RESEARCH ARTICLE

Aberrant DNA Methylation: Implications in Racial Health Disparity

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Abstract

Background

Incidence and mortality rates of colorectal carcinoma (CRC) are higher in African Americans (AAs) than in Caucasian Americans (CAs). Deficient micronutrient intake due to dietary restrictions in racial/ethnic populations can alter genetic and molecular profiles leading to dysregulated methylation patterns and the inheritance of somatic to germline mutations.

Materials and Methods

Total DNA and RNA samples of paired tumor and adjacent normal colon tissues were prepared from AA and CA CRC specimens. Reduced Representation Bisulfite Sequencing (RRBS) and RNA sequencing were employed to evaluate total genome methylation of 5′ regulatory regions and dysregulation of gene expression, respectively. Robust analysis was conducted using a trimming-and-retrieving scheme for RRBS library mapping in conjunction with the BStool toolkit.

Results

DNA from the tumor of AA CRC patients, compared to adjacent normal tissues, contained 1,588 hypermethylated and 100 hypomethylated differentially methylated regions (DMRs). Whereas, 109 hypermethylated and 4 hypomethylated DMRs were observed in DNA from the tumor of CA CRC patients; representing a 14.6-fold and 25-fold change, respectively. Specifically; CHL1, 4 anti-inflammatory genes (i.e., NELL1, GDF1, ARHGEF4, and ITGA4), and 7 miRNAs (of which miR-9-3p and miR-124-3p have been implicated in CRC) were hypermethylated in DNA samples from AA patients with CRC. From the same sample set, RNAseq analysis revealed 108 downregulated genes (including 14 ribosomal proteins) and 34 upregulated genes (including POLR2B and CYP1B1 [targets of miR-124-3p]) in AA patients with CRC versus CA patients.

PLOS ONE | DOI:10.1371/journal.pone.0153125 April 25, 2016 1/16
Conclusion

DNA methylation profile and/or products of its downstream targets could serve as biomarker(s) addressing racial health disparity.

Introduction

The incidence and mortality rates of colorectal cancer (CRC) in the United States are higher in African Americans (AAs) as compared to all other ethnic/racial groups [1]. One report illustrated a 30–50% higher rate of CRC mortality in AAs post-diagnosis compared to CAs. Moreover, this racial health disparity continues to expand despite increased CRC screening [2–7]. AAs also develop and are diagnosed with CRC at a younger age compared to CAs [8]. Cumulatively, it is hypothesized that 1) epigenetic or molecular differences elicit this prevalent racial disparity, and 2) socio-economic factors are at least partly responsible for these variances. In addition, the initiation and progression of CRC is linked to chronic intestinal inflammation. In North America, the risk of CRC in patients with inflammatory bowel disease is 2 times greater as compared to the general population [9].

It is well documented that factors such as diet and lack of preventive medical care are influential in incidence and early detection of disease. 12% of all CRC cases, regardless of ethnic background or other demographic factors, are attributed to a Western diet/nutrition [10]. Recent epidemiological studies have concluded that the abundance or deficiency of specific dietary micronutrients increases the risk for development and progression of CRC. For example, dietary folate levels regulate nucleotide synthesis and impact DNA methylation, which in turn alters cell proliferation, DNA repair and genomic stability [11]. Disparity in CRC incidence and ethnic genomic variation may have a direct correlation due in part to ethnic dietary patterns. Importantly somatic mutations may progressively become germline mutations [12]. For example, the p53 tumor suppressor gene, known to be mutated in over 50% of all human cancers [13], has unique polymorphisms within AAs further contributing to racial disparity seen in CRC patients [14].

Aberrant CpG island hypermethylation at the promoter of tumor suppressor transcription factors [15] and hypomethylation of oncogenes [16] are important mechanisms for gene inactivation or activation, respectively. This aberration is influential in accumulating genomic alterations leading to carcinogenesis. While many studies seek to define methylation patterns in CRC across the broad population, little is known about the role epigenetic differences play in racial/ethnic health disparity. For instance, sporadic CRC caused by promoter hypermethylation of the mismatch repair gene MLH1 could result in underlying genetic predisposition for AA in later generations [17]. Such is true in hereditary non-polyposis CRC with germline mutations in mismatch repair genes MLH1, MLH2, MSH6, and PMS2. Resulting microsatellite instabilities (MSIs) disproportionately occur in AAs compared to CAs which contribute to accelerated CRC progression [18]. In addition, dysregulation of microRNAs (miRNAs) are well-documented across many types of cancers and are potential biomarkers for cancer classification and prognosis [19]. The mechanism underlying miRNA dysregulation in cancer is not fully understood; however, recent studies have shown that epigenetic mechanisms play important roles in the regulation of miRNA expression [20]. Importantly, miRNAs can act as either tumor suppressors by inhibiting oncogenic gene expression or, conversely, as oncomirs by inhibiting tumor suppressor gene expression.

Here, we demonstrate that AA CRC specimens have significantly higher levels of hyper- and hypomethylation versus CA CRC specimens. Comparative analysis was conducted to elucidate epigenetic differences that may be drivers of racial disparity seen in incidence of CRC.
Association of aberrant methylation to its effects on downstream gene transcription was assessed by RNA sequencing. Here, we report that hypermethylation of the anti-inflammatory transcription factors NELL1, GDF1, ARHGEF4, and ITGA4 and multiple miRNAs including miR-9-3p and miR-124-3p may be factors driving the disparity observed in incidence of CRC between racial and ethnic groups. We also reveal, as determined by RNA sequencing analysis, that two targets of miR-124-3p (Polymerase (RNA) II Polypeptide B (POLR2B) and Cytochrome P450, Family 1, Subfamily B, Polypeptide 1 (CYP1B1)) are upregulated and 14 ribosomal proteins (RPs) are downregulated in tumors of AA CRC patients. This would suggest that functional and genetic studies are necessary to determine which hyper/hypomethylation events are truly relevant to human tumorigenesis and contribute to racial health disparity.

**Materials and Methods**

**Ethics statement**

This study was approved by the Washington University (WU) School of Medicine-St. Louis and Stony Brook University (SBU) Institutional Review Boards, approval #93677–16. Tissues were obtained from the WU (http://www.siteman.wustl.edu/ContentPage.aspx?id=243) and SBU human biobanks. The samples and clinical metadata were de-identified, assigned independent patient and sample codes prior to release to the researchers, and qualified for a waiver of consent per 45CFR46.116.d.

**Nucleic acid extraction from WU colon cancer tumor and adjacent normal colon tissue samples**

Total DNA and total RNA were prepared by and acquired from the Siteman Cancer Center Tissue Procurement Facility at Washington University (WU)-St. Louis for 6 AA and 7 CA colon cancer patients who underwent colon cancer surgery at Barnes Jewish Hospital. The total RNA was extracted from the 13 pairs of snap-frozen tumors and adjacent matching normal colon using TRIzol followed by lithium precipitation (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. RNA was qualitatively and quantitatively assessed using a NanoDrop 2000C (Thermo Scientific, Waltham, MA).

**DNA methylation at CpG islands**

Methylation of the 5’-regulatory region of the genome was analyzed by Reduced Representation Bisulfite Sequencing (RRBS) using the ABI 37370 (Applied Biosystems, Foster City, CA) with a 48 cm capillary array (Cold Spring Harbor Laboratory [CSHL] Core Facility).

**RNA sequencing**

RNA sequencing analysis was conducted by Illumina sequencing (CSHL core facility). A False Discovery Rate (FDR) of 0.05 was used to determine differentially expressed genes. Genes selected were analyzed for log2 transformed fold change of expression level in tumor tissues compared to adjacent normal tissues. Those $\leq 0.05$ were disregarded in the analyses.

**Statistical analysis**

A trimming-and-retrieving alignment scheme, recently described by our group, was use for accurate global profiling of DNA methylation. This algorithm is specifically designed for the mapping of bisulfite converted reads from RRBS libraries. The methylation percentage calling is performed by counting methylated and unmethylated bases (and their ratio) on each site.
that is a C in the reference genome. This strategy ensures both accurate bisulfite-converted read alignment and methylation calling [21].

Results

Demographics of colon cancer subjects

The clinical metadata available for the WU samples were limited to age (at time of surgical resection of the tumor), sex (male/female) and race (AA or CA). DNA and RNA samples isolated from paired tumor and adjacent normal colon were prepared from 6 AA and 7 CA subjects. The average ages of the AA subjects (72.7±3.6) and CA subjects (62.4±6.3) were not significantly different. Similarly, the sex distribution was comparable between AA (3 female, 2 male, 1 unspecified) and CA (4 female, 3 male) patients.

Increased genome-wide methylation in AA patients with colon cancer

The total DNA methylation profiles of AA and CA CRC patients were examined using RRBS and analyzed using the BStool toolkit with a trimming-and-retrieving scheme. In total, there was a significantly greater number of DMR regions across the AA colon cancer samples compared to that found in samples of CA colon cancer patients. In DNA samples examined from AA patients, 27,059 methylated CpG sites were detected in 1,688 DMRs. 93% (1,569 sites) of methylated CpG sites was located within the island regions whereas 4% (67 sites) was within the shore regions. Furthermore, 15.8% (266 DMRs) of methylated DMRs was contained within the promoter, 5.4% (91 DMRs) spanned the promoter and gene body, and a majority (58.9%; 995 DMRs) existed within the gene body (Fig 1A). In comparison, 764 methylated CpG sites

![Fig 1. Aberrant methylation in tumors of AA patients with CRC.](https://example.com)

- A: A total of 27,059 methylated CpG sites were detected in 1,688 DMRs in AA CRC specimens.
- B: 1,588 DMRs (94.1% of total DMRs) were hypermethylated.
- C: 100 DMRs (5.9%) were hypomethylated.

**Fig 1.** Aberrant DNA Methylation and Racial Health Disparity

PLOS ONE | DOI:10.1371/journal.pone.0153125 April 25, 2016 4/16
were identified across 113 DMRs of DNA from tumor samples of CA. 96.5% (109 sites) of methylated CpG sites was found within the island regions whereas 2.7% (3 sites) was within the shore regions. Furthermore, 21.2% (24 DMRs) of methylated DMRs was located within the promoter, 0.9% (1 DMR) spanned the promoter and gene body, and a majority (59.3%; 67 DMRs) existed within the gene body (Fig 2A). This represents a 35.4-fold and a 14.9-fold difference in methylated CpG sites and in DMRs in AA patients, respectively, with no observed differences in the percent composition of CpG sites (islands and shores) or DMRs (promoter, promoter and gene body, and gene body).

The rates of hyper- and hypomethylation were also analyzed for both sample sets. In DNA obtained from AA tumor samples, 1,588 DMRs were hypermethylated of which 95.8% (1,521 sites) was within CpG islands and 3% (48 sites) was within shores, and 16.7% (265 DMRs) was within the promoter, 5.7% (91 DMRs) spanned the promoter and gene body, and 58.4% (928 DMRs) was within the gene body (Fig 1B). In DNA from CA CRC specimens, 109 DMRs were hypermethylated of which 97.2% (106 sites) was within CpG islands and 1.8% (2 sites) was within shores, and 22% (24 DMRs) was within the promoter, 0.9% (1 DMR) spanned the promoter and gene body, and 57.8% (63 DMRs) was within the gene body (Fig 2B). Additionally, 100 DMRs were hypomethylated (5.9% of total DMRs) in AA CRC samples of which 48.8% (48 sites) was within CpG islands and 19% (19 sites) was within shores, and 1% (1 DMR) was within the promoter, 0% spanned the promoter and gene body, and 67% (67 DMRs) was within the gene body (Fig 1C). In contrast, 4 hypomethylated DMRs (3.5% of total DMRs) were observed in DNA obtained from CA tumor and compared to normal samples of which
75% (3 sites) was within CpG islands and 25% (1 site) was within shores, and 100% (4 DMRs) was within the gene body (Fig 2C).

Genes are differentially methylated in AA CRC compared to CA CRC

Genes were ranked by their statistical significance of differential methylation for both AA and CA specimens compared to their respective matched normal tissue samples. An abridged list contains 23 hypermethylated genes and 4 hypomethylated genes (CACNA2D4; LRTM2, ESPNL, SECTM1, PCDH8) for AA tumor samples; whereas, 29 hypermethylated genes and 1 hypomethylated gene (BRSK2) are listed for the CA tumor samples (Table 1). These lists were then cross-referenced in order to illustrate shared differentially methylated genes in the AA and CA samples. 2 hypermethylated genes (CCDC178 and FLI1) were common between the two sample sets. Of note, the hypermethylation of CHL1, a member of neuronal cell adhesion molecules involved in neuronal development and synaptic plasticity, was found in AA CRC but not CA CRC.

Table 1. Annotated list of the highest differentially methylated genes for AA and CA CRC specimens, ranked by statistical significance.

| African American Tumor Sample | Caucasian American Tumor Sample |
|-------------------------------|---------------------------------|
| **Gene**                      | **Gene**                        | **p-value** | **Gene**                      | **p-value** |
| CACNA2D4;LRTM2                | QKI;CAHM                        | 3.49E-25    | BRSK2                         | 3.88E-29    |
| FENDRR                       | BRSK2                           | 4.77E-18    | BRSK2                         | 3.21E-17    |
| APC2                         | NDRG4                           | 8.43E-18    | SDC2                          | 1.53E-16    |
| GSC                          | PHYHIPL                         | 1.16E-16    | PHYHIPL                       | 7.68E-15    |
| CHL1                         | VWC2                            | 4.64E-16    | VWC2                          | 2.01E-14    |
| KIAA1211L                    | GPR75-ASB3;GPR75                | 1.26E-15    | GPR75-ASB3;GPR75              | 3.22E-17    |
| KCNA1                       | FBLL1                           | 3.42E-15    | FBLL1                         | 2.19E-13    |
| ESPNL                       | LOC146880                       | 5.85E-15    | LOC146880                     | 2.19E-13    |
| LHX5                        | FGF14                           | 2.32E-14    | FGF14                         | 4.14E-13    |
| HOXA3                       | SH3GL3                          | 3.90E-14    | SH3GL3                        | 8.28E-12    |
| LINC01398                    | ESR1                            | 3.31E-13    | ESR1                          | 8.48E-12    |
| PTPRN2                      | NDRG4                           | 3.53E-13    | NDRG4                         | 3.28E-11    |
| ECEL1                       | GPR75                           | 1.23E-12    | GPR75                         | 7.20E-11    |
| GPR158;GPR158-AS1            | FLI1                            | 1.88E-12    | FLI1                          | 3.01E-10    |
| IFITM10                      | ERICH1-AS1                      | 1.91E-12    | ERICH1-AS1                    | 4.03E-10    |
| NELL1                       | NRG3                            | 2.39E-12    | NRG3                          | 5.61E-10    |
| NDRG4                       | CCDC178                         | 2.40E-12    | CCDC178                       | 9.01E-10    |
| MMD2                        | CHST2                           | 2.89E-12    | CHST2                         | 9.23E-10    |
| C9orf50; NTMT1               | KCNG3                           | 9.34E-12    | KCNG3                         | 1.28E-09    |
| CCDC178                     | GDF6                            | 1.05E-11    | GDF6                          | 6.50E-09    |
| FLI1; SENCRI                 | HS3ST2                          | 1.32E-11    | HS3ST2                        | 6.58E-09    |
| C8orf64;LOC286189            | FLI1                            | 2.42E-11    | FLI1                          | 8.38E-09    |
| MDFI;MDFI                   | ADAMTS2                         | 2.50E-11    | ADAMTS2                       | 1.57E-08    |
| GUCY2D                      | NKG6-2                          | 3.02E-11    | NKG6-2                        | 2.02E-08    |
| SECTM1                      | RASA3                           | 3.65E-11    | RASA3                         | 2.78E-08    |
| GDF1;CERS1                  | SLC6A2                          | 4.15E-11    | SLC6A2                        | 3.13E-08    |
| PCDH8;PCDH8                 | CDH4                            | 4.21E-11    | CDH4                          | 6.17E-08    |
|                              | ALX4                            |              | ALX4                          | 7.18E-08    |
|                              | ANKRD13B                        |              | ANKRD13B                      | 8.17E-08    |
|                              | FAM19A5                         |              | FAM19A5                       | 9.54E-08    |

doi:10.1371/journal.pone.0153125.t001
Racial disparity of differentially methylated microRNAs in CRC

Within the entire DNA methlyome of AA CRC specimens, 7 microRNAs were found to be hypermethylated compared to the respective matching adjacent normal tissue (Table 2) including miR-9-3p and miR-124-3p (S1 Fig). Likewise, two isoforms of miR-34 were the only miRNAs found to be hypermethylated in CA CRC samples. This study was unable to distinguish miRNAs that were significantly hypomethylated in either AA or CA tumors.

Differential gene expression in AA CRC

RNA sequencing was conducted using total RNA from the same patient CRC samples that were submitted for DNA methylation analysis. Analysis was first used to identify differentially expressed genes within the ethnic background for both AA and CA by evaluating CRC specimens against the respective matching adjacent normal tissues. For all analysis, only genes with \( p < 0.05 \) and a False Discovery Rate (FDR) \( < 0.05 \) were considered to be dysregulated. In AA CRC, 205 genes were downregulated (S1 Table) and 150 genes were upregulated (S2 Table) compared to the normal tissue (Fig 3A). Two miRNAs were reported within these differentially regulated genes; miR-4253 was upregulated and miR-3074 was downregulated. In the CA CRC specimens, 7 genes were upregulated (S3 Table) whereas no genes were reported to be downregulated when compared to normal adjacent tissue. Additionally, only SLCO4A1 and OXGR1 were upregulated in both AA and CA cases of CRC.

To evaluate the disparity of gene dysregulation between race, results obtained from AA CRC specimens were statistically analyzed against those results obtained from specimens of CA CRC. 108 genes were downregulated (S4 Table) and 34 genes were upregulated (S5 Table) in tumors of AA CRC patients versus CA CRC (Fig 3B). The top 15 downregulated and upregulated genes ranked by statistical significance are shown in Table 3 and Table 4, respectively. miR-1279 was found to be differentially upregulated in AA CRC compared to CA CRC, and was the only miRNA dysregulated between tumors of AA and CA CRC patients. Most strikingly, of the 108 genes downregulated in AA CRC tumors, 14 were ribosomal proteins including 10 members of the large 60S subunit (RPL7A, RPL8, RPL13, RPL13A, RPL18, RPL28, RPL29, RPL36, RPLP0, and RPLP1), 3 members of the small 40S subunit (RPS2, RPS15, and RPS19), and 1 mitochondrial ribosomal protein (MRPL12) of the large 39S subunit (Table 5). Additionally, two targets of miR-124-3p (POLR2B and CYP1B1) were upregulated.

Discussion

Chronic colonic inflammation from inflammatory bowel diseases (IBDs) results in a well-recognized increased risk of colon carcinogenesis [44–47]. As stated by Rubin et al, “It has become
increasingly clear that IBD is a polygenic, complex disorder with region- and ethnic-specific differences in genetic risk factors. In the past several years, progress in understanding the molecular basis of IBD has accelerated, beginning with the generation of animal models of colitis and progressing to the identification of specific genetic markers from candidate gene, gene linkage, and genome-wide association analyses [48]. Therefore suppression of anti-inflammatory transcription factors, as with hypermethylation, would promote the development and progression of CRC. Here, we observed the aberrant hypermethylation of several genes that are implicated in anti-inflammatory mechanisms including NEL-Like 1 (NELL1) [49], Growth Differentiation Factor 1 (GDF1) [50], and Rho Guanine Nucleotide Exchange Factor (ARH-GEF4) [51], and Integrin Alpha 4 (ITGA4) [52] in AA CRC but not CA CRC. Previous studies have demonstrated that ITGA4 is hypermethylated in inflamed colon tissue/colitis, and that the treatment of anti-ITGA4 antibodies further aggravate colitis by IL-1β, TNF-α, and IFN-γ recruitment [53]. Conversely, the treatment of ITGA4 antibodies in combination with
conventional therapies alleviated colitis by suppression of IL-1β and iNOS in mouse models [54]. If these anti-inflammatory genes are constitutively hypermethylated in tumors of AA CRC patients, these proteins could potentially drive CRC initiation and progression and thereby serve as targets for pharmaceutical intervention and therapy.

Similarly, examining differentially methylated miRNAs and their effects on presumptive downstream gene targets is also critical in understanding the epigenetic variances responsible for increased incidence and mortality of CRC in African Americans. Here, we demonstrate that 7 miRNAs are hypermethylated in AA CRC specimens via RRBS. Two of these miRNAs have been implicated in the initiation of CRC; miR-9 and miR-124. miR-9 is downregulated in

### Table 3. Annotated list of differentially downregulated genes in AA CRC compared to CA CRC, ranked by statistical significance.

| Gene   | Fold Change (log2) | p-value     | FDR        |
|--------|--------------------|-------------|------------|
| RPL13  | -2.650             | 6.91E-09    | 6.41E-05   |
| HMGCS2 | -4.244             | 1.55E-08    | 7.20E-05   |
| MYH14  | -2.591             | 4.15E-08    | 0.00013    |
| TFF3   | -3.381             | 1.13E-07    | 0.00023    |
| CES2   | -2.945             | 1.71E-07    | 0.00023    |
| KRT19  | -3.054             | 1.80E-07    | 0.00023    |
| RPS2   | -2.739             | 1.87E-07    | 0.00023    |
| FAM3D  | -3.447             | 2.02E-07    | 0.00023    |
| RPL36  | -2.350             | 2.39E-07    | 0.00025    |
| RPL28  | -2.351             | 4.69E-07    | 0.00037    |
| C10orf99| -3.804             | 8.22E-07    | 0.00051    |
| CDX1   | -3.132             | 1.08E-06    | 0.00060    |
| CHMP4B | -1.693             | 1.10E-06    | 0.00060    |
| CXCL14 | -2.548             | 1.20E-06    | 0.00062    |
| YBX1   | -1.786             | 1.65E-06    | 0.00081    |

doi:10.1371/journal.pone.0153125.t003

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### Table 4. Annotated list of differentially upregulated genes in AA CRC compared to CA CRC, ranked by statistical significance.

| Gene   | Fold Change (log2) | p-value     | FDR        |
|--------|--------------------|-------------|------------|
| THBS2  | 2.192              | 3.22E-07    | 0.00030    |
| MNS1   | 1.920              | 4.73E-07    | 0.00037    |
| BDNF-AS1| 3.451              | 5.83E-07    | 0.00041    |
| PCA3   | 3.530              | 6.24E-07    | 0.00041    |
| DNM1P46| 2.911              | 4.53E-06    | 0.00200    |
| CYP1B1 | 3.149              | 1.15E-05    | 0.00357    |
| OBSCN  | 2.066              | 1.61E-05    | 0.00428    |
| BCA1T1 | 2.980              | 3.63E-05    | 0.00823    |
| RNF224 | 2.672              | 4.27E-05    | 0.00863    |
| ZNF772 | 2.993              | 5.85E-05    | 0.01024    |
| MAP4K4 | 1.452              | 6.52E-05    | 0.01070    |
| EMB    | 2.660              | 8.19E-05    | 0.01226    |
| MIR1279| 1.563              | 8.65E-05    | 0.01249    |
| SLC2A3 | 2.647              | 8.74E-05    | 0.01249    |
| ZFHX4  | 3.021              | 0.00014     | 0.01745    |

doi:10.1371/journal.pone.0153125.t004
CRC resulting in the promotion of tumor survival and proliferation [55, 56]. Increased mortality rates are correlated with this inhibition [57]. miR-124 inhibits CRC progression in vitro and in vivo [58] and is suppressed in clinical specimens associated with IBD [59].

Analysis of altered downstream gene expression validates the observation of aberrant methylation. The upregulation of POLR2B and CYP1B1, 2 known targets of miR-124-3p [60], in AA CRC but not in CA CRC was the result of the hypermethylation of miR-124-3p. POLR2B, a DNA-dependent RNA polymerase, catalyzes the transcription of DNA to mRNA, microRNA, and small non-coding RNAs [61], and while POLR2B is not known to have a prominent role in the tumorigenesis and progression of cancer(s), a specific haplotype derived from single nucleotide polymorphisms (SNPs) is associated with an increased frequency of head and neck cancers [62]. CYP1B1, a member of the monooxygenase cytochrome P450 family, catalyzes drug metabolism and lipid synthesis. CYP1B1 localizes to the endoplasmic reticulum and metabolizes a variety of procarcinogens and xenobiotics [63]. Previous studies have demonstrated that CYP1B1 is overexpressed in colon cancers, and that the enzymatic activity is significantly higher in tumor specimens compared to normal colon tissue [64–66]. Interestingly, overexpression of CYP1B1 leads to the metabolism/biotransformation of docetaxel in in vitro models of breast cancer [67] and of flutamide, commonly prescribed in prostate cancer [68]; resulting in acquired chemotherapeutic resistance. Our preliminary data indicated that

Table 5. Ribosomal proteins are downregulated in AA CRC vs CA CRC.

| Ribosomal Subunit | Protein | Extraribosomal Function(s) in Homo Sapiens | Expression Profile in CRC |
|-------------------|---------|------------------------------------------|--------------------------|
| RPL7a             | Rearranges with the TRK proto-oncogene thus encoding an oncprotein consisting of the N-terminus of RPL7a fused to the receptor TRK domain [22] | Unknown |
| RPL8              | Unknown | Upregulated [23]                          | Upregulated [23]         |
| RPL13             | Unknown | Unknown; Overexpression in GI cancers leads to tumor growth and chemoresistance [24] | Downregulated [27] |
| RPL13a            | Reduces inflammation via IFN-γ-activated inhibitor of translation (GAIT) complex [25, 26] | Downregulated [27] |
| RPL18             | Prevents PKR activation when associated with the ribosome; | Upregulated [23, 28] |
| 60S               | Overexpression may promote protein synthesis and cell growth through inhibition of PKR activity [29] | Downregulated [27] |
| RPL28             | Unknown | Upregulated [30]                          | Downregulated [27]       |
| RPL29             | Unknown; Knockdown in HT-29 cells induces overexpression of p21 and p53 and cell differentiation in vitro [31] | Upregulated [23] |
| RPL36             | Unknown | Downregulated [32]                        | Hypermethylated [33]     |
| RPLP0             | Regulates tumor progression, invasion, metastasis, and differentiation by influencing p21 and p53 expression [34, 35] | Upregulated [28, 34] |
| RPLP1             | Induces immortalization and proliferation in MEFs via activation of E2F transcription factors [36] | Upregulated with an accumulation of mutant p53 [36] |
| RPS2              | Implicated in the regulation of cell growth and proliferation [37, 38] | Unknown |
| 40S               | Binds to MDM2 thus activating p53 and cell cycle arrest [39] | Unknown |
| RPS19             | In vitro knockdown 1) activates inflammation via p53-dependent TNF-α expression, and p38 MAPK expression leading to tumorigenesis, growth, and metastasis [40], and 2) decreases differentiation/maturation via GATA1 suppression [41] | Upregulated [23, 42] |
| Mito 39S          | MRPL12  | Binds to mitochondrial RNA polymerase POLRMT to promote transcription [43] | Unknown |

doi:10.1371/journal.pone.0153125.t005
chemoresponse to 5-Fluorouracil was lower in an in vivo patient-derived mouse xenograft model of AA CRC but not in that of CA CRC (data not shown). The overexpression of CYP1B1 and consequential chemotherapeutic resistance in AA CRC is potentially a new avenue of investigation for defining effective treatments for CRC.

In the same vein, the dysregulation of RPs is a fundamental observation in nearly all carcinomas [69]. Identifying and characterizing disease-specific expression profile aids in the understanding and treatment of disease. Although RPs are primarily involved in translation, many have known secondary extraribosomal functions in cell proliferation, differentiation, tumorigenesis, and/or metastasis [69]. Even more roles are likely to exist as many RPs are not fully characterized. Conflicting reports have been published on the expression patterns of RPs in CRC. It may be intuitive that RPs would be constitutively upregulated in carcinomas as hyperactive translation contributes to uncontrolled proliferation, however several findings including our current data conclude that specific RPs are in fact downregulated in CRC. We have illustrated that 14 RPs are downregulated in AA CRC versus CA CRC including 10 members of the large 60S subunit, 3 members of the small 40S subunit, and 1 mitochondrial ribosomal protein of the large 39S subunit. Experimental design (i.e. specimen collection, sample size) and demographic data (age, sex, race/ethnicity) may contribute to contradictory results, but a biological explanation may lie within the extraribosomal functions of these RPs. For example, RPL13a regulates IFN-γ-activated inhibitor of translation complex-mediated inflammation, and silencing of RPL13a in macrophages results in the overexpression of inflammatory chemokines and systemic macrophage infiltration [25, 26]. In vitro knockdown of RPS19 in hematopoietic progenitor cells activates inflammation via increased p53-dependent TNF-α expression. Downregulated GATA1 expression mediated by p38 MAPK preventing hematopoietic differentiation was also observed, which may promote tumorigenesis, growth, and metastasis [40, 41]. Decreased RPL13a and RPS19 expression in AA CRC may contribute to inflammation, thus predisposing AAs to increased incidence and severity of CRC as previously discussed. Furthermore, the suppressed expression of ribosomal subunits, together with the hypermethylation of miR-124-3p and resulting upregulation of POLR2B, suggests a key role for aberrant mRNA transcription in the incidence of CRC for AA that is altogether unique from CA CRC patients. Overall, dysregulated transcription levels could result in increased cell proliferation and growth, migration/metastasis to secondary tissues, and acquired chemoresistance.

An important mechanism of tumorigenesis is epigenetic silencing of selected genes such as tumor suppressor or inflammation genes, by promoter methylation or by miRNAs. Here, our results demonstrate that hypermethylation of CHL1 was found to be significantly increased in the CRC tumors of AA as compared to CA patients. Hypermethylation of CHL1 is reportedly associated with its downregulation of gene expression. Downregulation of CHL1 has been implicated in several cancers including 48% of all CRC cases [70], and hypermethylation of CHL1 is associated with increased rates of deletions and MSIs in Iranian CRC specimens [71]. Still, the role of CHL1 in CRC is not fully understood or characterized. Interestingly, microRNA-182 is a negative regulator of CHL1 in human papillary thyroid carcinoma (PTC) with overexpression of miR-182 suppressing CHL1 and therefore promoting PTC cell proliferation and invasion [72]. Indeed, our lab has previously demonstrated overexpression of miR-182 in AA compared to CA CRC tumor samples [73].

These, and our previous findings, have provided potential candidates for addressing racial disparity in CRC. Overall, understanding dysregulation of methylation patterns in CRC will provide us with the tools for preventive or therapeutic interventions.
Supporting Information

S1 Fig. Unique miRNAs are aberrantly methylated in CRC. (A) miR-9-3p and (B) miR-124-3p, two miRNAs identified in our RRBS analysis, are independently verified as having significantly increased methylation in CRC according to the MethHC database of DNA Methylation and gene expression in Human Cancer (http://methhc.mbc.nctu.edu.tw/php/index.php).

S1 Table. Downregulated genes in AA CRC compared normal adjacent tissue, ranked by statistical significance.

S2 Table. Upregulated genes in AA CRC compared normal adjacent tissue, ranked by statistical significance.

S3 Table. Upregulated genes in CA CRC compared normal adjacent tissue, ranked by statistical significance.

S4 Table. Downregulated genes in AA CRC compared to CA CRC, ranked by statistical significance.

S5 Table. Upregulated genes in AA CRC compared CA CRC, ranked by statistical significance.

Acknowledgments

The authors would like to thank Ms. Michele McTernan for editorial consultations and Drs. Richard McCombie and Eric Antoniou for acquisition of DNA methylation profiles.

Author Contributions

Conceived and designed the experiments: JLW. Performed the experiments: PJ. Analyzed the data: XW PJ YZ JFL XT EL JLW. Contributed reagents/materials/analysis tools: XW YZ EL JLW. Wrote the paper: JFL JLW. Interpretation of data: XW PJ YZ JFL XT EL JLW.

References

1. Lieberman DA, Holub JL, Moravec MD, Eisen GM, Peters D, Morris CD. Prevalence of colon polyps detected by colonoscopy screening in asymptomatic black and white patients. Jama. 2008; 300 (12):1417–22. PMID: 18812532. doi:10.1001/jama.300.12.1417
2. Chien C, Morimoto LM, Tom J, Li CI. Differences in colorectal carcinoma stage and survival by race and ethnicity. Cancer. 2005; 104(3):629–39. PMID: 15983985.
3. Clegg LX, Li FP, Hankey BF, Chu K, Edwards BK. Cancer survival among US whites and minorities: a SEER (Surveillance, Epidemiology, and End Results) Program population-based study. Arch Intern Med. 2002; 162(17):1985–93. PMID: 12230422.
4. Cooper GS, Yuan Z, Rimm AA. Racial disparity in the incidence and case-fatality of colorectal cancer: analysis of 329 United States counties. Cancer Epidemiol Biomarkers Prev. 1997; 6(4):283–5. PMID: 9107433.
5. Hodgson DC, Fuchs CS, Ayanian JZ. Impact of patient and provider characteristics on the treatment and outcomes of colorectal cancer. Journal of the National Cancer Institute. 2001; 93(7):501–15. Epub 2001/04/05. PMID: 11287444.
6. Hodgson DC, Zhang W, Zaslavsky AM, Fuchs CS, Wright WE, Ayanian JZ. Relation of hospital volume to colostomy rates and survival for patients with rectal cancer. Journal of the National Cancer Institute. 2003; 95(10):708–16. PMID 12759388.

7. Mayberry RM, Coates RJ, Hill HA, Click LA, Chen VW, Austin DF, et al. Determinants of black/white differences in colon cancer survival. Journal of the National Cancer Institute. 1995; 87(22):1686–93. PMID: 7473817.

8. Ries LAG, Kosary CL, Hankey BF, Miller BA, Harras A, Edwards BK, editors. SEER Cancer Statistics Review, 1973–1994. 1997.

9. Herrinton LJ, Liu L, Levin TR, Allison JE, Lewis JD, Velayos F. Incidence and mortality of colorectal adenocarcinoma in persons with inflammatory bowel disease from 1998 to 2010. Gastroenterology. 2012; 143(2):382–9. doi: 10.1053/j.gastro.2012.04.054 PMID: 22609382.

10. Slattery ML. Diet, lifestyle, and colon cancer. Semin Gastrointest Dis. 2000; 11(3):142–6. PMID: 10950460.

11. Salbaum JM, Kappen C. Genetic and epigenomic footprints of folate. Prog Mol Biol Transl Sci. 2012; 108:129–58. doi: 10.1016/B978-0-12-398397-8.00006-X PMID: 21427790; PubMed Central PMCID: PMC3978114.

12. Ashktorab H, Schaffer AA, Daremipour M, Smoot DT, Lee E, Brim H. Distinct genetic alterations in colorectal cancer. PloS one. 2010; 5(1):e8879. Epub 2010/02/04. doi: 10.1371/journal.pone.0008879 PMID: 20126641; PubMed Central PMCID: PMC2811180.

13. Rodrigues NR, Rowan A, Smith ME, Kerr IB, Bodmer WF, Gannon JV, et al. p53 mutations in colorectal cancer. Proceedings of the National Academy of Sciences of the United States of America. 1990; 87(19):7555–9. PMID: 21699228

14. Carethers JM. Racial and ethnic factors in the genetic pathogenesis of colorectal cancer. J Assoc Acad Minor Phys. 1999; 10(3):59–67. PMID: 10826011.

15. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med. 2003; 349(21):2042–54. doi: 10.1056/NEJMra023075 PMID: 14627790.

16. Ehrlich M. DNA hypomethylation in cancer cells. Epigenomics. 2009; 1(2):239–59. doi: 10.2217/epi.09.33 PMID: 20459664; PubMed Central PMCID: PMC2873040.

17. Samowitz WS. Genetic and epigenetic changes in colorectal cancer. Exp Mol Pathol. 2008; 85(1):64–7. Epub 2008/05/17. S0014-4800(08)00038-5 [pii] doi:10.1016/j.yexmp.2008.03.008 PMID: 18482722.

18. Kauh J, Brawley OW, Berger M. Racial disparities in colorectal cancer. Curr Probl Cancer. 2007; 31(3):123–33. Epub 2007/06/05. S0147-0272(07)00003-7 [pii] doi:10.1016/j.currproblcancer.2007.01.002 PMID: 17543944.

19. Iorio MV, Croce CM. Causes and consequences of microRNA dysregulation. Cancer journal. 2012; 18(3):215–22. doi:10.1097/PPO.0b013e318250c001 PMID: 22647357; PubMed Central PMCID: PMC3528102.

20. Okugawa Y, Grady WM, Goel A. Epigenetic Alterations in Colorectal Cancer: Emerging Biomarkers. Gastroenterology. 2015; 149(5):1204–25 e12. doi: 10.1053/j.gastro.2015.07.011 PMID: 26216839; PubMed Central PMCID: PMC4589488.

21. Wang X, Yu X, Zhu W, McCombie WR, Antoniou E, Powers RS, et al. A trimming-and-retrieving alignment scheme for reduced representation bisulfite sequencing. Bioinformatics. 2015. doi:10.1093/bioinformatics/btv089 PMID: 25681254.

22. Ziemiecki A, Muller RG, Fu XC, Hynes NE, Kozma S. Oncogenic activation of the human trk proto-oncogene by recombination with the ribosomal large subunit protein L17a. EMBO J. 1990; 9(1):191–6. PMID: 2409326; PubMed Central PMCID: PMC5550602.

23. Kitahara O, Furukawa Y, Tanaka T, Kihara C, Ono K, Yanagawa R, et al. Alterations of gene expression during colorectal carcinogenesis revealed by cDNA microarrays after laser-capture microdissection of tumor tissues and normal epithelia. Cancer research. 2001; 61(9):3544–9. PMID: 11325815.

24. Kobayashi T, Sasaki Y, Oshima Y, Yamamoto H, Mita H, Suzuki H, et al. Activation of the ribosomal protein L13 gene in human gastrointestinal cancer. Int J Mol Med. 2006; 18(1):161–70. PMID: 16786168.

25. Basu A, Poddar D, Robinet P, Smith JD, Febbraio M, Baldwin WM 3rd, et al. Ribosomal protein L13a deficiency in macrophages promotes atherosclerosis by limiting translation control-dependent retardation of inflammation. Arterioscler Thromb Vasc Biol. 2014; 34(3):533–42. doi: 10.1161/ATVBAHA.113.302573 PMID: 24436370; PubMed Central PMCID: PMC3954853.

26. Poddar D, Basu A, Baldwin WM 3rd, Kondratos RV, Barik S, Mazumder B. An extraribosomal function of ribosomal protein L13a in macrophages resolves inflammation. J Immunol. 2013; 190(7):3600–12. doi:10.4049/jimmunol.1201933 PMID: 23460747; PubMed Central PMCID: PMC3608820.
27. Kasai H, Nadano D, Hidaka E, Higuchi K, Kawakubo M, Sato TA, et al. Differential expression of ribosomal proteins in human normal and neoplastic colorectum. J Histochem Cytochem. 2003; 51(5):567–74. PMID: 12704204.

28. Barnard GF, Staniunas RJ, Mori M, Puder M, Jessup MJ, Steele GD Jr., et al. Gastric and hepatocellular carcinomas do not overexpress the same ribosomal protein messenger RNAs as colonic carcinoma. Cancer research. 1993; 53(17):4048–52. PMID: 8395335.

29. Kumar KU, Srivastava SP, Kaufman RJ. Double-stranded RNA-activated protein kinase (PKR) is negatively regulated by 60S ribosomal subunit protein L18. Mol Cell Biol. 1999; 19(2):1116–25. PMID: 9891046; PubMed Central PMCID: PMC116041.

30. Frigerio JM, Dagorn JC, Iovanna JL. Cloning, sequencing and expression of the L5, L21, L27a, L28, S5, S9, S10 and S29 human ribosomal protein mRNAs. Biochim Biophys Acta. 1995; 1262(1):64–8. PMID: 7772601.

32. Bertucci F, Salas S, Eysteriers S, Nasser V, Finetti P, Ginestier C, et al. Gene expression profiling of colon cancer by DNA microarrays and correlation with histoclinical parameters. Oncogene. 2004; 23 (7):1377–91. doi: 10.1038/sj.ong.1207262 PMID: 14973550.

33. Artero-Castro A, Castelvi L, Garcia A, Hernandez J, Ramon y Cajal S, Lleonart ME. Expression of the ribosomal proteins Rplp0, Rplp1, and Rplp2 in gynecologic tumors. Hum Pathol. 2011; 42(2):194–203. doi: 10.1016/j.humpath.2010.04.020 PMID: 21040949.

34. Teller A, Jechorek D, Hartig R, Adolf D, Reissig K, Roessner A, et al. Dysregulation of apoptotic signalling pathways by interaction of RPLP0 and cathepsin X/Z in gastric cancer. Pathol Res Pract. 2015; 211 (1):62–70. doi: 10.1016/j.prp.2014.09.005 PMID: 25433997.

35. Ludwig LS, Gazda HT, Eng JC, Eichhorn SW, Thiru P, Ghazvinian R, et al. Altered translation of GATA1 in Diamond-Blackfan anemia. Nature medicine. 2014; 20(7):748–53. doi: 10.1038/nm.3557 PMID: 24952648; PubMed Central PMCID: PMC4087046.

36. Bernstein CN, Blanchard JF, Kliever E, Wajda A. Cancer risk in patients with inflammatory bowel disease: a population-based study. Cancer. 2001; 91(4):854–62. PMID: 11241255.

37. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut. 2001; 48(4):526–35. PMID: 11247898; PubMed Central PMCID: PMC1728239.
46. Itzkowitz SH, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am J Physiol Gastrointest Liver Physiol. 2004; 287(1):G7–17. doi: 10.1152/ajpgi.00079.2004 PMID: 15194558.

47. Ulman TA, Itzkowitz SH. Intestinal inflammation and cancer. Gastroenterology. 2011; 140(6):1807–16. doi: 10.1053/j.gastro.2011.01.057 PMID: 21530747.

48. Rubin DC, Shaker A, Levin MS. Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer. Front Immunol. 2012; 3:107. doi: 10.3389/fimmu.2012.00107 PMID: 22586430; PubMed Central PMCID: PMC3347037.

49. Shen J, James AW, Zara JN, Asatrian G, Khadarian K, Zhang JB, et al. BMP2-induced inflammation can be suppressed by the osteoinductive growth factor NELL-1. Tissue Eng Part A. 2013; 19(21–22):2390–401. doi: 10.1089/ten.TEA.2012.0519 PMID: 23758588; PubMed Central PMCID: PMC3807546.

50. Bao MW, Zhang XJ, Li L, Cai Z, Liu X, Wan N, et al. Cardioprotective role of growth/differentiation factor 1 in post-infarction left ventricular remodelling and dysfunction. The Journal of pathology. 2015; 236 (3):360–72. doi: 10.1002/path.4523 PMID: 25726944.

51. Meng F, Melton A, Moldobaeva N, Mutlu G, Kawasaki Y, Akiyama T, et al. Asef mediates HGF protective effects against LPS-induced lung injury and endothelial barrier dysfunction. Am J Physiol Lung Cell Mol Physiol. 2015; 308(5):L452–63. doi: 10.1152/ajplung.00170.2014 PMID: 25539852; PubMed Central PMC: PMC4346776.

52. Gerecke C, Scholtka B, Lowenstein Y, Fait I, Gottschalk U, Rogoll D, et al. Hypermethylation of ITGA4, TFPi2 and VIMENTIN promoters is increased in inflamed colon tissue: putative risk markers for colitis-associated cancer. J Cancer Res Clin Oncol. 2015; 141(12):2097–107. doi: 10.1007/s00432-015-1972-8 PMID: 25902909.

53. Bjursten M, Bland PW, Willen R, Hornquist EH. Long-term treatment with anti-alpha 4 integrin antibodies aggravates colitis in G alpha i2-deficient mice. Eur J Immunol. 2005; 35(8):2274–83. doi: 10.1002/eji.200526022 PMID: 16052630.

54. Gillberg L, Berg S, de Verdier PJ, Lindbom L, Herr J, Hellstrom PM. Effective treatment of mouse experimental colitis by alpha 2 integrin antibody: comparison with alpha 4 antibody and conventional therapy. Acta Physiol (Oxf). 2013; 207(2):326–36. doi: 10.1111/apha.12017 PMID: 23009282.

55. Cekaite L, Rantala JK, Bruun J, Guriby M, Agesen TH, Danielsen SA, et al. MiR-9, -31, and -182 deregulation promote proliferation and tumor cell survival in colon cancer. Neoplasia. 2012; 14(9):868–79. PMID: 23019418; PubMed Central PMC: PMC3459289.

56. Oberg AL, French AJ, Sarver AL, Subramanian S, Morlan BW, Riska SM, et al. miRNA expression in colon polyps provides evidence for a multihit model of colon cancer. PloS one. 2011; 6(6):e20465. doi: 10.1371/journal.pone.0020465 PMID: 21694772; PubMed Central PMC3111419.

57. Slattery ML, Herrick JS, Mullany LE, Valeri N, Stevens J, Caan BJ, et al. An evaluation and replication of miRNAs with disease stage and colorectal cancer-specific mortality. Int J Cancer. 2015; 137(2):428–38. doi: 10.1002/ijc.29384 PMID: 25484364; PubMed Central PMC: PMC4428989.

58. Taniguchi K, Sugito N, Kumazaki M, Shinohara H, Yamada N, Nakagawa Y, et al. MicroRNA-124 inhibits cancer cell growth through PTB1/PKM1/PKM2 feedback cascade in colorectal cancer. Cancer Lett. 2015; 363(1):17–27. doi: 10.1016/j.canlet.2015.03.026 PMID: 25818238.

59. Koukos G, Polytarchou C, Kaplan JL, Morley-Fletcher A, Gras-Miralles B, Kokkotou E, et al. MicroRNA-124 regulates STAT3 expression and is down-regulated in colon tissues of pediatric patients with ulcerative colitis. Gastroenterology. 2013; 145(4):842–52 e2. doi: 10.1053/j.gastro.2013.07.001 PMID: 23856509; PubMed Central PMC: PMC4427058.

60. Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein output. Nature. 2008; 455(7209):64–71. doi: 10.1038/nature07242 PMID: 18668803; PubMed Central PMC: PMC2745094.

61. Ponicsan SL, Houel S, Old WM, Ahn NG, Goodrich JA, Kugel JF. The non-coding B2 RNA binds to the DNA cleft and active-site region of RNA polymerase II. J Mol Biol. 2013; 425(19):3625–38. doi: 10.1016/j.jmb.2013.01.035 PMID: 23416138; PubMed Central PMC: PMC3672349.

62. Michiels S, Danoy P, Dessen P, Bera A, Boulet T, Bouchardy C, et al. Polymorphism discovery in 62 DNA repair genes and haplotype associations with risks for lung and head and neck cancers. Carcinogenesis. 2007; 28(8):1731–9. doi: 10.1093/carcin/bgm111 PMID: 17490405.

63. Nebert DW, Dalton TP. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nat Rev Cancer. 2006; 6(12):947–60. doi: 10.1038/nrc2015 PMID: 17128211.

64. Androutsopoulos VP, Spyrou I, Ploumidis A, Papalampros AE, Kyriakakis M, Delakas D, et al. Expression profile of CYP1A1 and CYP1B1 enzymes in colon and bladder tumors. PloS one. 2013; 8(12):e82487. doi: 10.1371/journal.pone.0082487 PMID: 24358191; PubMed Central PMC: PMC3864999.
65. Chang H, Su JM, Huang CC, Liu LC, Tsai CH, Chou MC, et al. Using a combination of cytochrome P450 1B1 and beta-catenin for early diagnosis and prevention of colorectal cancer. Cancer Detect Prev. 2005; 29(6):562–9. doi: 10.1016/j.cdp.2005.09.007 PMID: 16289386.

66. Kumarakulasingham M, Rooney PH, Dundas SR, Telfer C, Melvin WT, Curran S, et al. Cytochrome p450 profile of colorectal cancer: identification of markers of prognosis. Clinical cancer research: an official journal of the American Association for Cancer Research. 2005; 11(10):3758–65. doi: 10.1158/1078-0432.CCR-04-1848 PMID: 15897573.

67. Martinez VG, O’Connor R, Liang Y, Clynes M. CYP1B1 expression is induced by docetaxel: effect on cell viability and drug resistance. Br J Cancer. 2008; 98(3):564–70. doi: 10.1038/sj.bjc.6604195 PMID: 18212750; PubMed Central PMCID: PMC2243158.

68. Rochat B, Morsman JM, Murray GI, Figg WD, McLeod HL. Human CYP1B1 and anticancer agent metabolism: mechanism for tumor-specific drug inactivation? J Pharmacol Exp Ther. 2001; 296(2):537–41. PMID: 11160641.

69. Lai MD, Xu J. Ribosomal proteins and colorectal cancer. Curr Genomics. 2007; 8(1):43–9. PMID: 18645623; PubMed Central PMCID: PMC2474683.

70. Senchenko VN, Krasnov GS, Dmitriev AA, Kudryavtseva AV, Anedchenko EA, Braga EA, et al. Differential expression of CHL1 gene during development of major human cancers. PloS one. 2011; 6(3):e15612. doi: 10.1371/journal.pone.0015612 PMID: 21408220; PubMed Central PMCID: PMC3049765.

71. Brim H, Abu-Asab MS, Nouraie M, Salazar J, Deleo J, Razjouyan H, et al. An integrative CGH, MSI and candidate genes methylation analysis of colorectal tumors. PloS one. 2014; 9(1):e82185. doi: 10.1371/journal.pone.0082185 PMID: 24475022; PubMed Central PMCID: PMC3903472.

72. Zhu H, Fang J, Zhang J, Zhao Z, Liu L, Wang J, et al. miR-182 targets CHL1 and controls tumor growth and invasion in papillary thyroid carcinoma. Biochemical and biophysical research communications. 2014; 450(1):857–62. doi: 10.1016/j.bbrc.2014.06.073 PMID: 24971532.

73. Li E, Ji P, Ouyang N, Zhang Y, Wang XY, Rubin DC, et al. Differential expression of miRNAs in colon cancer between African and Caucasian Americans: implications for cancer racial health disparities. International journal of oncology. 2014; 45(2):587–94. doi: 10.3892/ijo.2014.2469 PMID: 24865442; PubMed Central PMCID: PMC4091964.