Supplementary Material

Plasma-based digital PCR assay for early detection of gastric cancer using multiple methylation biomarkers

Yun Young Lee, Joon An, Jinil Han, Youngho Moon, and Sang-Il Lee

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SUPPLEMENTARY TABLES

Supplementary Table S1. Patient demographics and clinical characteristics for tissue-based validation

Characteristics	**Gastric Cancer[a]**	**Colorectal Cancer[b]**
Total, *N*	40	37
Sex, *N* (%)		
Male	24 (60.0)	20 (54.1)
Female	16 (40.0)	17 (45.9)
Age (years) - Median (range)	75.5 (49.0-90.0)	66.0 (43.0-97.0)
Stage, *N* (%)		
I	15 (37.5)	14 (37.8)
II	15 (37.5)	6 (16.2)
III	10 (25.0)	15 (40.6)
IV	0 (0.0)	2 (5.4)
Lauren's Classification, *N* (%)		
Intestinal Type	27 (67.5)	-
Diffuse Type	6 (15.0)	-
Mixed Type	5 (12.5)	-
Unknown	2 (5.0)	-
Grade of Differentiation, *N* (%)		
Well	5 (12.5)	-
Moderate	22 (55.0)	-
Poor	11 (27.5)	-
Unknown	2 (5.0)	-

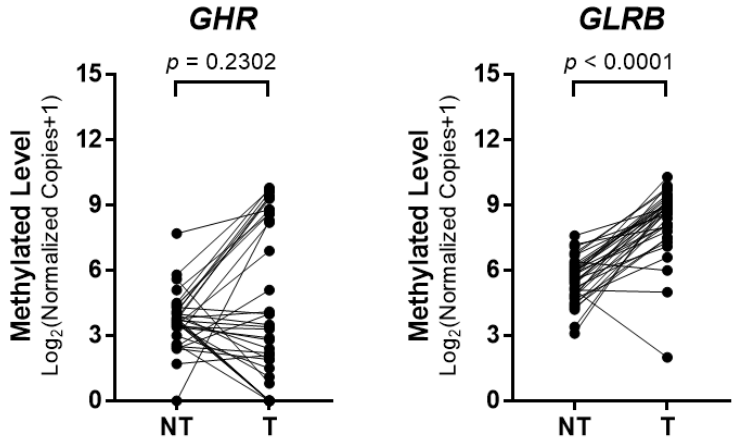
[a] Tissue obtained from the Chonnam National University Hwasun Hospital (Hwasun, Republic of Korea).
[b] Tissue obtained from the Ajou University Hospital (Suwon, Republic of Korea).

Supplementary Table S2. Target regions and biological functions of selected biomarker candidates

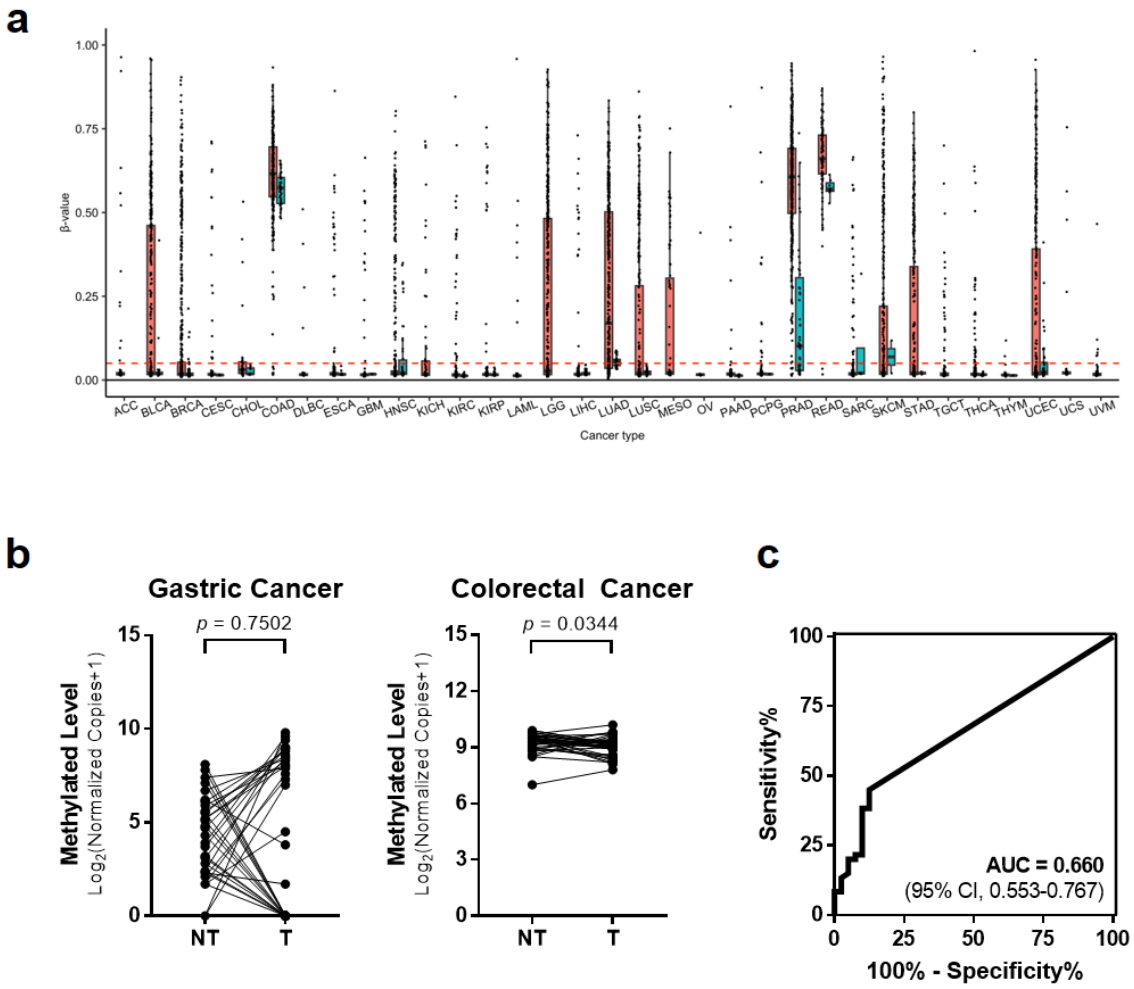
DNA Methylation Biomarker	**Biological Role**	**Genomic Coordinates** [a]
GHR (Growth Hormone Receptor)	Growth Hormone Receptor Activity	Chr5:42423946-42424524
GLRB (Glycine Receptor Beta)	Chloride Transport	Chr4:157997359-157997749
GATM (Glycine Amidinotransferase)	Creatine Biosynthesis	Chr15:45670525-45671027

[a]GRCh37/hg19 assembly

SUPPLEMENTARY FIGURES



Supplementary Fig. S1. DNA methylation status of *GHR* and *GLRB* in colorectal cancer tissues. Methylation levels of *GHR* and *GLRB* were analyzed in primary tumor tissues (T) and matched adjacent non-cancerous tissues (NT) from 37 colorectal cancer patients. Paired samples from the same patient are connected by lines. Statistical differences were evaluated using a paired *t*-test.



Supplementary Fig. S2. *GATM* methylation as a novel tissue-specific biomarker for colon and rectum. (**a**) DNA methylation levels of *GATM* across 33 cancer types and corresponding normal tissues. (β-values represent the methylation level, ranging from 0 to 1). (**b**) DNA methylation levels of *GATM* in primary tumor tissues (T) and matched adjacent non-cancerous tissues (NT) from 40 gastric cancer (GC) patients and 37 colorectal cancer (CRC) patients. Paired samples from the same patient are connected by lines. Statistical differences were evaluated using a paired *t*-test. (**c**) Receiver operating characteristic (ROC) curve for *GATM* in distinguishing GC patients from non-GC subjects based on plasma specimens, with area under the curve (AUC) values and 95% confidence intervals (CIs) shown.

DIGITAL MIQE CHECKLIST

Item to Check	Provided	Comment
1. SPECIMEN		
Detailed description of specimen type and numbers	**Y**	Explained in **Methods**, **Table 1**, and **Supplemental Table S1**
Sampling procedure (including time to storage)	**Y**	Explained in **Methods**
Sample aliquotation, storage conditions and duration	**Y**	Explained in **Methods**
2. NUCLEIC ACID EXTRACTION		
Description of extraction method including amount of sample processed	**Y**	Explained in **Methods**
Volume of solvent used to elute/resuspend extract	**Y**	Explained in **Methods**
Number of extraction replicates	**N**	Used up all of samples
Extraction blanks included?	**Y**	Explained in **Methods** (no-template control)
3. NUCLEIC ACID ASSESSMENT AND STORAGE		
Method to evaluate quality of nucleic acids	**Y**	Explained in **Methods** (DNA quantification using NanoDrop spectrophotometer)
Method to evaluate quantity of nucleic acids (including molecular weight and calculations when using mass)	**Y**	Explained in **Methods** (DNA quantification using NanoDrop spectrophotometer)
Storage conditions: temperature, concentration, duration, buffer, aliquots	**Y**	Explained in **Methods**
Clear description of dilution steps used to prepare working DNA solution	**N**	Not explained in the manuscript, but all samples used up
4. NUCLEIC ACID MODIFICATION		
Template modification (digestion, sonication, pre-amplification, bisulphite etc.)	**Y**	Explained in **Methods** (bisulfite conversion)
Details of repurification following modification if performed	**Y**	Explained in **Methods** (according to manufacturer's instructions)
5. REVERSE TRANSCRIPTION		
cDNA priming method and concentration	**N**	Not applicable
One or two step protocol (include reaction details for two step)	**N**	Not applicable
Amount of RNA added per reaction	**N**	Not applicable
Detailed reaction components and conditions	**N**	Not applicable
Estimated copies measured with and without addition of RT*	**N**	Not applicable
Manufacturer of reagents used with catalogue and lot numbers	**N**	Not applicable
Storage of cDNA: temperature, concentration, duration, buffer and aliquots	**N**	Not applicable

6. dPCR OLIGONUCLEOTIDES DESIGN AND TARGET INFORMATION		
Sequence accession number or official gene symbol	**Y**	Explained in **Supplementary Table S2**
Method (software) used for design and *in silico* verification	**N**	Not explained in the manuscript, but IDT website was utilized for *in silico* study
Location of amplicon	**Y**	Explained in **Supplementary Table S2**
Amplicon length	**N**	Only provided amplicon regions in **Supplemental Table S2**
Primer and probe sequences (or amplicon context sequence)**	**Y**	Explained in **Supplemental Table S2** (amplicon context regions)
Location and identity of any modifications	**N**	Not applicable
Manufacturer of oligonucleotides	**Y**	Explained in **Methods**
7. dPCR PROTOCOL		
Manufacturer of dPCR instrument and instrument model	**Y**	Explained in **Methods**
Buffer/kit manufacturer with catalogue and lot number	**Y**	Explained in **Methods**
Primer and probe concentration	**N**	Not explained in the manuscript, due to manufacturer's disclosure decision
Pre-reaction volume and composition (incl. amount of template and if restriction enzyme added)	**N**	Not applicable
Template treatment (initial heating or chemical denaturation)	**N**	Not applicable
Polymerase identity and concentration, Mg++ and dNTP concentrations***	**Y**	Explained in **Methods** (ddPCR master mix was provided by manufacturer)
Complete thermocycling parameters	**Y**	Explained in **Methods**
8. ASSAY VALIDATION		
Details of optimisation performed	**Y**	Explained in **Methods**
Analytical specificity (vs. related sequences) and limit of blank (LOB)	**N**	Not explained in the manuscript, but a no-template control was tested in all runs
Analytical sensitivity/LoD and how this was evaluated	**N**	Not applicable
Testing for inhibitors (from biological matrix/extraction)	**N**	Not applicable
9. DATA ANALYSIS		
Description of dPCR experimental design	**Y**	Explained in **Methods**
Comprehensive details negative and positive of controls (whether applied for QC or for estimation of error)	**Y**	Explained in **Methods**
Partition classification method (thresholding)	**Y**	Explained in **Methods**
Examples of positive and negative experimental results (including fluorescence plots in supplemental material)	**N**	Not explained in the manuscript, but plan to submit if required

Description of technical replication	**N**	Not applicable
Repeatability (intra-experiment variation)	**N**	Not applicable
Reproducibility (inter-experiment/user/lab etc. variation)	**N**	Not applicable
Number of partitions measured (average and standard deviation)	**Y**	Not explained in the manuscript (Average ± SD, 18482.3 ± 2314.4 in the validation study)
Partition volume	**N**	Not applicable
Copies per partition (λ or equivalent) (average and standard deviation)	**N**	Not applicable
dPCR analysis program (source, version)	**Y**	Explained in **Methods**
Description of normalisation method	**Y**	Explained in **Methods**
Statistical methods used for analysis	**Y**	Explained in **Methods**
Data transparency	**Y**	Available on request if corresponding author has given permission