Caudal-related homeoprotein CDX2 is expressed in intestinal epithelial cells, in which it is essential for their development and differentiation. A tumor suppressor function is suggested by evidence that CDX2 levels are decreased in human colon cancer specimens and that an inactivating mutation of Cdx2 in Apc<sup>D<sup>716</sup></sup> mice markedly increases the incidence of colonic polyps. In this study, we investigated roles for transcriptional and nontranscriptional functions of CDX2 in suppression of colonic tumorigenesis. Mutagenic analysis of CDX2 revealed that loss of function stabilizes CDK inhibitor p27<sup>Kip1</sup> by a nontranscriptional but homeodomain-dependent mechanism that inhibits cyclin E-CDK2 activity and blocks G0/G1-S progression in colon cancer cells. p27<sup>Kip1</sup> stabilization was mediated by an inhibition of ubiquitylation-dependent proteolysis associated with decreased phosphorylation of Thr187 in p27<sup>Kip1</sup>. siRNA-mediated knockdown of p27<sup>Kip1</sup> relieved the decrease in cyclin E-CDK2 activity and S-phase cell fraction elicited by CDX2 expression. Together, these results implicate a nontranscriptional function of CDX2 in tumor suppression mediated by p27<sup>Kip1</sup> stabilization. Up to approximately 75% of low-CDX2 human colon cancer lesions show reduced levels of p27<sup>Kip1</sup>, whereas approximately 68% of high-CDX2 lesions retain expression of p27<sup>Kip1</sup>. These results show that low levels of CDX2 accelerate colon tumorigenesis by reducing p27<sup>Kip1</sup> levels.
Materials and Methods

BrdU labeling, X-gal staining, determination of polyp distribution, and human tissue samples

The Apc\(^{+/−}\), Cdx2\(^{−/−}\)-Gal (Cdx2\(^{+/−}\)), and Apc\(^{+/−}\)/Cdx2\(^{+/−}\) mice have been described previously (5, 12, 13). Bromodeoxyuridine (BrdU) labeling and X-gal staining were carried out as described previously (5, 12). All animal experiments were approved by the Animal Care and Use Committee of Kyoto University. Colonic polyps in the Apc\(^{+/−}\)/C0 and Apc\(^{+/−}\)/Cdx2\(^{+/−}\)/C0 mice (4 mice at the age of 10 weeks, respectively) were scored as described previously (12). Human tissue samples and

Figure 1. CDX2 blocks cell proliferation of normal, adenoma, and carcinoma epithelia of colon. A, relationship between CDX2 expression (green) and proliferation (red) in colonic epithelial cells in a cross section at low magnification (left). The colon of Cdx2\(^{−/−}\)-Gal (β-galactosidase) knock-in mice (Cdx2\(^{−/−}\)-Gal) were stained with antibodies for β-Gal (green) and PCNA (a marker for cell proliferation, red), simultaneously. Right, a higher magnification of the boxed area in the left. Magnification bars, 400 µm (left) and 100 µm (right). B, representative photographs of LacZ-stained Cdx2\(^{−/−}\)-Gal colon (left), and nonstained colons of Apc\(^{+/−}\)/Cdx2\(^{−/−}\) (center) and Apc\(^{+/−}\)/Cdx2\(^{−/−}\)/C0 (right). Scale bars, 1 cm. C, representative photographs of colonic epithelial cells labeled with BrdU in wild-type (Cdx2\(^{+/−}\)) and Cdx2\(^{−/−}\)/C0 mice (top), and those of adenoma cells in nascent polyps of Apc\(^{+/−}\)/Cdx2\(^{−/−}\) mice (middle) at 10 weeks of age. Magnification bars, 100 µm. D, induction of Flag-tagged CDX2 in DLD-1–TetOff cells analyzed by Western blotting (WB; left), compared with that in lysates prepared from intestines (int.) of wild-type mouse. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. Note that induced Flag-CDX2 shows slower migration than endogenous (endog.) CDX2, due to its Flag tag. Proliferation profiles of DLD-1–TetOff and LS174T–TetOff cell clones that induced expression of CDX2 are shown with SD, compared with uninduced cells (right).
immunohistochemical analysis are described in Supplementary Data.

**TetOff system and introduction of small interfering RNA into TetOff cells**

TetOff cells were generated, using a pTetOff vector (Clontech). CDX2 and its mutants were expressed in the respective stable TetOff cell clones, using a pTRE-Tight vector (Clontech). Introduction of small interfering RNA duplexes (siRNA) into TetOff cells and siRNA used in this study, and cell-proliferation assay are described in Supplementary Data.

**Mutagenesis of CDX2 HD and construction of CDX2 deletion mutants**

Mutagenesis of the CDX2 HD was carried out using Quick-Change II Site-Directed Mutagenesis Kit (Stratagene), and confirmed by sequencing. Deletion mutants of CDX2 were constructed by PCR. Sequences of oligonucleotides used for the mutagenesis and for construction of the deletion mutants are provided in Supplementary Data. Construction of luciferase reporters and chromatin immunoprecipitation analysis are also described in Supplementary Data (15).

**Immunoprecipitation, Western blotting, and cycloheximide chase**

Analyses of the p27Kip1 complex and its subcellular localization are described in Supplementary Data. For analysis of the p27Kip1 ubiquitylation, cells were treated with 10 μmol/L of MG132 (Sigma-Aldrich) for 12 hours. For analysis of p27 Kip1 protein stabilization, cells were treated with 100 μg/mL of cycloheximide (CHX; Sigma-Aldrich). Quantitative RT-PCR analysis for the CDKN1B mRNA is also described in Supplementary Data.

**Statistical analysis**

Statistical analyses were carried out by the Student’s t test to compare the mean (Figs. 1, 2, and 4), or by the χ² test to compare the CDX2 and p27Kip1 levels in human colon cancer specimens (Fig. 6), using JMP software (SAS Institute Japan). P < 0.05 was considered significant.
Results

CDX2 blocks cell proliferation of normal, adenoma and carcinoma epithelia of colon

In the colonic epithelium, CDX2 was expressed strongly in the nonproliferating differentiated cells, whereas its level in the proliferating cells was much lower (Fig. 1A; ref. 6). The Cdx2+/-/C0 mutation increased proliferation of normal colonic epithelial cells by approximately 2 times (Fig. 1C, top) and of adenoma cells by approximately 3 times (Fig. 1C, middle). These results suggest that CDX2 normally suppresses proliferation of normal and adenoma epithelial cells of the colon. Consistently, most colonic polyps were found in the distal region where the level of CDX2 was low, in both Apc+/+ and Apc+/-Cdx2+/- mutant mice (Fig. 1B; 79 ± 8% and 92 ± 8%, respectively; mean ± SD).

To investigate the mechanism by which CDX2 suppressed the proliferation, we constructed doxycycline-controlled "Tet-Off-CDX2" cell clones, in which CDX2 was induced at levels comparable to those in the normal intestines (Fig. 1D, left). As test cells, we chose near-diploid human colon cancer lines DLD-1 and LS174T, which were used as models for the intestinal progenitor cells (16) and expressed low levels of CDX2 (Fig. 1D, left). Wnt signaling is activated through mutations in the APC and β-catenin genes, respectively. In addition, both Ras and phosphatidylinositol-3 kinase (PI3K)-Akt signaling pathways are also activated by mutations in KRAS and PIK3CA genes (17), whereas p53 is mutated only in DLD-1 cells.

Double bands of p27Kip1 protein have been described (42). D. Amount of p27Kip1 protein on day 2 after introduction of siRNA against CDX2 in human colon cancer cell lines DLD-1 and HCT-15, analyzed by Western blotting (left). Reduced level of CDX2 is shown on the top (left). Amount of p27Kip1 protein in colonic hamartomas of the Cdx2+/− mutant mice was analyzed by Western blotting (right). GAPDH was used as a loading control. Note essentially absent CDX2 in the hamartomas (right, top). Normal, the lysate derived from the surrounding normal mucosa.
and HEPH genes (Supplementary Fig. S1A), both controlled by CDX2 (18, 19). Importantly, induction of CDX2 arrested the TetOff cells in the G0/G1 cell-cycle phase and suppressed proliferation (Fig. 1D, right; Supplementary Figs. S1B and S2A). The same results were obtained with mouse colon cancer cells Colon26, in which Wnt signaling was not activated (Supplementary Fig. S1C and D). Consistently, knockdown of endogenous CDX2 expression increased the S-phase fraction by approximately 10% (Supplementary Fig. S2B and C). These results suggest that CDX2 suppresses proliferation of not only normal and adenoma epithelia but also colon cancer cells by blocking G0/G1-S progression.

Nontranscriptional activity of CDX2 in N-terminal and homeobox domains is sufficient to block cell proliferation

Because homeobox proteins function as transcription factors through their HDs (20), we investigated whether CDX2 blocked cell proliferation through its transcriptional activity. To this end, we constructed transcription-defective CDX2 mutants that contained point mutations in the HD (Fig. 2A, left). Mutations R189A, R237A, R237H, and R237P have been reported to eliminate the binding activity of HD to DNA, whereas Q234K alters its DNA-binding specificity (21). These HD mutations (R189A, Q234K, R237A, R237H, and R237P) lost the transcriptional activation for CDX2-target gene promoters (Fig. 2A, left, LI-cadherin gene promoter; Supplementary Fig. S3A, HEPH promoter), although all mutant proteins were stable, and localized predominantly to the nucleus (Supplementary Fig. S4A and B). Consistently, the HD mutations lost DNA-binding activity for the CDX2-target gene promoters (Fig. 2A, right, LI-cadherin gene promoter; Supplementary Fig. S3B, sucrase isomaltase promoter). Surprisingly, the transcription-defective CDX2 mutants (R189A, Q234K, R237A, and R237H) retained similar blocking activities on cell proliferation to that of the wild type (WT; Fig. 2B; Supplementary Figure 4.)

CDX2 stabilizes p27Kip1 protein by blocking its ubiquitylation. A, amount of p27Kip1 (CDKN1B) mRNA on day 2 of induction of wild-type CDX2 in DLD-1–TetOff cell clones, analyzed by quantitative PCR (qPCR). B, amount of p27Kip1 protein at the indicated hours after treatment with cycloheximide (CHX) on induction of wild-type CDX2 or C2HD-N1 protein, analyzed by Western blotting (top). GAPDH was used as a loading control. Quantified data are shown in bottom. C, the level of polyubiquitylated p27Kip1, analyzed by Western blotting, on expression of CDX2 in DLD-1 cells. DLD-1–TetOff cells were treated with 10 μM/L of MG132 for 12 hours to inhibit proteasome activity. p27Kip1 in the cell lysate was immunoprecipitated using an antibody for p27Kip1, and the level of polyubiquitylated p27Kip1 was analyzed by Western blotting, using an antibody for ubiquitin, D, phosphorylation levels of p27Kip1 at S10, T187, and T189 analyzed by Western blotting, on expression of CDX2 in DLD-1–TetOff cells. β-Actin was used as a loading control. Numbers for S10, T187, and T189 phosphorylation indicate relative ratios of the phosphorylated p27Kip1 to the total p27Kip1, whereas those for total p27Kip1 indicate relative levels of p27Kip1 to β-actin.
Fig. S3C). Importantly, these results suggest that both transcriptional and DNA-binding activities are dispensable for blocking proliferation by CDX2. On the contrary, another mutation R237P suppressed inhibition of cell proliferation by CDX2 (Fig. 2B), suggesting that HD was essential for the block of proliferation.

To localize in CDX2 the domains responsible for blocking cell proliferation, we then constructed deletion mutants "C2HDs" that retained its HD (Fig. 2C). Mutant C2HD-KK contained HD and its C-terminally adjacent lysyl-lysine (KK; Fig. 2C). Mutants C2HD-N1 and -N4 contained HD with C-terminal KK, and N-terminal extensions (Fig. 2C). Because mutant C2HD-N1 retained the transactivation domain (22) and activated the L1-cadherin and HEPH promoters, we also constructed its transcription-defective derivatives that carried HD mutations R237A and R237H (C2HD-N1:R237A and -N1:R237H, respectively; Fig. 2C). All C2HD proteins were expressed stably in DLD-1 cells (Supplementary Fig. S5A and B), whereas we failed to construct cells expressing CDX2 deletion mutant proteins lacking HD, due to their instability in vivo (data not shown). Like WT-CDX2 and C2HD-N1, cell proliferation was still blocked by induction of C2HD-N1 derivatives that lacked both the C-terminal domain and transcriptional activity (C2HD-N1:R237A and -N1:R237H; Fig. 2C and D; Supplementary Fig. S6A and B). These results indicate that CDX2 C-terminal domain as well as...
transcriptional activity was dispensable for the proliferation block. Although cell proliferation was also blocked by C2HD-KK and C2HD-N4 (Fig. 2D), the effect was only partial, suggesting that the HD alone was insufficient, and its N-terminal domain was needed for the full activity of CDX2.

**CDX2 increases amounts of p27\(^{kip1}\) protein and cyclin-CDK2/p27\(^{kip1}\) complex**

Progression from the G1 to S phase is positively regulated by the cyclin E-CDK2 complex (23). We found that the cellular cyclin E-CDK2 kinase activity was decreased by expression of WT-CDX2 or its mutants (CDX2:R237A and C2HD-N1), as assayed by histone H1 (HH1) phosphorylation (Fig. 3A; total CDK2 activity, Supplementary Fig. S7A, left). At the same time, induction of WT-CDX2 or mutant CDX2:R237A increased the amounts of cyclin E, cyclin A, and CDK2 coimmunoprecipitated with p27\(^{kip1}\), whereas the induction reduced the lysate levels of cyclin A and CDK2 (Fig. 3B). Induction of WT-CDX2 or CDX2:R237A also increased the level of total p27\(^{kip1}\) protein in the lysate of DLD-1 cells (Fig. 3C, left; for quantified data, Supplementary Fig. S7A, right, by approximately 3 times), without affecting the subcellular localizations of the cyclins, CDK2 and p27\(^{kip1}\) (Supplementary Fig. S7B, left). Also, in LS174T cells, the level of p27\(^{kip1}\) was increased on expression of CDX2 (Fig. 3C, right). These results suggest that expression of CDX2 increases the amounts of cyclin E-CDK2/p27\(^{kip1}\) and cyclin A-CDK2/p27\(^{kip1}\) complexes through raising the level of p27\(^{kip1}\). Consistently, the p27\(^{kip1}\) protein level was decreased in the reduced-CDX2 cells (by approximately 40%; Fig. 3D, left) and Cdx2\(^{+/−}\) colonic hamartomas where CDX2 was essentially absent (by approximately 90%; Fig. 3D, right).

Also, by expression of deletion mutant C2HD-N1 and its transcription-deficient derivatives (C2HD-N1:R237H and -N1:R237A), the level of p27\(^{kip1}\) in cell lysates was increased, as well as those of cyclin E- and cyclin A-CDK2/p27\(^{kip1}\) complexes (Fig. 3B and C; Supplementary Fig. S7B, right). On the contrary, mutation R237P suppressed increase in the level of p27\(^{kip1}\) (Supplementary Fig. S7C). These results show that the N-terminal and HD in CDX2 are sufficient for increasing the p27\(^{kip1}\) level and blocking proliferation.

**Expression of CDX2 stabilizes p27\(^{kip1}\) by blocking its ubiquitylation**

In contrast to the increased level of p27\(^{kip1}\) protein (Fig. 3C), its mRNA (CDKN1B) level remained unchanged on induction of CDX2 (Fig. 4A), suggesting that CDX2 stabilized p27\(^{kip1}\)
protein. To test this possibility, we treated CDX2-induced cells with CHX, an inhibitor of protein translation. In the CDX2-uninduced control cells, the level of p27^Kip1 protein decreased by approximately 50% in 3 hours after CHX treatment (Fig. 4B). However, the level decreased only by approximately 10% on induction of WT-CDX2 or C2HD-N1 protein (Fig. 4B), showing that CDX2 stabilized p27^Kip1 protein.

Stability of p27^Kip1 is controlled by SKP2 through ubiquitylation-mediated proteolysis (24–29). Because polyubiquitylated p27^Kip1 is detectable when its proteasomal degradation is inhibited, we treated CDX2-induced cells with MG132, a proteasome inhibitor. Notably, the level of polyubiquitylated p27^Kip1 was decreased on expression of CDX2 (Fig. 4C), whereas those of polyubiquitylated cyclins E and A, other SKP2 targets, were increased (Supplementary Fig. S7D). Because these results suggested that the p27^Kip1 ubiquitylation was specifically suppressed by CDX2 expression, we further investigated the p27^Kip1 phosphorylation that can control its ubiquitylation (24–31). The ratios of S10- and T187-phosphorylated p27^Kip1 to the total p27^Kip1 were reduced significantly to approximately 20% on induction of CDX2, whereas that of T198 was not affected (Fig. 4D). The phosphorylation of p27^Kip1 at T187 reduces the stability of p27^Kip1 through accelerating the ubiquitylation-mediated degradation, whereas that of S10 stabilizes p27^Kip1 (30–33). Thus, it is likely that the reduced level of the T187 phosphorylation is a main cause of the p27^Kip1 stabilization on expression of CDX2.

**p27^Kip1 is critical for CDX2 to block cell-cycle progression**

To investigate further the role of p27^Kip1 in the CDX2-mediated proliferation block, we simultaneously induced CDX2, and suppressed p27^Kip1 expression by introducing siRNA against CDKN1B (p27^Kip1 siRNA) into the TetOff-CDX2 cells (Fig. 5A and B). Notably, knocking down p27^Kip1 completely prevented the decrease in the S-phase fraction on expression of C2HD-N1-R237H (transcription-defective C2HD-N1 mutant; Fig. 5C, bottom; compare center and right). These results show that the increased level of p27^Kip1 is essential for CDX2 to block G0/G1-S phase progression. Consistently, reducing the level of p27^Kip1 completely blocked the decrease in the cyclin E–associated CDK2 activity on expression of C2HD-N1-R237H (Fig. 5D, right, top) or C2HD-N1-R237A (Supplementary Fig. S8). The reduced level of p27^Kip1 in C2HD-N1:R237H–induced cells also blocked the increase in the phosphorylation level of retinoblastoma protein (Rb; Fig. 5D, right). An endogenous target by cyclin E-CDK2 (34, 35). These results collectively indicate that CDX2 stabilizes p27^Kip1 through its nontranscriptional activity, which causes inhibition of cyclin E-CDK2 and blocks cell proliferation.

Furthermore, reducing the level of p27^Kip1 partially suppressed the decrease in the S-phase fraction on expression of WT-CDX2 or C2HD-N1 that retained transcriptional activity (Fig. 5C, top and middle). Consistently, the reduced level of p27^Kip1 partially suppressed the decreases in cyclin E-CDK2 activity and phosphorylation level of Rb (Fig. 5D, left and center). These results verify that p27^Kip1 is critical for CDX2 to block cell-cycle progression and suppress the cyclin E-CDK2 activity, although the transcriptional activity of CDX2 may also make some contribution to the cell-proliferation block.

**Correlation between CDX2 and p27^Kip1 levels in human colon cancer tissues**

Finally, we analyzed expression of CDX2 and p27^Kip1 proteins in 59 human colon cancer specimens, and investigated possible correlation between their levels. Among these tumors, 28 showed the lower level of CDX2 protein compared with that in normal mucosa (Nl; Fig. 6A and 6D), whereas the other 31 showed CDX2 at similar to those at higher levels (Fig. 6B–D). Notably, the p27^Kip1 level was reduced in 21 of the 28 low-CDX2 tumors (75%; Fig. 6A and D; Supplementary Fig. S9), whereas it was reduced in only 10 of the 31 high-CDX2 tumors (32%; P < 0.001, obtained from χ² test). These data are consistent with the results that the p27^Kip1 level was reduced in the low-CDX2 colon cancer cell lines and Cdx2+/−/− mutant mouse colonic tumors (Fig. 3C). Collectively, these results suggest that low levels of CDX2 contribute to the reduced levels of p27^Kip1 in human colon cancer tissues. On the contrary, p27^Kip1 protein in 21 of the 31 high-CDX2 tumors (68%; Fig. 6B–D; Supplementary Fig. S10), whereas it was retained in only 7 of the 28 low-CDX2 tumors (25%; Fig. 6D). These data are also consistent with the results that CDX2 increased the level of p27^Kip1 protein in colon cancer TetoOff cells (Fig. 3C), showing a positive correlation between CDX2 and p27^Kip1 levels.

**Discussion**

In human cancer cells of the colon and other epithelial tissues, the level of p27^Kip1 is frequently reduced by its accelerated degradation through proteasomes (36, 37). The reduced level of p27^Kip1 is also correlated with poor prognosis, suggesting its tumor-suppressing activity (25). Consistently, homozygous p27^Kip1 mutation increased the number of intestinal polyps in Apc mutant mice by 3 to 6 times (38). In colon cancer cells, however, the mechanism that stimulates the degradation of p27^Kip1 was unknown. Here we have shown that homeobox transcription factor CDX2 stabilizes p27^Kip1 by blocking its proteolysis (Figs. 3 and 4). Interestingly, we have found a significant correlation between CDX2 and p27^Kip1 protein levels in human colon cancer tissues (Fig. 6). Our present results suggest that the decreased level of CDX2 is one of the causes that stimulate the p27^Kip1 proteolysis in colon cancer cells.

During the G1 to S transition, phosphorylation of p27^Kip1 at T187 triggers its proteasomal degradation through the SCF-SKP2 E3 ubiquitin ligase complex (24, 26–29). The levels of SKP2 and Csk1 were not affected by expression of CDX2: R237A that increased p27^Kip1 (Fig. 3C, left), suggesting that CDX2 stabilizes p27^Kip1 protein independent of SKP2 and Csk1 levels. Notably, the extent of p27^Kip1 phosphorylation at T187 decreased on expression of CDX2 (Fig. 4D), suggesting that CDX2 suppressed the SKP2-dependent proteolysis of p27^Kip1, which caused p27^Kip1 accumulation (Fig. 3C). Consistently, the level of p27^Kip1 ubiquitylation was reduced on expression of CDX2 (Fig. 4C). On the contrary, the reduced activity of cyclin
E-CDK2 can stabilize p27Kip1 because cyclin E-CDK2 phosphorlates p27Kip1 at T187 and accelerates its proteosomal degradation (24, 26–29). Importantly, reducing the level of p27Kip1 blocked decreases in the cyclin E-CDK2 activity and S-phase fraction caused by CDX2 (Fig. 5C and Fig. 5D). These results indicate that p27Kip1 is a critical downstream target of CDX2 in regulating the cyclin E-CDK2 activity and blocking cell-cycle progression. Thus, p27Kip1 stabilization may precede the attenuated activity of cyclin E-CDK2 in CDX2-induced cells (Fig. 3A and C). It remains to be investigated how CDX2 reduces the level of p27Kip1 phosphorylation at T187.

The level of p27Kip1 phosphorylation at S10 was also decreased on expression of CDX2 (Fig. 4D). Because the S10 dephosphorylation promotes binding of p27Kip1 to cyclin-CDK complex, it helps p27Kip1 to inhibit the cyclin-CDK activity and suppress tumor development (30, 31). Thus, it is conceivable that the reduced p27Kip1 phosphorylation at S10 also contributes to the tumor-suppressive function of CDX2.

Interestingly, knocking down p27Kip1 completely blocked the decreases in the cyclin E-CDK2 activity and S-phase fraction on expression of the mutant C2HD-N1:R237A that lacked transcriptional activity and C-terminal domain of CDX2 (Fig. 5C and D; Supplementary Fig. S8). These results show that CDX2 stabilizes p27Kip1 through the nontranscriptional function mediated by its N-terminal and homeobox domains. The results have also prompted us to question whether the transcription-independent function of CDX2 is shared among the CDX family proteins. To address this issue, we constructed DLD-1–TetOff cells that expressed CDX1 (Supplementary Fig. S11A), the closest homolog of CDX2. The CDXs show a high similarity in their HDs (aa identity, 92%; and similarity, 97%; Supplementary Fig. S12A), but only low similarities in both N-terminal and C-terminal domains (aa similarities, 42% and 38%, respectively: Supplementary Fig. S12B and C). Expression of CDX1 did not increase the p27Kip1 level in the DLD-1 cells (Supplementary Fig. S11C), although the level of induced CDX1 and its transcriptional activity were comparable to those of CDX2 (Supplementary Fig. S11A and B). Thus, it appears that the transcription-independent block of proliferation is specific to CDX2. Because the HDs are almost identical between the CDXs (Supplementary Fig. S12A), the N-terminal domain can be critical for the nontranscriptional function of CDX2. This interpretation is consistent with the results that C2HD-N1 (N-terminal and HD) increases the level of p27Kip1 protein by approximately 5 times, whereas C2HD-KK alone does it only by approximately 1.5 times (Fig. 3C, left; Supplementary Fig. S7A, center). To understand the precise role of the N-terminal domain of CDX2 in stabilizing p27Kip1, it will be interesting to identify proteins that can bind to the domain.

Wnt, Notch, and PI3K-Akt signaling pathways are proposed as major mechanisms that stimulate proliferation of the colonic epithelial cells (16, 39, 40). On the contrary, the $Cdx2^{+/−}$ mutation stimulated proliferation of the Apc−/− adenoma epithelial cells in which Wnt and Notch signaling pathways were already activated (Fig. 1C; refs. 16, 39, 40). Expression of CDX2 also blocked the proliferation of colon cancer cells in which the Wnt and PI3K signaling pathways were activated through mutations in the APC, β-catenin, or $PIK3CA$ gene (Fig. 1D; refs. 16, 17). Collectively, these results may indicate that CDX2 functions as an additional layer of regulation that inhibits proliferation of the colonic epithelial cells, and ensures a quick halt of the cell cycle as soon as the transit-amplifying cells initiate differentiation.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest disclosed.

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Suppression of Colonic Polyposis by Homeoprotein CDX2 through its Nontranscriptional Function that Stabilizes p27Kip1

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