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MicroRNA expression in colorectal cancer

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1. Introduction

Colorectal cancer is a common disease entity with a multi-factorial aetiology which remains poorly understood. It is estimated that in 2008, colorectal cancer was responsible for 8% of all cancer deaths, making it the fourth most common cause of death from cancer [10]. Prognosis is heavily linked to stage at diagnosis. The major cause of death is development of metastasis in liver, abdominal lymph nodes, and lung, for which there is no cure [21]. According to the most recently defined American Joint Committee on Cancer (AJCC) system for colonic adenocarcinoma, 5-year stage-specific survivals were 93.2% for stage I disease compared with 8.1% for stage IV [34], highlighting a need for novel early detection strategies. Colorectal cancer, at least theoretically, is a disease entity which is amenable to early detection, since it exhibits a stepwise progression of carcinogenesis from benign polyps to adenocarcinoma, over a period of time. The majority of national screening programmes currently centre on the use of colonoscopy or faecal immunohistochemistry/occult blood testing. Colonoscopy is an expensive, invasive procedure which carries a significant risk of intestinal perforation (1:700). Faecal testing is more cost effective and less invasive but sacrifices sensitivity and specificity. Carcinoembryonic antigen, the only blood test currently available, exhibits low sensitivity and specificity. Hence, there is great need for new biomarkers for early detection of CRC.

2. MicroRNA biochemistry and function

First described by Lee et al. in 1993, microRNAs are a class of small (19–25 ribonucleotides) non-coding RNAs [25]. Upon discovery, each microRNA is assigned a numerical identifier and the number identified in humans to date approaches 1000 [12,30]. In brief, the maturation of microRNAs is thought to be triphasic [3]. Initially, transcription of pri-miRNA (primary transcripts) occurs in the nucleus [3,13]. They are then processed by an RNA specific ribonuclease enzyme complex (DROSHA) and cleaved into shorter 970 nucleotide precursor-microRNAs [41,46]. The final cleavage step occurs after transportation to the cytoplasm by exportin 5 [41].

MicroRNAs are known to regulate gene expression at a post-transcriptional level by acting on messenger RNA (mRNA) targets. Through this mechanism, they may induce degradation of mRNA or translational inhibition. Found in both prokaryotes and eukaryotes, microRNAs are also known to post-transcriptionally regulate a number of key cellular processes including differentiation, proliferation, progression and apoptosis [4]. In fact, it is now widely accepted that microRNAs are involved in almost all cellular processes to some degree. Since these processes represent central mechanisms of tumorigenesis, it is unsurprising that microRNAs became the subject of international interest in cancer detection and therapeutics. The focus of this review will remain on cancer detection and the use of microRNAs as biomarkers.

3. MicroRNAs in cancer

Almost a decade after microRNAs were first described, Calin et al. published the first study linking microRNAs and cancer [5]. The authors identified a translocation-induced deletion at chromosome 13q14.3 in a model of B-cell chronic lymphocytic leukaemia, with associated loss of miR-15a and miR-16-1 [5]. Since microRNAs represent key players in cellular processes crucial to carcinogenesis, over-expression of

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oncogenic microRNAs contributes to tumour development through promotion of migration, invasion and proliferation [30,36]. Conversely, increased expression of tumour suppressor microRNAs may have the opposite effect [30,36]. Aberrant expression of these small molecules has now been reported in many cancers, including colorectal [26]. Most importantly from a biomarker perspective, microRNA expression has recently been shown to be specific to tissue type and cancer type [15,44,46].

4. MicroRNAs in colorectal cancer

Soon after Calin et al. published their work linking microRNAs with cancer, researchers began to publish microRNA deletions associated with other haematological malignancies. Shortly afterwards, Michael et al. identified mir-143 and mir-145 as novel dysregulated microRNAs in colorectal cancer [29]. In the past decade, almost 400 further dysregulated microRNAs have been reported in colorectal cancer. These microRNAs are listed in Table 1. Ma et al. recently conducted a systematic review of candidate microRNA biomarkers in colorectal cancer and compiled a list of six microRNAs most consistently found to be dysregulated in colorectal cancer tissue in the literature. These microRNAs were mir-106a (up regulated), miR-125a, miR-133a, miR-145, miR-30a-3p and miR-139 (all down-regulated) [28]. The mechanism underpinning this dysregulation is not yet understood, however researchers have proposed that DNA hypermethylation may result in down-regulation of specific microRNAs. Histone acetylation and enzymatic mechanisms that influence microRNA processing have also been implicated [35]. Although the full extent of the human microRNA-ome has yet to be elucidated and although the mechanism of dysregulation of microRNAs in colon cancer remains largely unknown, a growing body of evidence supports the theory that specific microRNA expression patterns are associated with colorectal cancer. The potential implications of this association are far-reaching.

MicroRNAs have also been shown to correlate with clinicopathological features in colorectal cancer. MiR-143 and -145 were shown by Michael et al. to be consistently reduced in tissue during the early adenomatous stage of colorectal cancer development [29]. Furthermore, an association between miR-21 expression in tissue, clinicopathological stage and lymph node positivity has also been observed by Slaby et al. [40], with other studies supporting an association between high miR-21 expression and poor survival [38].

Table 1
MicroRNAs up-regulated and down-regulated in colorectal cancer

Collated list of dysregulated microRNAs in colorectal cancer with proposed target genes [2,6,8,28,33,46,47]	
let 7a	c-Myc, DLD-1, Ras
miR-1	FSCN1
miR-9	ACOT7, SLC45A1
miR-17	E2F1
miR-17-3p	MMP23B, CHD5
miR-17-5p	MMP23B, CHD5
miR-19a	KLHL17
miR-21	Cdc24A, Pcdc4, PTEN, Sprouty2
miR-25	CAMTA1, KIF1B
miR-29a	CCNL2, CAMTA1
miR-29b	CCNL2, CAMTA1
miR-31	FIH-1, KIF1B, ZUBR1
miR-34a	E2F1, SIRT1
miR-92	CAMTA1, KIF1B
miR-95	UBE4B
miR-100	TARDBP, FRAP1
miR-101	COX-2
miR-106a	E2F1
miR-124a	CAMTA1, RERE
miR-125a	TP73, E2F3
miR-126	P85beta, KIF1B, HP1BP3
miR-128a	CPSF3L
miR-128b	CPSF3L
miR-133b	c-Met
miR-135a	APC, GNB1, CAMTA1
miR-135b	APC, GNB1, CAMTA1
miR-137	Cdc42, LSD-1
miR-142-3p	CAMTA1
miR-143	Ras, DNMT3A, EN01, KIF1B, K-Ras
miR-145	IRS1, STAT1, YES, FLI1
miR-146a	SPEN
miR-148a	KLHL17, PRKCZ
miR-154*	GNB1, LZIC
miR-155	MSH2, MSH6, MLH1
miR-181b	CYLD
miR-182	CCNL2, KIF1B
miR-183	KLHL17, TNFRSF25
miR-192	CTNNBIP1
miR-195	Bcl-2
miR-200c	TGFβ2, ZEB1
miR-203	Sox2, Klf4
miR-328	ATAD3C
miR-335	CAMTA1, KIFIB
miR-338	WDR8, TNFRSF25

5. MicroRNAs and the colorectal tumour microenvironment

Investigation of colorectal carcinogenesis initially focussed solely on epithelial tumour cells but it is now known that epithelial cells exist within a supportive stromal network and that significant stromal dysregulation also occurs [17]. This dynamic crosstalk characterised by a bidirectional relationship between stromal and epithelial cells, impacts heavily on core tumour characteristics including degree of hypoxia, angiogen-

esis and inflammation [17,35]. These characteristics directly impact on prognosis and the level of angiogenesis has been reported as a survival predictor for patients with colorectal cancer [11,20]. Recent studies have implicated microRNAs in regulating hypoxia and interacting with inflammatory factors such as VEGF in the tumour microenvironment [35]. It has been proposed that the regulation of angiogenesis in colorectal tumours involves an interplay between anti-angiogenic microRNAs (miR-20a and mir-221) and pro-angiogenic microRNAs (let-7f and mir-126) [16,22,35].

6. MicroRNAs as biomarkers

An ideal biomarker should be sampled in as non-invasive a manner as possible and should display a good sensitivity and specificity profile [24]. Given that microRNAs are aberrantly expressed in colorectal cancer since colorectal cancer is a disease in great need of a biomarker, these candidates warrant extensive investigation. Furthermore, microRNAs are extremely stable molecules and have been shown to preserve their integrity for up to a decade in formalin-fixed paraffin embedded colorectal cancer tissue samples [33,45].

Cancer-associated RNAs have been described in the circulation of cancer patients for more than a decade and since patients routinely undergo blood test in the diagnostic work-up of even minor illnesses, this route holds potential in the pursuit of a minimally invasive sampling medium [42]. Recently, several groups have reported that circulating microRNAs exist and can be detected in the serum and plasma [31]. Expression profiles of circulating microRNAs have been more extensively investigated in breast and prostate cancer, however recent studies reveal potential promise in colorectal cancer [18]. Ng et al. report elevated miR-17-3p and miR-92 levels in the serum of patients with colorectal cancer with significant reduction in levels after tumour resection [32]. In the same study MiR-92 was also an effective discriminator between colorectal and gastric cancers [32]. Huang et al. examined plasma samples from over 150 colorectal cancer patients and report that plasma miR-29a and miR-92a have strong potential as novel non-invasive biomarkers for early detection of CRC. With combined analysis of both microRNAs they achieved a 83.0% sensitivity and 84.7% specificity in discriminating colorectal cancer from normal controls [18]. When variability of expression is taken into consideration, it appears that a tumour-specific panel of microRNAs, rather than the use of single microRNAs in isolation, may represent a more pragmatic opportunity for increased sensitivity and specificity in biomarker candidates [43].

In pursuit of a minimally-invasive biomarker, colorectal cancer presents the opportunity to use faeces as a potential source of a biomarker candidate. Recently, Link et al. reported that microRNAs are abundantly present in stool and can be easily and reproducibly detected in stool specimens using standard microRNA extraction techniques [27]. Furthermore, Ahmed et al. proposed that a panel of microRNA isolated from faecal samples may hold greater sensitivity and specificity than currently used screening genomic, methylomic or proteomic methods for colon cancer [1]. Interestingly, a recent study by Kalimutho et al. found miR-34b/c hypermethylation in 75% (21 out of 28) of faecal specimens examined indicating potential as a non-invasive screening tool [19]. Larger patient studies are required to validate and develop these novel strategies.

As our understanding of microRNA expression in colorectal cancer develops, it may be possible not simply to distinguish between cancers and controls, but to delineate degrees of disease activity, core tumour characteristics and predicted response to treatment. It is universally acknowledged that individualisation of therapy represents the future of cancer care. Early detection of tumours, as well as the ability to differentiate between cancers most likely to respond to certain therapies remains a key goal. For example, microRNAs have been shown to differentiate effectively between micro-satellite instability positive and negative tumours using formalin-fixed, paraffin-embedded tissue of resection specimens [8]. Genetic aberrations have a significant impact on core tumour characteristics and clinical behaviour with better prognosis associated with micro-satellite instability-associated tumours.

Furthermore, microRNAs may play a role in helping to identify response to treatment. In one study, expression of high levels of miR-196a was associated with chemosensitivity towards platin derivatives [39].

Certain microRNAs have been shown to correlate with poorer prognosis [37,40]. Tumours expressing high levels of mir-21 and mir-200 have been shown to represent poorer prognosis and more aggressive disease. Similarly, mir-29a expression is associated with greater degrees of nodal positivity [33,40]. While further studies are necessary, the identification of markers of more aggressive disease would allow tailoring of therapy with these patients receiving more aggressive therapy. Not only does this hold the potential to more effectively treat patients with more aggressive disease,

it also provides a potential opportunity to spare patients with less aggressive disease from undergoing unnecessarily overzealous treatment. Therefore, elucidation of microRNA markers of disease severity or activity is crucial in the pursuit of improved oncologic outcome, optimum patient quality of life and increased cost-effectiveness.

7. Current practical challenges

Continued investigation into elucidation of microRNA expression in colorectal cancer is crucial in exploring the potential benefits of these promising biomarker candidates. In the laboratory setting, it is important to encourage international standardisation and optimisation of techniques. Only through stringent controls can we hope to arrive at reliable conclusions amenable to translation from bench to bedside. For example, the evaluation of optimum endogenous controls for colorectal cancer blood and tissue samples is crucial. To correct for systematic variables such as the quality of RNA in a given sample or the amount of template available, RQ-PCR data is commonly normalised to an endogenous control (EC) gene which should be expressed in a stable manner across a sample set [7]. A universal endogenous control is unlikely to exist, so, to avoid introducing further error in the quantification of expression data it is necessary that candidate ECs be evaluated and validated in colorectal cancer tissue, blood and cells [7].

Exosomes are small nano-vesicles released by many cells which are known to be present in plasma and other body fluids [9]. Exosomes are known to contain RNA [9]. Therefore, in extraction of RNA from cells, blood and tissue, many researchers now agree that ultracentrifugation steps be performed to avoid the presence of confounding exosomal RNA. In an extensive systematic review of microRNA biomarker candidates in colorectal cancer, Ma et al. reported on 6 microRNAs differentially expressed in colorectal cancer which are inconsistently reported to be over or under expressed in 3 or more studies [28]. Optimisation of laboratory techniques such as exosome isolation and use of appropriate endogenous controls may help to improve accuracy of identification of RNA expression.

8. Conclusion

Despite improvement in early diagnosis, surgical techniques, and general patient care, a large disparity in 5-year survival exists between stage one and stage four colorectal cancer. Patient prognosis is highly dependent on stage at diagnosis. This highlights a need for better understanding of tumour biology in order to identify novel biomarkers for early disease detection and individualisation of therapy. MicroRNAs hold enormous potential to revolutionize diagnostics and screening in colorectal cancer. Not only have they have been shown to be differentially expressed in colorectal cancer, microRNAs may also be capable of providing crucial information regarding response to therapy and core tumour characteristics. Furthermore, the detection of microRNAs in the circulation and faecal matter of patients with colorectal cancer may facilitate minimally invasive disease detection and characterisation.

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