Cellular context-dependent consequences of *Apc* mutations on gene regulation and cellular behavior

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The spectrum of genetic mutations differs among cancers in different organs, implying a cellular context-dependent effect for genetic aberrations. However, the extent to which the cellular context affects the consequences of oncogenic mutations remains to be fully elucidated. We reprogrammed colon tumor cells in an *Apc*$^{Min/+}$ (adenomatous polyposis coli) mouse model, in which the loss of the *Apc* gene plays a critical role in tumor development and subsequently, established reprogrammed tumor cells (RTCs) that exhibit pluripotent stem cell (PSC)-like signatures of gene expression. We show that the majority of the genes in RTCs that were affected by *Apc* mutations did not overlap with the genes affected in the intestine. RTCs lacked pluripotency but exhibited an increased expression of *Cdx2* and a differentiation propensity that was biased toward the trophectoderm cell lineage. Genetic rescue of the mutated *Apc* allele conferred pluripotency on RTCs and enabled their differentiation into various cell types in vivo. The redisruption of *Apc* in RTC-derived differentiated cells resulted in neoplastic growth that was exclusive to the intestine, but the majority of the intestinal lesions remained as pretumoral microadenomas. These results highlight the significant influence of cellular context on gene regulation, cellular plasticity, and cellular behavior in response to the loss of the *Apc* function. Our results also imply that the transition from microadenomas to macroscopic tumors is reprogrammable, which underscores the importance of epigenetic regulation on tumor promotion.

iPS cell | cancer epigenetics | plasticity | colon cancer | mouse model

Genetic alterations are associated with the pathogenesis of various diseases, including cancer. Genotype-linked disease phenotypes are often observed in an organ-specific manner, suggesting that the correlation between the genotype and phenotype depends on the cell type (1, 2). However, there is limited direct evidence on the cell type-specific correlation between the genotype and phenotype, especially in diseases with multiple genetic alterations, such as cancer. Induced pluripotent stem cells (iPSCs), the generation of which enables the control of cell fate while retaining the original genetic information, are suitable for investigating the cell type-specific correlation between the genotype and phenotype (3). By establishing induced iPSCs from neoplastic cells, we can obtain various cell types that share genetic information associated with tumor development.

The *APC* (adenomatous polyposis coli) gene was initially discovered as a mutated gene in patients with familial adenomatous polyposis (4). The presence of this mutation predisposes the patient to the development of colon cancer. In addition, somatic *APC* mutations are found in the majority of sporadic colon cancers (5). *APC* mutations often result in the production of a truncated protein, which typically stabilizes β-catenin and causes the activation of β-catenin/Tcf–mediated transcription (6). Given that the stabilized form of β-catenin induces intestinal neoplasms (7, 8), it is suggested that *Apc* mutations induce intestinal tumors through the activation of β-catenin/Tcf–mediated transcription. Notably, *Apc* mutations are observed in various types of cancers, albeit at a low frequency. However, with the exception of the intestinal cell lineage, the consequences of *Apc* mutations have not been fully elucidated in most cell types.

Accordingly, we used iPSC technology and investigated the impact of the cellular context on the consequence of *Apc* mutations. We succeeded in obtaining various cell types from colon tumor cells by establishing reprogrammed tumor cells (RTCs). We provide direct in vivo evidence of the cell type-specific consequences of tumor-associated mutations.

Results

The Reprogramming of Macroscopic Colon Tumor Cells. We attempted to reprogram macroscopic colon tumor cells in *Apc* Min mice that were treated with a potent tumor promoter, dextran sodium sulfate (DSS) (Fig. S1*A*). In this study, we used a doxycycline (Dox)-inducible system to transduce reprogramming factors [octamer-binding transcription factor (*Oct3/4*), sex determining region Y-box 2 (*Sox2*), Kruppel-like factor 4 (*Klf4*), and avian myelocytomatosis viral oncogene homolog (*c-Myc*)]. *Apc*$^{Min/+}$ mice were crossed with in vivo reprogrammable mice (9). The macroscopic colon tumors in the DSS-treated compound mice were carefully resected, and tumor cells were cultured in Dox-containing ES cell (ESC) medium.

Significance

Genotype-linked disease phenotypes are often observed in a cell type-specific manner, implying a cellular context-dependent effect of the genetic aberrations. However, the extent to which cellular context affects the biological consequences of oncogenic mutations is unclear. Here, we reprogrammed colon tumor cells in an *Apc*$^{Min/+}$ (adenomatous polyposis coli) mouse model and showed the divergent in vivo consequences of *Apc* mutation that arise in different cellular contexts. We also showed that the reprogrammed tumor cells remain in a pretumoral microadenoma stage after differentiation into colonic epithelium, suggesting that macroscopic colon tumor cells are reprogrammable into microadenoma cells. Our results underscore the significance of epigenetic regulation on gene expression, cellular plasticity, and cellular behavior in response to cancer-causing mutations.

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Data deposition: The microarray data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE77202).

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After the transduction of reprogramming factors in the primary cultured cells, we obtained colonies that were morphologically similar to iPSCs. The iPSC-like colonies were picked up and expanded in the absence of Dox to establish iPSC-like cell lines (Fig. 1*A*). It is known that the neoplastic transformation of the colonic epithelium in *Apc* Min mice is initiated by a spontaneous loss of heterozygosity (LOH) at the *Apc* locus (10). To exclude the iPSC-like cell lines that are derivatives of nonneoplastic stromal cells within tumors, we next performed an exome analysis and determined the *Apc* status of the established iPSC-like cell lines (nos. 1–7). Three of seven iPSC-like cell lines exhibited mitotic recombination and consequently, lacked the WT allele (nos. 1–3), and one line showed a one-base deletion at the *Apc* allele (no. 4), indicating that they are derived from colon tumor cells (Fig. 1*B*). We designated the iPSC-like cells harboring *Apc* LOH or additional *Apc* mutation as RTCs (nos. 1–4) and performed additional analyses. Comparison of four RTC cell lines with the *Apc* LOH-negative cell lines (nos. 5–7) revealed a total of 43 potential tumor-specific mutation sites, including *Apc* mutations, in each of four RTC lines (Table S1), although we also found mutations that were specific to the *Apc* LOH-negative cell lines. A conventional direct sequencing analysis confirmed the presence of *Ascc3*, *Dlg2*, and *Plekho2* mutations in RTC 1. Consistent with the findings of previous studies, which reported that intestinal tumors arise with a normal karyotype in the *Apc* Min mouse model (11), a comparative genomic hybridization analysis revealed no abnormal karyotypes in three independent RTC lines (nos. 1–3 in Fig. S1*B*).

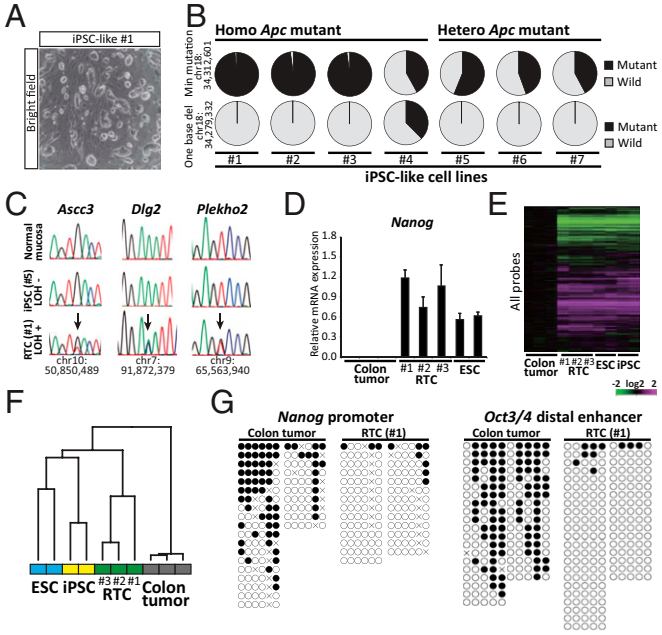

Fig. 1. Establishment of RTCs. (*A*) Morphology of iPSC-like cells. iPSC-like cell lines were maintained in the absence of Dox. (*B*) *Apc* status of established iPSC-like cell lines (nos. 1–7) by exome analysis. Three lines (nos. 1–3) show somatic recombination, resulting in the lack of WT allele at the Min mutation site. iPSC-like cell line no. 4 harbors a one-base deletion in Exon 7 of the *Apc* gene. (*C*) A direct sequencing analysis of normal colonic mucosa, iPSC-like, and RTC lines. RTC no. 1 harbors point mutations at *Ascc3*, *Dlg2*, and *Plekho2* gene loci, whereas normal colonic mucosa and iPSC-like line no. 5 do not possess any of these mutations. (*D*) qRT-PCR analysis for the expression of *Nanog* in colon tumors from *Apc* Min mice, RTCs (nos. 1–3), and ESCs. Data are presented as the mean ± SD. The mean expression level of RTCs was set to one. (*E*) Global transcriptional profiles of RTCs (nos. 1–3), colon tumors (GSE60620), ESCs, and iPSCs (GSE45916). (*F*) A hierarchical cluster analysis of the microarray data revealed that RTCs are clustered with PSCs. (*G*) Bisulfite sequencing analyses revealed that the *Nanog* promoter and the distal enhancer region of *Oct3/4* are demethylated in RTCs. White and black circles indicate nonmethylated and methylated cytosine at CpG sites, respectively.

RTCs Share Features with Pluripotent Stem Cells. We next examined whether these RTCs have similar characteristics to pluripotent stem cells (PSCs). The RTCs were positive for alkaline phosphatase (Fig. S1*C*), and a quantitative RT-PCR (qRT-PCR) revealed the high expression of pluripotency-related genes, such as *Nanog* and *Fbxo15* (Fig. 1*D*, Fig. S1*D*, and Table S2). A microarray analysis revealed that the global transcriptional profile of RTCs exhibits an ESCs/iPSCs-like signature (Fig. 1 *E* and *F*). Three modules for the ESC transcriptional program (12) were similarly activated in RTCs and PSCs (Fig. S1*E*), implying that the loss of the *Apc* gene does not have a large impact on the global gene expression in these cells. A DNA methylation analysis revealed that RTCs exhibit a loss of DNA methylation at *Oct3/4* distal enhancer and *Nanog* promoter, which is a critical event for successful reprogramming (Fig. 1*G*). Taken together, these results indicate that RTCs exhibit similar features to PSCs, despite the presence of genetic mutations that are associated with colon tumor development.

Cell Type-Specific Effects of the *Apc* Mutation on Gene Expression. The representative target genes of the *Apc* mutation in the intestine include *Axin2*, *Lgr5*, and *Ascl2* (13, 14). We next investigated the genes affected by *Apc* mutations in RTCs. For this purpose, we rescued one of the Apc^{Min} alleles in RTCs by homologous recombination with a targeting allele that contained the WT sequence of *Apc* (Fig. 2*A* and Fig. S2*A*). Two *Apc*-rescued RTC lines (nos. 45 and 9) were established from two independent RTC lines (nos. 1 and 3, respectively) (Fig. S2 *B* and *C*).

Although the morphology and global gene expression of the *Apc*-rescued RTC lines were similar to those of RTCs, we found a substantial difference in the expression of a subset of genes (Fig. 2*B*). Notably, genes that were up-regulated by the *Apc* mutation in the intestine (15) were hardly affected in RTCs with the mutation. Conversely, genes that were up-regulated by the *Apc* mutation in RTCs were not affected in intestinal cells with the mutation (Fig. 2*B*). Of particular note, the majority of up- and down-regulated genes did not overlap in the two different cell types (Fig. 2*C*).

Given that the *Apc* mutation exerts tumorigenic activity through the activation of β-catenin/Tcf–mediated transcription in the intestine, we compared genes that were affected by the *Apc* mutation in the RTCs and genes that were affected by the induction of *β-catenin* in ESCs. For this purpose, we induced a stabilized form of human *β-catenin* (S33 mutant) in ESCs (16) and examined their gene expression. Microarray analyses revealed that the set of genes up-regulated by the *Apc* mutation in RTCs was substantially different from the genes that were affected by ectopic induction of the mutant *β-catenin* in ESCs, whereas the target genes of the *Apc* mutation and the mutant *β-catenin* overexpression often overlap in the intestine (Fig. 2*D*). We further examined the effects of the induction of mutant *β-catenin* on gene expression in different cell types. We first confirmed that the previously reported target genes of the canonical Wnt pathway are often up-regulated by mutant *β-catenin* induction in the colonic epithelium. However, these Wnt target genes were hardly affected in ESCs and differentially regulated in mouse embryonic fibroblasts (MEFs) by the induction of mutant *β-catenin* (Fig. 2*E* and Fig. S2). A previous study showed that different levels of canonical Wnt signaling affect differentiation propensity of ESCs (17). Although the induction levels of mutant *β-catenin* did not affect the expression of *Ascl2* and *Lgr5*, well-known Wnt target genes, in either MEFs or ESCs (Fig. S2*D*), we cannot exclude the possibility that a dose-dependent effect may have a role in the different preferences of target gene expression. Taken together, these results indicate that the *Apc* mutation has remarkably distinct effects on gene regulation, which depend on the cellular context.

RTCs Lack the Ability to Form Teratomas but Exhibit the Propensity to Differentiate Along the Trophectoderm Cell Lineage. Given that the morphology and overall gene expression profiles of RTCs are similar to those of PSCs, we performed a teratoma assay and evaluated the differentiation propensity of RTCs. However, the

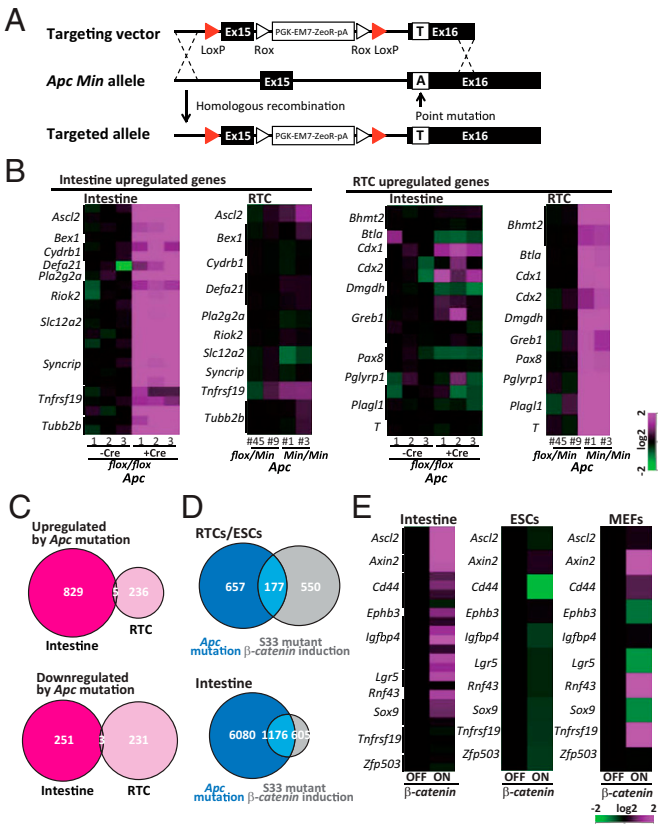

Fig. 2. Cell type-specific effect of *Apc* mutation on gene expression. (*A*) A schematic illustration of homologous recombination of the *Apc* Min allele with a targeting vector containing the WT *Apc* gene sequence. (*B*) Cell type-specific effect of the *Apc* mutation on gene expression. (*C*) Up-/down-regulated genes by the *Apc* mutation in the intestine and RTCs (nos. 1 and 45 were compared; cutoff value is threefold in the intestine and twofold in RTCs). GSE70262 was used for the gene expression data in the intestine. (*D*) Genes up-regulated by the *Apc* mutation or induction of S33 mutant *β-catenin*. *Apc* mutation affects a substantially different set of genes in RTCs (no. 1) than the ectopic induction of mutant *β-catenin* does in ESCs (cutoff value is 1.5-fold). Note that target genes of *Apc* mutation and those of the *β-catenin* induction often overlap in the intestine (cutoff value is 1.5-fold). (*E*) The effects of S33 mutant *β-catenin* induction on gene expression in different cell types. Previously reported target genes of the canonical Wnt pathway are often up-regulated in colonic crypts (GSE41688). However, the target genes were hardly affected in ESCs and differentially regulated in MEFs.

tumors that developed in immunocompromised mice were distinct from teratomas and mainly consisted of histologically undifferentiated cells, implying that RTCs lacked pluripotency (Fig. 3*A*). Notably, we found that the expression of *Cdx2* is up-regulated in RTCs (Fig. 2*B* and Fig. S3*A*), whereas it is often down-regulated in tumors of the colon (18, 19). A previous study showed that the overexpression of *Cdx2* in mouse ESCs is sufficient to generate proper trophoblast stem cells (TSCs), the precursors of the differentiated cells of the placenta (20). We, therefore, hypothesized that RTCs might have the propensity to differentiate into trophectoderm cells. Indeed, the RTC-derived tumors contained a small number of giant cells, which resembled trophoblast giant cells that are terminally differentiated, polyploid cells in the placenta (Fig. 3*A*). Some of these giant cells were positive for placental lactogen 1 (Pl1), a marker for trophoblast giant cells in the mouse placenta (Fig. 3*B*), suggesting that the differentiation of RTCs was biased toward the trophectoderm cell lineage. Despite the undifferentiated morphology, the RTC-derived tumor cells often expressed Cdx2 protein, and this expression was reciprocal to the expression of Oct3/4, which was reminiscent of early embryonic development (Fig. 3*B*) (20). Moreover, Pl1-positive giant cells were often surrounded by Cdx2-positive tumor cells (Fig. 3*B*), which resemble the expression patterns that are observed in the mouse placenta (Fig. S3*B*).

We next cultured RTCs in vitro under the TSC culture conditions. Morphologically, the RTCs changed into epithelium-like cells, which include giant cells (Fig. 3*C*). qRT-PCR showed the increased expression of *Cdx2* and *Eomes* in RTCs, both of which are important for the determination and maintenance of TSCs (Fig. 3*D*) (21). However, the expression of *Elf5* was not up-regulated in these cells (Fig. S3*C*). Similarly, we did not observe an increased expression of trophectoderm differentiation markers, such as *Gata3* and *Hand1*, after cultivation in TSC differentiation medium for 72 h (Fig. S3*D*). These results indicate that RTCs are not pluripotent; rather, it suggests that they have a biased but limited potential for differentiation into the trophectoderm cell lineage. To examine whether RTCs contribute to the trophectoderm cell lineage in vivo, we injected EGFP-labeled RTCs into the perivitelline space of eight-cell stage

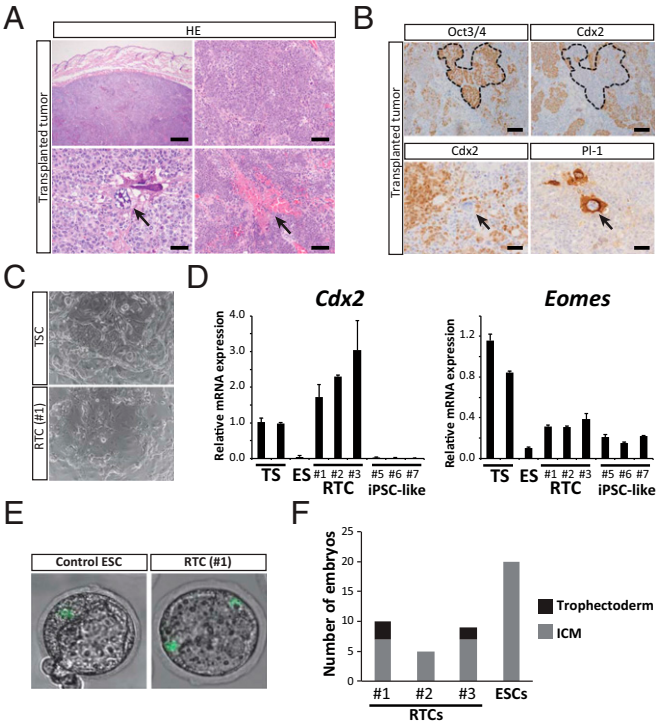

Fig. 3. RTCs lack the ability of teratoma formation but exhibit a biased differentiation propensity. (*A*) The histology of RTC-derived tumors developed after inoculation into s.c. tissue of nude mice. The tumors consist of mainly histologically undifferentiated cells (*Upper*), with a small number of giant cells that resemble trophoblast giant cells (*Lower Left*; arrow). Multiple hemorrhagic foci are observed in the tumors (*Lower Right*; arrow). (Scale bars: *Upper Left*, 500 μm; *Upper Right* and *Lower Right*, 200 μm; *Lower Left*, 50 μm.) (*B*) Immunohistochemistry for the RTC-derived tumors. The histologically undifferentiated cells often express Cdx2 (*Upper Right*), and this expression is often reciprocal with Oct3/4 expression (*Upper Left*; the dashed outline indicates the area containing Oct3/4-expressing cells, which lack Cdx2 expression). Some trophoblast giant cell-like cells are positive for Pl1 (*Lower Right*; arrow) and often surrounded by Cdx2-positive tumor cells (*Lower Left*; arrow indicates a trophoblast giant cell-like cell). (Scale bars: *Upper*, 200 μm; *Lower*, 50 μm.) (*C*) Morphology of RTCs cultured in TSC medium. RTCs change their morphology into epithelium-like cells and giant cells, which is reminiscent of TSCs. (*D*) qRT-PCR analysis for *Cdx2* and *Eomes* in TSCs, ESCs, RTCs (nos. 1–3), and iPSC-like cells ($Apc^{Min/+}$). ESCs, RTCs, and iPSC-like cells were cultured with TSC medium for 3 d. Data are presented as the mean ± SD. The mean expression level of TSCs was set at one. (*E*) Representative images of embryos after injection of EGFP-labeled ESCs and RTCs (no. 1). (*F*) A summary of the localization of EGFP-positive cells.

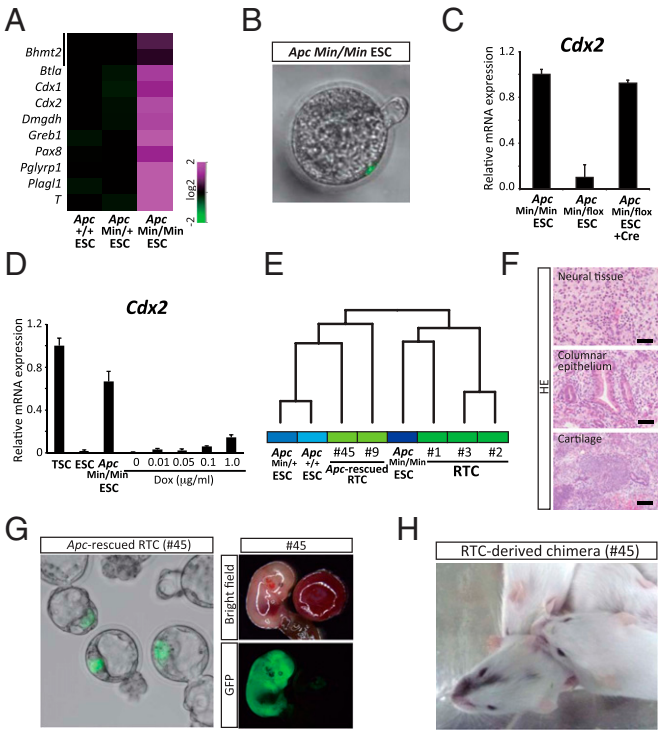

Fig. 4. Genetic rescue of the *Apc* gene confers pluripotency on RTC. (*A*) Up-regulated genes by *Apc* mutation in RTCs were similarly affected in $Apc^{Min/Min}$ ESCs compared with those in $Apc^{Min/+}$ or $Apc^{+/+}$ ESCs. (*B*) Representative images of a blastocyst after the injection of $Apc^{Min/Min}$ ESCs. (*C*) qRT-PCR analysis of *Cdx2* in $Apc^{Min/Min}$ ESCs. After rescue of the *Apc* gene, ESCs decrease *Cdx2* expression, whereas redisruption of the *Apc* gene with Cre recombinase causes up-regulation of *Cdx2*. (*D*) qRT-PCR analysis of the *Cdx2* expression in TSCs, ESCs, $Apc^{Min/Min}$ ESCs, and ESCs with Dox-inducible alleles for mutant *β-catenin*. ESCs expressing mutant *β-catenin* for 14 d show a slight up-regulation of *Cdx2*; however, the expression levels are far less than those in $Apc^{Min/Min}$ ESCs. Data are presented as the mean ± SD. The expression level of TSCs was set at one. (*E*) A hierarchical cluster analysis using microarray data. (*F*) Genetic rescue of the *Apc* gene leads to the acquisition of pluripotency in RTCs. *Apc*-rescued RTCs (no. 45) form teratoma containing various differentiated cells, including neuronal cells, chondrocytes, and columnar epithelium. (Scale bars: 50 μm.) (*G*) EGFP-labeled *Apc*-rescued RTCs were injected into eight-cell stage embryos. (*Left*) *Apc*-rescued RTCs lose the biased differentiation propensity to trophectoderm cell lineage and contribute exclusively to the ICM region. (*Right*) EGFP signals are observed exclusively in the embryo and are not observed in the placenta. (*H*) Adult chimeric mice derived from *Apc*-rescued RTCs (no. 45).

embryos and examined the distribution of EGFP-positive cells at the blastocyst stage. As reported previously, control ESCs exclusively contributed to the inner cell mass (ICM) region but failed to contribute to the trophectoderm layer (Fig. 3*E*). In contrast, we observed EGFP signals in the trophectoderm cell layer after injection of EGFP-labeled RTCs, albeit at a low frequency (Fig. 3 *E* and *F*), indicating that a subset of RTCs is indeed able to incorporate into the trophectoderm cell layer in vivo. Although we also observed EGFP signals in the ICM regions of some blastocysts, we never observed RTC-derived cells in the resulting embryonic day (E)11.5–E14.5 embryos, further supporting the notion that RTCs lack pluripotency.

Loss of the *Apc* Gene Is Responsible for Increased Expression of *Cdx2* and Biased Differentiation of RTCs. To examine the role of the *Apc* mutation on the biased differentiation of RTCs, we established $Apc^{Min/Min}$ ESCs and analyzed the gene expression and differentiation propensity. Genes up-regulated by the *Apc* mutations in RTCs, including *Cdx2*, were similarly affected in the $Apc^{Min/Min}$ ESCs compared with $Apc^{Min/+}$ or $Apc^{+/+}$ ESCs (Fig. 4*A*). Moreover, the $Apc^{Min/Min}$ ESCs occasionally incorporated into the trophectoderm layer of blastocysts after injection into eight-cell stage embryos (Fig. 4*B*).

Notably, the expression levels of *Cdx2* and *Eomes* were decreased in $Apc^{Min/flox}$ ESCs after the genetic rescue of the *Apc* mutation, affirming that the *Apc* mutation does indeed cause the increased expression of *Cdx2* in ESCs (Fig. 4*C* and Fig. S4*A*). This finding was further confirmed by an increased expression of *Cdx2* on redisruption of the rescued *Apc* gene in ESCs (Figs. 4*C* and 5*A*, redisruption of the rescued *Apc* allele is described). We also examined the effect of inducing a stabilized form of *β-catenin* in ESCs. Although the 2 wk of mutant *β-catenin* induction led to a slight up-regulation of *Cdx2*, the level remained far lower than that observed in $Apc^{Min/Min}$ ESCs (Fig. 4*D* and Fig. S4*B*), suggesting that the induction of mutant *β-catenin* is insufficient to cause increased expression of *Cdx2* in $Apc^{Min/Min}$ ESCs. A clustering analysis of global gene expression revealed that RTCs exhibit similar expression patterns to $Apc^{Min/Min}$ ESCs, whereas *Apc*-rescued RTCs clustered with the control ESCs (Fig. 4*E*), suggesting that the altered gene expression patterns in RTCs are largely attributable to loss of the *Apc* gene.

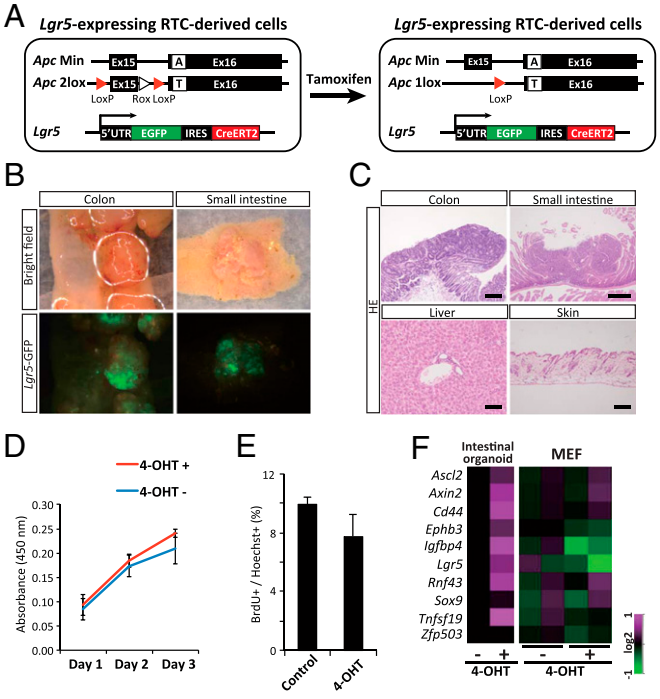

Fig. 5. Cell type-specific consequences of redisruption of the rescued *Apc* gene in vivo. (*A*) A schematic drawing of redisruption of the rescued *Apc* allele in *Lgr5*-expressing RTC-derived cells. (*B*) Macroscopic tumors develop in the colon and the small intestine of RTC-chimeric mice after tamoxifen administration. A strong *Lgr5*-EGFP signal is observed in RTC-derived secondary tumors. (*C*) The histology of the colon, small intestine, liver, and skin of RTC-derived mice after tamoxifen administration. Although neoplastic growth is observed in the colon and small intestine, the liver and skin show no histological abnormalities. (Scale bars: *Upper Left* and *Lower Right*, 200 μm; *Upper Right*, 500 μm; *Lower Left*, 100 μm.) (*D*) WST8 assay for RTC (no. 45)-derived MEFs with or without 4-OHT treatment (2 μg/mL). These MEFs were infected with constitutively active ERT2creERT2-expressing retrovirus in advance. No significant difference in the growth activity was observed between the two groups. (*E*) The number of BrdU-positive proliferating cells did not increase in the RTC (no. 45)-derived MEFs by 4-OHT treatment in vitro. (*F*) Expression of the previously reported target genes of the canonical Wnt pathway in RTC-derived intestinal organoids and MEFs (no. 45-derived). Two independent experiments were performed with MEFs. Note that the target genes are up-regulated in organoids but are not up-regulated in MEFs after 4-OHT exposure.

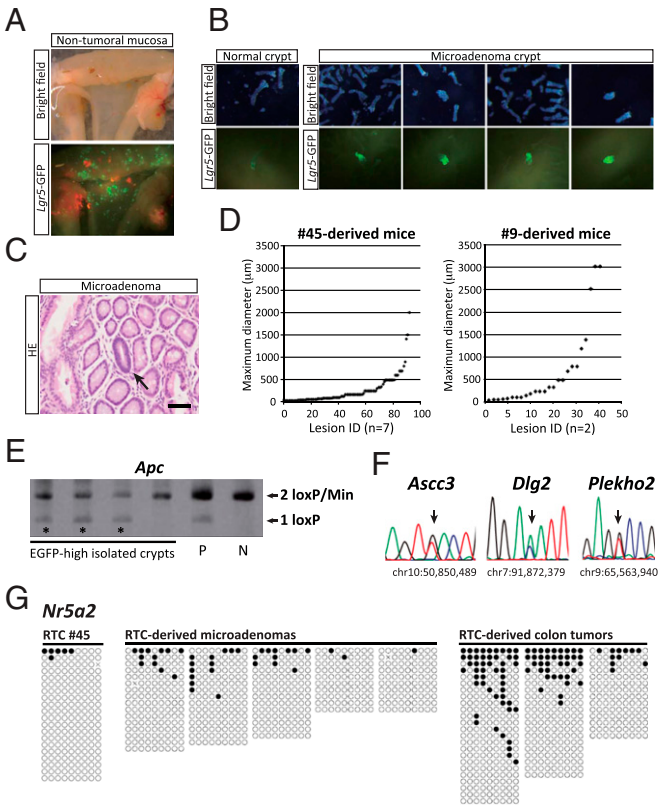

Fig. 6. The majority of colonic lesions remain in a pretumoral microscopic stage in RTC-derived mice. (*A*) Macroscopic images of nontumoral flat mucosa. Note the presence of EGFP-positive crypts in RTC-derived mouse. (*B*) Representative images of normal crypt and microadenoma crypts in the colon. Microadenoma crypts can be identified by an increased signal intensity for *Lgr5*-EGFP. (*C*) Representative histology of pretumoral microadenoma (arrow). (Scale bar: 50 μm.) (*D*) Distribution of the maximum diameter of neoplastic lesions developed in RTC-derived colon after tamoxifen treatment. The majority of neoplastic lesions exhibit a pretumoral microscopic size in both nos. 45- and 9-derived mice. (*E*) Genomic PCR identified deletion of *LoxP*-flanked Exon 15 in the rescued *Apc* allele in the majority of microadenomas. P, positive control; N, negative control. (*F*) Direct sequencing for RTC-derived microadenomas. The identical genomic mutations to RTC (no. 45) were detected in microadenomas ($n = 5$). (*G*) Bisulfite sequencing analysis at the *Nr5a2* locus in *Apc*-rescued RTCs (no. 45), RTC-derived microadenomas, and RTC-derived secondary colon tumors. Although *Nr5a2* methylation level is very low in *Apc*-rescued RTCs and RTC-derived microadenomas, *Nr5a2* is heterogeneously methylated in RTC-derived secondary colon tumors.

Genetic Rescue of the *Apc* Gene Results in the Acquisition of Pluripotency in RTCs. Importantly, both *Apc*-rescued nos. 45 and 9 RTCs formed teratomas containing various differentiated cells, including neural cells, chondrocytes, and ciliated columnar epithelia (Fig. 4*F*). In sharp contrast to RTCs, the *Apc*-rescued RTCs lost their bias toward differentiation into trophectoderm cells but contributed to the ICM region and embryos after injection into eight-cell stage embryos (Fig. 4*G*). Moreover, the *Apc*-rescued RTC lines contributed to a wide variety of organs in chimeric embryos and adult mice after blastocyst injection (Fig. 4*H* and Fig. S4*C*). Taken together, the results suggest that genetic rescue of the *Apc* mutation increased the plasticity of RTCs and that *Apc*-rescued RTCs can differentiate into the various cell types that constitute the adult mouse body.

Redisruption of the Rescued *Apc* Gene in RTC-Derived Differentiated Cells Leads to Neoplastic Growth in the Intestine in Vivo. To examine the influence of the colon tumor cell genome in the context of various cell types, we disrupted the rescued *Apc* allele in differentiated cells in RTC-derived chimeric mice. The targeting vector used to rescue the Apc^{Min} allele contained Exon 15, which was flanked by two *LoxP* sequences (Fig. 2*A*). Thus, the induction of Cre recombinase in these mice leads to excision of Exon 15 and redisruption of the *Apc* gene, resulting in the generation of a truncated Apc protein that is reminiscent of the Apc Min protein, which lacks β-catenin binding sites. We used an *Lgr5-EGFP*-ires-*CreERT2* allele to redisrupt the rescued *Apc* allele in vivo (Fig. 5*A*) (22), because *Lgr5* is expressed in the tissue stem/progenitor cells of various organs, including intestine, hair follicles, and the damaged liver (22–24).

Six-wk-old RTC-derived chimeric mice were treated with tamoxifen and examined 12 wk later ($n = 9$). We confirmed that the *LoxP*-flanked sequence was deleted in organs that contained *Lgr5*-expressing cells (Fig. S5*A*). After redisruption of the *Apc* gene, neoplastic growth was observed in the small intestine and colon (Fig. 5 *B* and *C*), resulting in the development of macroscopic tumors. In contrast to the lack of an *Lgr5*-EGFP signal in the RTCs (Fig. S5*B*), the *Lgr5*-EGFP signal of the macroscopic intestinal tumors was stronger than that of the control crypts (Fig. 5*B* and Fig. S5*C*), which confirmed that the *Apc* mutation affected different genes in the intestinal cells and RTCs. Notably, despite the presence of the disrupted *Apc* gene, no pathological evidence was detected in the liver or skin (Fig. 5*C* and Fig. S5*A*), suggesting that the colon tumor cell genome can only exert neoplastic activity in specific organs in vivo.

Cell Type-Specific Effects of the *Apc* Mutation in RTC-Derived Differentiated Cells in Vitro. Our in vivo experiments suggested that RTCs lose their neoplastic activity after differentiating into other lineages. We next aimed to distinguish whether this tumor suppressive effect is caused by a cell-autonomous phenomenon or in vivo environmental factors, such as an immune response. Accordingly, we performed an in vitro assay using differentiated cells derived from *Apc*-rescued RTCs (45). We first performed organoid culture of the intestinal crypts from adult RTC-chimeric mice (25). After exposure to tamoxifen [4-hydroxytamoxifen (4-OHT)], intestinal organoids changed into a cyst-like structure that was reminiscent of *Apc*-depleted intestinal organoids (Fig. S5 *D* and *E*) (14).

We next established MEFs from the *Apc*-rescued RTC-derived fetus (45) and then, transduced CreERT2 in vitro. Notably, tamoxifen treatment of *Apc*-rescued MEFs did not lead to a higher growth activity compared with nontreated cells (Fig. 5*D* and Fig. S5*F*). Similarly, cells that incorporated BrdU were unchanged after redisruption of the *Apc* gene (Fig. 5*E* and Fig. S5*G*). Our results show that RTCs do not exhibit enhanced cell growth in multiple lineages (other than the intestine) in vivo and in vitro.

Consistent with cellular context-dependent effects of the *Apc* mutation on gene expression, target genes of the canonical Wnt pathway in the intestine (14) were not up-regulated in MEFs after treatment with tamoxifen, whereas they were up-regulated in the intestinal organoids (Fig. 5*F*).

The Majority of Colonic Lesions Remain in a Pretumoral Microscopic Stage in RTC-Derived Chimeric Mice. Although we observed macroscopic tumors in RTC-derived colon, we also found that the majority of colonic lesions in both nos. 45- and 9-derived chimeric mice were macroscopically invisible microadenomas, which can be identified by higher expression of the *Lgr5*-EGFP reporter (Fig. 6 *A* and *B* and Fig. S6*A*). Histological analyses revealed that the maximum diameter of microadenomas in RTC-derived mice was often smaller than 500 μm (Fig. 6 *C* and *D*). Furthermore, a majority of the colonic lesions in RTC-derived mice remained as microadenomas, even in aged mice 20 wk after the administration of tamoxifen. Notably, microadenomas harbor the redisrupted *Apc* gene as well as *Ascc3*, *Dlg2*, and *Plekho2* mutations, indicating that these pretumoral lesions are indeed derived from RTCs (Fig. 6 *E* and *F*). We also established $Apc^{flox/Min}$ iPSCs (Het no. 18) from the iPSC-like cell line 5 (Fig. S6*B*). Control Het no. 18-derived chimeric mice similarly developed microadenomas as

well as a small number of macroscopic tumors, suggesting that loss of *Apc* function alone phenocopies the consequences observed in RTC-derived mice. These results also suggest that the genetic aberrations of colon tumor cells are not sufficient for full-blown tumor development, which is consistent with previous findings showing that de novo DNA methylation plays a pivotal role in the transition from pretumoral microadenomas to tumors in the colon of Apc^{Min} mice (18, 26). In agreement with this hypothesis, we confirmed that tumor-specific heterogeneous hypermethylation at *Nr5a2* (27) was absent in both RTCs and RTC-derived microadenomas but present in the secondary colon tumors that arose in RTC-derived mice (Fig. 6*G* and Fig. S6*B*).

Discussion

Cell type-specific cancer development has been recognized in many organs. Although patients with familial adenomatous polyposis harbor the *APC* gene mutation in cells throughout their bodies, they predominantly develop colon cancer (28). These observations suggest that genetic mutations require a specific cellular context or organ-specific environment to exert cancer properties. We herein showed that a cancer-causing *Apc* mutation indeed has distinct, cell-autonomous effects on gene expression in different types of cells. Surprisingly, there was little overlap between intestinal cells and RTCs with regard to the genes that were affected by *Apc* mutation. We also showed that RTCs confer neoplastic activity after differentiation into intestinal cells (but not into other cell types) in vivo and in vitro. Taken together, our results provided experimental evidence that the biological consequences of tumor-causing mutations are substantially different depending on the cellular context (Fig. S7).

The plasticity of the cancer cell genome is involved in various aspects of cancer biology, including the maintenance, progression, and recurrence of cancer cells. Pioneering studies that investigated the plasticity of the cancer cell genome used nuclear cloning experiments (29, 30). These studies showed that some cancer nuclei are competent for the initial developmental stage after nuclear transplantation. In this study, we showed that colon tumor cells can be reprogrammed into iPSC-like RTCs. However, with the exception of trophectoderm lineage cells, RTCs did not give rise to embryonic tissue, showing that the genomic information of colonic tumor cells does not allow for their differentiation into most cell lineages; rather, they harbor a biased and limited differentiation propensity. Importantly, genetic rescue of the mutated *Apc* gene, a driver mutation in this model, canceled this limited differentiation propensity and induced pluripotency in the RTCs, raising the possibility that the restoration of key genetic defects may confer plasticity on tumor cells. This notion is consistent with a recent study, which showed that *Apc* gene expression promotes the differentiation of colon tumor cells into the multiple cell types that constitute intestinal crypts/villi (14). Our results may provide an unappreciated link between oncogenic driver signals and the plasticity of cancer cells.

In summary, we showed divergent in vivo consequences of cancer-causing mutations that depend on different cellular contexts. We also showed that the majority of RTC-derived intestinal lesions are microadenomas, suggesting that macroscopic colon tumor cells are reprogrammable into pretumoral microadenoma cells. Given that epigenetic regulation plays a central role in cell type specification and cellular reprogramming, these results may underscore the significance of epigenetic regulation in interpreting the genetic information of cancer cells and the subsequent biological consequences in vivo.

Experimental Procedures

All experiments using animals were performed under the ethical guidelines of Kyoto University and approved by the Center for Induced Pluripotent Stem Cell Research and Application Animal Experiment Committee. Generation of chimeric mice, histological analysis, qRT-PCR, and microarray analysis were performed as described previously (9). The accession number for microarray data reported in this paper is GSE77202. Details on experimental procedures are provided in *SI Experimental Procedures*.

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