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EDITORIAL

MicroRNAs, development of Barrett's esophagus, and progression to esophageal adenocarcinoma

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Abstract

Barrett's esophagus is a premalignant condition caused by gastroesophageal reflux. Once developed, it can progress through varying grades of dysplasia to esophageal adenocarcinoma. Whilst it is well accepted that Barrett's esophagus is caused by gastroesophageal reflux, the molecular mechanisms of its pathogenesis and progression to cancer remain unclear. MicroRNAs (miRNAs) are short segments of RNA that have been shown to control the expression of many human genes. They have been implicated in most cellular processes, and the role of miRNAs in disease development is becoming increasingly evident. Understanding altered miRNA expression is likely to help unravel the molecular mechanisms that underpin the development of Barrett's esophagus and its progression to cancer.

Key words: Barrett's esophagus; MicroRNA; Esophageal adenocarcinoma; Transdifferentiation; Tumour suppressor

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INTRODUCTION

Barrett's esophagus is characterized by a metaplastic transition whereby a columnar-lined epithelium with intestinal metaplasia arises within the squamous epithelium of the distal esophagus[1,2]. The development of Barrett's esophagus appears to be a protective action, initiated in response to the esophagus being continually exposed to chronic gastroesophageal reflux[3,4]. The clinical significance of Barrett's esophagus lies with the increased risk for cancer development, as it can progress through varying grades of dysplasia to esophageal adenocarcinoma. Barrett's esophagus is the principle identifiable precursor to esophageal adenocarcinoma. The prevalence of esophageal adenocarcinoma has been increasing significantly in Western countries, and it has a poor prognosis, with the overall 5-year survival remaining less than 20%[5,6].

Due to the increased prevalence of esophageal adenocarcinoma, the precursor lesion, Barrett's esophagus, has come under the spotlight. Although a number of alterations in gene expression have been identified in its progression to cancer, the current sequence of molecular events that drive the development of Barrett's esophagus, and its subsequent progression to cancer, remains un-

clear[4]. Great interest surrounds the identification of early molecular changes which contribute to the pathogenesis of Barrett's esophagus, as these may present opportunities for therapeutic development, or new strategies for the prevention of esophageal adenocarcinoma. Of particular interest is the relatively newly identified class of molecules known as microRNAs (miRNAs), and the potential roles they play in the development of Barrett's esophagus and adenocarcinoma.

miRNAs

miRNAs are approximately 21 nucleotide long, non-coding segments of RNA that act to regulate gene expression[7]. Since their initial discovery in *Caenorhabditis elegans* in 1993, an enormous body of research has been published, implicating miRNAs in almost every cellular process investigated[7,8]. Both miRNA biogenesis and miRNA regulation of protein synthesis have been reviewed extensively[7,9-12]. In short, miRNA biogenesis begins with primary miRNAs (pri-miRs, inactive form), which are either transcribed by RNA polymerase Ⅱ or are excised as portions of introns[9,12]. Pri-miRs are processed in the nucleus by Drosha ribonuclease and the resultant precursor-miRNA (pre-miR) is then exported to the cytoplasm[9,12,13]. In the cytoplasm, Dicer ribonuclease processes the pre-miR and a single RNA strand is transferred to an argonaute protein within the RNA-induced silencing complex (RISC) complex[9,12]. The mature (active) miRNA-RISC complex targets complementary mRNA transcripts to repress translation[9,10].

THE ROLE OF miRNAs IN BARRETT'S ESOPHAGUS DEVELOPMENT

Key areas of interest for miRNAs in Barrett's esophagus include their involvement in transdifferentiation and its links with the development of columnar metaplasia, and also cancer development within Barrett's esophagus. miRNAs are useful biomarkers for tumor classification, and their expression pattern may have a role to play in the early detection of cancer development[14,15].

Can the developing esophagus provide insight?

To understand where miRNAs may fit into the development of Barrett's esophagus, it is important to consider how the normal esophagus develops and also how developmental mechanisms may drive pathogenesis. The esophagus begins to form during week four of embryonic development, with the formation of the foregut[16]. However, it is not until week five that the foregut can be visually divided into esophagus, stomach and duodenum[17]. Development of the esophageal lumen begins in weeks seven and eight, when the epithelium begins to proliferate, and by week 10 the lumen is enclosed and lined with a ciliated epithelium[16]. In the fourth month of gestation, the ciliated epithelium begins to be replaced by a squamous epithelium. Residual islands of ciliated epithelia remain, and these give rise to esophageal glands[16].

It is interesting that the human embryonic esophagus is initially lined with a columnar epithelium, which is then replaced by a stratified squamous epithelium[16]. A reversal of normal developmental mechanisms is observed in Barrett's esophagus where a columnar epithelium with intestinal metaplasia arises in the squamous-lined esophagus. Transdifferentiation involves a change from one differentiated cell type to another, occurring as metaplasia, resulting in a change in cell fate or a switch in phenotype[18]. The columnar-squamous transition that occurs in the developing esophagus is likely to result from a transdifferentiation event, as it is not dependent on cell division. In addition, some epithelial cells express both squamous and columnar markers during the transition period[19]. Although these two criteria are not a requirement for transdifferentiation, they do suggest that the epithelium is not being replaced with new epigenetically distinct cells. Therefore, gene expression profiles can be initiated to drive a squamous or columnar phenotype in the esophagus. As miRNAs function by regulating gene expression[20], it is likely that miRNAs are involved in directing gene expression in esophageal development, and they may contribute to the development of the columnar-lined epithelium which colonizes the luminal surface of the esophagus in Barrett's esophagus.

miRNAs can directly regulate transdifferentiation

What drives the formation of a columnar-lined epithelium with intestinal metaplasia in the esophagus? It is known that Barrett's esophagus is caused by chronic exposure of the esophagus to gastroesophageal reflux. Chang *et al*[21] showed that squamous epithelium exposed to all-trans retinoic acid (ATRA) drove a sequence of events, beginning with the removal of surface squamous epithelia, which allowed for the esophageal sub-mucosal glands to access the luminal surface. The evidence implicating miRNAs in transdifferentiation/metaplasia is limited. However, a study by Tsonis *et al*[22] implicated the let-7 miRNA family in regulating dedifferentiation (a critical event in transdifferentiation) in the lens and inner ear hair cell regeneration. In alveolar epithelial cells, miR-375 has been shown to regulate transdifferentiation *via* the Wnt/β-catenin pathway[23]. Finally, the miR-200 family and miR-205 have been implicated in driving epithelial to mesenchymal transdifferentiation, a crucial event in tumor metastasis[24]. These studies provide further evidence that fluctuations in miRNA expression play crucial roles in directing transdifferentiation.

ATRA interacts with retinoic acid receptors and retinoid X receptors (RXR) to mediate the suspected transdifferentiation response observed in the study by Chang *et al*[21]. Importantly, lithocholic acid (LCA), a component of gastroesophageal refluxate, has been shown to interact with human RXR-β[25], providing evidence that ATRA exposure is relevant to the conditions

experienced in patients with chronic gastroesophageal reflux. Interestingly, RXR-β expression is up-regulated in Barrett's esophagus compared with tissue from non-refluxing patients, and it also correlates with RXR-α expression[26]. This provides evidence that retinoid-induced differentiation is suppressed in normal squamous epithelia due to receptor down-regulation. In theory, following chronic exposure of the esophagus to refluxate, there could be a change in gene expression facilitated by differential expression of miRNAs targeting RXR receptor mRNA. Consequential up-regulation of RXR receptor translation would follow, allowing for increased binding of LCA to the RXR-β receptor.

Among the retinoic acid targets are the *CDX1/2* genes, transcription factors shown to play a major role in driving intestinal gene expression required for developing a Barrett's esophagus phenotype[4,27,28]. Therefore, the initiation of RXR expression could be a crucial step in the early development of Barrett's esophagus.

There are examples of miRNA interaction with transcription factors forming negative feedback loops to regulate gene expression. One example includes the miR-200 family and miR-205 targeting of *ZEB1* and *ZEB2* transcription factors in epithelial-mesenchymal transition. The miR-200 family and miR-205 have been shown to directly repress *ZEB1* and *ZEB2* while these transcription factors regulate the transcription of the miR-200 family[29]. A miRNA regulatory loop, similar to that described by Bracken *et al*[29], could be directing the expression of transcription factors such as *CDX1/2* genes that are critical to the development of Barrett's esophagus.

Chronic gastroesophageal reflux is often associated with esophagitis. This condition provides the earliest disease state available for analysis prior to the development of Barrett's esophagus. Our laboratory has shown that some miRNAs are differentially regulated in response to chronic gastroesophageal reflux (unpublished results). miRNAs shown to be differentially regulated during exposure to acid or specific bile components may be involved in directing early molecular events required for the development of Barrett's esophagus. It is possible that miRNA de-regulation observed in patients with chronic reflux and esophagitis may allow RXR expression and initiate the early events required for Barrett's esophagus to develop.

MAINTAINING A SPECIALIZED COLUMNAR EPITHELIUM

Whether miRNAs directly alter the gene expression profile in esophageal cells, thereby directing the development of a columnar epithelium with intestinal metaplasia, is unclear. Although the initial development of Barrett's esophagus may occur *via* transdifferentiation, it must be maintained. The sustained presence of the columnar epithelium suggests a continual gene expression profile is established in progenitor cells. It is possible that aberrant miRNA expression acts to establish the required gene expression that allows progenitor cells to differentiate to a columnar phenotype. The role of miRNAs in driving differentiation is well established. An excellent example of this is miR-203, a miRNA down-regulated in Barrett's esophagus[30,31].

miR-203 and squamous epithelia

miR-203 has been implicated in driving terminal differentiation in skin epithelial cells[32]. The luminal surface of the esophagus is continually replaced by cells that migrate from the basal layer[33]. Once these basal cells become suprabasal, proliferative capacity is lost and terminal differentiation is initiated[32]. Suprabasal cells in the esophagus undergo terminal squamous differentiation to maintain a stratified squamous epithelium[33]. In skin epithelia, miR-203 has been shown to target and repress the transcription factor p63[32,34]. p63 plays a crucial role in maintaining stem cells in stratified squamous epithelium[32,35]. miR-203-directed repression of p63 acts to repress a cell's proliferative capacity and induce cell cycle exit[32,34]. It is therefore likely that induction of miR-203 expression is a crucial checkpoint required for terminal squamous differentiation. Whether miR-203 expression is linked with replenishment of the esophageal epithelium is unclear, although miR-203 expression is lost in Barrett's esophagus, and therefore it is likely that the miR-203-directed mechanism of epithelial replacement is lost in this tissue.

miRNAs AND BARRETT'S ESOPHAGUS: CLINICAL INSIGHTS

Increasing evidence supports the application of knowledge about miRNAs to clinical settings. Recent publications have shown that miRNA expression patterns can be used as prognostic and pathogenic markers of numerous disease states, and can predict response to therapeutic strategies and outcomes from clinical procedures[36-40]. One clinically relevant example in carcinogenesis involves miR-21. miR-21 is reported to be up-regulated in a number of different solid tumors, and increased expression correlates with poor prognosis[38]. In the context of Barrett's esophagus, miR-196a expression has been shown to correlate with different disease states in the Barrett's metaplasia-dysplasia-carcinoma sequence[39]. Currently, Barrett's esophagus is the principle identifiable precursor to esophageal adenocarcinoma and, therefore, miR-196a expression may provide a valuable tool in early cancer detection. Standard clinical management of Barrett's esophagus involves continual endoscopic surveillance. Surveillance efficacy has been the topic of much scrutiny and a less invasive, more cost-effective patient management strategy is highly sought after[41,42]. Such an alternative management strategy could be one in which miRNA expression profiling may play a major role.

miRNAs AND CANCER DEVELOPMENT

Aberrant miRNA expression has been linked with the development and progression of almost all cancers studied to date. The influence of miRNAs in the regulation and control of crucial cellular processes, including signaling, proliferation, apoptosis, motility and angiogenesis, has implicated a number of miRNAs in cancer development and progression[43]. Functional studies of miRNAs in cancer development have identified miRNAs acting as both oncogenes (e.g. miR17-92 cluster) and tumor suppressors (let-7)[44-46]. Forced expression of the miR17-92 cluster in mice leads to the accelerated development of B-cell lymphoma[45]. Also, the miR17-92 cluster is over-expressed in lung cancer, and this is associated with an increase in cellular proliferation[45,47]. Let-7 acts as a tumor suppressor through negative regulation of Ras[44,46]. In lung cancer, decreased let-7 expression results in increased cellular proliferation[46]. Also, increased let-7 expression *in vivo* has been shown to inhibit lung cancer cell xenograft growth in mice[48].

Tumor profiling and classification

Advances in the ability to profile different aspects of tumorogenesis have led to the rapid identification of differentially expressed miRNAs in cancer[49]. Different miRNA expression profiles are observed for normal and tumorogenic tissues[49], where miRNA expression is generally down-regulated in tumor compared with normal samples[15]. Studies of miRNA expression in cancer have identified miRNA tumor profiles which can be used to classify different tumor subtypes[10,15]. Studies have also constructed miRNA expression profiles that can identify tumor origin[15]. This is particularly useful in the classification of poorly differentiated tumors.

CURRENT KNOWLEDGE OF miRNAs IN BARRETT'S ESOPHAGUS AND ESOPHAGEAL ADENOCARCINOMA

Recent reports describe altered miRNA expression in Barrett's esophagus and esophageal adenocarcinoma. Initial work performed by Feber *et al*[50] identified miRNA alterations in these conditions. This study[50] provided preliminary evidence for using miRNAs in the identification of patients at risk of esophageal adenocarcinoma development. Since then, several publications have described miRNA expression in Barrett's esophagus and esophageal adenocarcinoma[31,39,51,52]. In a further study, our laboratory performed miRNA microarray and q-PCR analysis of miRNA expression in squamous esophageal epithelia, normal gastric epithelia, Barrett's esophagus with intestinal metaplasia and esophageal adenocarcinoma[30]. Our analysis identified miRNAs differentially regulated in Barrett's esophagus development and its subsequent progression to esophageal adenocarcinoma. miRNAs with differential expression patterns included miR-21, miR-143, miR-145, miR-194, miR-203, miR-205 and miR-215. With respect to Barrett's esophagus and esophageal adenocarcinoma, current literature regarding miRNA expression allows for speculation about the potential molecular consequences of the aberrant miRNA expression identified in our study.

The oncomiR, miR-21

Our group's research has shown that miR-21 expression is up-regulated in Barrett's esophagus and esophageal adenocarcinoma, compared with squamous esophageal epithelia. This finding is in accord with other publications where miR-21 has been shown to be up-regulated in various solid tumors[38]. Elevated miR-21 has been implicated in many cellular processes required for neoplastic development and progression. Elevations in miR-21 have been shown to promote survival in myeloma cells[53], confer apoptotic resistance in prostate cancer cells[54], increase cell proliferation, migration and invasion in hepatocellular carcinoma cells[55], and increase invasion and metastasis in colorectal cancer cells[56]. Also, reduced miR-21 expression has been shown to reduce proliferation in MCF7 breast cancer cells and tumor growth in a mouse xenograft model[57], reduce anchorage-independent colony formation in hepatocellular carcinoma cells[58], and reduce invasion, intravasation and metastatic capacity of colon cancer cells[56]. It is possible that the observed up-regulation of miR-21 in esophageal adenocarcinoma may either provide a selective advantage to cells within a metaplastic columnar epithelium, increasing the chance for neoplastic development, or confer on esophageal adenocarcinoma similar molecular traits to those reported in the literature.

Dual roles for miR-194

miR-194 is up-regulated in Barrett's esophagus and esophageal adenocarcinoma[30]. miR-194 expression is regulated by HNF-1a, a transcription factor induced in Barrett's esophagus and esophageal adenocarcinoma[59]. A study by Hino *et al*[59] also showed that miR-194 expression is induced during intestinal epithelial cell differentiation. Furthermore, miR-194 expression is induced in metastatic pancreatic cell lines[60]. Taken together, these results could suggest that elevated miR-194 may be contributing to intestinal differentiation observed in Barrett's esophagus, and may also contribute to the molecular phenotype required for tumor metastasis.

miRNAs as tumor suppressors

miR-143, miR-145 and miR-215 are down-regulated in esophageal adenocarcinoma[30]. Similar alterations are observed in other expression profiling studies. miR-143, 145 and 215 are all down-regulated in colonic adenocarcinoma[61,62]. miR-143 and 145 have been shown to be down-regulated in gastric cancer[63] and miR-145 has been shown to be down-regulated in lung cancer[64].

More specific roles for miR-143, 145, and 215 in carcinogenesis have been elucidated. miR-143 has been

shown to target the *KRAS* oncogene, suppressing colorectal cancer cell growth *via* inhibition of *KRAS* translation[65]. Therefore, loss of miR-143 expression in esophageal adenocarcinoma could result in a loss of *KRAS* regulation contributing to neoplastic development. Also, miR-143 up-regulation in Jurkat T cells has been linked with the regulation of FAS-mediated apoptosis[66]. miR-145 has also been implicated in regulating apoptosis *via* a negative feedback loop involving TP53, and also *via* translational inhibition of RTKN in breast cancer cell lines[67,68]. Furthermore, miR-143 and miR-145 expression are induced by p53 in response to DNA damage[69]. Therefore, the loss of both miR-143 and miR-145 in the progression of Barrett's esophagus to esophageal adenocarcinoma may alter the cell's ability to direct the appropriate apoptotic responses.

miR-215 expression is induced by *p53*, and it acts in cooperation with miR-192 to regulate cell cycle events through their ability to induce cell cycle arrest[61,70]. Loss of miR-215 expression causes a reduction in the ability of cells to regulate proliferation, a key neoplastic attribute. It is therefore possible that miR-143, 145 and 215 may act as tumor suppressors, with loss of expression contributing to the development of esophageal adenocarcinoma.

CONCLUSION

It is now clear that altered regulation of miRNA expression results in numerous cellular consequences. Functional studies of miRNAs, which drive different disease states including neoplastic progression, are increasing in number. This literature is matched by an increase in studies evaluating miRNA expression in the clinical setting as a possible prognostic and diagnostic marker, with miRNA analysis of blood samples providing exciting potential for new avenues in disease diagnosis[71]. Although miRNA research in Barrett's esophagus and esophageal adenocarcinoma is in its early stages, increasing evidence allows for speculation about the potential roles of miRNAs in the development of Barrett's esophagus and its progression to esophageal adenocarcinoma.

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