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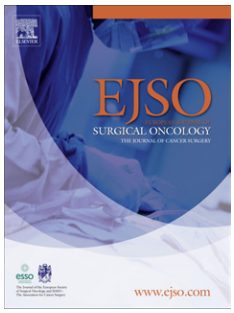

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Category: Review

MicroRNAs in colorectal cancer: function, dysregulation and potential as novel biomarkers

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Abstract

Background: MicroRNAs (miRNAs) are short non-coding segments of RNA which are involved in normal cellular development and proliferation. Recent studies have identified altered miRNA expression in both tumour tissues and circulation in the presence of colorectal cancer. These altered expression patterns may serve as novel biomarkers for colorectal cancer. This review explores recent developments in this rapidly evolving field.

Methods: A thorough literature search was performed to identify studies describing miRNA expression in colorectal cancer. Specific areas of interest included miRNA expression patterns in relation to development, diagnosis, progression and recurrence of disease, and potential future therapeutic applications.

Results: MiRNAs are associated with the development and progression of colorectal cancer. These may be either overexpressed or underexpressed (depending on the specific miRNA). Although there are fewer published studies regarding circulating miRNAs, these appear to be reflective of alterations in tissue expression and may have a potential role as minimally invasive biomarkers.

Conclusion: MiRNAs have immense potential for refinement of the current processes for diagnosis, staging and prognostic prediction. They may also provide potential future therapeutic targets in the management of colorectal cancer.

Introduction

Colorectal cancer is the third most common cancer worldwide and second only to lung cancer as the leading cause of death from cancer in Western countries.[1] Currently, the only curative treatment is surgical resection, with modest survival benefit from chemotherapy, particularly in Stage III disease.[2] The majority of patients who develop colorectal cancer have no specific risk factor for the disease and the best indicator of prognosis is stage at diagnosis. Therefore, early detection is crucial and new diagnostic markers are needed to identify disease at an earlier stage.

MicroRNAs

MicroRNAs (miRNAs) are small (19-25 ribonucleotides), single-stranded non-coding RNAs encoded in plant, invertebrate and vertebrate genomes.[3] They regulate gene expression at post-transcriptional level by acting on specific messenger RNA (mRNA) targets, inducing mRNA degradation or translational inhibition.[4 5] MiRNAs are involved in regulation of pathways in cell differentiation, cell cycle progression and apoptosis.[4] Each miRNA is assigned a numerical identifier and more than 900 have been identified in humans to date.[6]

MiRNAs and cancer

MiRNAs were first linked with cancer in 2002, initially chronic lymphocytic leukaemia (CLL), and subsequently many other types including colorectal cancer.[3 7-9] Expression of miRNAs is dysregulated (either overexpressed or silenced) in malignancy, with some thought to behave as tumour suppressor genes and others as oncogenes. Dysregulation of miRNA expression may therefore contribute to development of cancer through loss of these controls and miRNA expression

represents a means to detect or characterise specific cancers. Thus miRNA expression levels may have potential for use as biomarkers of tumour presence or activity.

MiRNA biochemistry

MiRNAs are the product of miRNA genes (found as independent transcripts or within introns of another gene). They are transcribed in the nucleus by RNA polymerase II as primary transcripts (pri-miRNAs), processed by an RNA specific ribonuclease enzyme complex (DROSHA) into short 70 nucleotide precursor-miRNAs (pre-miRNAs), and transported to the cytoplasm by exportin 5. In the cytoplasm pre-miRNAs are further cleaved by the endonuclease, Dicer, generating mature miRNAs.[10-12]

Mature miRNAs do not code for proteins but regulate protein production in the cell by binding to complementary 'target' mRNAs via the RNA-induced silencing complex (RISC).[12-14] With perfect pairing to target mRNAs, this complex inhibits protein expression by cleavage and degradation of mRNA.[10 15] MiRNAs can also bind to imperfect complementary sites within the 3'untranslated regions (3'UTR) of mRNA targets and act through translational inhibition of gene expression.[10 11] MiRNAs may also interfere with protein translation in polyribosomes.

Regardless of the mechanism employed, the final effect is reduction of protein production by genes regulated by that miRNA.[12]

Regulation of miRNA expression

Although miRNAs function as regulators of mRNA transcription and protein translation, their expression is itself regulated via several mechanisms including specific translational regulation, methylation and histone deacetylation, DNA copy alteration and gene mutations affecting proteins involved in processing and maturation.[10 16] Other proposed influences on miRNA expression include genetic polymorphisms on miRNA 3'UTR binding sites and p53 tumour suppressor gene expression.[17]

Methods of detection and quantification of miRNAs

Several methods of detection and quantification of miRNAs have been developed, including direct detection methods such as Northern blotting and in situ hybridization techniques, hybridisation based microarray platforms (allow high throughput miRNA profiling), and single miRNA approaches such as quantitative RT-PCR (reverse transcriptase/polymerase chain reaction).[18 19] Reliability of RT-PCR depends on correct normalization to stably expressed controls and endogenous controls have been identified for gene expression analysis and miRNA expression profiling in colorectal cancer.[20 21]

Most studies on miRNAs in colorectal cancer focus on miRNA expression in human or murine tissues or human colon cancer cell lines. However, recent advances have shown that they are also present and detectable in circulation. Circulating miRNAs appear to be stable and resistant to degradation by endogenous ribonucleases.[22]

Role of miRNAs in oncogenesis

MiRNAs play roles in the control of many cellular processes including development, differentiation, apoptosis and metabolism. Recent work has shown aberrant miRNA expression patterns in a range of human diseases including many cancers.[10]

Dysregulation of miRNA expression can influence carcinogenesis if target mRNAs are encoded by tumour suppressor genes or oncogenes. Relatively minor variations could have important consequences for the cell because of the large number of targets for each miRNA.[11 14] Many human miRNA genes are thought to be located in cancer-associated regions or at fragile sites of chromosomes which are prone to deletion, amplification and mutations in cancer cells.[10-12]

As miRNAs function as negative regulators of gene expression, overexpression of oncogenic miRNAs can contribute to tumorigenesis by promoting cellular proliferation and evasion of apoptosis. Reduced expression of tumour suppressive miRNAs may have similar effects.[3] Both increases and decreases in the expression of specific miRNAs have been demonstrated in cancer. These appear to be tissue-specific and characteristic of cancer type.[11 12]

MiRNA expression profiles in colorectal cancer

A wide range of specific miRNAs dysregulated in colorectal cancer have been identified in cell lines, tumour and normal tissues, with over 100 miRNAs currently implicated. The first study of miRNA expression in colorectal cancer, published in 2002, identified *miR-143* and *miR-145* as novel dysregulated miRNAs.[23] Since then, numerous individual miRNAs have been implicated and these are summarised in table 2.

Mechanisms of miRNA dysregulation in colorectal cancer

Although it remains unclear how miRNAs become dysregulated in cancer cells, a number of possible mechanisms have been proposed. Altered expression of miRNA in colorectal cancer may be due to epigenetic mechanisms, including hypermethylation of promoter regions (which can occur when the miRNA gene is located near a CpG island) or histone modifications.[16] Abnormal hypermethylation of promoters of *miR-9, miR-34a*, *miR-34b, miR-34c*, *miR-129* and *miR-137* is associated with reduced expression in colorectal cancer tissues, suggesting a contribution to transcriptional down-regulation of miRNAs.[16]

Transcriptional regulation of miRNA gene expression may also be achieved by epigenetic alterations of host gene regulatory elements located far from the miRNA locus, such as silencing of *miR-342* in colorectal cancers by methylation of a CpG island in the 5´ aspect of its host gene, Ena/Vasp-like (EVL).[24]

Inherited variation in both miRNAs and their binding sites provide another means of miRNA dysregulation. Evaluation of 57 single nucleotide polymorphisms (SNPs) in miRNA binding sites found eight common polymorphisms and a significant association was established between variant alleles of CD86 (affecting *miR-184, miR-212, miR-200a, miR-337* and *miR-582* binding sites) and INSR (affecting *miR-612* and *miR-618* binding sites) genes and colorectal cancer risk.[25] However, a recent Korean study of 426 patients with adenocarcinoma invesitgated 40miRNA-related gene polymorphisms and found none to be an independent prognostic marker in this cohort.[26]

Dysregulation of P53, a tumour suppressor gene commonly mutated in colorectal cancers, is associated with many abnormalities of miRNA expression.[7 11] Comparison of miRNA expression patterns in wild-type and p53-/- mutant HCT-116 colon cancer cell lines after treatment with DNA damaging agents showed several miRNAs (including *miR-34a*) induced in the wild-type but not the p53-/- mutant cells and experimentally overexpressing *miR-34a, miR-34b* or *miR-34c* allowed p53 effects like cell cycle arrest and apoptosis to be phenocopied.[27].

Several targets through which miRNA effects are mediated in colorectal cancer have been identified. These are summarised in table 1.

Role in disease progression, invasion & metastases

In addition to initiation of tumorigenesis, dysregulated miRNAs may have a role in disease progression through promotion of growth, invasion and metastasis. Involvement of miRNAs in metastasis was first reported in breast cancer by Ma *et al.* in 2007, who showed that *miR-10b* initiates breast cancer invasion and metatasis.[28]

Lujambio *et al.* subsequently identified a miRNA hypermethylation profile characteristic of human metastasis (including colorectal cancer) which suggested that DNA methylation-associated silencing of tumour suppressor miRNAs contributes to the development of human cancer metastasis.[29] In this study, which used colorectal cancer (SW620), melanoma (IGR37) and head and neck cancer (SIHN-011B) cell lines derived from lymph node metastases, reintroduction of *miR-148a, miR-34b* and *miR-34c* into cancer cells with epigenetic inactivation inhibited motility, tumour

growth, and metastasis formation in xenograft models, with associated downregulation of miRNA oncogenic target genes such as c-Myc, E2F3, CDK6 and TGIF2.[29]

A number of studies have shown differential miRNA expression between early and late stage disease in colorectal cancer, summarised in table 3.[8 30] However, these were small studies using tissues from cohorts of between 37 and 50 patients.

Downregulation of *miR-143* and *miR-145* is detectable at the pre-adenomatous polyp stage, supporting a role for these miRNAs in early stages of carcinogenesis.[30 31] While both appear to have tumour suppressor effects in normal tissue, with loss of this effect resulting from downregulation in the early stages of carcinogenesis, *miR-145* was found to have an oncogenic effect in metastatic colorectal cancer (associated with downregulation of the G1/S cell cycle checkpoint and neuregulin pathways) but not in an isogenically matched non-metastatic model. No such effect was seen with *miR-143* which showed a tumour suppressor effect in metastatic colorectal cancer cells.[30]

Microsatellite instability

Microsatellite instability is indicative of defective DNA mismatch repair and, in colorectal tumours, is associated with a poor response to chemotherapy but an overall better prognosis.[32] MiRNA expression profiles may allow high-microsatellite instability tumours to be distinguished from microsatellite stable tumours.[33-35] MiRNA profiles appear to separate microsatellite unstable colorectal cancer from microsatellite stable cancer with a specificity of around 80% and sensitivity of around 90%, and may be partly responsible for their differing clinical behaviour.[33 34]

MiRNAs in circulation

Although there are fewer published studies on circulating miRNA profiles, they appear to reflect tissue expression, and hence are altered in malignancy. This has been shown in several cancer types including prostate cancer and breast cancer.[22 36] Levels of specific miRNAs in serum or whole blood are stable, reproducible and consistent within species, with no significant differences between healthy males and females for most.[37 36]

Published reports of circulating miRNAs in colorectal cancer are limited, however Ng *et al.* found *miR-17-3p* and *miR-92* were significantly elevated in the serum of colorectal cancer patients with significant reduction following surgery.[38] Further validation with an independent set of 180 samples indicated that *miR-92* levels in plasma can distinguish between colorectal and gastric cancers, inflammatory bowel disease or normal subjects.[38] A separate study comparing plasma miRNA expression in 157 patients with advanced adenomas or carcinomas and 59 controls found significant upregulation of *miR-29a* and *miR-92a* in colorectal cancer patients.[39]

MiRNAs in faecal matter

Much of the attention to date has been focussed on miRNAs in tissue and, to a lesser extent, in circulation, however there are some recent reports of detection of miRNAs in stool samples indicating that miRNAs can be extracted from stool in a reproducible and predictable fashion and possibly distinguish between individuals with and without colorectal neoplasia, though this requires further investigation in a larger cohort.[40 41]

MiRNAs as tumour markers

It is likely that circulating or faecal miRNAs may present an opportunity to develop new, relatively non-invasive molecular markers of tumour activity. In view of the variability of expression, a tumour-specific profile based on a panel of miRNAs may have greater sensitivity and specificity than use of single miRNAs. [35 42]

MiRNAs as prognostic indicators

Current investigations for diagnosis and staging of colorectal cancer are based on histology and radiology. MiRNAs have immense potential to refine this process, providing better information regarding staging and prognosis, and perhaps identifying patients who are likely to respond well to therapy.

MiRNA expression has already been shown to correlate with disease outcomes. Tumours expressing high levels of *miR-21* or *miR-200* correlate with poorer prognosis regardless of stage and *miR-29a* expression is associated with more advanced tumours or positive nodes.[8 43] Patients with stage II colorectal carcinoma with high expression of *miR-320* or *miR-498* showed significantly shorter progression-free survival.[44] As described previously, miRNAs can also distinguish microsatellite unstable colon cancers from microsatellite stable colon cancers.

MiRNAs and therapeutic response

Tumour response to treatment may be affected by the miRNA expression profile. In one study, high levels of *miR-196a* promoted chemosensitivity towards platin derivatives.[45] Suppression of *miR-31* has recently been shown to increase sensitivity to 5-fluorouracil (5-FU) and thus inhibit proliferation in colon cancer cell lines.[46] 5-

FU leads to down regulation of *miR-200*, thought to inhibit protein tyrosine phosphatise nonreceptor type 12, a tumour-suppressor gene. It also induces upregulation of *miR-133a* (thought to inhibit KRAS) and many other miRNAs. 5-FU may act as a switch to turn on p53 and, through p53, a cascade of miRNAs that may act with or independently of p53.

The full extent of the human miRNA-ome has yet to be determined and their targets and roles in normal cell pathways and oncogenesis clarified, however, these small molecules possess enormous potential and may have wide-ranging future applications including utilisation as novel non-invasive biomarkers in screening, disease surveillance, monitoring of therapeutic response and patient selection for therapy.

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Table 1: MiRNA targets in colorectal cancer

Cyclooxygenase-2 (COX-2)	• Overexpressed in 40% of adenomas and 80% of adenocarcinomas • Strongly contributes to growth and invasiveness through inhibition of apoptosis and promotion of cell invasion via action on prostaglandin E_2 (PGE_2)[47] • Direct inhibition of COX-2 mRNA translation by *miR-101* demonstrated in vitro[47]
Adenomatous polyposis coli (APC)	• Inactivation is an initiating event in majority of colorectal carcinomas • *MiR-135a* and *miR-135b* decrease translation of APC transcript, inducing β-catenin signalling and activation of the *Wnt* pathway[11]
Kirsten retrovirus-associated sequences (KRAS) oncogene	• Direct target of *let-7a* family of miRNAs[13] • Reduced *let-7* levels shown in tumours and colon cancer cell lines • Transfection of cell lines with *let-7a-1* precursor miRNA results in growth suppression and decrease in KRAS protein levels • Also thought to be a target of *miR-143*
Epidermal growth factor receptor (EGFR)	• Phosphatidylinositol-3-kinase (PI-3-K) pathway is a central signalling pathway downstream from EGFR • p85ß regulatory subunit involved in stabilising and propagating the signal is suppressed by *miR-126*

Programmed cell death 4 (PDCD4) gene	• Tumour suppressor gene (inhibits transformation and invasion) • Has conserved *miR-21* binding site within 3'UTR • Inverse correlation between *miR-21* and PDCD4 protein amounts[48]
Other possible targets[1014]	• ERK5 (involved in the mitogen-activated protein-kinase pathway [MAPK]) • Insulin receptor substrate-1 (IRS-1) • C-Myc (oncogenic transcription factor) • DNA methyltransferase 3A • Tumour suppressor gene TP53 • Cell cycle regulator Cdc25A • Bcl-X_L oncogene

Table 2: MiRNAs associated with colorectal cancer

Overexpressed miRNAs[83449]	Underexpressed miRNAs[392349]
miR-15b	*miR-1*
miR-17-5p	*miR-9-1*
miR-19a	*miR-30c*
miR-20	*miR-30a-3p*
miR-21	*miR-30a-5p*
miR29a	*miR-34a*
miR-31	*miR-34b*

miR-92	*miR-34c*
miR-96	*miR-126*
miR-133b	*miR-129*
miR-135b	*miR-133a*
miR-148a	*miR-133b*
miR-181b	*miR-137*
miR-182	*miR-139*
miR-183	*miR-143*
miR-191	*miR-145*
miR-200b	*miR-195*
miR-200c	*miR-342*
miR-212	*miR-422a*
	miR-422b
	let-7a-1

Table 3: MiRNAs associated with invasion and metastasis

Increased[830]	Decreased[829-31]
miR-7	*miR-34b*
miR-21	*miR-34c*
miR-31	*miR-99b*
miR-196a	*miR-125*
	miR-133a
	miR-143

	miR-145
	miR-148a
	miR-378