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A microRNA Signature Associated With Metastasis of T1 Colorectal Tumors to Lymph Nodes

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Abstract

Most T1 colorectal tumors treated by radical surgery can now be cured by endoscopic submucosal dissection. Although 70%–80% of T1 colorectal tumors are classified as high risk, fewer than 16% of these patients actually have lymph node metastases. Biomarkers are needed to identify patients with T1 tumors with the highest risk of metastasis, to prevent unnecessary radical surgeries. We collected data from the Cancer Genome Atlas and identified 5 microRNAs (MIR32, MIR181B, MIR193B, MIR195, and MIR 411) with significant changes in expression in T1 and T2 colorectal tumors with vs without lymphatic invasion. Levels of the 5 microRNAs identified patients with lymph node invasion by T1 or T2 tumors with an area under the receiver operating characteristic curve (AUROC) value of 0.84. We validated these findings in 2 cohorts of patients with T1 tumors, using findings from histology as the reference. The 5-microRNA signature identified T1 tumors

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with lymph node invasion in cohort 1 with an AUROC value of 0.83, and in cohort 2 with an AUROC value of 0.74. When we analyzed biopsy samples from untreated patients, the 5-microRNA signature identified tumors with lymph node metastases with an AUROC value of 0.77. The 5-microRNA therefore identifies high-risk tumors with a greater degree of accuracy than currently used pathologic features.

Keywords

colorectal cancer; colon cancer; CRC; stratification

By virtue of recent improvements in colonoscopic devices and diagnostic approaches, majority of T1 colorectal cancers (CRCs) that were previously treated by radical surgeries can now be cured by endoscopic submucosal dissection¹. Currently used pathological criteria to detect lymph node metastases (LNM) in patients with T1 CRCs include: depth of submucosal invasion ($> 1000\mu\text{m}$), presence of lymphatic or vascular invasion, and poorly differentiated histology. When at least one of these factors is present, patients are deemed as being 'high-risk' for LNM and are recommended to undergo surgical treatment^{1–3}.

Using these criteria, approximately 70–80% of patients with T1 CRC are classified as high-risk, however, post-surgery pathology results suggest that in reality only 8–16% of these patients actually have LNM. This concern highlights the lack of availability for more appropriate biomarkers that can help to detect 'genuine high-risk' patients with LNM, which can facilitate reduction of unnecessary radical surgeries currently being performed in patients with T1 CRC^{2, 4–7}.

Herein, using genome-wide small RNA-Sequencing approach, we identified and established a novel microRNA (miRNA) signature for more accurately detecting LNM in patients with T1 CRC, followed by subsequent validation of its clinical significance in multiple patient cohorts with such cancers^{8,9}. The overall strengths of our study include; 1) the use of an unbiased, systematic and comprehensive biomarker discovery approach based on high-throughput miRNA-sequencing (miRNA-seq) data analysis; 2) analysis of large collection of patient specimens for biomarker validation; and 3) additional evaluation of the miRNA signature in colonoscopy-derived biopsy samples.

Using the Cancer Genome Atlas (TCGA) dataset as the discovery cohort, we established a panel of five miRNAs: MIR32, MIR181b-1, MIR193b, MIR195 and MIR411 (Figure 1A and B). A miRNA signature derived by combining the expression status of all 5 markers yielded an impressive area under the receiver operating characteristic curve (AUROC) value of 0.84 to detect LNM in patients with T1–2 CRC, and even though the sample size was rather modest, an AUROC value of 1.00 in patients with T1 CRC (Supplementary Figure 1).

In order to translate our early findings into the clinic, we next performed training and validation of our miRNA signature in two clinical cohorts of patients with T1 CRC (Supplementary Table 1 and 2). The miRNA signature significantly detected LNM in the training cohort (cohort-1; AUROC=0.83; Figure 2A), and by applying the same model and

the cut-off thresholds in a large, independent validation cohort (cohort-2), we were able to successfully validate our five-miRNA signature (AUROC=0.74; Figure 2B).

We next asked whether we can further improve the detective potential of our miRNA classifier by combining it with other clinicopathological features that are routinely evaluated in these patients. In this regard, we analyzed cohort-2, and applied backward step-wise elimination approach. Using this strategy, in addition to our five-miRNA signature, we selected the degree of lymphatic invasion and tumor depth for the development of a risk-classification model. This new model consisting of these three factors was even more superior in detecting LNM (AUROC=0.86), while the range of AUROCs for other conventional risk factors including computed tomography (CT) diagnosis and tumor size ranged from 0.51 to 0.60 (Figure 2C). We next categorized all patients with T1 CRC into high and low risk groups using the cut-off thresholds derived by Youden's index from this risk-classification model. Interestingly, the conventional risk factors classified only 13% of the patients into the low-risk category (with 0% LNM), and identified 87% as high-risk (with 19% LNM); however, our proposed new risk-classification model identified 78% of the patients as low-risk (with 5% LNM) and 22% as high-risk (with 50% LNM; Figure 2D).

While the availability of surgically resected tissues from patients with T1 CRC was essential towards developing this LNM detective signature, an ideal situation in the clinical setting would be if such a signature might also work on pre-treatment biopsy specimens. If successful, this would obviate unnecessary endoscopic treatment for genuine high-risk patients those who will eventually require a radical surgery. In order to address this clinical issue, we evaluated the performance of our five-miRNA signature in 83 matched, colonoscopy-derived biopsy specimens from the cohort-2 patients (Supplementary Table 1). Interestingly, the expression levels of four of the five miRNAs in these biopsy specimens significantly correlated with those in the surgical specimens, while expression status of MIR195 was marginally significant (Supplementary Figure 2). These results are indeed quite encouraging considering that biopsy specimens do not always adequately represent tumor heterogeneity, and can yield small variance. We next applied an independent logistic regression model to these biopsy specimens, which yielded an impressive AUROC value of 0.77 for distinguishing LNM-positive from negative cases (Figure 2E).

Finally, we were curious to understand the potential downstream gene targets of our discovered miRNAs, and hence conducted a miRNA-mRNA regulatory network analysis. Intriguingly, key cancer-related genes such as CCND1, CDK6, E2F3, and CDX2 emerged as potential miRNA targets, and pathway enrichment analysis indicated that cell proliferation and cell cycle were the main miRNA regulatory pathways (Supplementary Table 3).

Our study highlights the inadequacy of currently used clinicopathological features for identifying LNM in patients with T1 CRC, and demonstrates the potential clinical significance of our risk-classification model for dramatically reducing the number of "unnecessary" surgeries in these patients^{6,10}. Furthermore, we, for the first time, evaluated and demonstrated the LNM detective potential of our miRNA signature in the pre-treatment biopsies, which was comparable to that of the surgically resected specimens. These results further underscore the importance of our five-miRNA signature which can be successfully

applied to even tiny biopsy samples; hence facilitating the physicians with a more informed decision-making by identifying and performing endoscopic resection only in the patients that are true low-risk and spare the others from such unnecessary treatments, reducing patient complications, physician burden, and healthcare costs¹¹.

In conclusion, using a systematic, genome-wide biomarker discovery approach, we have developed a novel miRNA-based LNM detective signature, which is superior to currently used clinicopathological criteria in patients with T1 CRC. Our findings unravel a new paradigm for more adequate risk-assessment and identification of patients that are true candidates for endoscopic treatment or radical surgery and reduce unnecessary treatment and healthcare burden.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) Study design

(B) From an initially selected seven microRNAs (miRNAs), those which were highly correlated with the others were excluded, and finally five miRNAs were selected as candidates for the establishment of a miRNA signature.

Figure 2.

(A–B) The detection values of the five-microRNA (miRNA) signature in each patient (red line: positive for lymph node metastases (LNM), blue line: negative for LNM). The cut-off threshold was set as 0.06. The five-miRNA signature revealed an area under the receiver operating characteristic curve (AUROC) values of 0.83 in the training cohort (A) and 0.74 in the validation cohort (B) for discriminating LNM positive and negative patients.

(C) The AUROC values of each clinicopathological features and the risk model constructed with five-miRNA signature, lymphatic invasion, and tumor depth.

(D) Eighty-seven percent of the patients were classified as high-risk (LNM 19%) and 13% were low-risk (LNM 0%) by the conventional risk factors. While, new risk classification using our risk model identified 22% as high-risk (LNM 50%) and 78% as low-risk (LNM 5%).

(E) The detective values of the five-miRNA signature in each patient (red line: positive for LNM, blue line: negative for LNM). The cut-off value was set as 0.15. The five-miRNA signature showed an AUROC value of 0.77 for discriminating LNM positive and negative patients.