Mouse Embryos Cloned from Brain Tumors

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Abstract

Cancer cells escape from growth control by accumulating genetic and epigenetic alterations. In rare instances, epigenetic changes alone are oncogenic. Furthermore, agents that modify DNA methylation or chromatin structure can restore a normal phenotype to cells harboring oncogenic mutations. However, it is unclear to what extent epigenetic reprogramming can reverse oncogenesis. Using somatic nuclear transfer, we show that medulloblastomas arising in Ptc1+/− mice can direct preimplantation development. Additionally, blastocysts derived from medulloblastoma nuclei form postimplantation embryos with typical cell layers. Thus, tumor cells can be epigenetically reprogrammed into normal cell types. This approach could lead to a general strategy for assessing genetic and epigenetic contributions to tumorigenesis.

INTRODUCTION

Cancers arise through the accumulation of genetic mutations (1) and epigenetic modifications (2, 3). Although many proto-oncogenes and tumor suppressor genes are widely expressed, the mutation of these genes is associated with cancer of specific organs or cell types (4, 5). This suggests that, to some extent, malignant growth depends on epigenetic factors that are governed by the cellular context in which a tumor arises. Teratocarcinomas can arise through purely epigenetic changes and represent an extreme case in which the transformed phenotype can be abrogated under appropriate conditions (6–8). Indeed, in early chimera studies, it was demonstrated that teratocarcinoma cells are able to contribute to the germ line, ultimately giving rise to adult mice (6). In a frog renal carcinoma model, the transfer of nuclei from tumor cells into oocytes was reported to reverse oncogenesis and to direct development to the tadpole stage (9). Although this was suggested to occur through epigenetic reprogramming, subsequently it was found that the transforming gene, which was encoded by a Herpesvirus episome (10), was lost during the nuclear transfer procedure (11). Here, we investigate the possibility of whether a tumor cell nucleus, in which transformation is caused by somatic mutation, can be epigenetically reprogrammed into normal tissues.

MATERIALS AND METHODS

Tumor Cell Culture. Medulloblastomas were collected from Ptc1 heterozygous mice and minced, and dissociated cells were cultured on poly-L-lysine in DMEM supplemented with 2 mM L-glutamine and 10% fetal bovine serum at 37°C in 10% CO2 in air. Subsequently, cells were passaged in the same medium after detachment with trypsin. For immunohistochemistry, cells were fixed in 4% paraformaldehyde and were processed according to standard techniques with antibodies to medium and heavy chains of neurofilament (1:200; Zymed Laboratories, San Francisco, CA), synaptophysin (1:100; Zymed Laboratories), neuron-specific enolase (1:200; DAKO Corporation, Carpinteria, CA), or GFAP4 (1:200; DAKO Corporation). Bound antibodies were detected using appropriate secondary antibodies conjugated to Texas Red-X or Oregon Green 500 (each, 1:500; Molecular Probes, Eugene, OR).

Nuclear Transfer. Female B6D2F1 mice were superovulated, and oocytes were harvested according to standard techniques (12). An enucleation pipette attached to a PiezoDrill (Burleigh, NY) was used to cut through the zona pellucida, and the metaphase II spindle was aspirated (removal was confirmed by Hoechst 33258 staining). Subsequent transfer of medulloblastoma nuclei and embryo culture was as described by Nakayama et al. (13).

Genotyping. DNA was isolated from embryos, decidua, and surrounding uterine tissue by standard techniques. Genotyping was performed using PCR for lacZ and neo genes contained within the Ptc1 targeting vector (14). One set of primers (5’-GCTGGAATTCGCGGATCCGCCATTGTCAGACATG-3’ and 5’-GCTGGATCCGCCGATACTGAC-3’) amplified a 295-bp fragment of the lacZ gene whereas the other set amplified a 520-bp fragment of the neo gene. PCR for lacZ was run for 30 cycles with denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. PCR products were analyzed on a 1.0% agarose gel.

RESULTS AND DISCUSSION

Medulloblastoma, the most common malignant pediatric brain tumor, originates from granule neuron precursors in the developing cerebellum (15). Ptc1, a tumor suppressor gene, has been implicated in familial and sporadic medulloblastoma in humans (16). Furthermore, ~14% of Ptc1 heterozygous mice develop medulloblastoma (14, 17), but the incidence increases to ~95% in the absence of p53 (18), which suggests that additional mutations contribute to tumorigenesis. We cultured cells from tumors arising spontaneously in two female Ptc1+/− mice at 3 months (SJMM4) and 7 months (SJMM2) of age, respectively. These tumor cells were chosen for investigation, because their karyotypes were grossly normal during early passages, an important criterion for nuclear transfer. Transplantation of SJMM2 and SJMM4 directly into the flank of immunodeficient mice resulted in tumor formation; and SJMM2, at passage 10, formed colonies in soft agar. Although the levels of Ptc1 were low in the majority of cultures examined, SJMM2 expressed very high steady-state levels of protein.

Simultaneous expression of both neural and glial markers is a feature characteristic of medulloblastoma (15). After passage three, the great majority of cultured cells coexpressed glial and neuronal markers, including GFAP, neurofilament (Fig. 1), neuron-specific enolase, and synaptophysin. Although the tumors from which the cell cultures were derived could be grown in allografts and formed colonies in agar at passage 12 and 13 (SJMM2), it is formally possible that some of the cultured cells lost their tumorigenic capacity during monolayer culture. The cells were used for nuclear transfer between

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4 The abbreviations used are: GFAP, glial fibrillary acidic protein; ICM, inner cell mass.
passage 5 and 12 to completely exclude contamination by non-tumor-derived neuronal cells, which do not survive passage in culture, and to minimize the accumulation of mutations arising in culture. To assess the reproducibility of phenotypes, multiple batches of cells from each passage were used. Normal mouse spleen cells (from B6D2F1 mice) were used as a source of control nuclei to compare the efficiency of developmental progression after nuclear transfer.

The initial stage of preimplantation development involves nuclear remodeling and the formation of pronuclei (12). The order and timing of cell division and differentiation during preimplantation development is geared strictly to a zygotic clock (19). Therefore, if the transferred medulloblastoma nuclei do not cease uncontrolled cell proliferation, they would not be able to direct preimplantation development. After transfer (n = 1669), ~75% of medulloblastoma nuclei increased dramatically in size (Fig. 2, A–C) and subsequently formed pronuclei with one or more pronucleolus within 24 h (Fig. 2C; Table 1). For comparison, ~84% of spleen cell-derived nuclei (n = 212) progressed to the same stage (Table 1). 72 h after activation, ~27% of the transferred oocytes had progressed to the 2-cell stage (Fig. 2D; Table 1), 10% to the 4/8-cell stage (Fig. 2, E and F; Table 1), and 10% had developed into morulae or blastocysts (Fig. 2, G and H; Table 1). No substantial differences in cloning efficiency were observed between the two tumor cell cultures (Table 1) or among cells from different passage numbers. The equivalent results for spleen cell-derived nuclei were 35, 17, and 29%, respectively (Table 1). This apparent enhanced efficiency seems to be related to the increased number of cloned embryos that die soon after transfer (Table 1), possibly as a result of the more stringent methods required to remove nuclei from tumor cells. Noticeably, although medulloblastoma-derived embryos died, none exhibited uncontrolled proliferation resembling tumorigenesis.

Blastocysts derived from medulloblastoma were morphologically indistinguishable from those derived from spleen cell nuclei: they formed blastocyst cavities surrounded by trophoblast cells, and ICMs could be identified (Fig. 2H). Blastocyst cavities gradually accumulated fluid and expanded, and, when maintained for longer periods, blastocysts hatched from the zona pellucida (Fig. 2I). Again, there was no evidence of the uncontrolled cell growth characteristic of cultured tumor cells.

We cultivated medulloblastoma-derived blastocysts for extended periods under the same conditions that were used to propagate the parental medulloblastoma cells. After 2 days in culture, blastocysts hatched from the zona pellucida, trophoblast cells spread onto the culture dishes, and an ICM formed in the center of the expanded trophoblast regions (Fig. 2J). The ICM cells subsequently differentiated, and, in marked contrast to medulloblastoma cells, both trophoblasts and ICM cells ceased proliferation. Thus, reprogrammed medulloblastoma nuclei lost the capacity for extensive proliferation characteristic of tumor cells in vitro.

Postimplantation development occurs under conditions of even...
more rigid and complex control than preimplantation development (12). To establish whether medulloblastoma-derived embryos could direct later stages of development, we transferred cloned blastocysts into pseudo-pregnant females. Recipients were sacrificed at various stages of pregnancy, and decidua were fixed and sectioned or were processed for DNA analysis. Although evidence of implantation was observed even at late stages of gestation, no viable embryos were recovered subsequent to E8.5. Even when maintained well past term, none of the recipients developed tumors (n = 29). However, we were able to identify viable embryos and embryos undergoing resorption at E7.5 and E8.5. At 7.5 days of development, the embryos appeared grossly normal, and they contained embryonic ectoderm, mesoderm, endoderm, ectoplacental cones, chorion, amnion, Reichert’s membrane, yolk sac cavity, and amniotic cavity (Fig. 3). Later embryos (8.5 days of development) showed more extensive differentiation with cephalic vesicles and neural tube.

To confirm that the postimplantation embryos originated from transplanted medulloblastoma nuclei, we performed PCR to identify the β-galactosidase (lacZ) and neomycin resistance (neo) genes present in the targeting vector. A 295-bp amplicon from lacZ and a 520-bp amplicon from Ptc1-neo were detected in the decidua and embryos, although surrounding uterine tissues were negative for both genes (Fig. 4).

These results demonstrate that epigenetic reprogramming of medulloblastoma nuclei by somatic nuclear transfer abrogates the tumorigenic phenotype: uncontrolled proliferation is suppressed and normal patterns of differentiation are restored. Although transplanted medulloblastoma nuclei are less efficient than spleen cell-derived nuclei, they, nevertheless, directed all of preimplantation development. Moreover, they gave rise to postimplantation embryos that had undergone tissue differentiation and early stages of organogenesis. Remarkably, no malignancies were observed in any of the recipient mice, and normal proliferation control was observed in cultured blastocysts. Thus, the tumorigenic mutations that underlie medulloblastoma must act within the context of the cerebellar granule cell lineage, and those changes will not necessarily support malignant cell proliferation in the context of other cell lineages. It will now be important to establish whether the medulloblastoma nuclei preferentially produce tumors in specific tissue types. The successful reprogramming of medulloblastoma that preclude further embryonic development or the genesis of specific tissue types. The successful reprogramming of medulloblastoma nuclei by somatic nuclear transfer encourages exploration of the utility of anticancer agents that target chromatin and DNA methylation for the treatment of this devastating pediatric cancer.

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REFERENCES

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