Supporting Information for

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Modification of cysteine 457 in plakoglobin modulates the proliferation and migration of colorectal cancer cells by altering binding to E-cadherin/catenins

Suhee Kim[1,2], Sun Hee Ahn[1], Hee-Young Yang[1], Jin-Sil Lee[1], Hyang-Gi Choi[1,2], Young-Kyu Park[3], Tae-Hoon Lee[1, 2]

[1]Department of Oral Biochemistry, Dental Science Research Institute, Medical Research Center for Biomineralization Disorders, School of Dentistry, Chonnam National University, Gwangju, Republic of Korea

[2]Department of Molecular Medicine (BK21plus), Chonnam National University Graduate School, Gwangju, Republic of Korea

[3]Department of Surgery, Chonnam National University Hwasun Hospital, Hwasun, Korea

Correspondence to: Tae-Hoon Lee, Department of Oral Biochemistry, School of Dentistry, Chonnam National University, Gwangju 500-757, Republic of Korea.

E-mail address: thlee83@jnu.ac.kr

Supplementary materials and methods

Tissue sample preparation

Thirteen CRC patient samples were used for proteomic analysis. Detailed information about the samples are summarized in our previous report.[1]

Nano-UPLC MS^E shotgun proteomics

Details of the procedures can be found in our earlier reports.[1, 2]

Fibroblast growth factor (FGF)-2 treatment

MC38 cells expressing a WT or mutant Pg were serum-starved for 24 h and then maintained for 30 min, 3 h, or 24 h with FBS-reduced (1% FBS) media containing 0, 10, or 50 ng/ml recombinant human FGF-2 (Peprotech, Rocky Hill, NJ) and 1 μg/ml heparin to stimulate the cadherin/catenin axis. The proteins extracted from cells treated with different FGF-2 concentrations and incubation time were loaded onto SDS-PAGE gels and immunoblotted with anti-HA (CST) and anti-GFP (Abfrontier) to detect protein expression of Pg and E-cadherin. This was followed by incubation with HRP-conjugated secondary antibody and detected using an ECL system (iNtRON, South Korea).

Supplementary references

1 Yang HY, Kwon J, Park HR, Kwon SO, Park YK, Kim HS, *et al*. Comparative proteomic analysis for the insoluble fractions of colorectal cancer patients. J Proteomics 2012;75(12):3639-53.

2 Yang HY, Chay KO, Kwon J, Kwon SO, Park YK, Lee TH. Comparative proteomic analysis of cysteine oxidation in colorectal cancer patients. Mol Cells 2013;35(6):533-42.

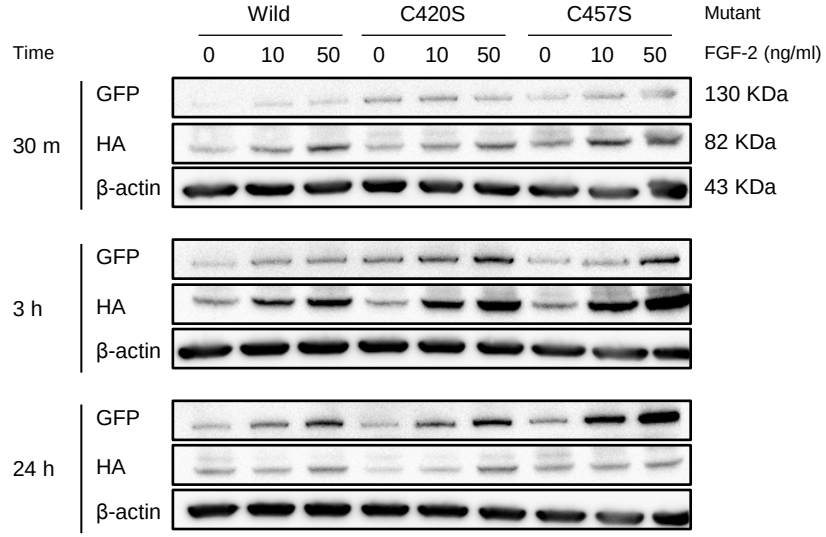
Table S1. Clinical information about the biopsies from CRC patients

Case No.	Age (years)	Gender	Colon site	Tumor stage	Pathologic grade	TNM (metastasis)
553	77	Male	Rectum	II	None	T3N0M0
350	67	Female	Upper rectum	II	Well	T3N0
1323	46	Female	Rectum	II	Moderate	T3N0M0
1367	76	Male	Sigmoid	III	Well	T3N1
948	57	Female	Rectum	III	Moderate	T3N2
1500	64	Male	Rectum	III	Moderate	T3N2M0

Table S2. Information on Pg peptide sequences containing variably modified cysteines in tumor and non-tumor tissues from CRC patients

IPI accession No	Peptide sequence[a]	Cys. local[b]	Cys. modification	Unique[c]	pT:pN ratio[d]
IPI00554711	VREAM**C**PGVSGEGQLALLATQVEGQ	90	Carbamidomethyl	pN	
IPI00554711	VREAM**C**PGVSGEGQLALLATQVEGQ	90	Sulfinic	pT	
IPI00554711	VREAM**C**PGVSGEGQLALLATQVEGQ	90	Carbamidomethyl		0.13
IPI00554711	VREAM**C**PGVSGEGQLALLATQVEGQ	90	Carbamidomethyl		1.57
IPI00789324	**C**TTSILHNLSHHR	204	Carbamidomethyl	pN	
IPI00554711	**C**TTSILHNLSHHR	204	Carbamidomethyl	pT	
IPI00554711	**C**TTSILHNLSHHREGLLAIFK	204	Sulfinic	pT	
IPI00789324	**C**TTSILHNLSHHREGLLAIFK	204	Sulfenic		1.39
IPI00554711	FLAITTD**C**LQLLAYGNQESK	291	Carbamidomethyl	pN	
IPI00554711	FLAITTD**C**LQLLAYGNQESK	291	Sulfenic	pN	
IPI00789324	FLAITTD**C**LQLLAYGNQESK	291	Sulfenic	pT	
IPI00554711	FLAITTD**C**LQLLAYGNQESK	291	Sulfenic		0.93
IPI00789324	FLAITTD**C**LQLLAYGNQESK	291	Carbamidomethyl		0.49
IPI00789324	ILVNQLSVDDVNVLT**C**ATGTLSNLT**C**	410, 420	Sulfinic	pT	
IPI00789324	ILVNQLSVDDVNVLT**C**ATGTLSNLT**C**	410, 420	Sulfinic		1.42
IPI00789324	AGDKDDITEPAV**C**ALR	457	Carbamidomethyl	pN	
IPI00554711	AGDKDDITEPAV**C**ALR	457	Sulfinic	pT	
IPI00554711	AGDKDDITEPAV**C**ALR	457	Carbamidomethyl		0.56
IPI00554711	NLAL**C**PANHAPLQEAAVIPR	511	Carbamidomethyl	pN	
IPI00554711	NLAL**C**PANHAPLQEAAVIPR	511	Carbamidomethyl	pT	
IPI00554711	NLAL**C**PANHAPLQEAAVIPR	511	Sulfinic		1.13
IPI00554711	VAAGVL**C**ELAQDK	609	Carbamidomethyl	pN	
IPI00789324	VAAGVL**C**ELAQDK	609	Sulfinic	pN	

[a]**C** is a cysteine exhibiting variable modification; carbamidomethylation (IAM binding) or oxidation (sulfinic or sulfenic)
[b]Cys local indicates the cysteine position within the protein sequence.
[c]Unique indicates tissue-specific cysteine modification; pN (patient non-tumor) or pT (patient tumor) tissue
[d]pT:pN ratios were calculated for cysteine modifications without tissue-specificity



Supplementary Figure 1. Protein expression level of Pg and E-cadherin after FGF-2 treatment. MC38 cells co-transfected with E-cadherin plus WT or mutant Pg were incubated with different concentrations (0, 10, or 50 ng/ml) and incubation time (30 m, 3 h, or 24 h) of FGF-2, after which Pg (HA) and E-cadherin (GFP) expression was evaluated.