Original Article

Analysis of poliovirus receptor, CD155 expression in different human colorectal cancer cell lines: Implications for poliovirus virotherapy

ABSTRACT

Context: Poliovirus (PV) receptor (CD155) is expressed on several kinds of cells and exerts diverse functions. Various investigations have confirmed that changes in CD155 expression in cancer cell lines affect metastasis, proliferation, and migration.

Aims: The purpose of the present study was to investigate the CD155 transcript and protein expression in human colon adenocarcinoma cell lines in comparison to normal fetal human colon (FHC) cells.

Materials and Methods: The CD155 expression level in four human adenocarcinoma cell lines and normal colon cell line were assessed using the SYBR green quantitative real-time polymerase chain reaction (PCR) and flowcytometry.

Results: The results of real-time PCR indicated that CD155 was significantly overexpressed in all human adenocarcinoma cell lines ($P = 0.000$). The highest and the lowest expression level of CD155 messenger RNA was observed in SW480 and HT29 cell lines by 491.14, and 12.04 fold changes, respectively, in comparison with the human normal cell line (FHC). Results of flowcytometry indicate that protein was strongly expressed in cancer cell lines. SW480 cells showed the highest CD155 protein expression level of 98.1%, whereas this protein expression was 1.3% in human normal colon cell line (FHC). Totally, these data indicate that CD155 expression is significantly elevated in cancer cell lines.

Conclusions: The preferential expression of CD155 on cancer cell lines rather than on normal cell line suggests that CD155 could be targeted for future PV virotherapy.

KEY WORDS: CD155, colorectal cancer, flowcytometry, overexpression, poliovirus receptor, real-time polymerase chain reaction

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INTRODUCTION

Colorectal cancer is the third leading frequent cancer in men and the second in women global.[1] Approximately 55% of the cases happen in developed countries.[2] There is a geographical variety in incidence worldwide, and the geographic models are very alike in males and females.[3] The various therapeutic approaches such as chemotherapy, surgery, and radiotherapy, employed separately or in various mixtures do not eliminate tumors or their progression all the time. Advanced molecular biomarkers for recruitment of new cancer virotherapy method are needed as treatment decisions should be based on cell-based biological indicators.

The CD155 protein has been stated to be expressed in many kinds of cells with different functions[4,5] and was recognized as a receptor for polioviruses, which produces the typical flaccid paralysis of poliomyelitis[4,6] Studies have been shown the ubiquitous expression of CD155 in several human tissues, containing the brain, liver, ileum, leukocytes, placenta, and lung.[4,7] CD155 is an immunoglobulin-like molecule.[8] The amino-terminal immunoglobulin region of the fundamental membrane graft alternatives of CD155 works as the poliovirus (PV) required moiety. Receptor coupling results in virion destabilization and cells.[9-13] Selective targeting by PV is most likely determined by the distribution of its cellular

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receptor, the Ig superfamily molecule CD155.[14] CD155 were stated to be included in cell–cell adhesion by a heterophilic through nectin-3. The stimulation of this receptor was shown to repress cell adhesion and become cell movement,[15,16] while the loss of CD155 is leading to the inhibition of migration and induction of cell spreading. Cross-linking of CD155 and extracellular matrix have led to reduced adhesion to fibronectin, a reduction in some focal adhesions, and a rise in migration.[17] Overexpression of CD155 in cancer cells in the absence of ligand probably derives dimerization of CD155 and results in reduced adhesion and raised movement.[16] Downregulation of CD155 in cancer cell lines reduces their movement,[16,18] proliferation,[19] and metastasis.[20] Earlier investigations have determined that representation of CD155 be practically undetectable in nontransformed healthy cells. It can be attributable to the evidence that the promoter for the receptor is sufficient just over a limited period of growth.[21]

Higher expression level of CD155 in the sera of cases with breast, gynecologic, gastrointestinal, and lung cancers in comparison with sera of healthy donors has been reported. In addition, available evidence shows high expression level of CD155 in human cancer tissues.[22] It has been shown that CD155 was broadly expressed in several personal bones and soft-tissue sarcoma cell groups.[14]

The objective of the present research is to represent the quantitative expression level of CD155 in four human colon adenocarcinoma cell lines and its normal counterpart in both messenger RNA (mRNA) and protein level using SYBR green real-time polymerase chain reaction (PCR) and flowcytometer.

MATERIALS AND METHODS

Cell lines

Four human colon adenocarcinoma cell lines including HCT116 (C570), SW480 (C506), HT29 (C466), and Caco-2 (C139) were obtained from National Cell Bank of Iran, and fetal human colon (FHC) (CRL-1831) cell line was obtained from ATCC used in this study. The features of all colon adenocarcinoma cell lines are shown in Table 1.[23] The purpose of using four different cell lines is to investigate CD155 gene and protein expression level and its ability as potential cancer biomarker in cell lines with different stages of cancer from A to D. All cell culture mediums were supplemented through 10% heat-inactivated fetal bovine serum (Gibco, Sigma-Aldrich, Germany) and penicillin (100 units/ml), and streptomycin (0.1 mg/ml) (Gibco, Sigma-Aldrich, Germany). The suitable medium for HCT116 and Caco-2 Cell lines is Dulbecco's modified eagle's medium (DMEM): F12 and for SW480 and HT29 cell lines are RPMI 1640 containing sodium bicarbonate and L-glutamine (Gibco, Germany). An additional 10 mM HEPES (for a final conc. of 24 mM), 10 ng/ml cholera toxin, 0.005 mg/ml transferrin, 0.005 mg/ml insulin, and 100 ng/ml hydrocortisone were added to FHC cell culture medium (DMEM: F12). All cells were grown at 37°C under humidified 5% CO_2 atmosphere. Cancer cell lines are passaged twice a week. However, the normal cell line (FHC) was passaged once per 14 days (because of slow doubling time).

Extraction of total RNA and SYBR green real-time polymerase chain reaction

Total RNA was extracted from 3×10^6 of each cell lines according to the TRI Reagent (Sigma-Aldrich, Germany) protocol, and reverse transcriptase (RT) was done with Revert Aid First Strand complementary DNA Synthesis Kit from Thermo Scientific (Lithuania) using 4 μg of RNA as template and 1 μl of random hexamer primer in a reaction volume of 12 ml. The mixture was heated to 65°C for 5 min and then allowed to cool on ice. Four microliters of 5x reaction buffer, 2 μl of 10 mM dNTP mix, 1 μl of RT Revert Aid M-MuLV RT (200 U/μl), and 1 μl of Ribo Lock RNase Inhibitor (20 U/μL) were added and the reaction incubated for 5 min at 25°C followed by 60 min at 42°C. The reaction was stopped by heating at 70°C for 5 min and the RT stocks frozen at −80°C. SYBR green real-time quantitative PCR analysis was carried out using an ABI Prism 7300 sequence detector system (Applied Biosystems, USA). The SYBR green real-time quantitative PCR condition was as follows: add 4 μg of RT stock and mixed with 12.5 μl of RealQ Plus 2x Master Mix Green (High Rox) Ampliqon (Denmark), 0.2 pmol of each primer and water to a total volume of 25 μl. Beta-actin gene was used as an endogenous housekeeping gene for normalization. All primer sets were listed in Table 2[24] and purchased from Bioneer (South Korea). The primers used to quantify expression of CD155 gene were located in a region corresponding to the extracellular domain of the protein, which is conserved in all spliced variants. Real-time quantitative PCR Cycling conditions were as follows: an initial step at 95°C for 15 min, followed by forty cycles of 95°C for 15 s, and 60°C for 30 s. All assays were performed in triplicate and repeated three times.

Detection of CD155 protein expression in adenocarcinoma and normal human colon cell lines by flowcytometry

All cell lines were trypsinized, and counted as $5–10 \times 10^5$ cells/tube was suitable for flowcytometry analysis.

Table 1: Features of four different colon adenocarcinoma cell lines studied

Cell line	Patient	Organ	Stage
Caco-2	72-year-old male	Colon	C
HCT116	48-year-old male	Colon ascendens	D
HT29	44-year-old female	Colon	C
SW480	50-year-old male	Colon	B

Table 2: Primer sequences and their nucleotide binding site for expression of CD155 using real-time polymerase chain reaction

Primer name	Sequence	Nucleotide
Pol/F	5'-TGGACGGCAAGAATGTGACC-3'	940–959
Pol/R	5'-ATCATAGCCAGAGATGGATACC-3'	1055–1034
β-actin/F	5'-GTCTGCCTTGGTAGTGGATAATG-3'	120–142
β-actin/R	5'-TCGAGGACGCCCTATCATGG-3'	203–222

The pellets were washed with 1 ml of phosphate-buffered saline (PBS) and homogenized by pipetting. Each 500 µl of the cell suspension was placed in separate tube and centrifuged for 3 min at 664 xg. Supernatant was discarded, and pellets in the first tube were suspended in 300 µl cell staining buffer supplemented with 3 µl of phycoerythrin (PE) antihuman CD155 (PV receptor [PVR]) antibody (BioLegend, San Diego, CA, USA), and the second tube was suspended in 300 µl cell staining buffer supplemented with 3 µl of PE Mouse IgG2a, κ Isotype Control (BioLegend, San Diego, CA, USA). Tubes were incubated in dark chamber for 20 min. The cell suspension was washed twice with 1000 µl of PBS, centrifuged for 3 min at 664 xg, and then cell pellets were suspended with 200 µl of PBS and analyzed by Flow Cytometer (BD Accuri C6).

Statistical analysis

Comparison of CD155 expression level on different human colon cancer and normal cell lines was analyzed by two-way analysis of variance (*post hoc*) test. Statistical significance was determined to be $P < 0.05$. All statistical analyses were performed using SPSS.22 (IBM SPSS Statistics V22.0, IBM United States Software).

RESULTS

Expression of CD155 mRNA is upregulated in human colorectal cancer cell lines than normal cell line.

Expression of the CD155 gene was significantly ($P = 0.000$) higher in all human colon cancer cell lines compared with the normal cell line as illustrated in Table 3. Quantification of obtained signals in real-time PCR indicated that there were a 491.14, 461.44, 24.25, and 12.04 fold increases in CD155 mRNA expression in SW480, HCT116, HT29, and Caco-2 cell lines, respectively, compared with normal cell line (FHC). Comparison chart of CD155 gene expression in colorectal cancer and normal cell line is shown in Figure 1. As it shown, the highest expression level of CD155 mRNA was observed in SW480 cell line by 491.14 fold changes, while the lowest expression was belonged to HT29 cell line by 12.04 fold changes in comparison with human normal FHC cell line.

Logarithmic fold change among human colon adenocarcinoma cell lines showed that SW480 cell line has the highest (2.69) expression of CD155 gene Log_{10} fold in comparison with three other human colon adenocarcinoma cell lines ($P = 0.000$). HCT116 was shown significantly the next highest (2.66) expression of CD155 gene log 10-fold ($P = 0.000$), and the lowest Log 10-fold change in CD155 gene expression was significantly belong to Caco-2 (1.38) ($P = 0.026$) and HT29 (1.08) ($P = 0.009$) cell lines, respectively [Figure 2].

CD155 protein expression level is higher on colon adenocarcinoma cell lines in comparison to normal cell line

To examine CD155 protein expression across four different human colon adenocarcinoma cell lines and a normal

Table 3: Expression of CD155 in different adenocarcinoma and normal human colon cell lines using SYBR green real-time polymerase chain reaction

Cell lines	Normalized gene expression	Fold change	SD	Log_{10} of fold change
FHC	1.31	1	0	0
SW480	648.06	491.1432	0.056569	2.691208
HCT116	608.87	461.4402	0.070711	2.664115
Caco-2	32	24.25147	0	1.384738
HT29	15.88	12.04197	0.692965	1.080698

Normalized gene expression=$2^{-\Delta CT}$, Fold change=$2^{-\Delta\Delta CT}$. SD=Standard deviation, FHC=Fetal human colon

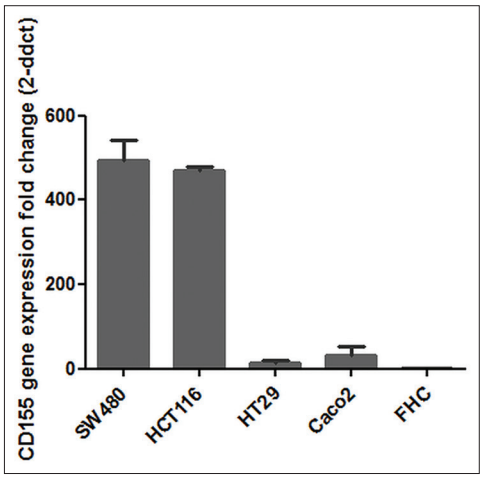

Figure 1: Comparison of CD155 messenger RNA expression fold changes in four human adenocarcinoma cell clines and normal colon cell line (fetal human colon) using SYBR green real-time polymerase chain reaction. Bars represent the mean ± standard error of the mean. ***Indicates $P = 0.0001$. *Post hoc* test is applied

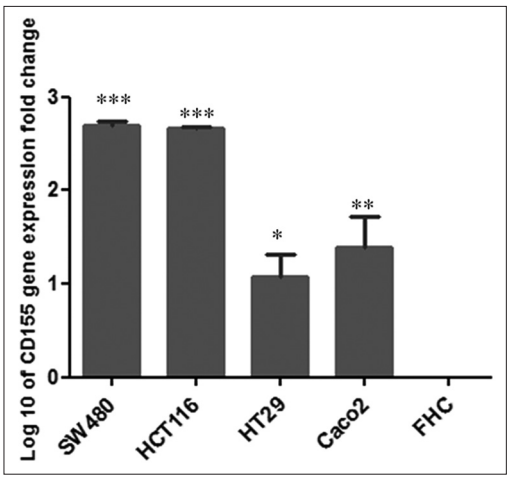

Figure 2: Comparison of log 10 of CD155 messenger RNA expression fold changes in four human adenocarcinoma cell clines and normal colon cell line (fetal human colon) using SYBR green real-time polymerase chain reaction. Bars represent the log 10 mean ± standard error of the mean. ***Indicates $P = 0.0001$, **Indicates $P = 0.001$, and *Indicates $P = 0.0001$ *post hoc* test is applied

human colon cell line as a control, flowcytometry analysis has performed. CD155 was detected by PE antihuman PVR CD155 monoclonal antibody, and an extensive level of expression was observed in adenocarcinoma cell lines. CD155 protein was expressed on 98.1%, 96.7%, 87.7%, and 57.9% of SW480, HCT116, HT29, and Caco-2 cell lines, respectively, whereas the CD155 protein was expressed 1.3% in normal FHC cell line [Figure 3]. Collectively, these data indicate a frequently

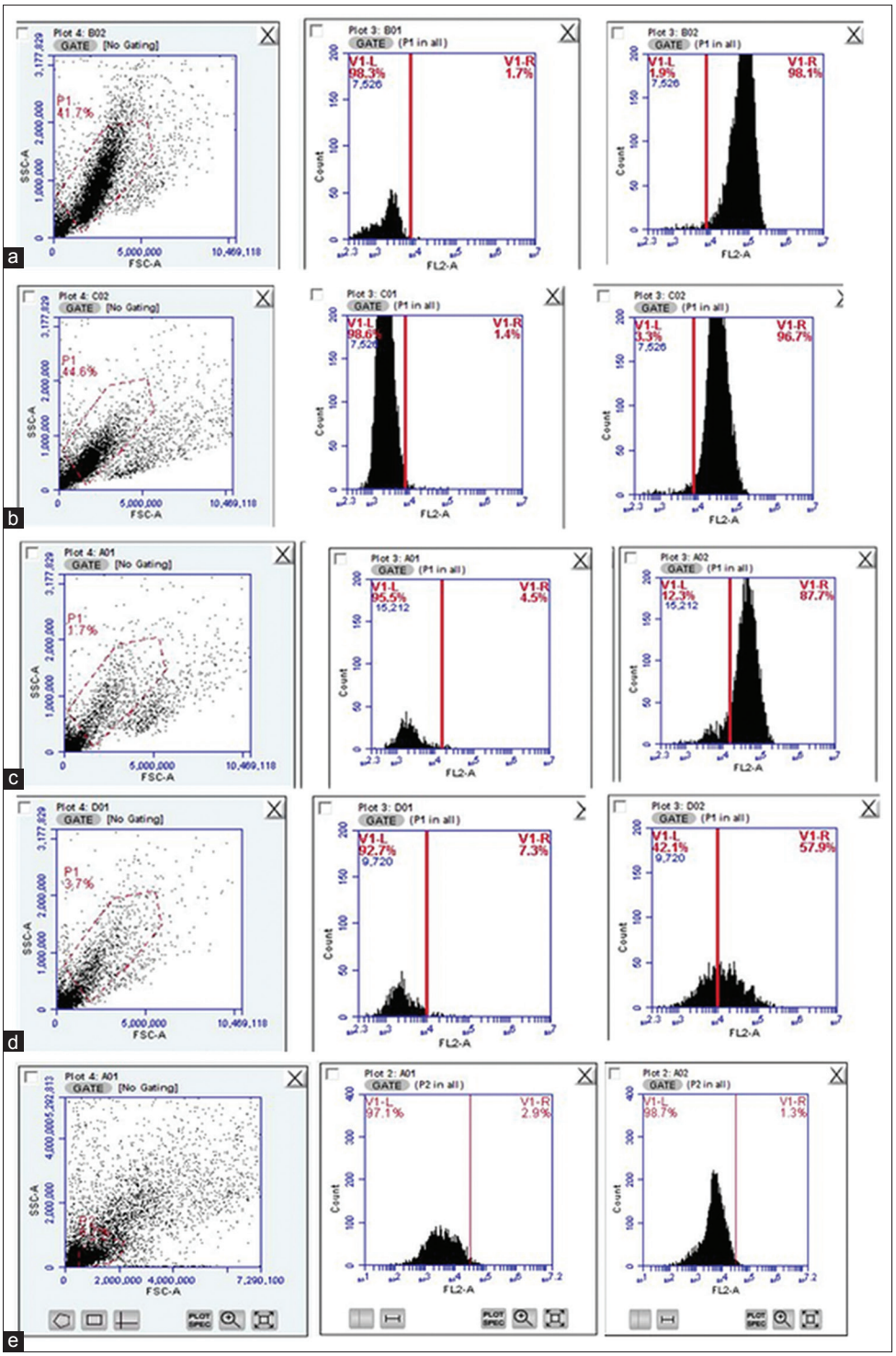

Figure 3: Flowcytometry analysis of CD155 protein in adenocarcinoma and normal human colon cell lines; (a) SW480 cell line, (b) HCT116 cell line, (c) HT29 cell line, (d) Caco-2 cell line, and (e) fetal human colon cell line. In each case, the left panel shows the distribution of fluorescence from individual cells plotted as a dot plot; and the middle and right histogram panels show images obtained from phycoerythrin mouse IgG2a, κ isotype control using FL2-A channels background, and phycoerythrin antihuman poliovirus receptor CD155 monoclonal antibody, respectively. FSC: Forward scatter. SSC: Side scatter

elevated CD155 expression in the primary adenocarcinoma cell lines.

DISCUSSION

The immunoglobulin-like surface molecule, CD155 expression, is common in cell lines established from ectodermal/neuroectodermal tumors.[25] It has been stated for glial,[21,26,27] colorectal,[24] lung,[28] breast,[29] and hepatocellular carcinoma[30] cancers through the privilege of selected lymphoma cell lines.[31]

Overexpression of CD155 was reported in cultured breast cancer cell line using western blotting and immunohistochemistry, by our result, they have reported an upregulation of the CD155 expression.[29]

Bioinformatics evaluation of the SAGE and EST library databases maintained through the Cancer Genome Anatomy Project (http://www.cgap.nci.nih.gov) using the unique identifier AACCACCCAG moreover promotes the idea that expression of the CD155 gene might be elevated in various cancer types containing brain, colon, kidney, lung, stomach, and pancreas.[16]

The results of this study revealed that CD155 gene overexpressed at both mRNA and protein stages in different colorectal cancer cell lines originated from different stages of cancer[23] (SW480, HCT116, HT29, and caco-2) in comparison with the normal colon cell line (FHC). As previously reported the CD155 level is significantly higher in patients with gastric cancer than controls and also patients with advanced stages (Stages 3 and 4) disease possessed more expression level of CD155 than early-stage disease.[22] Nakai *et al*. showed CD155 plays a role in mediating pulmonary tumor cell invasion with clinical significance for prognosis of primary pulmonary adenocarcinoma outcome.[28] Moreover, Qu *et al*. suggested that the loss of CD155 expression might play a significant role in the immune escape of HCC cells, and therefore, CD155 might serve as a prognostic marker as well as a potential therapeutic target for HCC.[32] Therefore, the CD155 expression level might be potentially beneficial as a biomarker for cancer development and progress.[22]

In another hand, the CD155/PVR expression level is determinant in the prediction of the viral therapy strategy result of colorectal cancer. Since here in the current research, we intended to investigate the different types of colorectal cancer cell lines originated from various tumor grades with different cellular characteristics. As of our knowledge, this is the first report on CD155 expression level in HT29, Caco-2, and HTC116. The results of this study revealed that different colorectal cancer cell lines (SW480, HCT116, HT29, and Caco-2) similarly express more CD155 at both protein and mRNA levels in comparison with the normal colon cell line (FHC). In accordance with our results, Zhang *et al*. reported the CD155 protein upregulation in 3 colon cancer cells Colo205, SW480, and SW116 likewise the colon carcinoma tissues. However, they used the normal colon tissue as a reference instead of the normal colon cell line, FHC, which utilized in the current study. As we have shown that CD155 is expressed in 98.4% of the SW480 colon adenocarcinoma cells, they have also reported the approximately same rate of 95.1% for SW480 cells.[33] Priority of our data is based on the detection of CD155 transcript mRNA expression as well as the determination of protein expression level.

Our findings showed the upregulation of CD155 at both transcription and translation level in colorectal cancer cells, suggesting that upregulation of the CD155 starts from gene transcription process. It was stated and confirmed that CD155 is linked through more advanced stages of HCC,[33] gastric[22] and pulmonary tumors,[28] indicating its value as a potential prognostic marker. In addition, there is no evidence supporting its association in highly advanced colorectal cancer tumors; our data showed that CD155 mRNA is mostly expressed in SW480 cell line which is originated from Grade 3–4 primary tumor, while the minimum CD155 expression observed in HT29 cells derived from Grade 1 tumor. Concordantly, it has been reported that CD155 representation is linked with the tumor stage with more expression in advanced metastatic tumors.[34]

Both *et al*. has compared the migration and invasion capacity of different colorectal cancer cells (including SW480, HT29, and Caco-2) with Matrigel assay, as their report SW480 acquires the most migration ability (58.3%), while Caco-2 cells represent less migration score (4.4%).[35] Regarding the CD155 function in cell adhesion and migration enhancement, it is reasonable to compare the CD155 expression level with the migration capacity of these cells. Interestingly, the highly migratory SW480 cells have the highest level of CD155 among the studied colorectal cancer cells in the current study, as well as the Caco-2 colorectal cancer cell line expressing less migration and invasion ability and concordantly the minimum CD155 expression.[35]

Of notice, the HCT116 which is known as more enriched for cancer stem cell and high clonogenic features[36] possess the high expression of CD155 mRNA and protein in comparison to the well-differentiated Caco-2 cell line. Indeed, there is evidence supporting the function of alpha V integrin,[37] the associated concomitant of CD155 in cell adhesions in clonogenicity and self-renewal regulation of cancer cells. It may suggest a potential role for CD155 in clonogenicity regulation, but more investigation is needed.

Altogether these finding supports the upregulation of CD155 in colorectal cancer cells (SW480, HCT116, HT29, and Caco-2). However, the cellular characteristics including the original tumor grade, the migratory capacity of the cell and the invasion potential have to be considered.

Our finding suggested that detection of CD155 overexpression in human colon adenocarcinoma cell lines rather than normal cell line may hold promise for using this receptor for PV virotherapy candidate. Since our team is still working on this project (data not published), further fundamental studies are needed to clarify.

CONCLUSION

The overexpression of CD155 was recognized in all 4 Human adenocarcinoma cell lines. However, the magnitude of overexpression was different according to the type of cell lines. These results indicate that the selective overexpression of CD155 in cancer cell lines compare to normal cell line may more support the idea of CD155 targeted therapy of colon cancer by PV. Furthermore, our finding suggests that it is crucial to measure the level of CD155 overexpression in target cancer cells before PV virotherapy strategy.

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Conflicts of interest

There are no conflicts of interest.

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