SUPPORTING INFORMATION

A novel therapeutic anti-CD55 monoclonal antibody inhibits the proliferation and metastasis of colorectal cancer cells

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Materials included:

Data S1

Supporting materials and methods

Animal studies. Male BALB/c nu/nu mice (n=10, weight range: 23.6-26.5 g) were purchased at 6 weeks of age from Nara Biotec, Inc., quarantined and allowed acclimatize for 1 week. The animals were housed in a room that was maintained at 23±2˚C and 50±5% relative humidity, with artificial lighting from 8:00 a.m. to 8:00 p.m. and 13-18 air changes per hour. Four animals were housed per cage with access to tap water and commercial rodent chow (Samyang Feed) ad libitum. The health and well-being of the mice was monitored once per day. Xenograft experiments were performed as previously described (1). Briefly, LoVo cells ($1x10^7$ cells) were implanted subcutaneously into female BALB/c mice (n=10). When tumor volumes reached ~30 mm^3, mice were randomly distributed. Mice received normal human IgG (5 mg/kg) or chimeric anti-CD55 antibody (5 mg/kg) intravenously twice a week. Tumor volumes were measured and calculated by the following formula: 4/3 x pi x (length (mm)/2 x width (mm) 2 x height (mm)/2). Animal care and experimental protocols were approved by the Institutional Animal Care and Use Committee at Korea Atomic Energy Research Institue (KAERI) (KAERI-IACUC-2017-025). Analgesics were not used as they may have affected the results of the experiment. All animals were sacrificed by using a 60~70% CO_2 chamber. All methods were carried out in accordance with the relevant regulations and guidelines.

Immunohistochemistry (IHC) and immunoblotting. Tissues were fixed in 10% neutral buffered formalin at room temperature for 24 h, dehydrated with ethanol, cleared with xylene and embedded in paraffin. Tissue sections (4-μm-thick) were cut, mounted onto silane-coated slides, incubated at 60˚C for 2 h, deparaffinized in xylene and rehydrated. Antigen retrieval was performed in citrate buffer (0.01 M; pH 8.4) at 100˚C for 24 min. Tissue microarray slides (CDA3, human colorectal cancer-metastasis-normal; BC8, human normal organs and cancers; SuperBioChips Laboratories) from colorectal cancer patients were used for IHC. The tissue sections were stained with anti-CD55 polyclonal antibody (AP14798A; Abgent; 1:200) at 37˚C for 60 min and horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:200; cat. no. A0545; Sigma-Aldrich; Merck KGaA) at 37˚C for 30 min. IHC staining was performed using the UltraVision Quanto Detection System HRP DAB kit (TL-060-QHD; Thermo Fisher Scientific, Inc.), according to the protocol of the manufacturer. Images of the stained slides were captured using an Olympus light microscope (magnification, x200) and Olympus OlyVIA software (version 2.6). All the IHC processes were performed by SuperBioChips Laboratories as described previously (2). Positively stained cells were counted and classified as follows: >50% positive staining, strong; 10-50% positive staining, moderate; <10% positive staining, negative. The following antibodies were used for immunoblotting: Anti-CD55 (1:1,000; cat. no. ab54595; Abcam) and anti-tubulin (1:1,000; cat. no. sc-9104; Santa Cruz Biotechnology).

Cell culture. Percent survival was quantified by a microplate reader using the Cell counting kit-8 (Dojindo Molecular Technologies). For this assay, the human complement system (S1764; Sigma-Aldrich; Merck KGaA) was applied to detect complement-dependent cytotoxicity. Invasion and migration assays were carried out as described previously (1,2).

Supporting references

1. Dho SH, Kim JY, Lee KP, Kwon ES, Lim JC, Kim CJ, Jeong D and Kwon KS: STAT5A-mediated NOX5-L expression promotes the proliferation and metastasis of breast cancer cells. Exp Cell Res 351: 51-58, 2017.
2. Dho SH, Lee KP, Jeong D, Kim CJ, Chung KS, Kim JY, Park BC, Park SS, Kim SY and Kwon KS: GPR171 expression enhances proliferation and metastasis of lung cancer cells. Oncotarget 7: 7856-7865, 2016.

Figure S1. Effect of the anti-CD55 antibody on cell viability of DLD-1 cells. Cell viability assays of DLD-1 cells treated with IgG and anti-CD55 antibody.

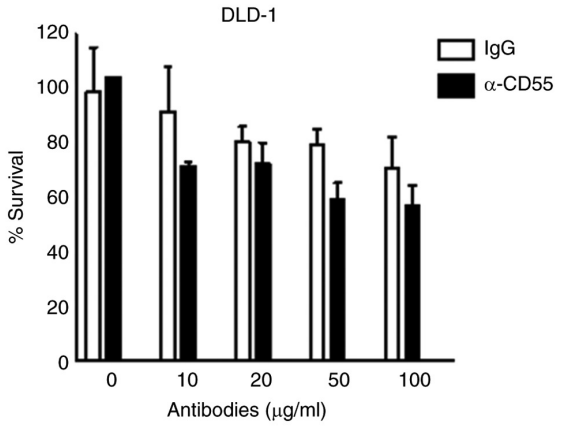

Figure S2. Combinatorial effect of the anti-CD55 antibody and 5-FU *in vitro*. Cell viability assays of LoVo cells treated with anti-CD55 in the presence or absence of 5-FU. 5-FU, 5-fluorouracil.

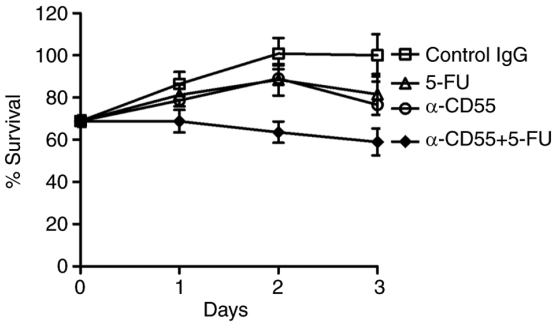

Table SI. Immunohistochemical quantification of CD55 in colorectal cancer and normal tissues[a].

Clinical types of colorectal tissues	Numbers of stained samples/examined samples		
	Strong	Moderate	Negative
Cancer	23/51 (45.1%)	14/51	14/51
Adenocarcinoma	21/41 (51.2%)	12/41	8/41
Metastasis	2/10	2/10	6/10
Normal	1/11	2/11	8/11

[a]CD55 is upregulated in colorectal cancer tissues. Strong, Moderate and Negative indicate >50%, 10-50% and <10% positive cells, respectively.

Table SII. Increased levels of CD55 in progressive stages of colorectal cancer[a].

Clinical stages of colorectal cancer	Numbers of stained samples/examined samples		
	Strong	Moderate	Negative
Cancer	21/41 (45.1%)	12/41	8/41
Stage I	0/2	2/2	0/2
Stage II	6/11 (54.4%)	3/11	2/11
IIA	5/10	3/10	2/10
IIB	1/1	0/1	0/1
Stage III	7/15 (46.7%)	5/15	3/15
IIIB	4/9	3/9	2/9
IIIC	3/6	2/6	1/6
Stage IV	8/13 (61.5%)	2/13	3/13
IVA	4/8	1/8	3/8
IVB	4/5	1/5	0/5

[a]CD55 is upregulated in a subset of colorectal cancer tissues. Strong, Moderate and Negative indicate >50%, 10-50% and <10% positive cells, respectively.