**MAPRE1 as a plasma biomarker for early-stage colorectal cancer and adenomas**

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**SUPPLEMENTARY METHODS**

**Mouse models and mass spectrometry analysis of mouse plasma samples**

*Adenoviral-Cre* (Ad5CMVCre)-infected mice carrying conditional alleles for the mouse *Apc* and *Msh2* genes (*ApcloxP/loxP;Msh2loxP/loxP* mice) develop large intestinal tumors. Therefore, to create intestinal cancer models, *ApcloxP/loxP;Msh2loxP/loxP* mice were infected with Ad5CMVCre through laparotomy to delete *Apc* and *Msh2* ([1](#_ENREF_1)). Plasma samples were collected from tumor-bearing mice aged for 7-8 months following Ad5CMVCre infection and from control mice that were Ad5CMVCre-infected. Plasma samples from the disease groups (low-grade adenoma, high-grade adenoma, and adenocarcinoma) and from the controls were pooled separately by combining aliquots of plasma from 4-7 mice.

A tamoxifen-inducible *KrasG12D* colon cancer mouse model has been generated (unpublished) using the tamoxifen-inducible VillinCreERT2, which deletes *APCLoxP/LoxP* and *P53LoxP/LoxP* while activating oncogenic KrasG12D. Plasma samples were retro-orbitally collected every 2 weeks after treatment with tamoxifen until sacrifice. Serial plasma samples collected at 0 weeks, 2 weeks, and 4 weeks after tamoxifen treatment from a female mouse in which advanced colon adenocarcinoma was observed at 5 weeks () were compared with a pool of plasma samples from five female mice without tumors collected 4 weeks after tamoxifen treatment.

Samples were individually immunodepleted of the three most abundant proteins (albumin, IgG, and transferrin). Samples were then reduced with DTT and alkylated with isobaric iodoTMT Reagents (Thermo Scientific), which enable up to six different protein samples. For each sample, a unique reporter mass (i.e., 126-131 Da) in the low-mass region of the MS/MS spectrum was used to measure relative protein expression levels during peptide fragmentation and tandem mass spectrometry. After labeling, the samples were mixed and fractionated by anion-exchange chromatography and reversed-phase chromatography. For protein identification, individual fractions were digested with trypsin and finally grouped into 96 pools. Pools were analyzed in a SYNAPT G2-S mass spectrometer (Waters) coupled to a nanoACQUITY nanoflow chromatography system (Waters). The liquid chromatography separation was performed in a nanoACQUITY UPLC column (75 µm x 150 mm column) using a 90-minute linear gradient from 5% to 40% of acetonitrile in 0.1% formic acid at 500 nl/minute. The spectra were acquired in a data-independent mode in a m/z range of 50 to 1,800 with ion-mobility function.

The acquired data were automatically processed by the PLGS. For the identification of proteins with a false discovery rate of <4%, LC/MS/MS spectra were subjected to tryptic searches against a database consisting of UniProt databases using the PLGS. The database search results were then analyzed by an in-house program for peptides and proteins. The quantitative approach consisted of differential labeling of peptides containing cysteine with isobaric iodoTMT Reagents. The reporter-ion ratios were plotted on a logarithmic scale in a histogram, and the median of the distribution was centered at zero for normalization. All normalized reporter-ion ratios for a specific protein were averaged to compute an overall protein ratio. The statistical significance of the protein quantitative information was obtained via the following procedures: for proteins with multiple peptides quantified, a P value for the mean log-ratio, which has a mean of zero under the null hypothesis, was calculated using a t-test; for proteins with a single paired MS event, we give them annotation.

**REFERENCE**

1. Kucherlapati MH, Esfahani S, Habibollahi P, Wang J, Still ER, Bronson RT, et al. Genotype directed therapy in murine mismatch repair deficient tumors. PLoS One. 2013;8:e68817.