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ORIGINAL ARTICLE

Lectin drug conjugate therapy for colorectal cancer

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Abstract

Drug resistance represents an obstacle in colorectal cancer (CRC) treatment because of its association with poor prognosis. rBC2LCN is a lectin isolated from *Burkholderia* that binds cell surface glycans that have fucose moieties. Because fucosylation is enhanced in many types of cancers, this lectin could be an efficient drug carrier if CRC cells specifically present such glycans. Therefore, we examined the therapeutic efficacy and toxicity of lectin drug conjugate therapy in CRC mouse xenograft models. The affinity of rBC2LCN for human CRC cell lines HT-29, LoVo, LS174T, and DLD-1 was assessed in vitro. The cytocidal efficacy of a lectin drug conjugate, rBC2LCN-38 kDa domain of pseudomonas exotoxin A (PE38) was evaluated by MTT assay. The therapeutic effects and toxicity for each CRC cell line-derived mouse xenograft model were compared between the intervention and control groups. LS174T and DLD-1 cell lines showed a strong affinity for rBC2LCN. In the xenograft model, the tumor volume in the rBC2LCN-PE38 group was significantly reduced compared with that using control treatment alone. However, the HT-29 cell line showed weak affinity and poor therapeutic efficacy. No significant toxicities or adverse responses were observed. In conclusion, we demonstrated that rBC2LCN lectin binds CRC cells and that rBC2LCN-PE38 significantly suppresses tumor growth in vivo. In addition, the efficacy of the drug conjugate correlated with its binding affinity for each CRC cell line. These results suggest that lectin drug conjugate therapy has potential as a novel targeted therapy for CRC cell surface glycans.

KEYWORDS

colorectal cancer, lectin, lectin drug conjugate, rBC2LCN, targeted therapy

1 | INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide,[1,2] and the 5-year survival rate of patients with stage IV CRC is barely 10%. Unfortunately, drug resistance remains a challenge and is associated with low survival rates in CRC. Thus, new therapeutic options are required, especially for patients with late-stage and drug-resistant CRC.

Abnormal surface glycan expression is a key feature of various types of cancers, including CRC. It is believed to play a key role in tumorigenesis and metastasis and represents an emerging area of investigation with excellent diagnostic and therapeutic potential.[3] We have

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previously shown that the rBC2LCN lectin has a specificity for pancreatic cancer cell surface glycans (fucosylated glycan epitopes of H typess 1/3/4 trisaccharide).[4] rBC2LCN is a tumor necrosis factor-like lectin molecule that binds fucose. It was isolated from the gram-negative bacterium *Burkholderia cenocepacia*.[5] Previously, we conjugated the rBC2LCN lectin with the 38-kDa domain of pseudomonas exotoxin A containing domains Ib, II, and III (PE38) chemotherapeutic.[6] We showed that this rBC2LCN-PE38 fusion protein was both safe and effective in treating pancreatic cancer in mouse models.

Increased levels of fucose are frequently found in the serum and urine of cancer patients, suggesting the presence of increased fucosylation rates in cancer cells.[7,8] In addition, enhanced fucosyltransferase (FUT) expression has been reported in various cancers. FUT are key enzymes that accelerate malignant transformation through fucosylation of different sialylated precursors. Enhanced FUT activity is associated with amplified metastatic potential in various cancer cells through enhancement of epithelial to mesenchymal transition by transforming growth factor-ß.[9,10] FUT also mediate cancer cell migration and metastasis, suggesting that fucosylation may play an important role in disease progression.[11-19]

Based on our previous results, we hypothesized that rBC2LCN could target other cancers that express high levels of fucose. The aim of the present study was to test the affinity of rBC2LCN lectin for different CRC cell lines and to evaluate the therapeutic efficacy and toxicity of lectin drug conjugate therapy for CRC in vivo.

2 | MATERIALS AND METHODS

2.1 | Patient tissue sample collection

A total of 25 human clinical CRC tissues, including 24 CRC tissues and 1 colon adenoma tissue, were collected for the study from patients who underwent colorectal surgery at the University of Tsukuba Hospital, Japan. We obtained the patients' data from clinical and pathological records. Postoperative pathological staging was determined according to the 8th edition of the Union for International Cancer Control tumor node metastasis staging system for CRC. The patient characteristics are summarized in Table S1.

Informed consent was obtained from all participants in the form of an opt-out option, in accordance with the Good Clinical Practice Guidelines of the Ministry of Health and Welfare of Japan. Tumor tissues were not obtained from patients who declined participation. The study protocol was approved by the ethics committee of the University of Tsukuba Hospital (registration no. H28-90).

2.2 | Cell culture and reagents

Four human CRC cell lines were used in this study. HT-29, LoVo, and LS174T cell lines were purchased from ATCC. The identity of all cell lines was confirmed by short tandem repeat (STR) testing. STR DNA profiling aids in the identification of human cell lines derived from individual tissue, ensuring the purity of cultures and preventing cross-contamination. The DLD-1 cell line was obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank (National Institutes ofS Biomedical Innovation, Health and Nutrition). The cell lines were selected based on a previous report describing their histology after establishment as xenografts in nude mice.[20] SUIT-2, a pancreatic cancer cell line without affinity for rBC2LCN,[4] was also obtained from the JCRB Cell Bank for use as a negative control in each experiment. HT-29, LoVo, and LS174T were cultured in McCoy's 5a, F-12K, and E-MEM (all ATCC-formulated media), respectively. DLD-1 and SUIT-2 were cultured in RPMI 1640 and D-MEM (both from FUJIFILM Wako Pure Chemical), respectively. Each medium was supplemented with 10% FBS (Thermo Fisher Scientific) and 1% penicillin-streptomycin (FUJIFILM Wako Pure Chemical).

2.3 | Lectin staining

Histochemical staining: Antigen retrieval in 3-μm slide sections of formalin-fixed and paraffin-embedded (FFPE) tissues was performed by autoclaving for 20 minutes at 120°C. Endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol. HRP-labeled rBC2LCN (1 μg/mL) was applied and incubated for 60 minutes at room temperature, then visualized by diaminobenzidine chromogen (Nichirei Biosciences) application. We classified the pattern of staining as negative, weak positive, or strong positive according to the percentage of positive cells and staining intensity, as described by Wang et al[21] with minor modifications.

Live-cell staining: Live cells were incubated at room temperature for 1 hour in medium containing fluorescein isothiocyanate-labeled rBC2LCN (1 μg/mL); images were captured using a BIOREVO BZX-710 fluorescence microscope (KEYENCE).

2.4 | Lectin affinity assay

The affinity of rBC2LCN for each CRC cell line was analyzed by flow cytometry. Cells were plated and grown for 48 hours in culture and then harvested with trypsin. Live cells were washed and incubated with phycoerythrin-labeled rBC2LCN (1 μg/mL) for 60 minutes on ice in darkness and then washed with buffer (1% BSA/PBS). Typically, 10 000 cells per sample were analyzed on a CytoFLEX (Beckman Coulter), and data were processed using FlowJo software.

2.5 | Protein extraction and lectin microarray

The lectin microarray was performed according to the manufacturer's protocol.[22] After washing three times with PBS, tissue pellets were collected by centrifugation (10 000 *g*, 5 minutes, 4°C) and the protein was extracted using a CelLytic MEM Protein Extraction Kit (Sigma-Aldrich). Protein concentrations were determined with

a Micro BCA Protein Assay Reagent Kit (Thermo Fisher Scientific). Glycoprotein fractions (0.4 μg each) were labeled with Cy3 NHS Mono-reactive Ester (GE Healthcare Life Sciences) using a probing solution in a 100-μL volume, applied to each well of a high-density microarray plate, and then incubated overnight at 20°C. Fluorescence images were then acquired using a Bio-REX Scan200 evanescent-field activated fluorescence scanner (Rexxam). The fluorescence signal of each spot was quantified using Array-Pro Analyzer version 4.5 (Media Cybernetics), mean-normalized, log-transformed, and analyzed via the average linkage method using Cluster 3.0 (yellow: high; black: intermediate; blue: low).

2.6 | In vitro quantitative cell viability assay

We purchased rBC2LCN-PE38 from FUJIFILM Wako Pure Chemical under the trade name: Stem Sure Human Pluripotent Stem Cell (hPSC) Remover. The viability of cells treated with rBC2LCN-PE38 was verified using Cell Counting Kit WST-8 Assay (Dojindo Molecular Technologies). All cell lines were plated at 5000 cells per well in 96-well plates and incubated at 37°C for 48 hours prior to treatment. rBC2LCN-PE38 was diluted in complete medium and added to wells at the indicated concentrations (1-100 000 pg/mL) for 48 hours at 37°C. Cells were then tested for viability with the WST-8 assay reagent as per the manufacturer's instructions. Plates were incubated at 37°C for 2 hours, and the absorbance at 450 nm was measured. Biological and technical triplicates were performed to minimize errors. The data were plotted in Microsoft Excel and GraphPad Prism 6 for curve fitting and the half-maximal inhibitory concentration (IC_{50}) value was interpolated.

2.7 | Mouse xenograft tumor model

Animal experiments were performed in accordance with approval from the Institutional Animal Care and Use Committee and the Regulation for Animal Experiments at the University of Tsukuba. The study also conformed to the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology (Japan).

Colorectal cancer cells (3×10^6) from each cell line were resuspended in PBS (100 μL) and injected subcutaneously into the right flank of 6-week-old female BALB/c athymic nude mice. Tumors were allowed to grow for 10-14 days prior to treatment initiation. rBC2LCN-PE38 was diluted in PBS (1 μg/300 μL/mouse) and administered by intraperitoneal injection a total of six times, on Days 1, 3, 5, 8, 10, and 12. Plain PBS (300 μL/mouse) was also administered to the control group by intraperitoneal injection following the same schedule as the intervention group. Tumor size was measured in two dimensions by digital calipers on the same days, and the volume was calculated using the following formula: $0.5 \times \text{width}^2 \times \text{length}$. The tumor volume on Day 1 was defined as the standard volume (100%), and the change of relative tumor volume (RTV) was recorded on the same schedule as the intraperitoneal injection. Animals were regularly killed on Day 15, and tumors were collected and weighed. Animals were killed in cases in which the tumor volume reached 1000 mm^3 or the tumors developed necrotic ulcerations, even if this occurred before Day 15. Tumor growth curves were plotted in Microsoft Excel.

2.8 | Toxicity evaluation of rBC2LCN-PE38

To evaluate the toxicity of rBC2LCN-PE38 and any adverse responses caused by intraperitoneal injection, the body weight change of all the mice during the experimental period was measured. In addition, whole blood, peritoneum, and 10 major organs, including the heart, lung, liver, kidney, spleen, pancreas, stomach, duodenum, small intestine, and colon, were collected from tumor xenograft mice when they were killed. Hematological parameters were measured, including complete blood count (white blood cells, red blood cells, hemoglobin, platelets, and hematocrit), aspartate aminotransferase, alanine aminotransferase, total bilirubin, amylase, and creatinine. The major organs and peritoneal surface were histologically evaluated using 3-μm slide sections of FFPE tissues.

2.9 | Statistics

The results are presented as the mean (± standard error) for each sample. Data were compared using Student's *t* test. All tests were two-sided, with the level of significance set at $P < 0.05$. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (R Foundation for Statistical Computing). EZR is a modified version of R Commander designed for statistical functions frequently used in biostatistics.[23]

3 | RESULTS

3.1 | Histochemical lectin staining for colorectal cancer patient tissue samples

We observed a positive affinity between rBC2LCN lectin and the CRC cell surface (as evidenced by brown histochemical staining in Figure 1A) in 25 patients. According to the staining intensity, rBC2LCN affinity was strongly positive in 21 patients (84%) and weakly positive in 4 patients (16%), among whom 3 had moderately differentiated adenocarcinomas and 1 had an adenoma.

3.2 | High-density lectin microarray

Figure 1B shows the results of the lectin array binding in each cell line. Of the 96 lectins, 36 (37.5%) showed high-to-intermediate

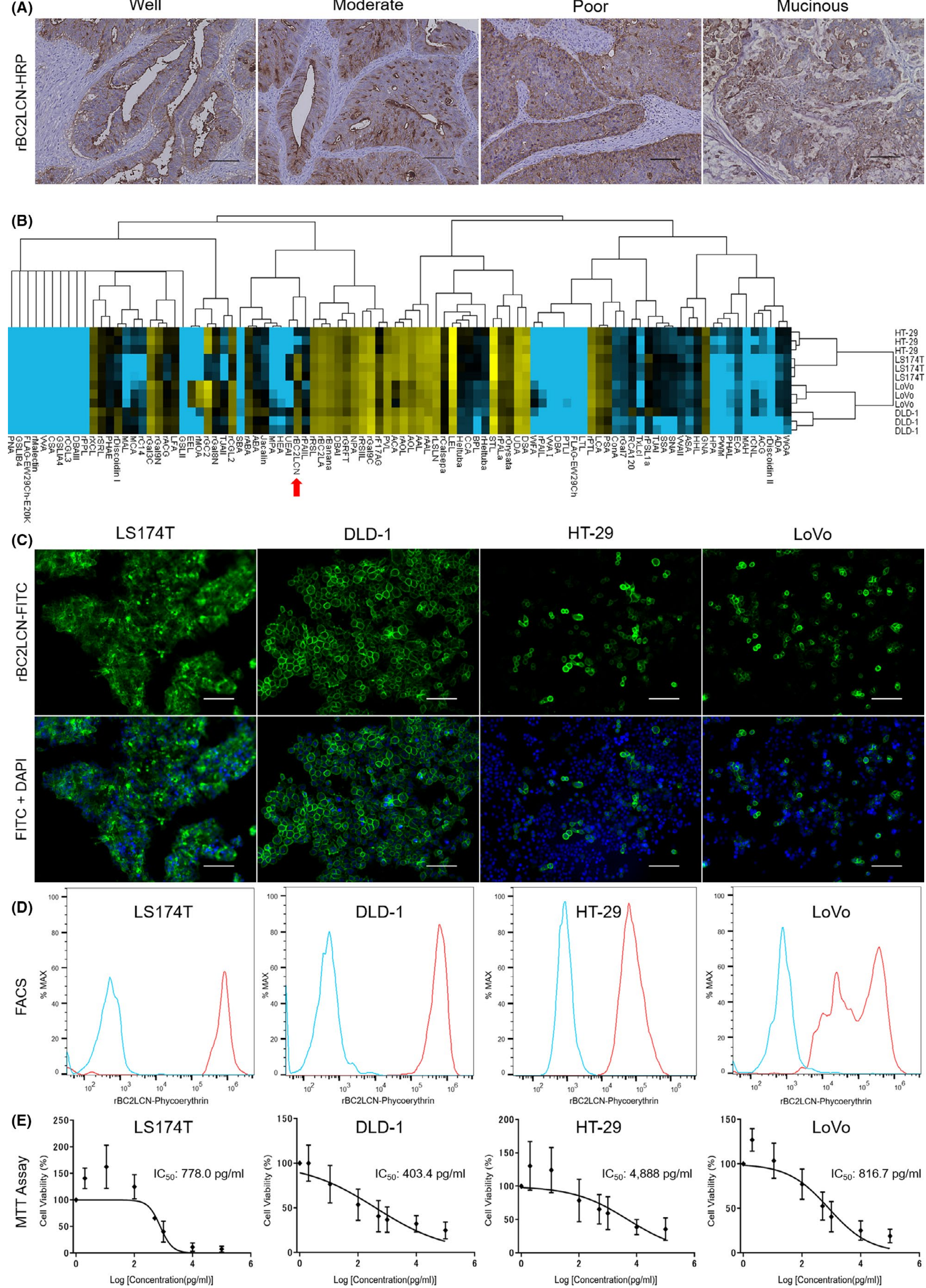
(A)
Well
Moderate
Poor
Mucinous
rBC2LCN-HRP
(B)
HT-29
HT-29
HT-29
LS174T
LS174T
LS174T
LoVo
LoVo
LoVo
DLD-1
DLD-1
DLD-1
rBC2LCN
(C)
LS174T
DLD-1
HT-29
LoVo
rBC2LCN-FITC
FITC + DAPI
(D)
FACS
LS174T
DLD-1
HT-29
LoVo
% MAX
rBC2LCN-Phycoerythrin
(E)
MTT Assay
LS174T
DLD-1
HT-29
LoVo
Cell Viability (%)
IC_{50}: 778.0 pg/ml
IC_{50}: 403.4 pg/ml
IC_{50}: 4,888 pg/ml
IC_{50}: 816.7 pg/ml
Log [Concentration(pg/ml)]

FIGURE 1 Evaluation of the affinity between rBC2LCN and colorectal cancer cell lines in vitro. A, rBC2LCN lectin staining for clinical colorectal cancers showing representative findings of cancers with diverse levels of cell differentiation (scale bar: 100 μm). B, Quantification of lectin ligands present in cell lysates binding to 96 lectins arrayed on a high-density microarray (yellow: high; black: intermediate; blue: low). The red arrow notes the position of rBC2LCN. C, Microscopy images of live-cell staining with FITC-labeled rBC2LCN (1 μg/mL; scale bar: 100 μm). D, Flow cytometric analysis of rBC2LCN-PE (1 μg/mL) binding to live cells. E, Cytocidal effect of rBC2LCN-PE38 on each cell line was evaluated using Cell Counting Kit WST-8 Assay. PE, phycoerythrin

binding in the cell lysates. The remaining 60 showed negative binding. rBC2LCN was included in the positive group.

Different levels of rBC2LCN binding were observed across the cell lines. LS174T and DLD-1 cells demonstrated strong binding signals to rBC2LCN. LoVo cells had moderate binding levels to rBC2LCN. HT-29 cells showed weak binding to rBC2LCN.

3.3 | Lectin affinity assay

Binding of rBC2LCN to each CRC cell line was assessed by live-cell staining (Figure 1C). LS174T and DLD-1 cells were extensively and strongly stained by rBC2LCN, while HT-29 and LoVo were partially stained.

We confirmed cell surface binding with flow cytometry (Figure 1D). All the cell lines were positive for BC2LCN surface binding. However, the level of surface binding differed between the cell lines. As with the microarray and microscopy results, the HT-29 cells had the lowest level of rBC2LCN surface binding in comparison with the other CRC lines.

3.4 | Sensitivity of colorectal cancer cell lines to rBC2LCN-PE38 in vitro

Cell viability assays were performed to evaluate the cytocidal effect of rBC2LCN-PE38 on each CRC cell line (Figure 1E). The IC_{50} values for LS174T, DLD-1, and LoVo were similar, at 778.0, 403.4, and 816.7 pg/mL, respectively. The IC_{50} value for HT-29 was 4888 pg/mL, supporting the low binding results we identified earlier. The SUIT-2 cells, which we have previously shown to not bind rBC2LCN,[6] had an IC_{50} with rBC2LCN-PE38 of 93 161 pg/mL.

3.5 | Histochemical staining for mouse xenograft tumor

To assess whether in vitro rBC2LCN binding patterns were maintained in vivo, we assessed xenograft tumors derived from each CRC cell line for rBC2LCN binding by histochemistry (Figure 2A). Tumors derived from DLD-1, HT-29, and LoVo cells showed poor differentiation, while tumors derived from LS174T cells showed well-to-moderate differentiation. Consistent with our in vitro results, the xenograft tumors derived from LS174T, DLD-1, and LoVo were strongly stained for lectin; while those derived from HT-29 cells were weakly stained.

3.6 | Therapeutic efficacy of rBC2LCN-PE38 in vivo

LS174T-, DLD-1-, and HT-29-derived mouse xenograft models were used for experiments in vivo. LoVo was excluded due to engraftment-related difficulties. The RTV change and tumor weight in each CRC cell line are shown in Figure 2B,C.

3.6.1 | LS174T

The mean RTV on Day 15 in the intervention group was significantly smaller than that in the control group (247% [±18%] vs 589% [±90%] $P = 0.0172$). The mean tumor weight in the intervention group was significantly lower than that in the control group (0.85 g [±0.17 g] vs 1.89 [±0.21 g], $P = 0.00832$; n = 5 each).

3.6.2 | DLD-1

The mean RTV on Day 15 in the intervention group was significantly lower than that in the control group (359% [±105%] vs 1300% [±237%], $P = 0.0128$). The mean tumor weight in the intervention group was significantly lower than that in the control group (0.21 g [±0.03] g vs 0.40 g [±0.05 g], $P = 0.0106$; n = 6 each).

3.6.3 | HT-29

The mean RTV on Day 15 was 324% (±135%) in the intervention group and 578% (±32%) in the control group; no statistically significant differences were observed. The mean tumor weight was 0.49 g (±0.09 g) in the intervention group and 0.49 g (±0.04 g) in the control group; there were no statistically significant differences (n = 5 each).

3.7 | Toxicity evaluation associated with drug administration

Figure 3A-D shows the body weight transition, hematological findings, and histological findings of the collected major organs and the peritoneum. Neither body weight (n = 16 each) nor any hematological parameter (n = 11 each) differed between the intervention and control groups. The creatinine value in the intervention group tended to be slightly higher than that in the control group (0.35 mg/

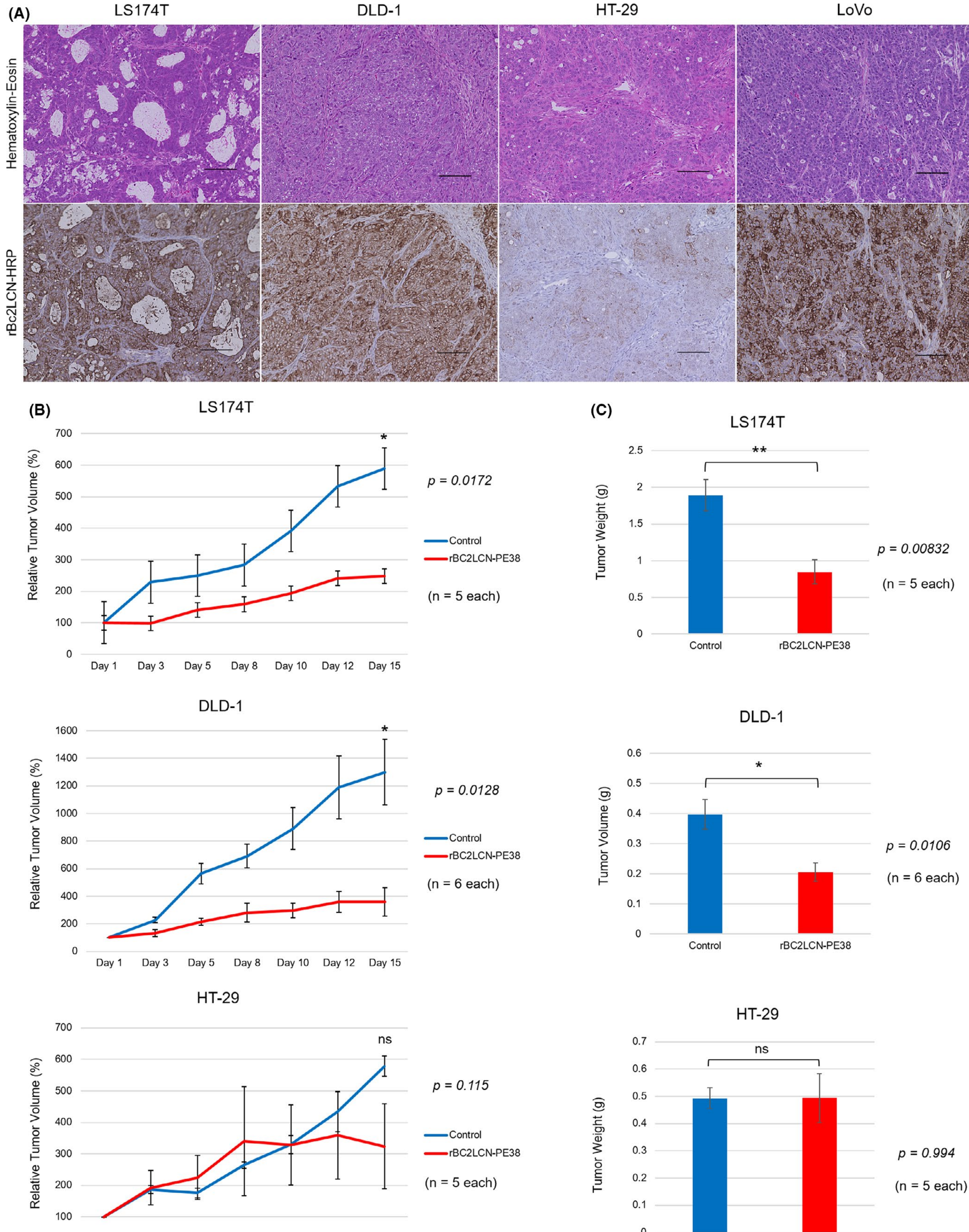
(A)
LS174T
DLD-1
HT-29
LoVo
Hematoxylin-Eosin
rBc2LCN-HRP
(B)
LS174T
Relative Tumor Volume (%)
p = 0.0172
Control
rBC2LCN-PE38
(n = 5 each)
Day 1 Day 3 Day 5 Day 8 Day 10 Day 12 Day 15
DLD-1
p = 0.0128
(n = 6 each)
HT-29
ns
p = 0.115
(n = 5 each)
(C)
LS174T
**
Tumor Weight (g)
p = 0.00832
(n = 5 each)
Control
rBC2LCN-PE38
DLD-1
*
Tumor Volume (g)
p = 0.0106
(n = 6 each)
HT-29
ns
p = 0.994
(n = 5 each)

FIGURE 2 Evaluation of the therapeutic efficacy of rBC2LCN-PE38 in cell line-derived mouse xenograft models in vivo. (A) Findings of histochemical staining for each cell line-derived subcutaneous tumor collected from mouse xenograft models (scale bar: 100 μm). Histological differentiation/rBC2LCN expression were: LS174T, well-to-moderate/strong; DLD-1, poor/strong; HT-29, poor/weak; and LoVo, poor/strong. (B) Change of relative tumor volume during the experimental period. Tumor size was measured in two dimensions by digital calipers, and the volume was calculated using the following formula: $0.5 \times width^2 \times length$. The tumor volume on Day 1 was defined as the standard volume (100%). (C) Excised tumor weight from mouse xenograft models. *$P < 0.05$, **$P < 0.01$. ns, not significant

dL [±0.10 mg/dL] vs 0.16 mg/dL [±0.02 mg/dL]); however, the differences were not significant ($P = 0.07$) and the control group was within the normal range.

No abnormal findings such as inflammatory cell infiltration or tissue damage were observed at the surface of the peritoneum when comparing the intervention and control groups. Histological examination of the major organs collected from the intervention group also showed no abnormal findings.

4 | DISCUSSION

In this study, we demonstrated that rBC2LCN lectin binds to CRC cells and that rBC2LCN-PE38 significantly reduces the growth of tumor xenografts. This indicates that lectin drug conjugate rBC2LCN-PE38 has potential as a novel therapeutic for CRC. In addition, we demonstrated the specificity of tumor killing with rBC2LCN targeting. Using several different methods, we demonstrated that the HT-29 cells have a low level of rBC2LCN binding and were resistant to killing with the drug conjugate. We demonstrated this with a very high IC_{50} value in vitro and with no reduction in tumor growth following in vivo administration. These results suggest that the therapeutic effect of rBC2LCN-PE38 was due to its ability to target and bind the cancer cells. Therefore, because lectin can bind with cell surface glycans, rBC2LCN drug conjugate may be a robust targeted therapy for cancer cell surface glycans detected using rBC2LCN.

rBC2LCN was originally identified as a lectin probe specific for hPSC, such as human embryonic stem cells and human-induced pluripotent stem cells.[24-26] rBC2LCN recognizes unique Fucα1-2Galβ1-3GlcNAc/GalNAc-containing glycans, such as H type-1, H type-3, and Globo-H (H type-4), expressed on undifferentiated cells.[27,28] Fucosylation by FUT1 and FUT2 is known to be crucial for generating H type-1/3/4 trisaccharides,[26] and FUT1/2 also synthesizes the aforementioned hPSC markers, such as SSEA-3/4/5 and Tra-1-60/81.[29] Using this characteristic, an rBC2LCN lectin drug conjugate could specifically eliminate tumorigenic hPSC without killing differentiated cells.[30]

The issue to be discussed is the heterogeneous effect of rBC2LCN-PE38 among the clinical cases. Our previous report analyzing human pancreatic cancer demonstrated that poorly differentiated cancer tissues tended to show a weak affinity for rBC2LCN, whereas moderately or well-differentiated tissues tended to show a strong affinity[4]; however, the affinity of rBC2LCN lectin in CRC seems generally moderate to strong regardless of cancer cell differentiation. In fact, one poorly differentiated human CRC tissue (Figure 1A) and one poorly differentiated human CRC cell line (DLD-1) showed strong affinities for rBC2LCN lectin. We indeed encountered 3 weak staining cases among the 25 clinical cases; however, the diversity of rBC2LCN affinity seems small in CRC, and the majority may be the candidate for rBC2LCN-PE38 therapy. We understand that rBC2LCN affinity (ie, the effect of rBC2LCN-PE38) is heterogeneous across individual cancers; although it might depend on the expression of FUT1/2 (Figure S1), it does not depend solely on tissue differentiation.

Fucosylated antigens are often used as tumor biomarkers for detection and therapeutic monitoring. For example, the level of fucosylated cancer antigen (CA) 19-9 is frequently elevated in patients with CRC (approximately 60%) and is used as a marker of disease.[31] Elevated CA19-9 levels are associated with a lower postoperative survival rate among CRC patients.[32-34] High sLeX expression also signifies poor prognosis in CRC.[35,36] Enhanced FUT activity is associated with increased metastatic potential of CRC cells,[37-39] suggesting that fucosylation and L-fucose may play an important role in disease progression.[40] Mawaribuchi et al[41] reported that prostate cancer cells have rBC2LCN-positive and rBC2LCN-negative subpopulations and that rBC2LCN-positive cancer cells have cancer stem-like and metastatic features. These results suggest that both fucosylation and rBC2LCN positivity relate to stemness. Therefore, rBC2LCN-PE38 may specifically target the most difficult to kill cancer cells, highlighting its therapeutic potential.

In this study, we analyzed the toxicity and adverse response associated with rBC2LCN-PE38 administration. No abnormal findings, such as body weight loss, myelosuppression, hepatic dysfunction, or inflammatory cell infiltration to organs, were observed. Histochemical rBC2LCN staining of the major organs was performed, and except in the kidney, no significant staining was detected. Renal tubular epithelial cells were positive for rBC2LCN staining (Figure 3E), consistent with decreased renal function in the intervention group. Further examination is required to evaluate the adverse effect on renal function.

Lectins generally occur as multimers and because of their multivalency, often form cross-linkages between cells.[42] An example of this occurs during hemagglutination, when lectins interact with sugar moieties on the surface of blood cells. The cross-linking that occurs between them causes the formation of multi-cellular aggregates. We have previously verified that the rBC2LCN lectin does not induce hemagglutination in human erythrocytes of every blood type even at extremely high concentrations.[4] As such, rBC2LCN-PE38 is considered to have potential for human administration.

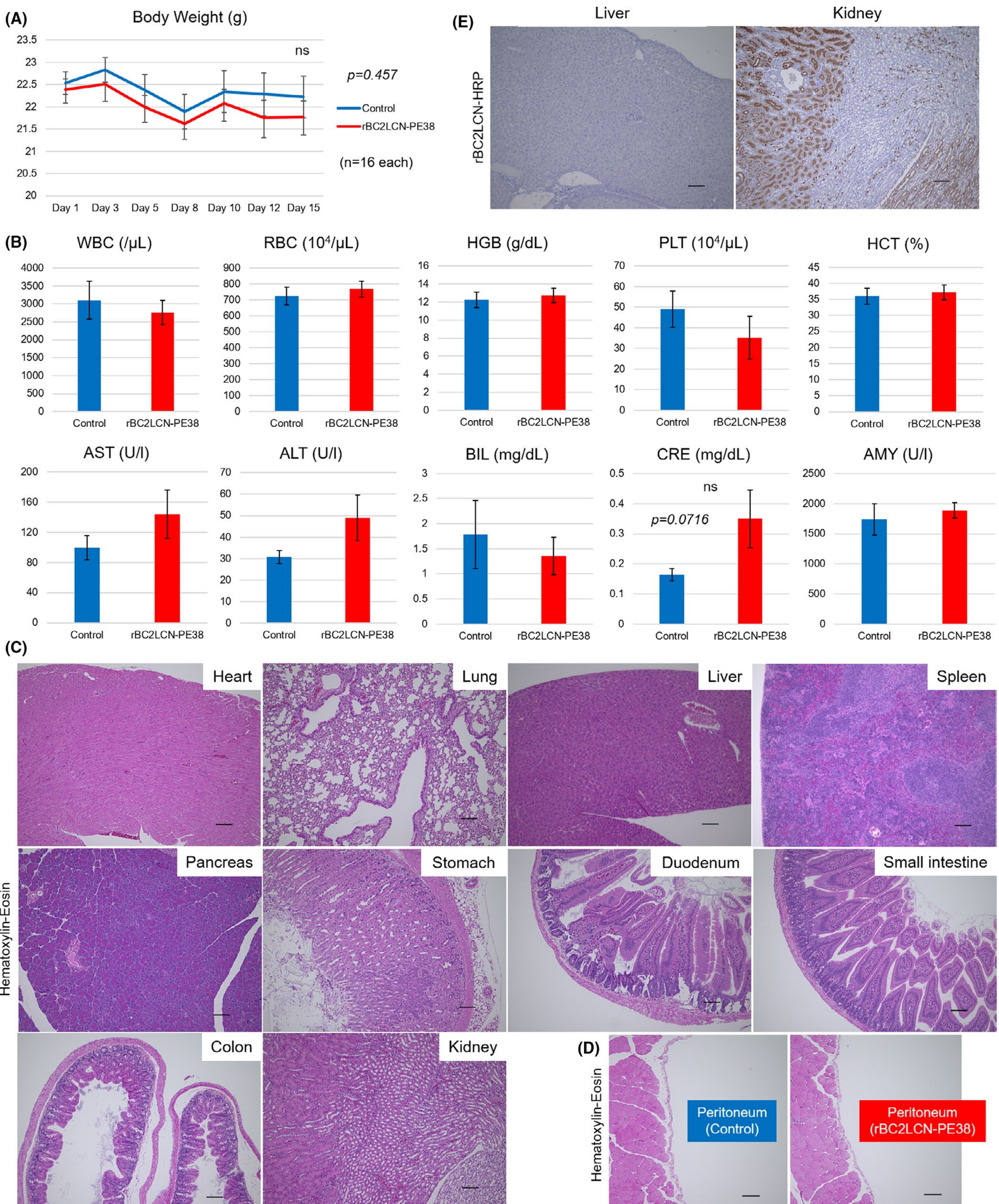

FIGURE 3 Evaluation of toxicity and adverse effects of rBC2LCN-PE38 in in vivo mouse xenograft models. A, Body weight change during experimental period. B, Hematological findings on Day 15. The levels of 10 hematological examination parameters, including complete blood count (white blood cells [WBC], red blood cells [RBC], hemoglobin [HGB], platelets [PLT], and hematocrit [HCT]), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (BIL), amylase (AMY), and creatinine (CRE) were measured. ns, not significant. C, Histological analysis of major organs, including heart, lung, liver, kidney, spleen, pancreas, stomach, duodenum, small intestine, and colon (scale bar: 100 μm). D, Histological analysis of the peritoneum. E, Histochemical staining for rBC2LCN in the major organs (scale bar: 100 μm)

This study has several limitations. First, as it used mouse xenograft models, it is impossible to elaborate on the therapeutic effect and safety of rBC2LCN-PE38 in human CRC patients. Second, because the numbers of CRC cell lines and mouse xenograft models used in the study were not large, the statistical accuracy of our outcome analyses may be limited. However, we expect that the growing incidence of CRC will increase the demand for new therapeutic options, in particular for those with resistance to existing drugs. Future research must focus on the therapeutic effect of rBC2LCN-PE38 when used in combination with existing anticancer drugs as a new regimen and it of when rBC2LCN is conjugated to other chemotherapeutics instead of PE38.

In brief, we used in vivo animal models to demonstrate the first usage of a rBC2LCN lectin drug conjugate as a targeted therapy for CRC. Low toxicity and no adverse responses were observed. This therapy has potential as a targeted therapy for several types of cancer cells that have elevated cell surface expression of H type 1/3/4 glycans that can be detected by rBC2LCN lectin. To evaluate the feasibility of rBC2LCN therapy, preclinical and clinical studies of rBC2LCN for human administration are urgently required.

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DISCLOSURE

All the authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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