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A pilot study comparing protein expression in different segments of the normal colon and rectum and in normal colon versus adenoma in patients with Lynch syndrome

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Abstract

Purpose—Lynch syndrome (LS) is a common inherited predisposition to colorectal cancer (CRC). In LS patients, CRC is predominantly located in the right colon, as opposed to sporadic CRC, which usually affects the left colon or rectum. Previous studies have demonstrated a clear distinction in gene expression between sporadic CRC and normal colon at different locations in the colorectum. However, little is known about LS gene expression profiles in different areas of the colorectum. Here, we compared the protein expression profiles for normal colorectal samples among different locations as well as between adenomas and matched normal tissue in LS.

Methods—Protein from 33 tissue samples (27 normal tissues and 6 adenomas) from 9 patients with LS was extracted for reverse-phase protein array (RPPA) analysis. The antibody panel used for RPPA included 109 key proteins involved in various cancer-related pathways. Cluster 3.0 was used for unsupervised and supervised clustering analysis.

Results—IGF1R and COL6A1 were expressed significantly differently between the normal right and normal left colon ($q<0.05$); FN1, COL6A1, and IGF1R were expressed significantly differently between the normal right colon and normal rectum ($q<0.05$). In the adenomas and matched normal tissue, PEA-15 was the only protein with significantly different expression ($q<0.05$).

Conclusion—We found differences in protein expression between normal tissues from the right colon, left colon, and rectum as well as between adenomas and matched normal tissue. However, those differences should be further confirmed in a larger sample size.

Keywords

Lynch syndrome; Reverse phase protein array; PEA15; COL6A1; IGF1R; and FN1

Introduction

Lynch syndrome (LS), also known as hereditary non-polyposis colorectal cancer (HNPCC), is an autosomal dominant disorder caused by germline mutations in mismatch repair (MMR) genes (Lynch et al. 1991), which include *MLH1*, *MSH2*, *MSH6*, and *PMS2* (Lynch et al.

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1991; Marra and Boland 1995). In addition, a deletion in the 3' region of *EPCAM/TACSTD1* (not an MMR gene), which is located immediately upstream of *MSH2*, causes a transcriptional read-through into *MSH2*, which silences the gene (Ligtenberg et al. 2009; Niessen et al. 2009). Patients with LS are at high lifetime risk for a variety of different cancers, with colorectal cancer (CRC) and endometrial cancer being the most common (Aarnio et al. 1995; Mecklin and Jarvinen 1991). There is considerable variation in the reported median age of onset of CRC in LS (44–52 years) (Hampel et al. 2008; Vasen 2005); in contrast, sporadic CRC has a median age of onset of 69 years (Howlader 2012). In contrast to familial adenomatous polyposis (FAP), another hereditary colorectal cancer predisposition syndrome where patients develop thousands of colonic polyps or adenomas, patients with LS do not develop a large number of adenomas. However, in LS, adenomas undergo accelerated malignant transformation compared with sporadic CRC development (Rijcken et al. 2002).

CRC is typically categorized into rectal, left colon and right colon cancers. In LS patients, CRC is predominantly located in the ascending and transverse colon (right side). In contrast, the sporadic or non-syndromic form of CRC, which usually affects the descending colon (left side) or rectum but can affect any part of the colorectum. Almost 25% of right-sided colon tumors exhibit high microsatellite instability (MSI) which results from an accumulation of insertion/deletion lesions in regions of DNA repeat sequences called microsatellites, compared to only 2% of left-sided tumors (Iacopetta 2002). CRCs in LS patients typically exhibit high MSI, as a consequence of deficient mismatch repair (Boland et al. 1998). This suggests that there may be inherent differences between the right and left colon that predispose the right colon to initiation of adenoma or CRC through a DNA mismatch repair–dependent pathway. The inherent differences between the left and right colon include different embryological origin; the right colon derives from the embryonic midgut and the left colon and rectum from the hindgut. The 2 sides of the colon also have different blood supplies; the superior mesenteric artery supplies the right colon and the inferior mesenteric artery supplies the left colon (Iacopetta 2002). Recently, Ramírez-Ramírez et al reported a higher frequency of aberrant crypt foci (ACF) in right segment of the colon of LS patient in contrast to a higher frequency of ACF in the left segment of the colon of sporadic CRC (Ramirez-Ramirez et al. 2012). There may, therefore, be different pathways of transformation associated with right- and left-sided colorectal carcinogenesis that are dependent on inherent biological characteristics of the right versus left colon or acquired through a differential response to the influence of pro-carcinogenic risk factors. It has been proposed that right-sided and left-sided CRC represent distinct disease entities (Carethers 2011; Gervaz et al. 2004; Jensen 1984). Several researchers (Bara et al. 1984; Glebov et al. 2003; Komuro et al. 2005) have demonstrated a clear distinction in the gene expression profiles of sporadic CRC arising from different locations in the colorectum and between normal colon tissue and adenomas; however, little has been done to characterize the gene expression patterns of LS in different parts of the colorectum.

Understanding the pattern of gene expression in normal and premalignant tissue is vital to understanding the gene expression changes in diseased tissue. To examine whether there are differences in gene expression between different colonic and rectal locations in LS, we used reverse-phase protein array (RPPA) analysis to examine the gene expression profiles at the protein level for important molecules involved in cell death and survival, cellular growth and proliferation, cellular development, cell cycle regulation, and cellular movement signaling. We compared the expression profiles between the left- and right-sided normal colon, left- or right-sided normal colon and rectum, and adenoma and matched normal colon samples. These comparisons will provide insights for a better understanding of protein expression variation in both normal and premalignant tissues. The differences identified may

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be useful as prognostic biomarkers for early detection and/or novel targets for the prevention of LS-associated CRC.

Materials and Methods

Patients and samples

Adenomas and normal-appearing tissues from the left colon, right colon, and rectum were collected during endoscopies performed at The University of Texas MD Anderson Cancer Center in patients with LS prior to any treatment. These patients had consented to participate in an ongoing Institutional Review Board (IRB)-approved study, "Molecular Genetics of HNPCC," and were recruited between September 1994 and June 2011 at MD Anderson Cancer Center. The current study was approved by and conducted in accordance with the policies of the IRB at MD Anderson Cancer Center. Thirty-three tissue samples, including 27 normal- tissues and 6 adenomas, from 9 Caucasian patients with LS were obtained for analysis.

Protein extraction

Protein was extracted as follows. For each patient, a biopsy of normal-appearing tissue was taken from each of the following locations: right-sided colon, left-sided colon, and rectum. In addition, adenomas were removed from 6 of the 9 patients, as described in Table 1. The tissues were cut into small pieces on dry ice and then homogenized with ice-cold RIPA buffer (Cell Signaling #9806, Boston, MA) containing freshly added protease/phosphatase inhibitors (Cell Signaling #5872). Homogenization was set on ice to prevent heating. The lysate was centrifuged for 10 minutes at 14,000 x g in a cold microfuge, and the supernatant was collected for reverse-phase protein array (RPPA) analysis. Protein concentrations were determined using bicinchoninic acid (BCA) protein assay reagents (Pierce, Rockford, IL).

Reverse-phase protein array analysis

RPPA was performed as previously described (Iadevaia et al. 2010). The antibody panel used for RPPA includes the proteins involved in cell death and survival, cellular growth and proliferation, cellular development, cell cycle regulation, and cellular movement. Briefly, a series of two fold-diluted lysates was spotted onto nitrocellulose-coated slides (Grace Biolab, Bend, OR) using an Aushon 2470 Arrayer (Aushon Biosystems, Billerica, MA). Each slide was probed with a primary antibody and then a biotin-conjugated secondary antibody. The antibody-binding signal was amplified using a Dako Cytomation–catalyzed system (Dako, Carpinteria, CA) and visualized by DAB colorimetric reaction. The slides were scanned, analyzed, and quantified using the customized software Microvigene (VigeneTech, Inc., Carlisle, MA) to generate spot intensity. Each dilution curve was fitted with a logistic model ("Supercurve Fitting," developed by the Department of Bioinformatics and Computational Biology at MD Anderson Cancer Center, "http://bioinformatics.mdanderson.org/OOMPA"). All the data were normalized for protein loading and transformed to linear values.

Antibodies

A panel of 133 antibodies, which correspond to 109 key proteins involved in the various pathways and networks, is included in RPPA. These antibodies were used to detect the expression level for total protein and/or its activated form, such as phosphorylated or cleaved protein. (Appendix will be provided upon request)

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Statistical analysis

Unsupervised and supervised clustering analysis was done using Cluster 3.0 to classify the samples into statistically similar groups, and the resulting heatmaps were visualized in TreeView 1.6 (www.eisenlab.org/eisen). Proteins were classified according to their relevant signaling pathways using Ingenuity Pathway Analysis (Ingenuity Systems, Redwood, CA). The differences in protein expression among matched different locations were analyzed using one-way repeated measures analysis of variance or the paired t-test when appropriate. The differences in expression between paired normal mucosa and adenomas were analyzed using the paired t-test. To account for multiple comparisons in our studies, we calculated and reported false discovery rate (FDR)–adjusted P-values by using the Benjamini-Hochberg method to determine whether the observed P-values were still significant after taking into account multiple comparisons. An FDR cut-off of 0.05 was applied ($q<0.05$). Analyses were performed using STATA software (version 10, StataCorp LP, College Station, TX).

Results

Protein expression at different locations in the normal colon

To explore variations in protein expression along the normal colon in LS patients, we used protein expression data collected using RPPA. First, clustering analysis showed distinct variations in expression along the normal colon. As shown in Figure 1A, 31 proteins were expressed at significantly different levels among the person-matched right colon, left colon, and rectum samples ($p<0.05$). After the multiple-testing correction, 7 proteins remained significant at the FDR cut-off of 5%: COL6A1, IGF1R, EEF2K, RPS6, KIT, EIF4EBP1, and RPS6KB1 (Figure 2). Interestingly, the protein expression levels in the right colon were consistently lower than those in the left colon and rectum for these 7 protein markers. Next, we compared the 3 different regions of the colon and rectum in a pairwise fashion (summarized in Table 2). Seventeen proteins showed significant differences between the right and left colon at the $p<0.05$ level, and 2 proteins remained significant after multiple-test correction. Likewise, 20 proteins showed significant differences between the right colon and rectum at the $p<0.05$ level, and 3 of these proteins remained significant after multiple-test correction at the FDR cut-off of 5%. Seventeen proteins showed significant differences between the left colon and rectum at the $p<0.05$ level, but none of these remained significant after multiple-test correction.

Protein expression between normal colon and adenomas

We also compared protein expression between person-matched, location-matched normal colon samples and adenomas. Clustering analysis showed variation between protein expression in the normal colon and adenomas (Figure 1B). Seventeen markers were significantly differentially expressed between the person-matched normal colon and adenomas at the same location ($p <0.05$) (Table 2). One marker, the protein PEA15, remained significant at the FDR cut-off of 5% (Figure 1B).

Pathway analysis

We further used Ingenuity Pathway Analysis to explore whether a particular pathway was significantly associated with protein expression in different colon locations and adenomas.

We selected 31 proteins with p value <0.05 in the test comparing expression levels in normal- tissues at different locations to perform pathway analysis. The top canonical pathways to which these proteins belong are the p53 signaling ($p =1.19E-08$), ovarian cancer signaling ($p =9.76E-08$), and ERK/MAPK signaling ($p =6.69E-07$) pathways. We found that 6 significant proteins, CTNNB1, CHEK2, RB1, SNAI2, CHEK1, and PTEN, were part of

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the p53 signaling pathway; 5 significant proteins, CTNNB1, SRC, RAD51, RB1, and PTEN, fell into the ovarian cancer signaling pathway; and 6 significant proteins, EIF4EBP1, SRC, EIF4E, PXN, ESR1, and RPS6KA1, fell into the ERK/MAPK signaling pathway. We also selected 17 proteins with p value <0.05 in the comparison test between paired normal colon and adenomas for pathway analysis. The top canonical pathways to which these proteins belong are the hereditary breast cancer signaling (p =3.98E-06), endometrial cancer signaling (p =1.67E-05), and ATM signaling (p =2.20E-05) pathways. Three significant proteins, TP53, PTEN, and CCNB1, fell into the hereditary breast cancer signaling pathway; 3 significant proteins, ERBB2, TP53, and PTEN, fell into the endometrial cancer signaling pathway; and 2 significant proteins, TP53 and MRE11A, fell into the ATM signaling pathway.

Discussion

It has long been appreciated that developmental, physiological, genetic and epigenetic difference exists between different segments of the colorectum (Bufill 1990; Gervaz et al. 2004; Iacopetta 2002). In this pilot study, we examined protein expression patterns along the normal colorectum and between normal tissues and adenomas in LS. Our results suggest that there are some differences in protein expression profiles between normal tissues from different locations as well as between adenomas and matched normal tissue. However, the number of proteins statistically significantly different in expression after the multiple-test correction was very minimal, and the fold change difference in expression level was subtle. In the normal LS colorectum, although there are distinctions in protein expression profiles between the right colon and either the left colon or rectum, the expression level appears to change gradually along the colorectum from the right to left and the rectum. In addition, there was virtually no difference between the left colon and rectum. In the normal vs. premalignant adenoma comparison, only the PEA15 protein expression level was expressed differentially. Nevertheless, the location-specific or tumor-specific protein expression profiles observed here might provide valuable insight into understanding variations along the LS colorectum in either the nonneoplastic or neoplastic setting and could be relevant for the clinical management of LS.

Specifically, in the normal LS colorectum, 2 proteins (IGF1R and COL6A1) and 3 proteins (FN1, COL6A1, and IGF1R) remained statistically significantly different at the FDR cut-off of 5% (q<0.05) between the right colon and left colon and the right colon and rectum, respectively. These differences in protein expression profiles may be due to anatomic and/or physiological differences between the right colon, left colon, and rectum, considering the distinct regional embryological origins and the different biological characteristics of the segments. Among these proteins, IGF1R, the insulin-like growth factor 1 receptor, is involved in cell growth and survival control. It plays a critical role in transformation events and is highly overexpressed in most malignant tissues, where it functions as an anti-apoptotic agent by enhancing cell survival. The protein encoded by *COL6A1* is the alpha 1 subunit of collagen VI, which is a major structural component of microfibrils. These collagens play a role in maintaining the integrity of various tissues. Previously, a gene expression microarray study by Glebov et al (Bara et al. 1984; Glebov et al. 2003; Komuro et al. 2005) showed COL6A1 expressed significantly less at transcriptional level in right versus left colon of LS patients. Here, we observed the same trend in reduction of COL6A1 expression at protein level. Put it together, the comparison of COL6A1 at transcriptional level corresponds to its comparison at translational level in LS colorectum. Interestingly, in this same study by Glebov et al (Bara et al. 1984; Glebov et al. 2003; Komuro et al. 2005), differential expression of COL6A1 at the transcriptional level in the right versus left colon was also observed in non-LS CRC patients, suggesting that LS and non-LS colorectums share some common molecular features. However, IGF1R and FN1 were not included in this

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study. *FN1* encodes fibronectin, which is involved in cell adhesion and migration processes. In our present study, we observed the differential lower expression of FN1 at the protein level in the normal right colon relative to normal rectum in the LS colorectum. Interestingly, in a gene expression microarray study of 184 normal human colon specimens by LaPointe et al. (LaPointe et al. 2008), FN1 was reported to be expressed transcriptionally lower in the right vs. left colon. Although they didn't specify whether FN1 was also expressed transcriptionally lower in the right colon vs. rectum, the authors pointed out that no transcripts were expressed significantly differently between any two adjacent segments such as the rectum and left colon. If that is the case, the differential lower expression pattern of FN1 we observed here is not specific to the LS colorectum.

Understanding the protein expression in normal tissue is vital to understanding the expression changes in diseased tissue. Correspondingly, 2 studies on the expression patterns of sporadic CRC arising from different locations showed that there were significant regional differences in CRC using gene expression array and/or immunohistochemical staining (Minoo et al. 2010; Sanz-Pamplona et al. 2011). However, due to our limited sample size, we could not compare the expression patterns of adenomas from patients with LS among the different locations in the colorectum. Instead, we compared patient-matched, location-matched normal colon tissue and adenomas to minimize bias. Interestingly, we observed that PEA-15 was the only protein with significantly different expression between adenomas and adjacent normal- tissue after FDR justification, regardless of the location of the adenoma. PEA-15 is a small death effector domain–containing protein that is expressed ubiquitously. It is involved in the regulation of fundamental cellular functions, including apoptosis, proliferation, and glucose metabolism (Fiory et al. 2009)). High levels of PED/PEA-15 expression have been reported in gliomas (Hao et al. 2001) and (Xiao et al. 2002) and mammary carcinomas (Hwang et al. 1997; Stassi et al. 2005; Tsukamoto et al. 2000), suggesting a role in cancer development. In addition, transgenic mice with PEA-15 overexpression have an increased occurrence of skin papillomas upon sequential treatment with 7,12-dimethylbenz[a]anthracene (Formisano et al. 2005). In a more recent study by Sun et al (Sun et al. 2010), the expression level of PEA-15 was significantly higher in hepatocellular carcinoma (HCC) than in the adjacent normal tissues, and the level of expression was significantly associated with the pathological grade and clinical stage of HCC. To our knowledge, this is the first observation of overexpression of PEA-15 in adenomas from patient with LS. One of the common issues with array data is over interpretation. For this reason, we tested whether differences in expression level of PEA15 could be confirmed by western blotting. We were unable to ascertain the difference by western blotting (data not shown), perhaps because the limited sensitivity of this semi-quantification method did not allow us to detect the subtle difference between adenomas and matched normal tissue we observed in the RPPA. Considering that adenomas are premalignant, it will be interesting to determine whether PEA-15 is dramatically overexpressed in carcinmoas from LS patient when tissue becomes available.

Most recently, Yamauchi et al (Yamauchi et al. 2012b) assessed molecular features along colorectum segments in a study of 1443 cases of sporadic CRCs. They found that the frequencies of CpG island methylator phenotype (CIMP), MSI, and BRAF mutation increased gradually (statistically linearly) from the rectum to the ascending colon rather than changing abruptly at the splenic flexure. The pattern of gradual change was previously observed by LaPointe et al (LaPointe et al. 2008). A similar gradual transition along the normal colon for ESR1, HILC1, and APBA2 methylation markers was observed by Worthley et al (Worthley et al. 2010). All together, these findings challenge the common conception of distinct dichotomy of proximal vs distal colorectum and support the necessity for a paradigm shift from the established dichotomous model to a continuum model (Yamauchi et al. 2012a).

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Among the 17 proteins we identified that did not pass the multiple-comparison test, ERBB2, TP53, and PTEN play important roles in the endometrial cancer signaling pathway, and TP53, PTEN, and CCNB1 play important roles in the breast cancer signaling pathway. It has been reported that in addition to CRC, patients with LS have an increased risk of developing various extra-colonic malignancies (Pande et al. 2012). For example, women with LS have a 50–60% risk of developing endometrial cancer, which is considered part of the LS spectrum. In addition, an increased risk of breast cancer has been observed in patients with LS. Our study included 4 adenomas from 4 female patients with LS; 2 of these patients developed endometrial cancer in their 20s and 40s, respectively, and 1 had breast cancer in her 40s. Our observation of differential expression in those key proteins between tumor and adjacent normal tissue might provide a molecular basis for understanding why LS patients have an increased risk of developing endometrial cancer and breast cancer. Therefore, our findings might be of clinical relevance for the early detection or diagnosis of cancers at extra-colonic sites during follow-up for patients with LS.

There are several major limitations of our study. First, our sample size was very limited. However, our pilot study does shed light on the protein expression profiles of LS in the colorectum, which has not been examined by RPPA previously. Second, the panel of antibodies used in RPPA mainly focuses on the key proteins involved in several signaling pathways, such as cell death and survival, cellular growth and proliferation, cellular development, cell cycle regulation, and cellular movement. Therefore, the number of antibodies is very limited and not able to provide a whole-spectrum protein expression profile. Third, it has been reported that the risk of LS tumors appears different between *MLH1* and *MSH2* mutation carriers (Lindor et al. 2008). *MLH1* mutation carriers have a somewhat higher CRC risk than *MSH2* mutation carriers (OR, 1.3; 95% CI, 1.0–1.7) (Kastrinos et al. 2008) and extracolonic non-endometrial cancers are more prevalent in *MSH2* carriers than in *MLH1*. In the present study, although 5 out of 6 tumors were with *MSH2* mutation, using mixed samples of *MLH1* and *MSH2* mutation might cause some selection bias. In addition, the observation of ACF in the normal colon tissue of LS patients suggests that those normal appearing tissue might not be normal (Ramirez-Ramirez et al. 2012), therefore, the normal tissue used in our study might have some early transformations that cause bias for analysis. Further confirmation with a larger, homogenous sample size and a wider selection of antibodies will be more informative.

In conclusion, we found different protein expression profiles between different regions of the colon in patients with LS. Understanding the protein expression in normal tissue is vital to understanding the expression changes in tumor tissue. In addition, we observed significant differences between adenomas and matched normal colon samples. These results may have important implications for diagnostic and preventive strategies for patient with LS.

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Reference List

Aarnio M, Mecklin JP, Aaltonen LA, Nystrom-Lahti M, Jarvinen HJ. Life-time risk of different cancers in hereditary non-polyposis colorectal cancer (HNPCC) syndrome. International journal of cancer Journal international du cancer. 1995; 64 (6):430–433. [PubMed: 8550246]

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Bara J, Nardelli J, Gadenne C, Prade M, Burtin P. Differences in the expression of mucus-associated antigens between proximal and distal human colon adenocarcinomas. British journal of cancer. 1984; 49 (4):495–501. [PubMed: 6324842]

Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer research. 1998; 58 (22):5248–5257. [PubMed: 9823339]

Bufill JA. Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location. Annals of internal medicine. 1990; 113 (10):779–788. [PubMed: 2240880]

Carethers JM. One colon lumen but two organs. Gastroenterology. 2011; 141(2):411–412.10.1053/j.gastro.2011.06.029 [PubMed: 21708155]

Fiory F, Formisano P, Perruolo G, Beguinot F. Frontiers: PED/PEA-15, a multifunctional protein controlling cell survival and glucose metabolism. American journal of physiology Endocrinology and metabolism. 2009; 297(3):E592–601.10.1152/ajpendo.00228.2009 [PubMed: 19531639]

Formisano P, Perruolo G, Libertini S, Santopietro S, Troncone G, Raciti GA, Oriente F, Portella G, Miele C, Beguinot F. Raised expression of the antiapoptotic protein ped/pea-15 increases susceptibility to chemically induced skin tumor development. Oncogene. 2005; 24(47):7012–7021.10.1038/sj.onc.1208871 [PubMed: 16044159]

Gervaz P, Bucher P, Morel P. Two colons-two cancers: paradigm shift and clinical implications. Journal of surgical oncology. 2004; 88(4):261–266.10.1002/jso.20156 [PubMed: 15565587]

Glebov OK, Rodriguez LM, Nakahara K, Jenkins J, Cliatt J, Humbyrd CJ, DeNobile J, Soballe P, Simon R, Wright G, Lynch P, Patterson S, Lynch H, Gallinger S, Buchbinder A, Gordon G, Hawk E, Kirsch IR. Distinguishing right from left colon by the pattern of gene expression. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2003; 12 (8):755–762.

Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Clendenning M, Sotamaa K, Prior T, Westman JA, Panescu J, Fix D, Lockman J, LaJeunesse J, Comeras I, de la Chapelle A. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2008; 26(35):5783–5788.10.1200/JCO.2008.17.5950 [PubMed: 18809606]

Hao C, Beguinot F, Condorelli G, Trencia A, Van Meir EG, Yong VW, Parney IF, Roa WH, Petruk KC. Induction and intracellular regulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated apotosis in human malignant glioma cells. Cancer research. 2001; 61 (3):1162–1170. [PubMed: 11221847]

Howlader, N.; Noone, AM.; Krapcho, M.; Neyman, N.; Aminou, R.; Altekruse, SF.; Kosary, CL.; Ruhl, J.; Tatalovich, Z.; Cho, H.; Mariotto, A.; Eisner, MP.; Lewis, DR.; Chen, HS.; Feuer, EJ.; Cronin, KA., editors. SEER Cancer Statistics Review, 1975–2009 (Vintage 2009 Populations). 2012. Based on November 2011 SEER data submission, posted to SEER website

Hwang S, Kuo WL, Cochran JF, Guzman RC, Tsukamoto T, Bandyopadhyay G, Myambo K, Collins CC. Assignment of HMAT1, the human homolog of the murine mammary transforming gene (MAT1) associated with tumorigenesis, to 1q21.1, a region frequently gained in human breast cancers. Genomics. 1997; 42 (3):540–542. [PubMed: 9205133]

Iacopetta B. Are there two sides to colorectal cancer? International journal of cancer Journal international du cancer. 2002; 101(5):403–408.10.1002/ijc.10635 [PubMed: 12216066]

Iadevaia S, Lu Y, Morales FC, Mills GB, Ram PT. Identification of optimal drug combinations targeting cellular networks: integrating phospho-proteomics and computational network analysis. Cancer research. 2010; 70(17):6704–6714.10.1158/0008-5472.CAN-10-0460 [PubMed: 20643779]

Jensen OM. Different age and sex relationship for cancer of subsites of the large bowel. British journal of cancer. 1984; 50 (6):825–829. [PubMed: 6498080]

Kastrinos F, Stoffel EM, Balmana J, Steyerberg EW, Mercado R, Syngal S. Phenotype comparison of MLH1 and MSH2 mutation carriers in a cohort of 1,914 individuals undergoing clinical genetic testing in the United States. Cancer epidemiology, biomarkers & prevention : a publication of the

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American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008; 17(8):2044–2051.10.1158/1055-9965.EPI-08-0301

Komuro K, Tada M, Tamoto E, Kawakami A, Matsunaga A, Teramoto K, Shindoh G, Takada M, Murakawa K, Kanai M, Kobayashi N, Fujiwara Y, Nishimura N, Hamada J, Ishizu A, Ikeda H, Kondo S, Katoh H, Moriuchi T, Yoshiki T. Right- and left-sided colorectal cancers display distinct expression profiles and the anatomical stratification allows a high accuracy prediction of lymph node metastasis. The Journal of surgical research. 2005; 124(2):216–224.10.1016/j.jss.2004.10.009 [PubMed: 15820251]

LaPointe LC, Dunne R, Brown GS, Worthley DL, Molloy PL, Wattchow D, Young GP. Map of differential transcript expression in the normal human large intestine. Physiological genomics. 2008; 33(1):50–64.10.1152/physiolgenomics.00185.2006 [PubMed: 18056783]

Ligtenberg MJ, Kuiper RP, Chan TL, Goossens M, Hebeda KM, Voorendt M, Lee TY, Bodmer D, Hoenselaar E, Hendriks-Cornelissen SJ, Tsui WY, Kong CK, Brunner HG, van Kessel AG, Yuen ST, van Krieken JH, Leung SY, Hoogerbrugge N. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3′ exons of TACSTD1. Nature genetics. 2009; 41(1):112–117.10.1038/ng.283 [PubMed: 19098912]

Lindor NM, McMaster ML, Lindor CJ, Greene MH. National Cancer Institute DoCPCO, Prevention Trials Research, G. Concise handbook of familial cancer susceptibility syndromes - second edition. Journal of the National Cancer Institute Monographs. 2008; 38:1–93.10.1093/jncimonographs/lgn001 [PubMed: 18559331]

Lynch HT, Lanspa S, Smyrk T, Boman B, Watson P, Lynch J. Hereditary nonpolyposis colorectal cancer (Lynch syndromes I & II). Genetics, pathology, natural history, and cancer control, Part I. Cancer genetics and cytogenetics. 1991; 53 (2):143–160. [PubMed: 1648437]

Marra G, Boland CR. Hereditary nonpolyposis colorectal cancer: the syndrome, the genes, and historical perspectives. Journal of the National Cancer Institute. 1995; 87 (15):1114–1125. [PubMed: 7674315]

Mecklin JP, Jarvinen HJ. Tumor spectrum in cancer family syndrome (hereditary nonpolyposis colorectal cancer). Cancer. 1991; 68 (5):1109–1112. [PubMed: 1913482]

Minoo P, Zlobec I, Peterson M, Terracciano L, Lugli A. Characterization of rectal, proximal and distal colon cancers based on clinicopathological, molecular and protein profiles. International journal of oncology. 2010; 37 (3):707–718. [PubMed: 20664940]

Niessen RC, Hofstra RM, Westers H, Ligtenberg MJ, Kooi K, Jager PO, de Groote ML, Dijkhuizen T, Olderode-Berends MJ, Hollema H, Kleibeuker JH, Sijmons RH. Germline hypermethylation of MLH1 and EPCAM deletions are a frequent cause of Lynch syndrome. Genes, chromosomes & cancer. 2009; 48(8):737–744.10.1002/gcc.20678 [PubMed: 19455606]

Pande M, Wei C, Chen J, Amos CI, Lynch PM, Lu KH, Lucio LA, Boyd-Rogers SG, Bannon SA, Mork ME, Frazier ML. Cancer spectrum in DNA mismatch repair gene mutation carriers: results from a hospital based Lynch syndrome registry. Familial cancer. 201210.1007/s10689-012-9534-6

Ramirez-Ramirez MA, Sobrino-Cossio S, de la Mora-Levy JG, Hernandez-Guerrero A, de Macedo-Reyes VJ, Maldonado-Martinez HA, Alonso-Larraga JO, Ramirez-Solis ME. Loss of expression of DNA mismatch repair proteins in aberrant crypt foci identified in vivo by magnifying colonoscopy in subjects with hereditary nonpolyposic and sporadic colon rectal cancer. Journal of gastrointestinal cancer. 2012; 43(2):209–214.10.1007/s12029-011-9303-z [PubMed: 21744313]

Rijcken FE, Hollema H, Kleibeuker JH. Proximal adenomas in hereditary non-polyposis colorectal cancer are prone to rapid malignant transformation. Gut. 2002; 50 (3):382–386. [PubMed: 11839719]

Sanz-Pamplona R, Cordero D, Berenguer A, Lejbkowicz F, Rennert H, Salazar R, Biondo S, Sanjuan X, Pujana MA, Rozek L, Giordano TJ, Ben-Izhak O, Cohen HI, Trougouboff P, Bejhar J, Sova Y, Rennert G, Gruber SB, Moreno V. Gene expression differences between colon and rectum tumors. Clinical cancer research : an official journal of the American Association for Cancer Research. 2011; 17(23):7303–7312.10.1158/1078-0432.CCR-11-1570 [PubMed: 21976543]

Stassi G, Garofalo M, Zerilli M, Ricci-Vitiani L, Zanca C, Todaro M, Aragona F, Limite G, Petrella G, Condorelli G. PED mediates AKT-dependent chemoresistance in human breast cancer cells. Cancer research. 2005; 65(15):6668–6675.10.1158/0008-5472.CAN-04-4009 [PubMed: 16061647]

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Sun DG, Wu YF, Gao ZM, Liang R, Wang LM. Expression and clinical significance of anti-apoptotic factors PED/PEA-15 and XIAP in hepatocellular carcinoma. Zhonghua yi xue za zhi. 2010; 90 (24):1660–1664. [PubMed: 20979872]

Tsukamoto T, Yoo J, Hwang SI, Guzman RC, Hirokawa Y, Chou YC, Olatunde S, Huang T, Bera TK, Yang J, Nandi S. Expression of MAT1/PEA-15 mRNA isoforms during physiological and neoplastic changes in the mouse mammary gland. Cancer letters. 2000; 149 (1–2):105–113. [PubMed: 10737714]

Vasen HF. Clinical description of the Lynch syndrome [hereditary nonpolyposis colorectal cancer (HNPCC)]. Familial cancer. 2005; 4(3):219–225.10.1007/s10689-004-3906-5 [PubMed: 16136381]

Worthley DL, Whitehall VL, Buttenshaw RL, Irahara N, Greco SA, Ramsnes I, Mallitt KA, Le Leu RK, Winter J, Hu Y, Ogino S, Young GP, Leggett BA. DNA methylation within the normal colorectal mucosa is associated with pathway-specific predisposition to cancer. Oncogene. 2010; 29(11):1653–1662.10.1038/onc.2009.449 [PubMed: 19966864]

Xiao C, Yang BF, Asadi N, Beguinot F, Hao C. Tumor necrosis factor-related apoptosis-inducing ligand-induced death-inducing signaling complex and its modulation by c-FLIP and PED/PEA-15 in glioma cells. The Journal of biological chemistry. 2002; 277(28):25020–25025.10.1074/jbc.M202946200 [PubMed: 11976344]

Yamauchi M, Lochhead P, Morikawa T, Huttenhower C, Chan AT, Giovannucci E, Fuchs C, Ogino S. Colorectal cancer: a tale of two sides or a continuum? Gut. 2012a; 61(6):794–797.10.1136/gutjnl-2012-302014 [PubMed: 22490520]

Yamauchi M, Morikawa T, Kuchiba A, Imamura Y, Qian ZR, Nishihara R, Liao X, Waldron L, Hoshida Y, Huttenhower C, Chan AT, Giovannucci E, Fuchs C, Ogino S. Assessment of colorectal cancer molecular features along bowel subsites challenges the conception of distinct dichotomy of proximal versus distal colorectum. Gut. 2012b; 61(6):847–854.10.1136/gutjnl-2011-300865 [PubMed: 22427238]

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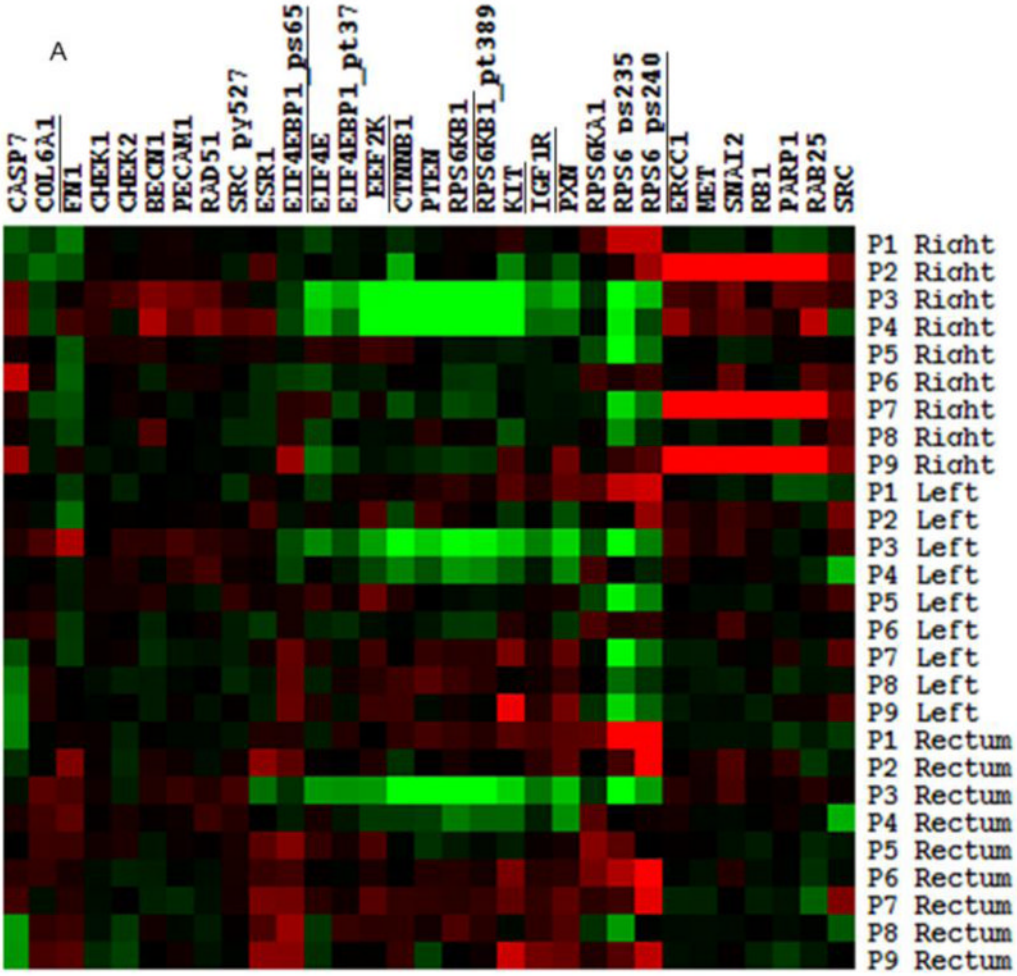

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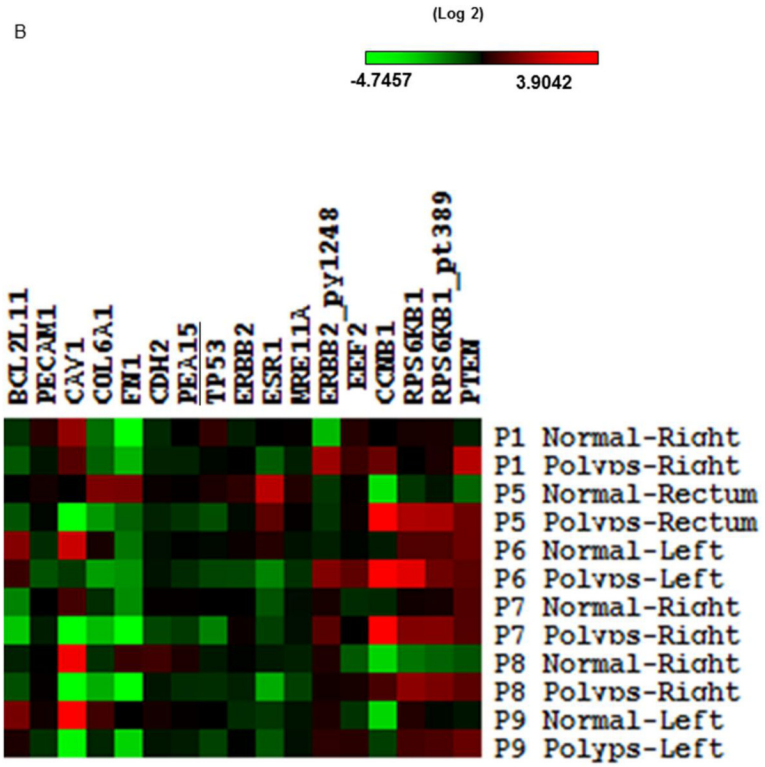

Figure 1.
Heatmap of RPPA. A, Protein expression was compared among different regions of the colon. B, Protein expression was compared between normal colon and adenomas. The vertical axis shows the proteins tested in the RPPA, and the horizontal axis shows the location/type of samples. The significant proteins after multiple test correction ($q < 0.05$) were underlined.

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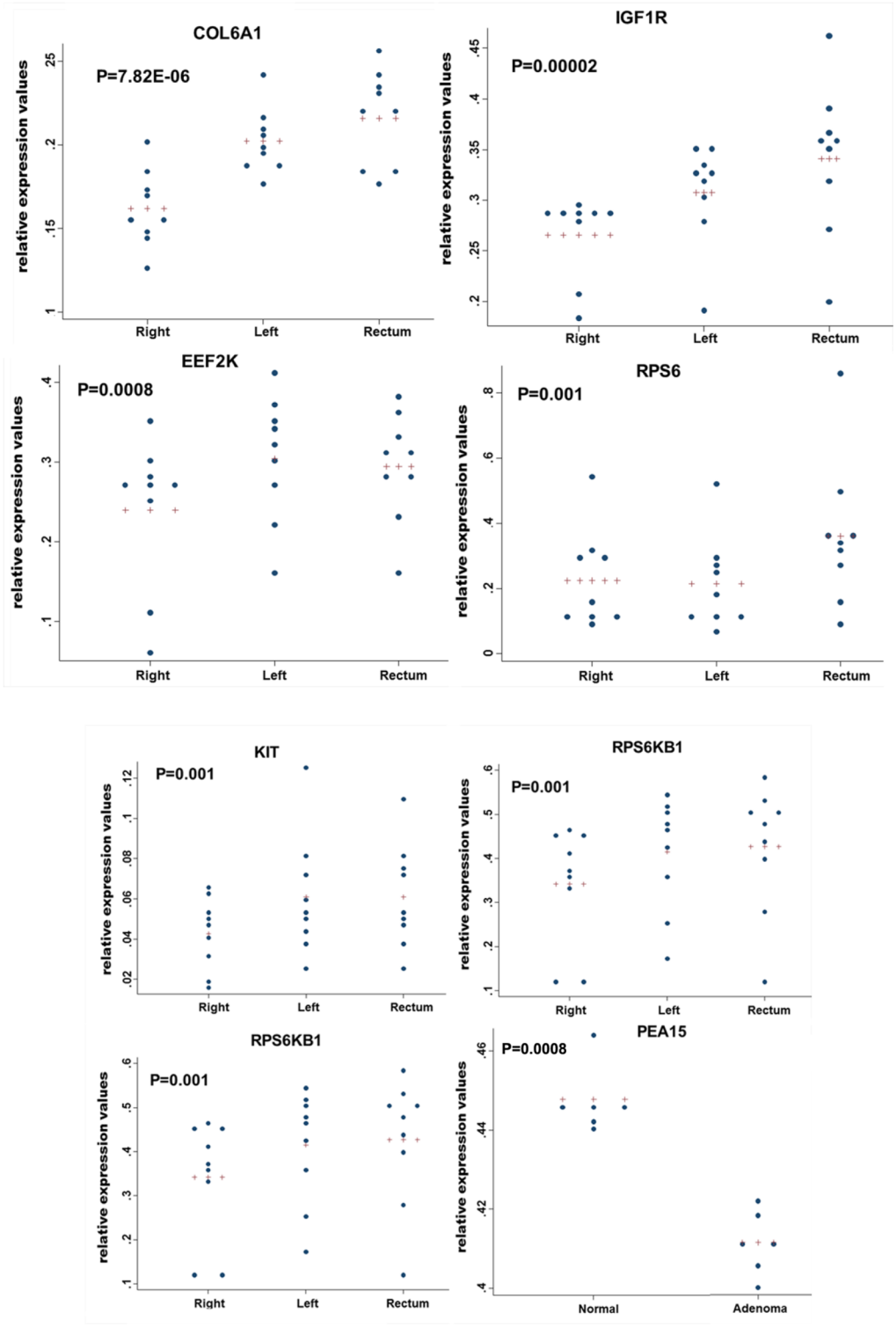

Figure 2.

Identification of proteins differentially expressed among different locations/types of colon samples. The horizontal lines of pluses (+) represent the mean relative protein expression values.

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Table 1

Sample description

	No. of patients (%)	No. of normal tissues (%)	No. of adenomas (%)
Sex			
Male	3 (33.3)	9 (33.3)	2 (33.3)
Female	6 (66.7)	18 (66.7)	4 (66.7)
MMR gene mutated			
MLH1	2 (28.6)	6 (28.6)	1 (16.7)
MSH2	7 (71.4)	21 (71.4)	5 (83.3)
Tissue location			
Right colon		9 (33.3)	3 (50.0)
Left colon		9 (33.3)	2 (33.3)
Rectum		9 (33.3)	1 (16.7)

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Table 2

Differences in protein expression by colon location (right-side, left-side, or rectum) or between normal colon and adenomas

Right vs. left	P-value	Fold change
IGF1R	**0.0002**	0.87
COL6A1	**0.0005**	0.80
EEF2K	0.002	0.77
RPS6KB1	0.006	0.80
AKT1/AKT2/AKT3	0.009	0.84
CTNNB1	0.009	0.70
PEA15	0.010	1.07
KIT	0.012	0.72
CAV1	0.015	0.88
PTEN	0.018	0.76
RPS6KB1_pt389	0.023	0.85
PECAM1	0.024	1.07
EIF4EBP1_pt37	0.030	1.10
BCL2L11	0.031	0.85
CDH3	0.032	1.14
EIF4EBP1	0.037	0.90
EIF4E	0.038	0.83

Right vs. rectum	P-value	Fold change
FN1	**0.0002**	0.68
COL6A1	**0.0003**	0.76
IGF1R	**0.0007**	0.79
AKT1/AKT2/AKT3	0.002	0.79
KIT	0.003	0.71
EIF4EBP1	0.004	0.86
CHEK1	0.006	1.06
RPS6KB1	0.007	0.81
EIF4EBP1_ps65	0.008	0.78
RPS6KB1_pt389	0.010	0.82
EEF2K	0.010	0.79
RPS6_ps240_s244	0.011	0.64
RPS6_ps235_s236	0.011	0.69
SRC_py416	0.012	1.32
PXN	0.027	0.84
CASP7	0.029	1.50
PRKAA1	0.030	1.50
CTNNB1	0.030	0.71
RAD51	0.041	1.09
EIF4E	0.047	0.85

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Left vs. rectum	P-value	Fold change
EIF4EBP1_ps65	0.003	0.84
INPP4B	0.004	1.06
RB1	0.005	1.04
RPS6_ps240_s244	0.009	0.63
RPS6_ps235_s236	0.012	0.67
PTK2	0.013	0.89
IRS1	0.018	0.94
VASP	0.018	1.06
IGF1R	0.024	0.91
MSH2	0.027	1.07
CHEK2	0.029	1.11
CCNB1	0.030	1.19
SRC_py527	0.031	0.93
PIK3R1	0.039	1.08
BIRC2	0.039	1.04
EIF4EBP1_pt37	0.041	0.93
ESR1	0.049	0.86

Adenomas vs. Normal	P-value	Fold change
PEA15	**0.0008**	1.09
BCL2L11	0.002	1.12
TP53	0.005	1.14
CAV1	0.014	1.89
CCNB1	0.017	0.53
EEF2	0.019	0.89
PECAM1	0.021	1.06
ERBB2_py1248	0.022	0.89
COL6A1	0.022	1.25
MRE11A	0.026	1.04
RPS6KB1_pt389	0.028	0.83
ESR1	0.029	1.15
RPS6KB1	0.032	0.79
ERBB2	0.039	0.85
CDH2	0.040	1.08
PTEN	0.041	0.81
FN1	0.045	1.33

The significant results after multiple test correction ($q < 0.05$) are shown in bold.