and methods. Isolation of embryonic fibroblasts, cell culture and flow cytometry. Inter-crossing of Parp-1−− progeny and Parp-1+− progeny with 129Sv/ICR mixed-genetic background was carried out and embryos were isolated at embryonic day 13.5. Mouse embryonic fibroblasts were propagated in DMEM (Gibco BRL, Rockville, MD, U. S. A.) supplemented with 10% fetal calf serum and antibiotics, and were continuously cultured for two months. Genotype of cells was confirmed by Southern blot analysis as described previously.

For flow cytometry, cultured cells were trypsinized and fixed in 70% ethanol and DNA was stained with 5 μg/mL propidium iodide (PI) (Sigma, St. Louis, MO, U. S. A.). Ten thousand cells were analyzed with FACScan (Beckton-Dickinson, Franklin Lakes, NJ, U. S. A.) without filtration. The number of cells with a single nucleus and multiple nuclei were counted under a fluorescence microscope after fixation of the cells with 3% formaldehyde in phosphate-buffered saline and staining with 4',6-diamidino-2-phenylindole, DAPI (Sigma).

Karyotype analysis. Cells were arrested in metaphase with colcemid and prepared metaphase spreads were stained with quinacrine-Hoechst and chromosomes were counted under a fluorescence microscope.

Results and discussion. From the Parp-1−− and the Parp-1+− mice intercross progeny, mouse embryonic fibroblasts were isolated as described in Materials and methods. The growth of both Parp-1−− and Parp-1+− embryonic fibroblasts in vitro was initially relatively slow. These cells were continuously cultured for two months, and the growth rates of Parp-1−− and Parp-1+− cells gradually became faster, and the cells were immortalized spontaneously after two months of culture. The size of the Parp-1−− cells were...
markedly larger than that of Parp-1\(^{+/+}\) cells, and the saturation density of Parp-1\(^{+/+}\) cells was approximately 4-fold lower than that of Parp-1\(^{-/-}\) cells (data not shown). Karyotype analysis revealed that Parp-1\(^{+/+}\) cells possessed 108–580 chromosomes, whereas Parp-1\(^{-/-}\) cells possessed 47–150 chromosomes (Fig. 1). Since normal mouse diploid cells contain 40 chromosomes, Parp-1\(^{+/+}\) cells consisted of a population with extensive hyperploidy, although the presence of fragmented chromosomes could not be excluded by this analysis. Parp-1\(^{-/-}\) cells also exhibited hyperploidy and the majority of Parp-1\(^{+/+}\) cells are either near triploidy or tetraploidy (Fig. 1). The microphotograph of representative metaphase spreads of Parp-1\(^{+/+}\) and Parp-1\(^{-/-}\) cells are shown in Fig. 2. Most of the Parp-1\(^{+/+}\) and Parp-1\(^{-/-}\) cells contained a single nucleus, and a few were multinucleated. The percentage of multi-nucleated cells was higher in the Parp-1\(^{+/+}\) cells than that in the Parp-1\(^{-/-}\) cells, being 13% and 4%, respectively (data not shown). This implies that some of the cells re-enter the S phase after nuclear division without undergoing subsequent cytokinesis.

To confirm that Parp-1\(^{+/+}\) cells possess hyperploidy, but not extensive chromosome fragmentation, DNA contents in cells were measured by flow cytometry. A relative DNA content for diploid cells was estimated to be around 90, and the majority of Parp-1\(^{+/+}\) cells were over 300 as shown in Fig. 3. On the other hand, most of the Parp-1\(^{-/-}\) cells were less than 300. Taking all these results together, Parp-1\(^{+/+}\) embryonic fibroblasts possess extensively higher ploidy compared to Parp-1\(^{-/-}\) embryonic fibroblasts, and the ploidy increase could have occurred through endoreduplication. The present result is in line with the previous observation that the immortalized fibroblasts derived from exon 2-disrupted Parp-1\(^{+/+}\) mice showed a population of tetraploid cells along with a diploid cell population.\(^{13}\) Most of Parp-1

![Fig. 1. Karyotype analysis of Parp-1\(^{+/+}\) and Parp-1\(^{-/-}\) mouse embryonic fibroblasts. The numbers of chromosomes in 37 and 50 metaphase spreads from Parp-1\(^{+/+}\) (A) and Parp-1\(^{-/-}\) (B) cells were counted, respectively.](image1)

![Fig. 2. Microphotographs of representative metaphase spreads of Parp-1\(^{+/+}\) and Parp-1\(^{-/-}\) mouse embryonic fibroblasts. A, Parp-1\(^{+/+}\); B, Parp-1\(^{-/-}\).](image2)
molecules are localized in nuclei, but recently Parp-1 is also shown to be localized to the centrosome at cell-division phase and interphase. Since centrosome is an important component for the regulation of ploidy, Parp-1 localized to the centrosome could possibly be engaged in the chromosomal ploidy control of the cells. We previously observed preferential induction of trophoblast giant cells (TGCs) during the development of teratocarcinoma-like tumors from Parp-1-/- mouse ES cells in nude mice. Various types of cancer cells exhibit unstable and increased ploidy, although the underlying mechanism is not fully understood. The present study supports the possible involvement of Parp-1 in the chromosomal ploidy control, and it needs to be investigated whether the loss of Parp-1 could be one of the factors that would result in hyperploidy in cancer cells or not.

Acknowledgements. This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan and by a Grant-in Aid for the Second Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare of Japan. T. N. is a recipient of a Research Grant from the Sankyo Foundation of Life-Science.

References
13) Simbulan-Rosenthal, C. M., Haddad, B. R., Rosenthal, D. S.,


