


Transduced caudal-type homeobox (CDX) 2/CDX1 can induce growth inhibition on CDX-deficient gastric cancer by rapid intestinal differentiation

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Intestinal metaplasia induced by ectopic expression of caudal-type homeobox (CDX)2 and/or CDX1 (CDX) is frequently observed around gastric cancer (GC). Abnormal expression of CDX is also observed in GC and suggests that inappropriate gastrointestinal differentiation plays essential roles in gastric tumorigenesis, but their roles on tumorigenesis remain unelucidated. Publicly available databases show that GC patients with higher CDX expression have significantly better clinical outcomes. We introduced CDX2 and CDX1 genes separately into GC-originated MKN7 and TMK1 cells deficient in CDX. Marked suppression of cell growth and dramatic morphological change into spindle-shaped flat form were observed along with induction of intestinal marker genes. G0-G1 growth arrest was accompanied by changed expression of cell cycle-related genes but not with apoptosis or senescence. Microarray analyses additionally showed decreased expression of gastric marker genes and increased expression of stemness-associated genes. Hierarchical clustering of 111 GC tissues and 21 non-cancerous gastric tissues by selected 18 signature genes based on our transcriptome analyses clearly categorized the 132 tissues into non-cancer, "CDX signature"-positive GC, and "CDX signature"-negative GC. Gene set enrichment analysis indicated that "CDX signature"-positive GC has lower malignant features. Immunohistochemistry of 89 GC specimens showed that 50.6% were CDX2-deficient, 66.3% were CDX1-deficient, and 44.9% were concomitant CDX2/CDX1-deficient,

Chiemi Nakayama and Nobutake Yamamichi contributed equally to this work.

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suggesting that potentially targetable GC cases by induced intestinal differentiation are quite common. In conclusion, exogenous expression of CDX2/CDX1 can lead to efficient growth inhibition of CDX-deficient GC cells. It is based on rapidly induced intestinal differentiation, which may be a future therapeutic strategy.

KEYWORDS

CDX, gastric cancer, hierarchical clustering, intestinal differentiation, transcriptome

1 | INTRODUCTION

Despite the gradually decreased prevalence in most countries, gastric cancer is still the third most common cause of death from cancer worldwide.¹ Although the risk of gastric cancer is becoming reduced by improved cancer detection and decreased prevalence of *Helicobacter pylori* (*H. pylori*) infection, prognosis of advanced gastric cancer is still very poor as a result of insufficient treatment options.² It is broadly accepted that gastric cancer usually develops from gastric mucosa with atrophic change and intestinal metaplasia, both of which are mostly caused by chronic *H. pylori* infection.^{3,4} Despite the rather homogeneous condition of *H. pylori*-induced chronic gastritis, gastric cancer presents various clinicopathological features.⁵⁻⁷ Such diversity makes it difficult to plan a strategy against gastric cancer, and consequently leads to poor prognosis of the disease.

Intestinal metaplasia frequently observed around gastric cancer suggests that inappropriate gastrointestinal differentiation plays essential roles in gastric tumorigenesis.^{8,9} Our previous studies indicated that a disrupted balance between gastric and intestinal differentiation affects gastric oncogenesis.^{5,6,10,11} Ectopic expression of caudal-type homeobox genes (CDX families) is thought to be indispensable for "intra-gastric intestinal metaplasia," because CDX2 and/or CDX1 almost always express on the gastric mucosa with intestinal metaplasia, and also because exogenous CDX transduction induces intestinal metaplasia in the stomach of model mice.^{8,12-15} Of the three genes of human CDX families, *CDX4* expresses only at embryonic stage and its function is poorly understood.¹⁶ On the contrary, CDX2 and CDX1 are well known to play important roles not only in early embryonic development but also in regulating proliferation and differentiation of intestinal epithelial cells in adults.¹⁴ Both CDX2 and CDX1 function as transcription factors, and they are thought to be able to compensate each other.^{14,16} Although neither CDX2 nor CDX1 is originally expressed in the stomach; they are often induced in a morbid condition such as chronic atrophic gastritis and intestinal metaplasia.^{8,17}

It remains controversial as to how ectopic expression of CDX influences the initiation and progression of stomach cancer. In a mouse model, after long-term observation, Mutoh et al¹⁸ showed that intestinal-type adenocarcinoma frequently arose from intestinal metaplasia in the CDX2 transgenic mice. In Mutoh's study, most gastric tumors had some mutation of *p53* and/or *APC*, which

suggests that overexpression of *CDX2* is one of the multiple steps in gastric carcinogenesis. On the contrary, Liu et al¹⁹ showed that *CDX2* expression in gastric dysplasia/cancer progressively decreased over time. Mizoshita et al²⁰ reported that *CDX2*-positive gastric cancer showed a significantly better outcome compared with *CDX2*-negative gastric cancer. These pathological studies suggest a tumor-suppressive activity of *CDX2*, which is contradictory to Mutoh's result.¹⁸ We have previously reported that *CDX2* and Brm-type SWI/SNF chromatin remodeling complex cooperatively regulate *villin1* expression in gastrointestinal cells,¹⁰ and also found that Brm deficiency in gastric cancer is negatively associated with differentiation status of gastric malignancy.⁵ According to the accumulated results, including ours, we believe that CDX plays pivotal roles through interaction with the SWI/SNF complex upon determining differentiation status of gastric cancer. We also speculate that CDX expression can promote intestinal differentiation in gastric cancer and consequently reduce the malignant properties.

Based on this background, we tried to evaluate the effect of exogenous CDX (*CDX2* and *CDX1*) expression in gastric cancer cells. Recently, Dang et al²¹ reported that disruption of *CDX2* did not significantly affect tumorigenic potential in MKN45, a gastric adenocarcinoma cell line strongly expressing *CDX2*. In the present study, we used other gastric cancer-originated cell lines, MKN7 and TMK1, both of which lack expression of *CDX2* and *CDX1*.^{10,22} We believe our results can shed light on the controversial effect of CDX on gastric tumorigenesis, and further lead to a new therapeutic approach of gastric malignancy based on the control of disrupted gastrointestinal differentiation.

2 | MATERIALS AND METHODS

2.1 | Kaplan-Meier plot analyses

Publicly available KM plotter²³ and TCGA (The Cancer Genome Atlas) data set at the cBioPortal^{24,25} were used to plot disease-free survival curves and overall survival curves of gastric cancer patients. Regarding the TCGA data, the patients were divided into two groups based on the level of *CDX2* and *CDX1* expression which was shown as Z-scores (>0.65 for "CDX high" group and ≤0.65 for "CDX low" group). According to the log-rank test, *P* values <0.05 were considered statistically significant.

2.2 | Cell culture, retrovirus vectors, and cell proliferation assay

For the stable transduction of *CDX* genes, we used VSVG-pseudotyped pMXs-IRES-puro retrovirus vectors.¹⁰ To evaluate cell proliferation, we used MTT assay. Detailed information of cell lines used and cell-related experimental procedures are described in Doc S1.

2.3 | Western blot analysis and reverse transcriptase-PCR analysis

Western blotting and RT-PCR were carried out as we previously reported.²⁶ Antibodies used and primer sequences of 16 gene transcripts are described with detailed experimental procedures in Doc S1.

2.4 | Tumor samples and immunohistochemistry

We randomly selected 89 gastric adenocarcinoma samples surgically resected at the Fujita Health University Hospital. This study was approved by the ethics committees of the University of Tokyo and the institutional ethical review board for human investigation at Fujita Health University. Immunohistochemistry to examine expression of *CDX2* and *CDX1* is described in detail in Doc S1.

2.5 | Cell cycle analysis

MKN7 and TMK1 cells were grown semi-confluent in 100-mm culture dishes and infected with *CDX*-expressing retrovirus vectors (*CDX2*, *CDX1*, and mock) at appropriate MOI. After confirming the morphological changes in both cells infected with *CDX* expression vectors (6 days post-infection for MKN7 and 9 days post-infection for TMK1), staining with propidium iodide was carried out (using Cycletest plus DNA reagent kit; Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). Cell cycle was then analyzed using FACS Calibur flow cytometer (Becton Dickinson Immunocytometry Systems) and ModFit LT version 3.0 software (Verity Software House, Topsham, ME, USA).

2.6 | Microarray gene expression analysis and selection of gene probes for *CDX2/CDX1* common signatures, *CDX2* signature, and *CDX1* signature

We examined total RNA obtained from MKN7 cells at 6 days post-infection and from TMK1 cells at 8 days post-infection with *CDX*-expressing retrovirus vectors. After extraction with RNeasy Plus Mini Kit (Qiagen, Venlo, the Netherlands), total RNA was analyzed on GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's protocols. After normalization of the raw data, fold changes in gene expression in *CDX*-transduced cells were calculated relative to the mock-infected control cells. Raw data are available in NCBI's

Gene Expression Omnibus (GEO; series accession number is GSE102208).

To decide signature gene sets for *CDX2/CDX1* combined, *CDX2*, and *CDX1* expression, genes were selected based on more than threefold upregulation or downregulation compared with mock-infected control.

2.7 | Latest comprehensive gene expression data of 132 gastric tissues

Latest comprehensive gene expression data of 111 gastric cancer tissues and 21 noncancerous gastric tissues were obtained from GSE54129 from NCBI's GEO.

3 | RESULTS

3.1 | Gastric cancer patients with a higher level of *CDX* expression have significantly better clinical outcomes

Using the data set from KM plotter²³ and TCGA,²⁴ clinical outcomes of gastric cancer patients were analyzed focusing on the expression of *CDX2* and *CDX1* (Figure 1). According to the data of KM plotter,²³ overall survival curves and disease-free survival curves showed that gastric cancer patients with higher expression of *CDX* have significantly longer overall survival and disease-free periods compared to those without (Figure 1A,B). According to the data of TCGA,²⁴ disease-free survival curves also showed that gastric cancer patients with higher expression of *CDX* have significantly longer disease-free periods (Figure 1D). For overall survival curves based on TCGA data (Figure 1C), gastric cancer patients with a high level of *CDX* expression have better survival, although we could not detect a significant association between overall survival and *CDX* expression. In total, higher expression of *CDX* in gastric cancer generally shows a tendency to be associated with better prognosis.

3.2 | Exogenous expression of *CDX2* and *CDX1* induces dramatic morphological change and severe growth inhibition in *CDX*-deficient gastric cancer cells

To evaluate the effect of *CDX* transcription factors on gastric cancer cells, we introduced human *CDX2* and *CDX1* genes separately into MKN7 and TMK1 cells which are both deficient in *CDX* expression.^{10,22} After infection with retrovirus vectors and selection with puromycin, we discovered that transduction of either *CDX2* or *CDX1* gave rise to dramatic morphological change on these *CDX*-deficient gastric cancer cells (Figure 2A). At 7 days post-infection, both MKN7 and TMK1 showed a flatter morphology accompanied by marked suppression of cell growth. Transformation of MKN7 occurred earlier and more drastically compared with that of TMK1. Namely, we observed complete growth arrest with no surviving cells for *CDX*-transduced MKN7 cells. On the contrary, a very small fraction of TMK1 cells survived 10 or more days after transduction of *CDX*,

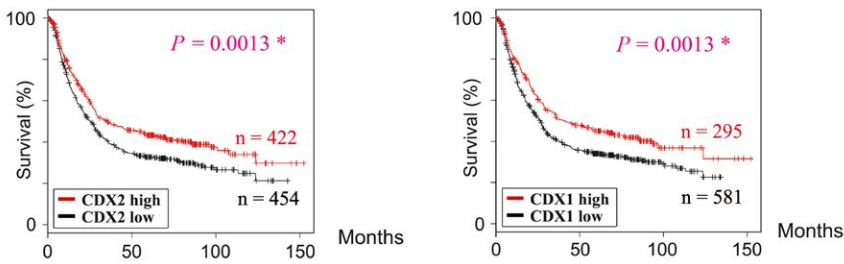
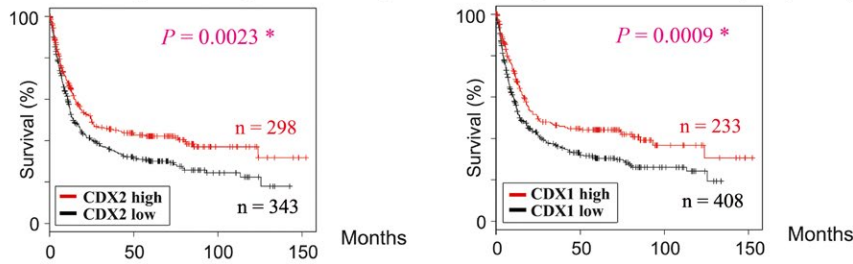
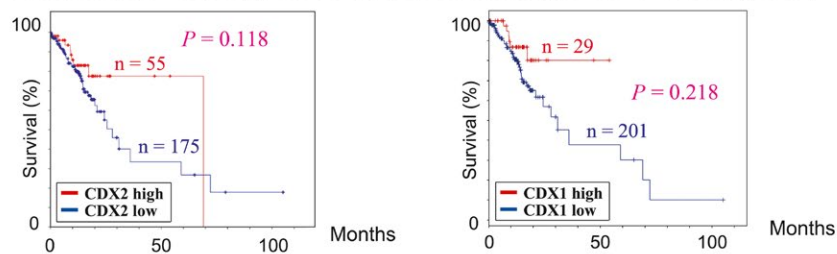
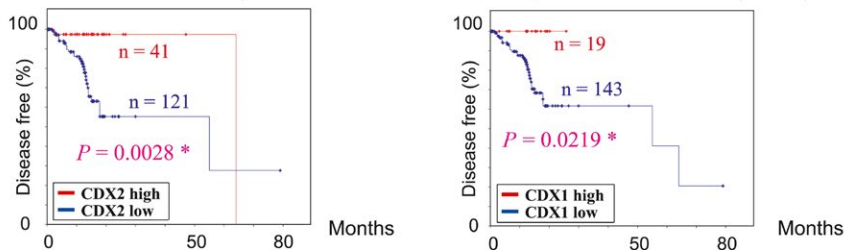
(A) Overall survival curve of gastric cancer patients focusing on CDX2 or CDX1 (KM plotter)**(B) Disease-free periods of gastric cancer patients focusing on CDX2 or CDX1 (KM plotter)****(C) Overall survival curve of gastric cancer patients focusing on CDX2 or CDX1 (TCGA)****(D) Disease-free periods of gastric cancer patients focusing on CDX2 or CDX1 (TCGA)**

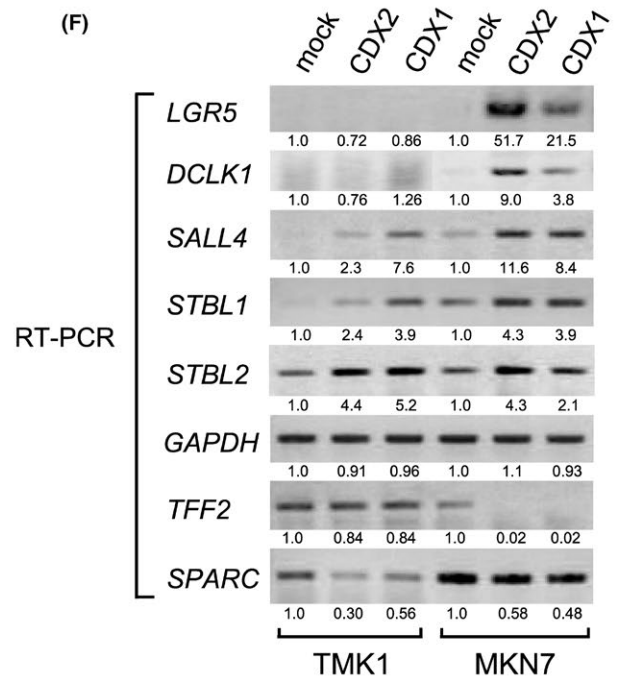
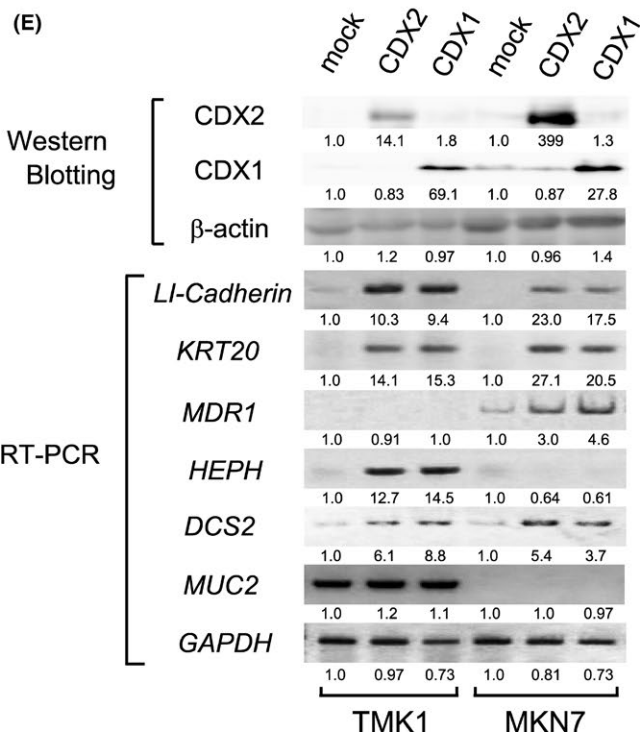
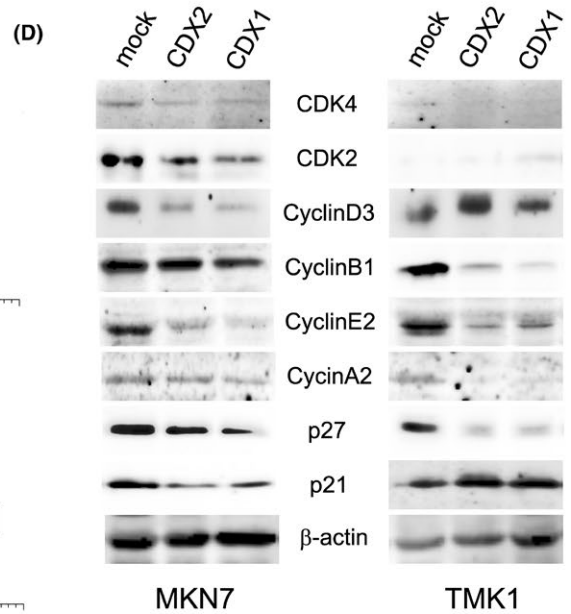
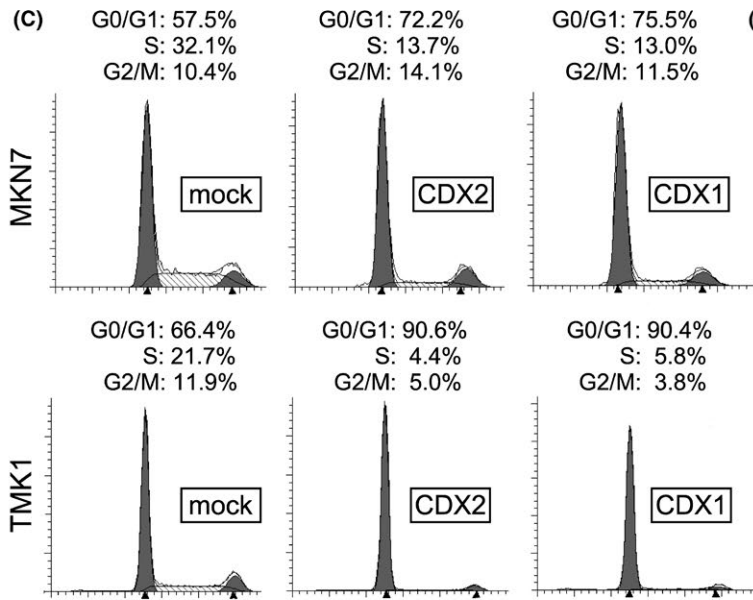
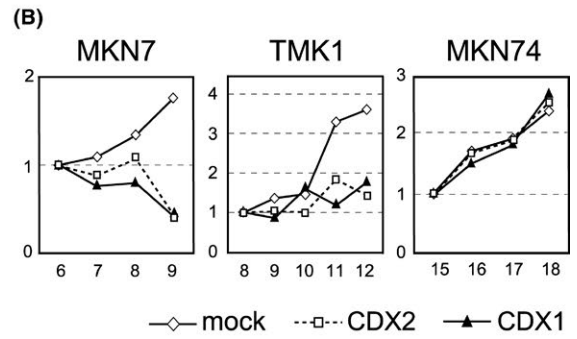
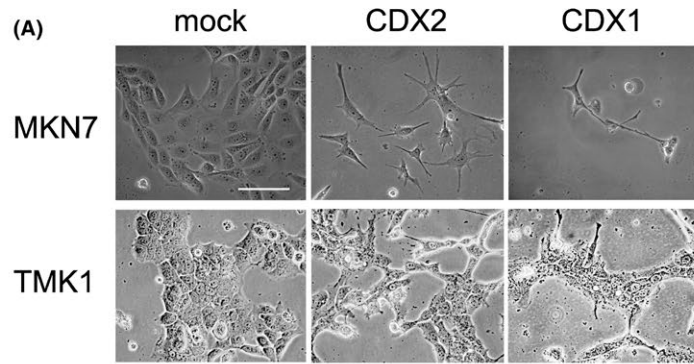
FIGURE 1 Overall survival curves and disease-free survival curves of gastric cancer patients focusing on the expression of caudal-type homeobox (CDX)2 or CDX1. Publicly available large-scale data sets were obtained from KM plotter (A,B) or The Cancer Genome Atlas (TCGA) by cBioPortal for Cancer Genomics (C,D). All the survival curves were analyzed by the log-rank test or Kaplan-Meier method, and P values <0.05 (*) were considered statistically significant

but the growth speed of surviving cells was much slower than for mock-infected cells. Such CDX-induced arrest or severe delay of cell growth was validated by MTT assay (Figure 2B), in which the effects of CDX2 and CDX1 were almost equivalent in both cell lines. Such suppressive effects on cell growth could not be detected in CDX-transduced MKN74 (Figure 2B), AGS, SW480, and LoVo cells (data not shown), all of which originally express CDX.¹⁰

3.3 | CDX-induced growth inhibition in gastric cancer cells occurs through G0-G1 cell cycle arrest

To elucidate the growth suppression in CDX-transduced MKN7 and TMK1, cell cycle analysis was carried out by flow cytometry and western blotting. As shown in Figure 2C, exogenous expression of CDX2 and CDX1 caused a substantial decrease in

FIGURE 2 Growth inhibition and intestinal differentiation observed in caudal-type homeobox (CDX)-deficient gastric cancer cells after transduction with CDX (CDX2 and CDX1) expression retrovirus vectors. A, Morphological changes in MKN7 and TMK1 gastric cancer cells after exogenous expression of CDX2 or CDX1 gene. B, MTT assay using MKN7, TMK1, and MKN74 cells stably infected with a retrovirus vector carrying CDX2 or CDX1 (6-9 days post-infection for MKN7; 8-12 days post-infection for TMK1; and 15-18 days post-infection for MKN74). C, Phase distribution of cell cycle in CDX-transduced MKN7 and TMK1 analyzed by flow cytometry. D, Western blotting analysis of cell cycle-related protein expression in MKN7 and TMK1 cells transduced with a retroviral vector carrying CDX2 or CDX1 sequence. E, RT-PCR analyses of six intestinal differentiation marker genes and *GADPH* (internal control) in MKN7 and TMK1 cells, which were stably transduced with a retrovirus vector carrying CDX (CDX2 or CDX1). Expression of CDX2, CDX1, and β -actin (internal control) was evaluated by western blotting. F, RT-PCR analyses of five genes related to stemness (*LGR5*, *DCLK1*, *SALL4*, *STBL1*, and *STBL2*) and two genes related to gastric differentiation (*TFF2* and *SPARC*) in the same MKN7 and TMK1 cells with exogenous CDX (CDX2 or CDX1) expression



S-phase populations and a concomitant increase in G0/G1-phase populations, indicating that the transduced CDX gene led to G0-G1 growth arrest in both cell lines. Western blot analyses of cell cycle-related proteins showed that expression of cyclin E2, CDK4 and p27/Kip1 was markedly decreased in both CDX-transduced cell lines (Figure 2D). In contrast, reduction of cyclin D3, CDK2 and p21/Waf1/Cip1 was observed in MKN7 only, and reduction of cyclin A2 and cyclin B1 was observed in TMK1 only (Figure 2D). Although there was some difference between the two CDX-deficient gastric cancer cell lines, expression of several cell cycle-related proteins was mostly decreased after exogenous expression of CDX. This was probably as a result of suppressed intracellular metabolism and reduced protein production, which simultaneously occurred with G0/G1 growth inhibition. We speculate that these are the reasons why p27 in CDX-transduced MKN7/TMK1 and p21 in CDX-transduced MKN7 were reduced, both of which usually increase along with G0/G1 growth arrest.

We further carried out a senescence-associated β -galactosidase assay (Figure S1) and two independent assays to detect apoptosis (cleaved caspase-3 immunohistochemistry in Figure S2A and TUNEL assays in Figure S2B), but we could not detect any difference between the CDX-transduced MKN7/TMK1 and the mock-infected control cells. It is indicated that severe growth suppression observed in CDX-transduced MKN7 and TMK1 was neither due to apoptosis nor rapid senescence.

3.4 | Differentiation status is considerably changed in CDX-deficient gastric cancer cells after transduction with CDX2/CDX1 expression vector

Reverse transcription-PCR was next carried out to evaluate transcription of several genes related to intestinal differentiation.^{12,27-30} As shown in Figure 2E, *LI-cadherin* and *KRT20* (*cytokeratin 20*) transcripts were strongly upregulated in CDX-transduced MKN7 and TMK1. Induction of these typical intestinal marker genes indicates that exogenous expression of CDX2 and CDX1 can lead to intestinal differentiation in gastric cancer cells. Furthermore, we also found that other known downstream genes of CDX such as *HEPH*,²⁷ *MDR1*,²⁹ and *DCS2* (*desmocollin2*)³⁰ were similarly transactivated by exogenous expression of CDX2 and/or CDX1 (Figure 2E). Although we could not detect activation of *MUC2* transcription,³¹ we concluded that retrovirally transduced CDX2 and CDX1 certainly

functioned as transcription factors^{12,14,28} and induced intestinal differentiation through transactivation of gut-associated genes.

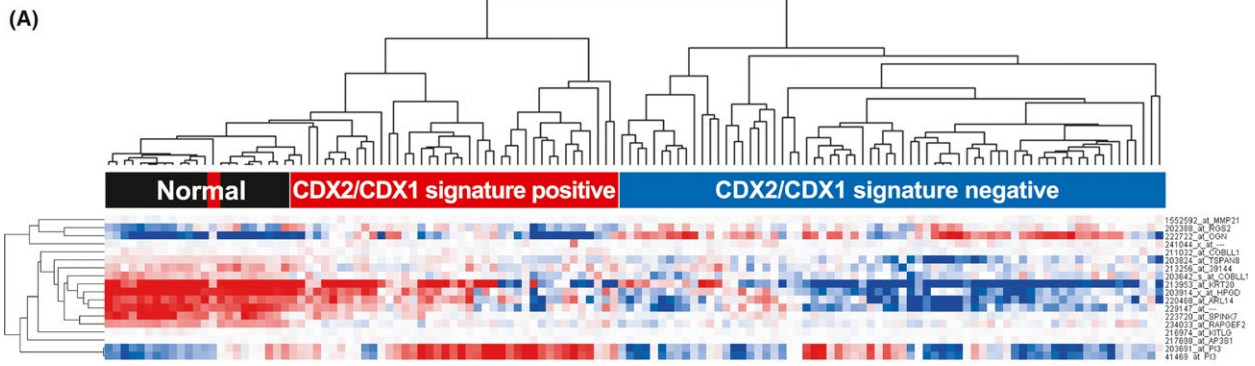
We further carried out RT-PCR to evaluate expression of genes related to stemness and gastric differentiation. Expression of *LGR5* and *DCLK1* (*DCAMKL1*), both of which are well-known marker genes of intestinal stemness,³²⁻³⁴ was induced in CDX-transduced MKN7 cells (Figure 2F). Although such induction was not detected in CDX-transduced TMK1 cells, possible transactivation of intestinal stemness-related genes by CDX should be worthy of note. Furthermore, we found that expression of several stemness-related genes such as *Sall4*,³⁵ *Satb2*,³⁶ and *Satb1*³⁶ was upregulated in response to exogenous expression of CDX (Figure 2F), although the meaning of induced stemness in gastric cancer cells remains unclear. Regarding expression of gastric marker genes, *TFF2/SP* (trefoil factor 2)^{37,38} was downregulated in MKN7 cells, and *SPARC* (osteonectin)^{39,40} was downregulated in TMK1 cells. Changed status of differentiation in CDX-transduced gastric cancer cells must have some association with the observed severe G0/G1 growth arrest in them.

To investigate the altered gene expression accompanied by CDX-induced growth arrest more broadly, mRNA microarray analyses using CDX-transduced MKN7 and TMK1 cells were carried out (Tables S1 and S2 for upregulated genes; Tables S3 and S4 for downregulated genes). From the view of gut differentiation, microarray data expectedly showed that exogenous CDX induced several intestinal marker genes such as *KRT20*,²⁸ *DSC2*,³⁰ *TSAPN8*,⁴¹ *CEACAM6*,⁴² and *MME* (*CD10*)⁴³ (Tables S1 and S2). Microarray data also showed that several gastric marker genes such as *TFF2/SP*,^{37,38} *TFF1/pS2*,⁴⁴ *KCNJ15*,⁴⁵ *SPARC*,^{39,40} and *CDA* (cytidine deaminase)⁴⁶ were contrastively downregulated (Tables S3 and S4). These results reinforced our hypothesis that rapidly induced "intestinal differentiation" accompanied by weakened "gastric differentiation" is a critical mechanism underlying the G0-G1 growth arrest of CDX-transduced MKN7 and TMK1.

3.5 | Signature genes related to CDX expression based on our transcriptome analysis clearly categorize gastric tissues into non-cancerous mucosa, "CDX signature"-positive cancer, and "CDX signature"-negative cancer

To examine the meaning of CDX expression in gastric cancer, the signatures related to CDX2/CDX1 expression were analyzed using

FIGURE 3 Hierarchical clustering and gene set enrichment analysis (GSEA) of 111 gastric cancer samples and 21 non-cancerous gastric tissues by 18 selected genes based on our transcriptome analysis of MKN7 and TMK1 gastric cancer cells stably transduced with a retrovirus vector carrying caudal-type homeobox (*CDX*)2 and *CDX*1. A, Hierarchical clustering of 132 gastric tissues by 18 selected signature gene probes, which showed more than threefold up-/downregulation in both TMK1 and MKN7 with retroviral introduction of both *CDX*2 and *CDX*1. Black area denotes the 21 non-cancerous gastric tissues, whereas non-black (red or blue) area denotes the 111 gastric cancer tissues. Non-black area can be clustered into two groups: red area consisting of "CDX2/CDX1 signature"-positive gastric cancer cases and blue area consisting of "CDX2/CDX1 signature"-negative gastric cancer cases. B, Top 25 gene sets enriched in gastric cancer tissues being positive for "CDX2/CDX1 signature" (left) and those being negative for "CDX2/CDX1 signature" (right). C, Four typical gene sets enriched in gastric cancer tissues being positive for "CDX2/CDX1 signature". D, Eight typical gene sets enriched in gastric cancer tissues being negative for "CDX2/CDX1 signature"

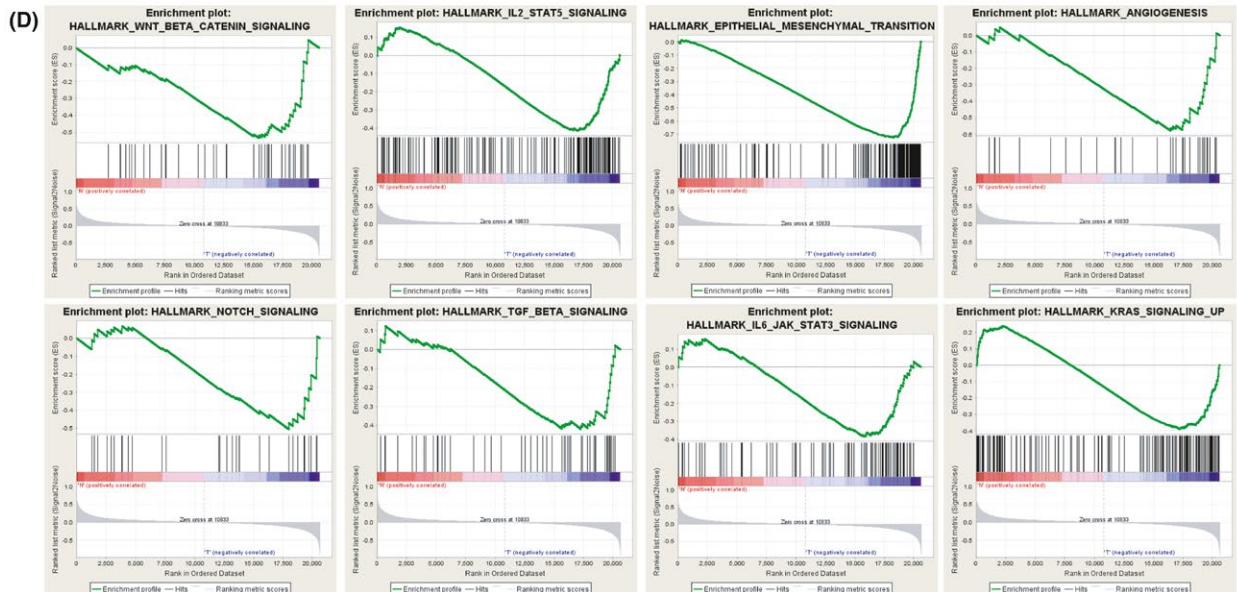
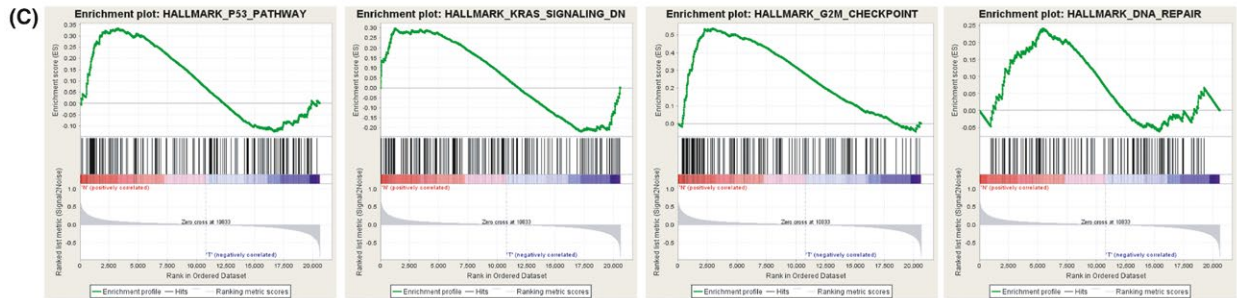


(B) Gene sets enriched in CDX2/CDX1 signature positive gastric cancer

NAME	SIZE	ES	NES	NOM p-val	FDR q-val
HALLMARK_CHOLESTEROL_HOMEOSTASIS	71	0.616	1.672	0.000	0.208
HALLMARK_PEROXISOME	98	0.491	1.626	0.000	0.153
HALLMARK_OXIDATIVE_PHOSPHORYLATION	188	0.468	1.593	0.093	0.143
HALLMARK_GLYCOLYSIS	186	0.461	1.586	0.016	0.113
HALLMARK_FATTY_ACID_METABOLISM	150	0.460	1.538	0.000	0.130
HALLMARK_XENOBIOTIC_METABOLISM	194	0.486	1.511	0.000	0.132
HALLMARK_ESTROGEN_RESPONSE_LATE	194	0.501	1.430	0.000	0.214
HALLMARK_MTORC1_SIGNALING	183	0.480	1.408	0.109	0.226
HALLMARK_BILE_ACID_METABOLISM	110	0.442	1.387	0.020	0.245
HALLMARK_ANDROGEN_RESPONSE	94	0.416	1.351	0.058	0.272
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY	46	0.372	1.336	0.133	0.269
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	104	0.393	1.288	0.175	0.316
HALLMARK_ESTROGEN_RESPONSE_EARLY	183	0.370	1.243	0.074	0.359
HALLMARK_MYC_TARGETS_V1	173	0.437	1.212	0.383	0.393
HALLMARK_PROTEIN_SECRETION	88	0.353	1.208	0.241	0.373
HALLMARK_HEME_METABOLISM	364	0.295	1.197	0.182	0.363
HALLMARK_MYC_TARGETS_V2	47	0.511	1.166	0.350	0.391
HALLMARK_P53_PATHWAY	182	0.333	1.128	0.328	0.415
HALLMARK_SPERMATOGENESIS	124	0.345	1.128	0.297	0.394
HALLMARK_G2M_CHECKPOINT	178	0.537	1.125	0.403	0.379
HALLMARK_E2F_TARGETS	173	0.585	1.122	0.393	0.365
HALLMARK_PANCREAS_BETA_CELLS	36	0.460	0.963	0.522	0.576
HALLMARK_KRAS_SIGNALING_DN	188	0.297	0.926	0.659	0.610
HALLMARK_DNA_REPAIR	145	0.242	0.839	0.672	0.697
HALLMARK_MITOTIC_SPINDLE	185	0.264	0.833	0.667	0.678

Gene sets enriched in CDX2/CDX1 signature negative gastric cancer

NAME	SIZE	ES	NES	NOM p-val	FDR q-val
HALLMARK_WNT_BETA_CATENIN_SIGNALING	40	-0.536	-1.560	0.038	0.756
HALLMARK_APICAL_JUNCTION	193	-0.495	-1.453	0.000	0.770
HALLMARK_MYOGENESIS	196	-0.671	-1.449	0.000	0.516
HALLMARK_ALLOGRAFT_REJECTION	191	-0.512	-1.433	0.208	0.424
HALLMARK_HEDGEHOG_SIGNALING	35	-0.583	-1.422	0.019	0.360
HALLMARK_UV_RESPONSE_DN	135	-0.555	-1.407	0.125	0.335
HALLMARK_IL2_STAT5_SIGNALING	182	-0.414	-1.369	0.083	0.344
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	192	-0.726	-1.336	0.022	0.356
HALLMARK_ANGIOGENESIS	34	-0.580	-1.257	0.240	0.449
HALLMARK_NOTCH_SIGNALING	29	-0.505	-1.234	0.170	0.449
HALLMARK_TGF_BETA_SIGNALING	50	-0.420	-1.218	0.226	0.434
HALLMARK_APOPTOSIS	159	-0.332	-1.200	0.174	0.432
HALLMARK_HYPOXIA	188	-0.372	-1.185	0.240	0.434
HALLMARK_IL6_JAK_STAT3_SIGNALING	85	-0.383	-1.139	0.267	0.467
HALLMARK_ADIPOGENESIS	184	-0.323	-1.138	0.333	0.440
HALLMARK_KRAS_SIGNALING_UP	192	-0.390	-1.137	0.222	0.413
HALLMARK_INFLAMMATORY_RESPONSE	189	-0.396	-1.105	0.273	0.441
HALLMARK_APICAL_SURFACE	42	-0.360	-1.096	0.276	0.431
HALLMARK_TNFA_SIGNALING_VIA_NFKB	95	-0.359	-0.998	0.442	0.559
HALLMARK_PI3K_AKT_MTOR_SIGNALING	188	-0.214	-0.993	0.513	0.541
HALLMARK_COMPLEMENT	192	-0.300	-0.987	0.409	0.521
HALLMARK_INTERFERON_GAMMA_RESPONSE	187	-0.390	-0.977	0.525	0.517
HALLMARK_COAGULATION	133	-0.339	-0.958	0.500	0.522
HALLMARK_UV_RESPONSE_UP	150	-0.248	-0.923	0.561	0.556
HALLMARK_INTERFERON_ALPHA_RESPONSE	88	-0.203	-0.458	0.952	0.980



the latest comprehensive gene expression data of 132 gastric tissues (111 gastric cancer tissues and 21 noncancerous gastric tissues). Using our microarray gene expression data of MKN7 and TMK1 with exogenous CDX2 and CDX1 expression, 18 common signature genes were selected based on more than threefold up-regulation or downregulation (common for CDX2 and CDX1) compared with mock-infected control. The 18 selected signature genes for CDX2/CDX1 expression were then applied to the gene expression data of a total of 132 total gastric cancerous/non-cancerous tissues (Figure 3A). Hierarchical clustering showed that this "CDX2/CDX1 (common) signature" can clearly distinguish gastric cancer from non-cancerous gastric mucosa. In addition, 111 gastric cancer lesions were plainly categorized into two categories: positive for "CDX2/CDX1 signature" and negative for "CDX2/CDX1 signature." The gene expression pattern of non-cancerous gastric mucosa is obviously close to that of "CDX2/CDX1 signature"-positive gastric cancer rather than "CDX2/CDX1 signature"-negative gastric cancer (Figure 3A).

We additionally selected 112 signature genes for CDX2 expression and 118 signature genes for CDX1 expression based on more than threefold up/downregulation in CDX-transduced MKN7 and TMK1 cell lines and carried out hierarchical clustering in the same way (Figures S3 and S4). Similar to the "CDX2/CDX1 (common) signature" (Figure 3A), both "CDX2 signature" and "CDX1 signature" can clearly distinguish gastric cancer from non-cancerous gastric mucosa and can also clearly categorize the 111 gastric cancer cases into "CDX signature"-positive and "CDX signature"-negative tumors.

3.6 | Gene set enrichment analysis indicates a significant association between cancer-associated properties and expression status of CDX in gastric cancer

We further carried out gene set enrichment analysis (GSEA) to understand the molecular background of "CDX signature" in gastric cancer.⁴⁷ For "CDX2/CDX1 signature"-positive gastric cancer (left panel of Figure 3B,C), we identified several pathways related to tumor-suppressive function including p53_PATHWAY, KRAS_SIGNALING_DN, G2M_CHECKPOINT, and DNA_REPAIR. For "CDX2/CDX1 signature"-negative gastric cancer (right panel of Figure 3B,D), we conversely identified several oncogenic pathways such as WNT_BETA_CATENIN_SIGNALING, IL2_STAT5_SIGNALING, EPITHELIAL_MESENCHYMAL_TRANSITION, ANGIOGENESIS, NOTCH_SIGNALING, TGF_BETA_SIGNALING, IL6_JAK_STAT3_SIGNALING, and KRAS_SIGNALING_UP. These results could partly explain the meaning of CDX2/CDX1 expression in gastric cancer, which was suggested by hierarchical clustering analyses (Figure 3A, Figures S3 and S4). In short, CDX-positive gastric cancer tends to have fewer malignant features, and CDX-negative gastric cancer conversely tends to have more malignant features.

3.7 | Approximately half of gastric cancer lesions surgically resected neither express CDX2 nor CDX1

To examine expression of CDX in gastric cancer, immunohistochemistry was carried out using 89 randomly selected gastric cancer specimens surgically resected. As shown in Figure 4A, 50.6% of gastric cancer lesions did not express CDX2, and 66.3% of them did not express CDX1. Concerning CDX2, our result was similar to the previous report showing that about half of gastric cancer tissues lack CDX2 expression.²⁰ Expression of CDX1 has not been adequately evaluated so far, but our result indicated that the rate of CDX1-deficient gastric cancer is similarly high (Figure 4A, Table S5). Our immunohistochemical analysis also showed that decrease or absence of CDX expression was observed not only in intestinal-type gastric cancer but also in diffuse-type gastric cancer (Figure 4A, Table S5).

Our data also showed that expression of CDX2 and CDX1 tends to be concomitantly reduced in gastric cancer lesions regardless of histological type (Figure 4B). Of the total 89 gastric cancer cases, as many as 40 lesions (44.9%) expressed neither CDX2 nor CDX1. Correlation coefficients of CDX2 and CDX1 expression were rather high (Figure 4B; all *P* values <0.001), which indicated that coexpression or codeficiency of CDX2 and CDX1 is frequently observed in gastric cancer. The high prevalence of CDX2/CDX1 double-negative tumors suggested that induced intestinal differentiation may be a novel strategy against gastric cancer in the future.

3.8 | Clinicopathological and molecular features of gastric cancer focusing on CDX2/CDX1 expression

Using the TCGA data set, we further analyzed CDX2-positive (*N* = 55), CDX2-negative (*N* = 175), CDX1-positive (*N* = 29), and CDX1-negative (*N* = 201) gastric cancer patients. As shown in Figure 4D, we analyzed associations between the expression status of CDX and 24 various variables and found four clinicopathological/molecular features significantly associated with expression of CDX in gastric cancer.

First, Epstein-Barr virus (EBV)-positivity is negatively associated with CDX-positivity with statistical significance, probably because CDX-positive gastric cancer mostly originated from *H. pylori*-induced chronic gastritis. Second, DNA hypermethylation status is frequently observed in CDX-positive gastric cancer compared with CDX-negative cancer, probably reflecting the fact that chronic infection of *H. pylori* induces intestinal metaplasia of gastric mucosa with high levels of aberrant DNA methylation.⁴⁸ Third, microRNA expression clusters denote significantly different patterns between CDX-positive gastric cancer and CDX-negative gastric cancer, possibly reflecting the transcription of miRNAs regulated by CDX2/CDX1. And, finally, lymphocyte infiltration was more frequently observed in CDX-negative gastric cancer, probably reflecting the abundant lymphocyte infiltration of CDX-negative and EBV-associated gastric cancer.²⁵

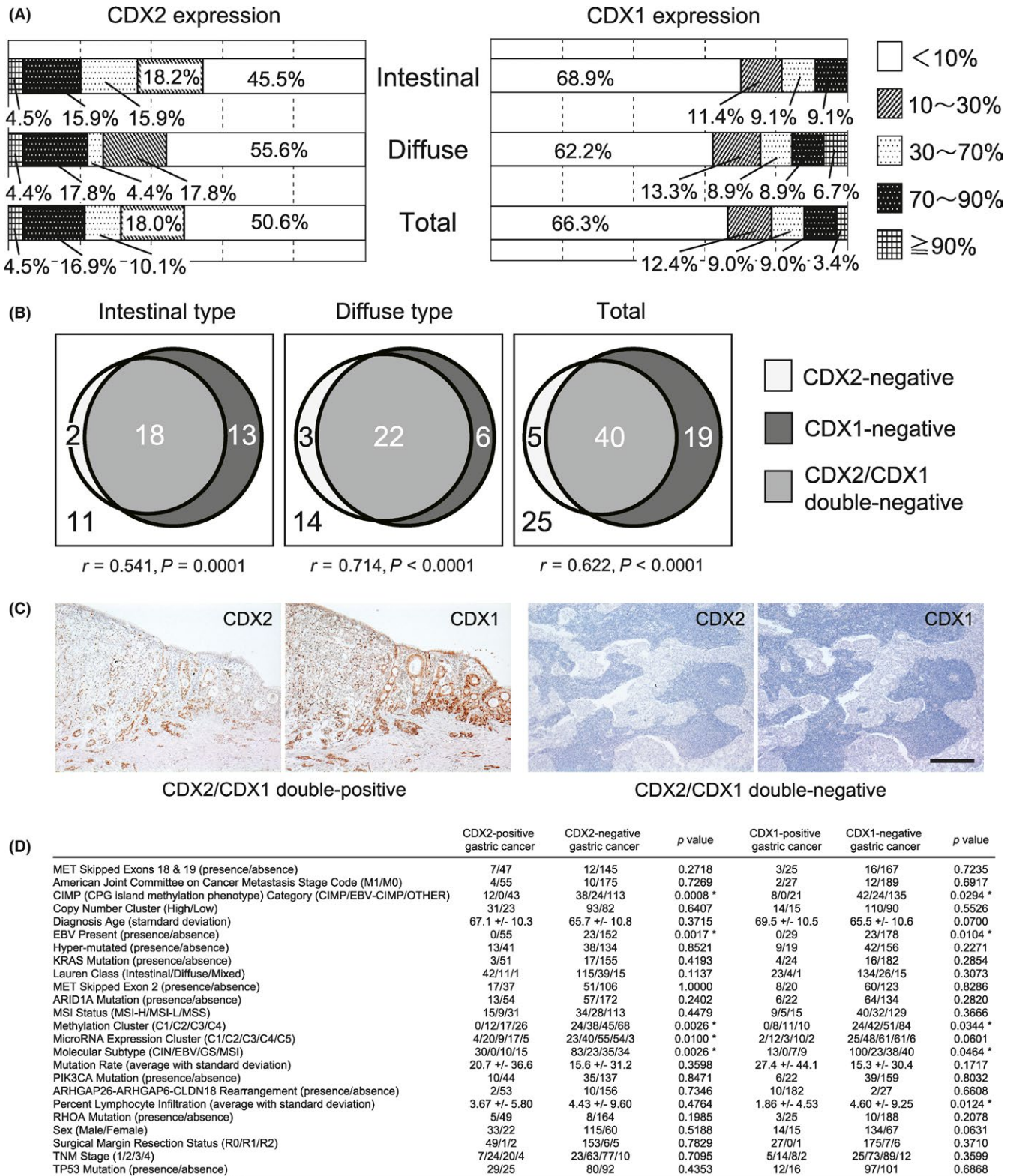


FIGURE 4 Distribution of caudal-type homeobox (CDX)2 and CDX1 expression in the 89 gastric cancer cases surgically resected. A, Five-grade expression pattern of CDX2 and CDX1 in the 89 gastric cancer cases surgically resected (44 cases of intestinal type and 45 cases of diffuse type). B, Venn diagrams showing the relation of CDX2 and CDX1 expression in gastric cancer. Results of Pearson's correlation coefficient (*r* with *P* value) to evaluate the concomitant expression of CDX2 and CDX1 in the 44 intestinal-type lesions, the 45 diffuse-type lesions, and the 89 total gastric cancer samples are shown. C, Typical immunohistochemical images showing concomitant expression and non-expression of CDX2/CDX1 in gastric cancer lesions. D, Clinicopathological and molecular features of CDX-positive and CDX-negative gastric based on the data set of the TCGA

4 | DISCUSSION

Although intestinal metaplasia of gastric mucosa is broadly accepted as a premalignant condition of the stomach,^{8,9} how to understand the progression of ectopic intestinal differentiation is a matter of debate. Abnormal expression of CDX2/CDX1 is an essential trigger of intestinal metaplasia in the stomach,^{8,13,15} but many results including ours (Figure 1) showed that CDX-expressing gastric cancer tends to have less malignant potential and better clinical outcome.^{19,20} Hierarchical clustering and GSEA also indicated that signatures related to CDX expression denote lower malignant features among the gastric cancer cases (Figure 3, Figures S3 and S4). Intestinal metaplasia is typically classified into two categories: mixed gastric-and-intestinal type (incomplete type expressing both gastric and intestinal marker mucin) and solely intestinal type (complete type expressing intestinal but not gastric marker mucin).⁷ Filipe's monumental study reported that the solely intestinal type showed a smaller risk of gastric oncogenesis compared with the mixed gastric-and-intestinal type.⁹ Together, these results suggested that CDX-induced intestinal differentiation may reduce the malignant potential of gastric mucosa.

Taking this into consideration, intestinal metaplasia (ectopic intestinal differentiation) cannot simply be regarded as an unfavorable event. Unstable differentiation status between "gastric" and "intestinal" property probably shows a higher risk of malignancy in the stomach.^{6,9} Therefore, properly accomplished intestinal differentiation could decrease the risk of gastric cancer. In the present study, we discovered that some gastric cancer cells can be treated by forced induction of intestinal differentiation (Figure 2). Of the various strategies against malignancy, regulating the disordered differentiation status of cancer is the smartest one. Because maturation of tumors generally leads to the lower malignant property of cancer, we hypothesize that CDX-induced intestinal differentiation can attenuate the malignant potential of gastric cancer. We hope that a personalized therapy for gastric cancer based on induced intestinal differentiation may be realized in the future.

Many previous studies showed that CDX directly controls transcription of various intestinal marker genes,^{10,12,28,31} which was reproduced in the present study (Figure 2E). In addition, we found a tendency that expression of gastric marker genes is suppressed in CDX-transduced gastric cancer cells (Figure 2F, Tables S3 and S4). Although associations between CDX and transcription of non-intestinal genes are probably indirect, such associations can partly explain the decreased expression of gastric markers accompanied by ectopic CDX expression observed in chronic gastritis.

Our results also suggest that CDX has the potential to promote de-differentiation by induction of stemness-associated genes (Figure 2F, Tables S1 and S2). Recently, Fujii et al⁴⁹ reported that CDX1 confers an intestinal phenotype on gastric epithelial cells based on increased expression of two stemness-associated factors. Simmini et al⁵⁰ reported that CDX2 is essential to maintain identity of the intestinal stem cell. These reports suggested that CDX not only promotes intestinal differentiation but also works to maintain the stemness of the intestine. Such paradoxical functions

of simultaneous differentiation and de-differentiation may be one of the reasons for the controversial aspects concerning CDX expression and gastric malignancy.¹⁸⁻²¹ The meaning of induced stemness in gastric cancer cells remains unclear. It may undesirably function by diminishing intestinal differentiation and consequently leading to instability of the gastric mucosa. Conversely, it may play preferable roles in recovery from the unstable differentiation status and subsequent appropriate reprogramming of the disordered gastric mucosa.

We are now planning to establish the dozens of primary culture cells from gastric cancer lesions surgically resected and will try to examine the effects of CDX transduction on these cells. The difference between CDX-expressing and CDX-deficient primary gastric cancer cells can elucidate the above-described disputable matters^{10,18-20} and also give us more accurate guiding principles concerning our hypothesis. In addition, we are searching new reagents which induce intestinal differentiation in CDX-deficient gastric cancer cells. On the basis of controlling disrupted gastrointestinal differentiation, such reagents may be a novel therapeutic choice against gastric cancer in the future. To avoid the confusing effect regarding differentiation and de-differentiation, we hope to identify novel reagents that can give rise to rigid intestinal differentiation without inducing unstable stemness.

In conclusion, we discovered that exogenous expression of CDX2/CDX1 can lead to G0-G1 growth arrest in CDX-deficient gastric cancer cells, accompanied by induction of intestinal genes and decreased expression of gastric genes. Publicly available databases showed that gastric cancer with CDX (CDX2 and/or CDX1) expression has significantly better clinical outcomes. Both hierarchical clustering of 132 gastric tissues based on our transcriptome analyses and gene set enrichment analyses indicated that "CDX signature"-positive gastric cancer has significantly lower malignant features compared with "CDX signature"-negative gastric cancer. A novel therapy against gastric cancer based on induced intestinal differentiation may be possible in the future.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest for this article.

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REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136:E359-E386.
2. Thrumurthy SG, Chaudry MA, Hochhauser D, Mughal M. The diagnosis and management of gastric cancer. *BMJ*. 2013;347:f6367.
3. Kuipers EJ, Uytterlinde AM, Pena AS, et al. Long-term sequelae of *Helicobacter pylori* gastritis. *Lancet*. 1995;345:1525-1528.
4. Hatakeyama M. Oncogenic mechanisms of the *Helicobacter pylori* CagA protein. *Nat Rev Cancer*. 2004;4:688-694.
5. Yamamichi N, Inada K, Ichinose M, et al. Frequent loss of Brm expression in gastric cancer correlates with histologic features and differentiation state. *Cancer Res*. 2007;67:10727-10735.
6. Konno-Shimizu M, Yamamichi N, Inada K, et al. Cathepsin E is a marker of gastric differentiation and signet-ring cell carcinoma of stomach: a novel suggestion on gastric tumorigenesis. *PLoS ONE*. 2013;8:e56766.
7. Tsukamoto T, Mizoshita T, Tatematsu M. Gastric-and-intestinal mixed-type intestinal metaplasia: aberrant expression of transcription factors and stem cell intestinalization. *Gastric Cancer*. 2006;9:156-166.
8. Almeida R, Silva E, Santos-Silva F, et al. Expression of intestine-specific transcription factors, CDX1 and CDX2, in intestinal metaplasia and gastric carcinomas. *J Pathol*. 2003;199:36-40.
9. Filipe MI, Munoz N, Matko I, et al. Intestinal metaplasia types and the risk of gastric cancer: a cohort study in Slovenia. *Int J Cancer*. 1994;57:324-329.
10. Yamamichi N, Inada K, Furukawa C, et al. Cdx2 and the Brm-type SWI/SNF complex cooperatively regulate villin expression in gastrointestinal cells. *Exp Cell Res*. 2009;315:1779-1789.
11. Kageyama-Yahara N, Yamamichi N, Takahashi Y, et al. Gli regulates MUC5AC transcription in human gastrointestinal cells. *PLoS ONE*. 2014;9:e106106.
12. Hinoi T, Lucas PC, Quirk R, Hanash S, Cho KR, Fearon ER. CDX2 regulates liver intestine-cadherin expression in normal and malignant colon epithelium and intestinal metaplasia. *Gastroenterology*. 2002;123:1565-1577.
13. Silberg DG, Sullivan J, Kang E, et al. Cdx2 ectopic expression induces gastric intestinal metaplasia in transgenic mice. *Gastroenterology*. 2002;122:689-696.
14. Yuasa Y. Control of gut differentiation and intestinal-type gastric carcinogenesis. *Nat Rev Cancer*. 2003;3:592-600.
15. Mutoh H, Sakurai S, Satoh K, et al. Cdx1 induced intestinal metaplasia in the transgenic mouse stomach: comparative study with Cdx2 transgenic mice. *Gut*. 2004;53:1416-1423.
16. Guo RJ, Suh ER, Lynch JP. The role of Cdx proteins in intestinal development and cancer. *Cancer Biol Ther*. 2004;3:593-601.
17. Silberg DG, Swain GP, Suh ER, Traber PG. Cdx1 and cdx2 expression during intestinal development. *Gastroenterology*. 2000;119:961-971.
18. Mutoh H, Sakurai S, Satoh K, et al. Development of gastric carcinoma from intestinal metaplasia in Cdx2-transgenic mice. *Cancer Res*. 2004;64:7740-7747.
19. Liu Q, Teh M, Ito K, Shah N, Ito Y, Yeoh KG. CDX2 expression is progressively decreased in human gastric intestinal metaplasia, dysplasia and cancer. *Mod Pathol*. 2007;20:1286-1297.
20. Mizoshita T, Tsukamoto T, Nakanishi H, et al. Expression of Cdx2 and the phenotype of advanced gastric cancers: relationship with prognosis. *J Cancer Res Clin Oncol*. 2003;129:727-734.
21. Dang LH, Chen F, Knock SA, et al. CDX2 does not suppress tumorigenicity in the human gastric cancer cell line MKN45. *Oncogene*. 2006;25:2048-2059.
22. Naito Y, Oue N, Hinoi T, et al. Reg IV is a direct target of intestinal transcriptional factor CDX2 in gastric cancer. *PLoS ONE*. 2012;7:e47545.
23. Lanczky A, Nagy A, Bottai G, et al. miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients. *Breast Cancer Res Treat*. 2016;160:439-446.
24. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2:401-404.
25. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*. 2014;513:202-209.
26. Yamamichi N, Yamamichi-Nishina M, Mizutani T, et al. The Brm gene suppressed at the post-transcriptional level in various human cell lines is inducible by transient HDAC inhibitor treatment, which exhibits antioncogenic potential. *Oncogene*. 2005;24:5471-5481.
27. Hinoi T, Gesina G, Akyol A, et al. CDX2-regulated expression of iron transport protein hephaestin in intestinal and colonic epithelium. *Gastroenterology*. 2005;128:946-961.
28. Chan CW, Wong NA, Liu Y, et al. Gastrointestinal differentiation marker Cytokeratin 20 is regulated by homeobox gene CDX1. *Proc Natl Acad Sci USA*. 2009;106:1936-1941.
29. Takakura Y, Hinoi T, Oue N, et al. CDX2 regulates multidrug resistance 1 gene expression in malignant intestinal epithelium. *Cancer Res*. 2010;70:6767-6778.
30. Funakoshi S, Ezaki T, Kong J, Guo RJ, Lynch JP. Repression of the desmocollin 2 gene expression in human colon cancer cells is relieved by the homeodomain transcription factors Cdx1 and Cdx2. *Mol Cancer Res*. 2008;6:1478-1490.
31. Mesquita P, Jonckheere N, Almeida R, et al. Human MUC2 mucin gene is transcriptionally regulated by Cdx homeodomain proteins in gastrointestinal carcinoma cell lines. *J Biol Chem*. 2003;278:51549-51556.
32. Barker N, van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature*. 2007;449:1003-1007.
33. Nakanishi Y, Seno H, Fukuoka A, et al. Dcl1 distinguishes between tumor and normal stem cells in the intestine. *Nat Genet*. 2013;45:98-103.
34. Itzkovitz S, Lyubimova A, Blat IC, et al. Single-molecule transcript counting of stem-cell markers in the mouse intestine. *Nat Cell Biol*. 2011;14:106-114.
35. Xiong J, Todorova D, Su NY, et al. Stemness factor Sall4 is required for DNA damage response in embryonic stem cells. *J Cell Biol*. 2015;208:513-520.
36. Savarese F, Davila A, Nechanitzky R, et al. Satb1 and Satb2 regulate embryonic stem cell differentiation and Nanog expression. *Genes Dev*. 2009;23:2625-2638.
37. Farrell JJ, Taupin D, Koh TJ, et al. TFF2/SP-deficient mice show decreased gastric proliferation, increased acid secretion, and increased susceptibility to NSAID injury. *J Clin Invest*. 2002;109:193-204.
38. Peterson AJ, Menheniott TR, O'Connor L, et al. *Helicobacter pylori* infection promotes methylation and silencing of trefoil factor 2, leading to gastric tumor development in mice and humans. *Gastroenterology*. 2010;139:2005-2017.
39. Wang CS, Lin KH, Chen SL, Chan YF, Hsueh S. Overexpression of SPARC gene in human gastric carcinoma and its clinic-pathologic significance. *Br J Cancer*. 2004;91:1924-1930.
40. Zhao ZS, Wang YY, Chu YQ, Ye ZY, Tao HQ. SPARC is associated with gastric cancer progression and poor survival of patients. *Clin Cancer Res*. 2010;16:260-268.
41. Le Naour F, Andre M, Greco C, et al. Profiling of the tetraspanin web of human colon cancer cells. *Mol Cell Proteomics*. 2006;5:845-857.
42. Scholzel S, Zimmermann W, Schwarzkopf G, Grunert F, Rogaczewski B, Thompson J. Carcinoembryonic antigen family members CEACAM6 and CEACAM7 are differentially expressed in normal tissues and oppositely deregulated in hyperplastic colorectal polyps and early adenomas. *Am J Pathol*. 2000;156:595-605.

43. Groisman GM, Amar M, Livne E. CD10: a valuable tool for the light microscopic diagnosis of microvillous inclusion disease (familial microvillous atrophy). *Am J Surg Pathol*. 2002;26:902-907.
44. Ribieras S, Tomasetto C, Rio MC. The pS2/TFF1 trefoil factor, from basic research to clinical applications. *Biochim Biophys Acta*. 1998;1378:F61-F77.
45. Yuan J, Liu W, Karvar S, et al. Potassium channel KCNJ15 is required for histamine-stimulated gastric acid secretion. *Am J Physiol Cell Physiol*. 2015;309:C264-C270.
46. Matsumoto Y, Marusawa H, Kinoshita K, et al. *Helicobacter pylori* infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. *Nat Med*. 2007;13:470-476.
47. Curtis RK, Oresic M, Vidal-Puig A. Pathways to the analysis of microarray data. *Trends Biotechnol*. 2005;23:429-435.
48. Maekita T, Nakazawa K, Mihara M, et al. High levels of aberrant DNA methylation in *Helicobacter pylori*-infected gastric mucosae and its possible association with gastric cancer risk. *Clin Cancer Res*. 2006;12:989-995.
49. Fujii Y, Yoshihashi K, Suzuki H, et al. CDX1 confers intestinal phenotype on gastric epithelial cells via induction of stemness-associated reprogramming factors SALL4 and KLF5. *Proc Natl Acad Sci USA*. 2012;109:20584-20589.
50. Simmini S, Bialecka M, Huch M, et al. Transformation of intestinal stem cells into gastric stem cells on loss of transcription factor Cdx2. *Nat Commun*. 2014;5:5728.

SUPPORTING INFORMATION

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