Editing and Expression of miR-589-5p as an Important Biomarker in Colorectal Cancer

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Research article

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Abstract

Objectives: Colorectal cancer (CRC) is recognized as the third most common cancer worldwide. Recently, emerging evidence showed that microRNA (miRNA) A-to-I editing plays crucial roles in cancer prognosis as well as cancer therapy. However, the relationship between CRC and miRNA editing remains not fully understood. Herein, we presented the first comprehensive analysis of miRNA editing events in CRC.

Methods: Using patient data from The Cancer Genome Atlas (TCGA), we performed editing events and detected the expression levels of miR-589-5p.

Results: we identified both editing events and the expression levels of miR-589-5p are significantly associated with Consensus Molecular Subtyping (CMS), especially for CMS4. The editing and expression levels of miR-589-5p impact almost completely different sets of gene expression, indicating the edited miR-589-5p plays a different role in CRC progression, compared to the wildtype miR-589-5p.

Conclusions: In conclusion, our study provided the first association of miRNA editing and CRC. We demonstrated that expression and editing level of miR-589-5p may work as a novel biomarker for both prognosis and diagnosis in CRC.

1. Introduction

Colorectal cancer (CRC), which is one of the most common malignant tumors in digestive tract, accounts for nearly 10% of the overall newly-discovered cancer patients around the world [1]. Advanced clinical stages are associated with prominently worse prognosis and lower survival rate [2], highlighting the significance of early diagnosis, treatment and prevention. Though the pathogenesis of CRC still remains incompletely understood, a variety of genes and gene-related factors have been reported to be involved in the tumorigenesis and metastasis of CRC, including microRNAs (miRNA) [3, 4].

MicroRNA is fully abundant and highly conserved in various tissues, which directly participating in posttranscriptional regulation of gene expression via binding to the three prime untranslated regions (3'UTR) of the target gene. miRNAs were shown to regulate biological processes of cell division, differentiation, apoptosis, etc., and also play important roles in the metastasis, reoccurrence and chemotherapy resistance of certain types of tumors [5]. As the development of high-throughput sequencing, detailed information behind the subtle gene regulation networks have been revealed, as more miRNA are indicated to influence the tumorigenesis of CRC by targeting distinct genes like miR-22/HuR [6], miR-19a/TIA1 [7], miR-4775/Smad7/TGF-beta [8], miR-625-3p/MAP2K6-p38 [9], miR-638/Sox2 [10] and other regulatory axis. Meanwhile, evidence also has been shedding light on the diversified relationship between microRNA and chemotherapy resistance of CRC. For instance, miR-128-3p has been proved to be transmitted through exosome and participated in the intercellular communication which enables colorectal cancer cells to regain the sensitivity of oxaliplatin [11].

The diversity of non-coding RNAs including miRNA are partially on account of RNA editing. RNA editing, a kind of post transcription modification, refers to the phenomenon of base insertion, deletion and substitution of RNA after transcription, occurring not only in mRNA, but also in miRNA and other types of noncoding RNA [12, 13]. Adenosine to inosine (A to I) convert is widely reported in miRNA editing because the antisense transcript of miRNA is prone to form a hairpin site that is suitable for adenosine deaminase, RNA specific (ADARs), which combines the double stranded RNA region of coding or noncoding gene and deaminates adenosine into inosine [14]. MiRNA editing was discovered in more than 80% of the precursor miRNAs. It regulates the asymmetric strand selection process of miRNAs with tissue and developmental stage specificity [15]. The editing process of miRNAs was demonstrated to be highly related to the recognition of target genes of miRNAs. In addition, edited mature miRNA was also reported to protect antisense transcript of miRNA from degeneration via directly combining with the latter [16].

Recently, multiple studies have suggested that miRNA editing be closely related to the pathogenesis of cancer development. A systematic characterization of A-to-I miRNA editing events was performed in human cancers, suggesting miRNA editing's potential as a biomarker for cancer prognosis and therapy [17]. Nigita et al. demonstrated that miRNA editing in non-small cell lung cancer (NSCLA) tissue and exosome between NSCLA patients and healthy control group are dysregulated, especially the miR-411-5p [18]. The A-to-I edition of miR-379-5p, which contains an ADAR2 sensitive site, was found to be downregulated in tumor tissue samples from TCGA (The Cancer Genome Atlas) [19]. The underlying mechanism behind edited miR-379-5p for its negative correspondence with TCGA growth might be relevant to its potential targeted genes CD97, which was reported to be overexpressed in many cancers and had a potential effect on preventing apoptotic cell death [19]. Han et al. also analyzed thousands of miRNA editing events from over 6000 patients globally and identified some of which clinically relevant to proteomic diversity in 17 cancer types [20]. Interestingly, the peptide diversity corresponded with miRNA editing may serve as self-antigen to activate immune system. The proliferation and infiltration of T cells specific answered to edited peptide were found in melanoma tumors and consequently resulted in tumor cell killing [21]. Moreover, certain editing events of miRNA might be promising biomarkers for the diagnosis and prognosis prediction of cancer. MiR-200b, well known for its key function on suppressing cancer cell migration and invasion, was reported to be highly edited across different types of cancers. Systematic reviews revealed its significant correlation with malignant progression and poor overall survival, suggesting its potential for early detection of cancer [17].

However, less is known about the relationship between miRNA editing events and CRC progression. Herein, we provided the first comprehensive characterization of miRNA editing events in CRC patients regarding Consensus Molecular Subtypes (CMS) classification as well as relevant clinical outcomes. We identified miR-589-5p as the most important biomarker among all miRNA candidates with editing events for its variations on both editing and expression levels across all patients and association with CMS and clinical outcome.

2. Materials And Methods

2.1 Data preparation

We obtained both miRNA and mRNA expression data from The Cancer Genome Atlas (TCGA) [22]. The miRNA expression dataset includes 423 patients with 743 miRNAs with quantified expression. mRNA sequencing dataset contains 454 patients with 20,531 genes with quantified expression. We used the computational miRNA editing calling pipeline described in [17] to quantify the editing level of each miRNA from the raw miRNA sequence reads also available through TCGA. After merging with different clinical outcomes or selecting meaningful editing hotspots, the resulted number of patients varies from 350 to 391. Only major editing types in humans with validated editing enzymes, A-to-I and C-to-U, were considered [23]. The merged miRNA editing level data have 49 miRNAs with at least one A-to-I or C-to-U editing event in at least one patient.

2.2 Consensus molecular subtyping (CMS)

CMSCaller [24] was used to identify the CMS classes of all colon cancer patients. Except 42 patients, all other patients were classified into one of the four CMS classes with p-values < 0.05. Significantly related miRNA editing, and expression levels were identified using empirical Bayes statistics for differential expression in the R package limma (Linear Models for Microarray Data). Each CMS class was compared against all others' classes during the analysis.

2.3 Pathway analysis

Pathway analysis was performed using Enrichr with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway library. Only pathways with p-value no more than 0.05 were considered to be related. Gene ontology analysis was performed using STRING with Gene Ontology (GO) library. Only GO terms with false discovery rate (FDR) of no more than 0.05 were considered to be related.

3. Results

3.1 miRNA editing and expression levels

There are 1,544 A-to-I editing hotspots and 403 C-to-U editing hotspots identified in all patients. Most of the editing events are concentrated at three miRNAs, miR-589-5p, miR-381-3p, and miR-411-3p. Figure 1A shows the editing levels of the top-2 miRNA with most non-zero editing level across all patients. In fact, only 7 miRNAs have more than 25% of non-zero editing level across all patients (Fig. 1B). miR-589-5p and miR-381-3p also have significant variations in log expression level across all patients (Fig. 1C), although much larger variations were seen for other miRNA (Fig. 1D). No apparent hierarchical clusters were identified from the heatmap. The log expression levels of all miRNA fit to a normal distribution after normalization to maximum (Fig. 1E). We further performed principal component analysis of the log expression level, and similarly no clusters can be identified in this unsupervised manner.

3.2 Relation to CMS

We performed CMS classification based on the gene expression data. 412 patients were classified into the four CMSs with p-values < 0.05 (Fig. 2A). The identified CMS classes of each patient were then mapped back to the miRNA editing and expression data and differences were analyzed. No miRNA editing levels were significantly related with CMS1, CMS2, and CMS3, but the editing levels of both miR-411-5p and miR-589-5p are significantly related to CMS4 (Fig. 2B). Since the expression data contain much more miRNAs with non-zero log expression values, for all CMS classes, more than a hundred miRNAs have expression levels that are significantly related to each CMS. Table 1 shows the relation of expression level of the three top-edited miRNAs with CMS. The expression levels of miR-589-5p and miR-381-3p are significantly related to 3 CMS classes with relatively large fold changes, while miR-411-5p is significantly related to 2 CMS classes with a relatively small fold change. In conclusion, we found that both editing and expression levels of miR-589-5p are significantly associated with CMS.

Table 1 Fold changes and p-values of expression levels of top-edited miRNAs in CMS

A. Log Fold Change	CMS1	CMS2	CMS3	CMS4
miR-589-5p	12.5	5.9	-4.0	-14.5
miR-381-3p	-7.4	4.7	-6.8	9.5
miR-411-5p	-0.8	1.1	-2.1	1.8
B. - Log10 of p-value	**CMS1**	**CMS2**	**CMS3**	**CMS4**
miR-589-5p	3.6	1.4	0.6	6.2
miR-381-3p	2.0	1.3	1.6	4.0
miR-411-5p	0.6	1.1	2.2	2.5

3.3 Relation to Clinical Outcome

We first studied the relationship of the miRNA editing with pathologic stage and tumor status after treatment. miR-589-5p was identified to be the only miRNA whose editing level is significantly related (Mann-Whitney U test p-value < 0.05) to pathologic stage as well as tumor status (Fig. 3A and 3B). Its log expression level was also found to be significantly related to pathologic stage but not status (Fig. 3C). The log expression level of miR-21-5p has the most significant relation with tumor status (Fig. 3D). In fact, quite a few miRNA expression levels are significantly related to pathologic stage and tumor status (Fig. 3E and 3F). For clarity purposes, only miRNAs with p-value < 0.001 are shown, while in total 111 and 127 miRNAs are significantly related (p-value < 0.05) to pathologic stage and tumor status, respectively. We then studied the relation of the miRNA editing and expression levels with progression-free survival (PFS). None of the miRNA editing levels was found to be significantly related to PFS whether log-likelihood ratio test p-value from Cox proportional hazards model or log-rank test p-value was considered. The log expression levels of 60 miRNAs were identified to be associated with survival, among which miR-589-5p does not show significant association but miR-381-3p shows significant association in log-rank test with a p-value of 0.013 (Fig. 3G).

3.4 Pathway analysis

From the aforementioned analyses, we have identified miR-589-5p to be the most important biomarker among all miRNA candidates with editing events for its variations of both editing and expression levels across all patients and association with CMS and clinical outcome. There are 171 genes of which expression levels are significantly correlated (Spearman correlation p-value < 10^{-7}) with the editing level of miR-589-5p and 641 genes, of which expression levels are significantly correlated with the log expression level of miR-589-5p. The editing and expression levels of miR-589-5p influence almost completely different sets of gene expression with an intersection of only five (Fig. 4A). The KEGG pathways associated with the related genes are also different. The top pathways related to miR-589-5p editing level include cell cycle, pyrimidine metabolism, RNA transport, spliceosome, and focal adhesion, which impact local control of transcription and translation. The top pathways related to miR-589-5p expression level are mostly cancer and signaling pathways related (Fig. 4A). Notably, cGMP-PKG pathway and hippo signaling pathway have been known to be associated with major cancers [25, 26]. We also analyzed the protein-protein interactions and identified gene ontology (GO) terms that are associated with the editing and expression levels of miR-589-5p. For editing level of miR-589-5p, we found metabolic process, gene expression, and organelle organization is the three representative terms that are ranked top-20. They are able to cover most of the protein-protein interaction networks. Similarly, we also found development processes and biological regulation to be the representative biological processes terms. Those related GO terms are also completely different between editing level and expression level of miR-589-5p, but are consistent with identified KEGG pathways, respectively.

4. Discussion

Recently, miRNA editing has been recognized as a novel potential cancer biomarker for major cancer prognosis and therapy [17]. However, still very little is known regarding the relationship between CRC and miRNA editing. As the CMS groups are now widely acknowledged as a standard which enables oncologists to treat CRC patients case by case [27], herein we present the first comprehensive analysis of miRNA editing events on different CMS classification. We identified both editing and expression levels of miR-589-5p are significantly associated with CMS, especially CMS4 cancers, which are often diagnosed at advanced stages with poor prognosis [28].

Moreover, genes' expression which were affected by miR-589-5p editing and expression levels of miR-589-5p were more than 98% different, indicating that edited miR-589-5p plays a completely different role in regulating cancer cell transcriptome. MiR-589-5p expression was previously established to be a predictor for hepatocellular carcinoma outcomes and a potential molecular targets for hepatocellular carcinoma treatment [29]. Another study also demonstrated that inhibition of miR-589-5p may be a feasible approach for inhibiting hepatocellular carcinoma [30]. For other tumors, a recent study showed that miR-589-5p may also work as a tumor suppressor in prostate cancer by targeting CCL-5 protein [31]. However, it was never established that miR-589-5p plays roles in CRC, nor was its editing events. MiR-589-5p was recognized as a tumor suppressor in hepatocellular carcinoma and prostate cancers. We also noticed a significant decrease of miR-589-5p expression in CMS4 cancers, highlighting its tumor suppressor role in

advanced stage cancers. Notably, we found miR-589-5p editing level is significantly higher in CMS4 compared to CMS1, 2, though its overall editing level is lower in CRC patients compared to the healthy cohort. As it was previously reported that wildtype and edited miRNAs may target different gene sets and play completely different roles in certain biological process [19], we reasoned that edited miR-589-5p may also have similar effects, which could regulate different cellular functions. Indeed, we revealed that GO-terms correlated with edited miR-589-5p are completely different to the wildtype miR-589-5p's targets (Fig. 4A).

Although we presented the first comprehensive analysis of miRNA editing in CRC, provided a relationship between CMS classification and miRNA editing, and also identified both expression of editing level of miR-589-5p could be a novel biomarker for CRC prognosis, especially for CMS4, our conclusion still requires further experimental validations. As we only included one cohort which is from TCGA, miRNA expression of the second CRC cohort should be used for further validating the universal roles of miR-589-5p editing in CRC. Moreover, edited miR-589-5p mimics treatment experiments should also be performed both *in vitro* and *in vivo* for further confirming the biological function of miR-589-5p in tumor progression.

5. Conclusion

In conclusion, our data suggested a role of miRNA editing in CRC CMS classification and forecasting the clinical outcome.

6. List Of Abbreviations

Colorectal cancer CRC

microRNA miRNA

The Cancer Genome Atlas TCGA

Consensus Molecular Subtyping CMS

adenosine deaminase, RNA specific ADARs

non-small cell lung cancer NSCLA

Kyoto Encyclopedia of Genes and Genomes KEGG

Gene Ontology GO

false discovery rate FDR

progression-free survival PFS

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interest

The authors declare that they have no competing interests.

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Authors' contributions

Conceived and designed the experiments: Li Xiao

Analyzed the data: Qiang Zhang and Yuanyi Yue

Manuscript writing and revision: Qiang Zhang and Li Xiao

All authors read and approved the final manuscript.

Acknowledgments

No

8. References

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Figures

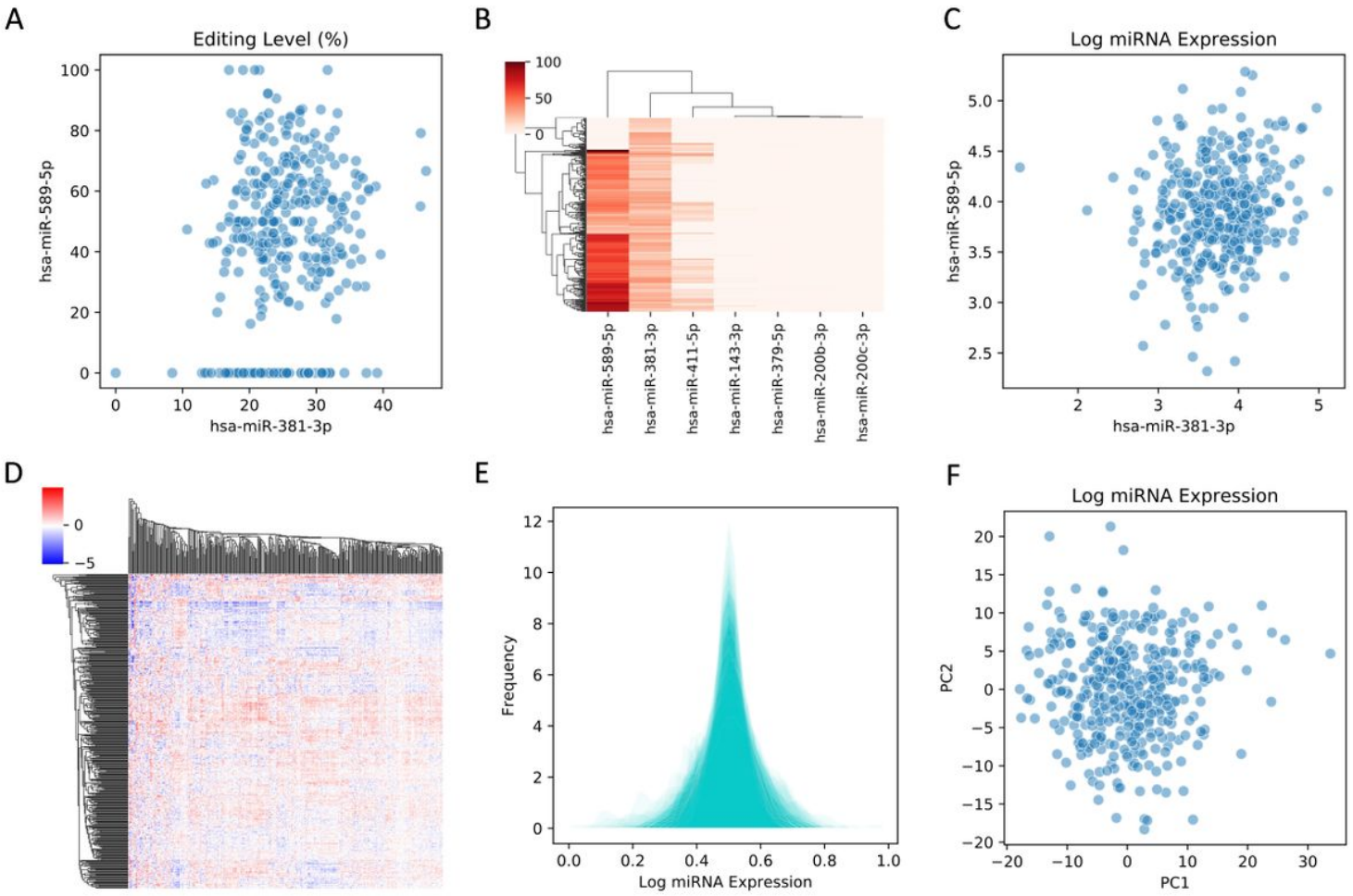

Figure 1

miRNA editing and expression. (A) Editing levels of miRNA with most non-zero editing level across all patients, miR-589-5p and miR-381-3p. (B) Heatmap of editing levels of the miRNA across all patients. Only miRNAs with 25% of non-zero editing level across all patients are considered for clarity purposes. (C) Log expression level of miR-589-5p and miR-381-3p. (D) Heatmap of log expression levels of the miRNA across all patients. Median was subtracted from each log expression level. (E) Histogram of log expression level of each miRNA normalized to maximum. (F) Principal component analysis of all miRNA expressions.

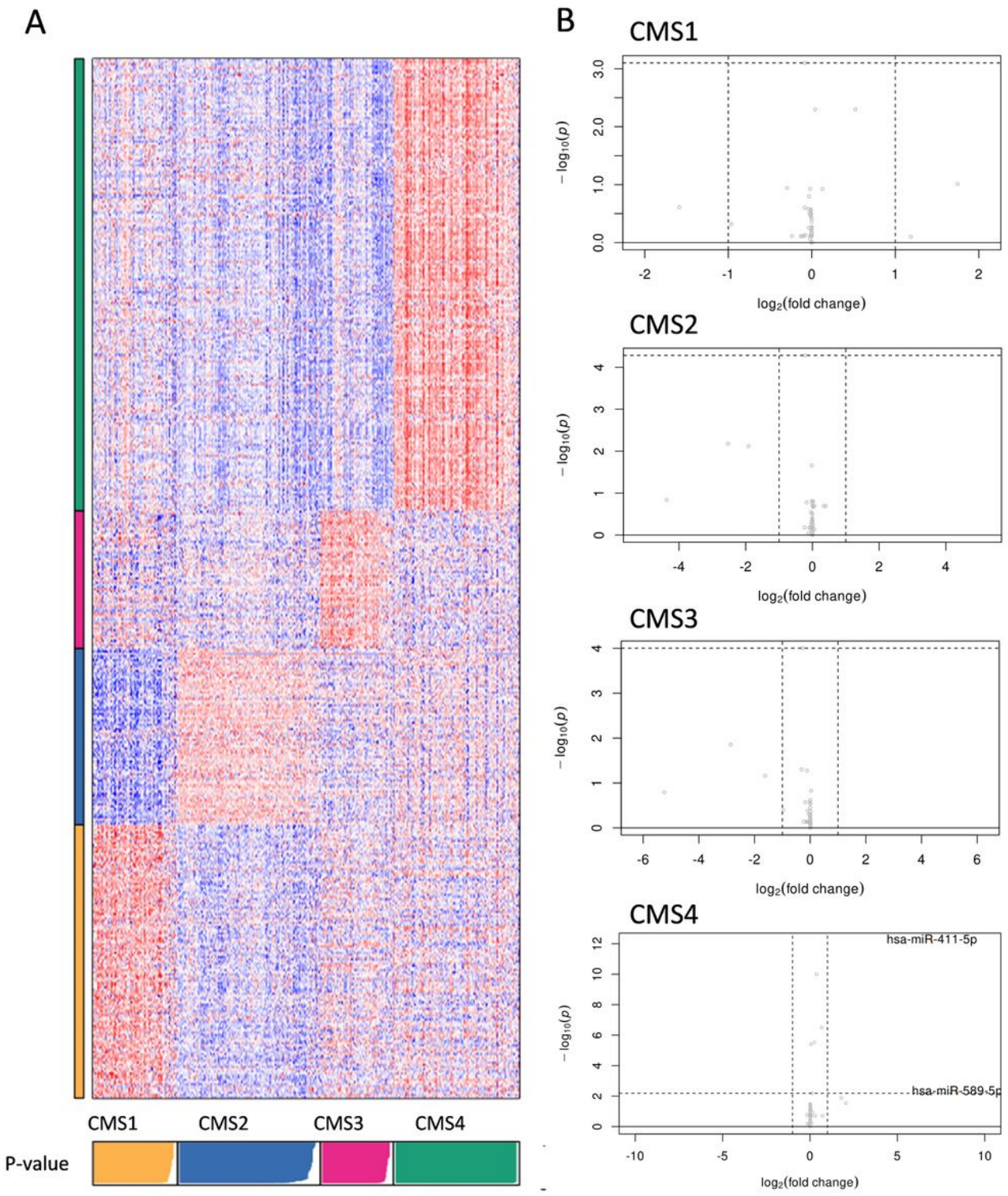

Figure 2

Relation to CMS of colon cancer. (A) CMS classification and corresponding p-values. (B) Volcano plots of significantly related miRNA editing levels. p-value of 0.05 and fold change of 2 are used as cutoffs.

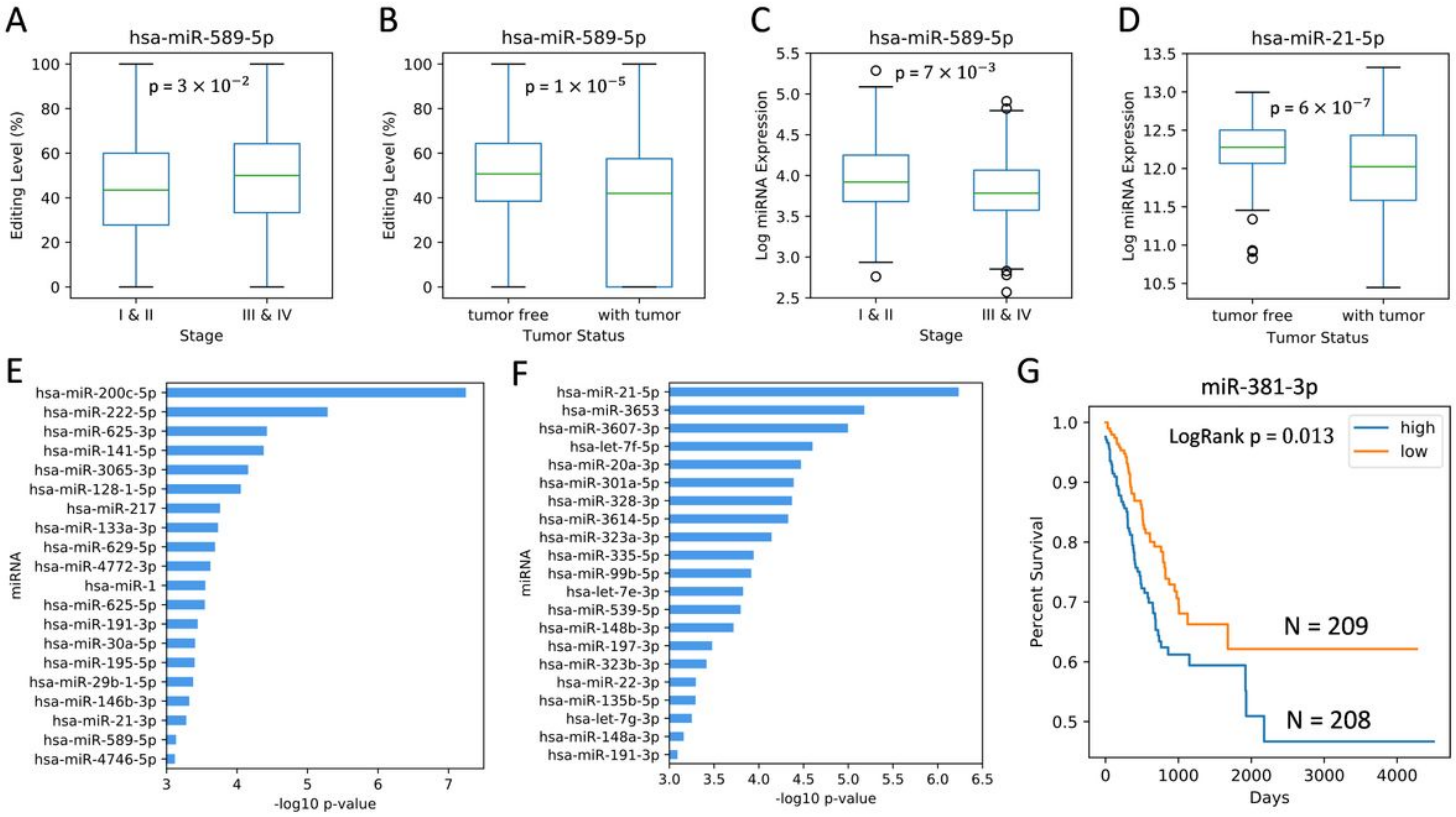

Figure 3

Relationship of miR-589-5p and miR-381-3p to clinical outcome (A-C) Relationship of editing and expression levels of miR-589-5p with pathologic stage and tumor status. (D) Relationship of expression level of miR-21-5p with tumor status. (E-F) Top miRNAs whose expression levels are associated with pathologic stage and tumor status, respectively. (G) Kaplan-Meier curve showing relationship of miR-381-3p expression level with progression-free survival.

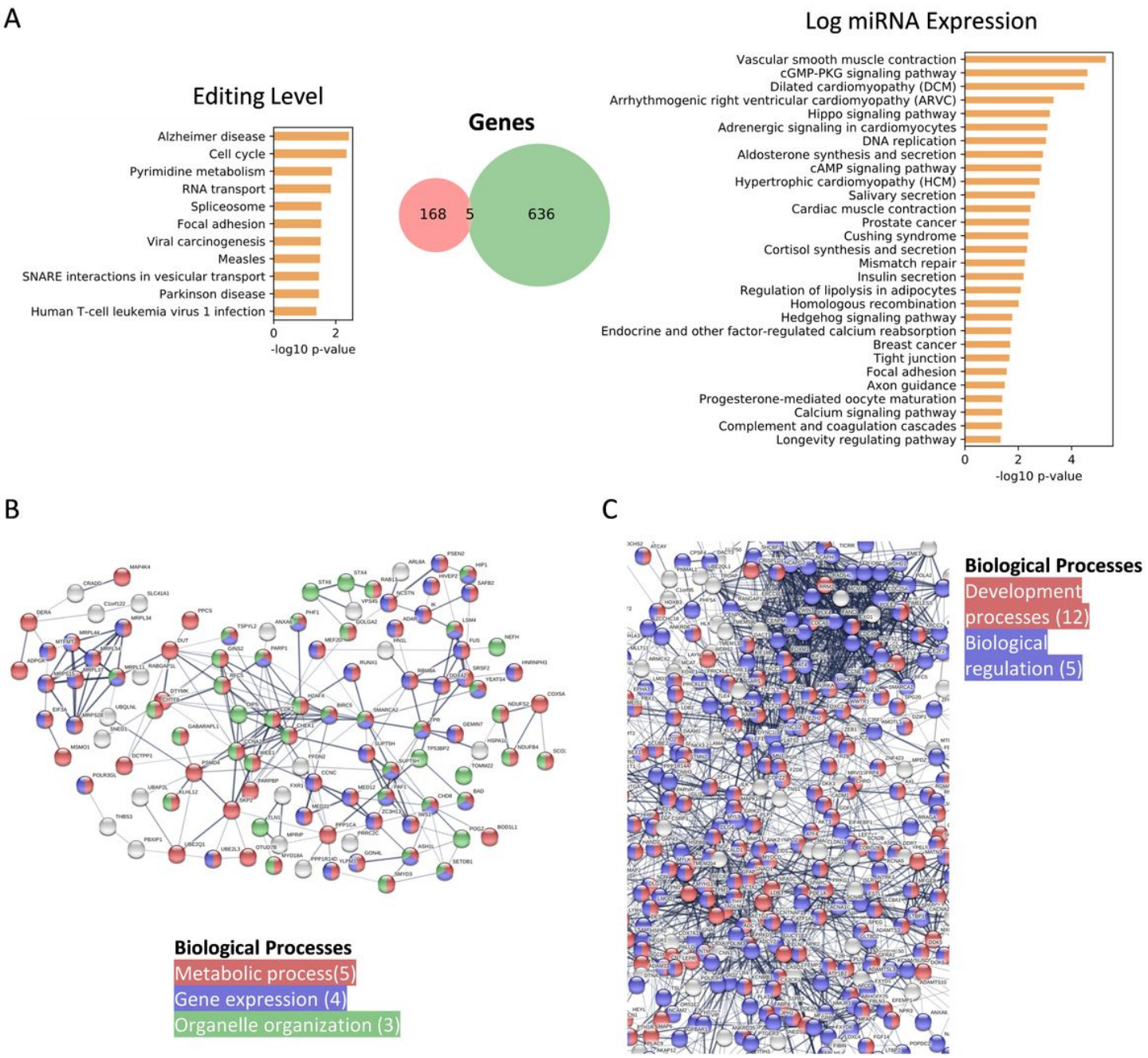

Figure 4

Pathway associated with miR-589-5p. (A) KEGG pathways associated with genes that are related to editing and expression levels of miR-589-5p respectively. (B) Protein-protein interaction network of genes related to editing level of miR-589-5p. (C) Protein-protein interaction network of genes related to log expression level of miR-589-5p. For (B) and (C), colors correspond to top GO terms. Colors correspond to top GO terms. The numbers in the brackets correspond to the nearest integer of the negative log value of false discovery rate.