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Review

A review of the current understanding and clinical utility of miRNAs in esophageal cancer

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ABSTRACT

Background: MicroRNAs (miRNAs) are a class of small, well-conserved, non-coding RNAs that regulate the translation of RNAs. They have a role in biological and pathological process including cell differentiation, apoptosis, proliferation and metabolism. Since their discovery, they have been shown to have a potential role in cancer pathogenesis through their function as oncogenes or tumor suppressors. A substantial number of miRNAs show differential expression in esophageal cancer tissues, and so have been investigated for possible use in diagnosis. Furthermore, there is increasing interest in their use as prognostic markers and determining treatment response, as well as identifying their downstream targets and understanding their mode of action.
Methods: We analyzed the most recent studies on miRNAs in esophageal cancer and/or Barrett's esophagus (BE). The publications were identified by searching in PuBMed for the following terms: Barrett's esophagus and microRNA; esophageal cancer and microRNA.
Results: Four miRNAs (mi-R-25, -99a, -133a and -133b) showed good potential as diagnostic markers and interestingly five (mi-R-21, -27b, -126, - 143 and -145) appeared to be useful both as diagnostic and prognostic/predictive markers.
Conclusion: The data so far on miRNAs in esophageal carcinogenesis is promising but further work is required to determine whether miRNAs can be used as biomarkers, not only in the clinical setting or added to individualized treatment regimes but also in non-invasive test by making use of miRNAs identified in blood.

1. Introduction

Esophageal cancer is the eighth most common cancer worldwide and the sixth most common cause of cancer death [1]. There are two main types of esophageal cancer – adenocarcinoma and squamous cell carcinoma, which have quite distinct etiology and epidemiology. Esophageal adenocarcinoma (EAC) is more prevalent in Western countries, with a dramatic increase in incidence observed in the last twenty years [2–4]. The reasons for the increasing incidence are not entirely clear but the main risk factors appear to be male sex, Caucasian race, a history of chronic reflux disease [5] which may or may not be symptomatic and there is recent evidence for a role of obesity [6]. The precursor condition for EAC is Barrett's esophagus (BE), which can proceed through dysplastic stages – from low-grade dysplasia (LGD) to high-grade dysplasia (HGD) to adenocarcinoma at an overall rate of progression to cancer of 0.33% per person per year [7]. In contrast, esophageal squamous cell carcinoma (ESSC) is more prevalent in the developing world with very high incidence areas found in East Asia and in the Caspian belt (also known as the "Central Asian Esophageal Cancer Belt") [8]. The incidence rates for this subtype are relatively stable from a

Abbreviations: miRNA/miR, MicroRNA [ribonucleic acid]; EAC, Esophageal adenocarcinoma; BE, Barrett's esophagus; LGD, Low grade dysplasia; HGD, High grade dysplasia; ESCC, Esophageal squamous cell carcinoma; NE, Normal epithelium; qRT-PCR, Quantitative reverse transcription polymerase chain reaction; FSCN1, ascin homolog 1, actin bundling protein; p53, Protein 53; LATS2, large tumor suppressor, homolog 2; LNM, Lymph node metastasis; RNA, ribonucleic acid; IRS, inflammatory risk score; RNASEN, Ribonuclease 3 enzyme gene; DGCR8, Pasha protein; DICER1, endoribonuclease of RNase III family; 5-FU, 5-fluorouracil; P27, protein 27; Bcl-2, B cell lymphoma 2; MDR1, multi drug resistance 1; PPP2R1B, protein phosphatase 2 regulatory subunit beta isoform; IGF1R, Insulin like growth factor receptor 1; CD47, Cluster of differentiation 47; CDH1, E-Cadherin; Rap1b, Ras related protein 1b; Rap1a, Ras related protein 1a; FBXW7, F box/WD repeat containing protein 7; MISH, miRNA in situ hybridization; LDHB, lactate dehydrogenase B.

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global perspective, although rates have been declining in the western world presumably due to a reduction in smoking which is a major risk factor [9,10].

Despite these striking differences in the two main subtypes of esophageal cancer, the clinical presentation and management options are broadly similar. Patients tend to present with dysphagia at a late stage and as a result the overall 5-year survival is less that 15% [11]. In contrast, tumors detected at an early stage prior to lymph node spread and when the disease is confined to the mucosa or submucosa have a survival in excess of 80% [12,13]. As a result there has been interest in screening to detect asymptomatic precursor lesions namely Barrett's esophagus and squamous cell dysplasia. However, endoscopic screening is not feasible on a population scale and therefore identifying blood borne biomarkers could constitute a significant advance. Furthermore, biomarkers could play a role in determining the optimal treatment algorithms for patients in terms of likely prognosis and response to therapy.

MicroRNAs (miRNAs) are small, well-conserved, non-coding RNAs of 20–24 nucleotides that regulate the translation of mRNAs [14]. They have a role in biological and pathological processes such as cancer, including cell differentiation, apoptosis, proliferation and metabolism [15]. miRNAs have also been shown to have the potential for a causal role since they can function as oncogenes or tumor suppressors [16]. The number of human miRNAs reported so far (August 2012, release of miRBase, Sanger Institute, Cambridge, UK [17]) is in excess of 21,000. MiRNAs are emerging as highly specific biomarkers with the potential for use in identification and classification of tumors with recently emerging data on their role in esophageal cancer. Furthermore, it has been recently demonstrated that miRNAs are present in circulating blood plasma, protected from degradation in a highly stable, cell-free form by inclusion in lipid or lipoprotein complexes [18,19]. This makes them ideal candidates for diagnostic screening in blood and, for example, characteristic changes in the serum or plasma miRNA profiles of pancreatic cancer have identified unique signatures that could be exploited as novel biomarkers in the clinic [20]. Recent studies have started to investigate miRNA profiles in esophageal cancer in order to determine their utility for use in diagnosis and assessment of prognosis.

The specific aims of this review are therefore to examine what is known about the expression and function of miRNAs in squamous cell cancer and adenocarcinoma of the esophagus compared to normal and pre-cancerous esophageal tissues and in so doing to examine the utility of these changes as biomarkers to aid in diagnosis, prognosis and predicting response to therapy.

2. Use of miRNAs in the diagnosis of ESCC and EAC

In order to use miRNAs as a diagnostic tool for esophageal cancer, differential level of expression must be found in cancer compared to normal or pre-malignant cells and specific miRNAs that are significantly up-regulated or down-regulated between these groups must be identified. miRNAs can be profiled on a genome wide scale using array or sequencing technologies but they can also be examined on a single candidate basis by quantifying, for example, the expression of known miRNAs of interest [21]. For genome-wide assessments, unsupervised hierarchical clustering can then be used to determine whether the different tissue or disease types can be distinguished based on their miRNA expression. In the esophagus, differential miRNA expression has been investigated with the aim of distinguishing EAC and ESCC from normal epithelium (NE). It is also hoped that miRNA expression may be useful in identifying dysplastic changes in BE (through LGD and HGD) in order to identify those patients who are at higher risk of progressing to EAC.

Guo et al. [22] were able to separate 90% of their 31 paired samples (ESCC and NE) into two disease specific groups. In another study that analyzed 35 samples (10 EAC, 10 ESCC, 5 BE, 1 HGD, 9 NE), Feber et al. [23] found that unsupervised hierarchical clustering was able to separate them into 4 distinct groups. In a larger study of 91 pairs of disease tissues (included BE and EAC) and adjacent non-cancerous tissue, Yang et al. [24] were able to differentiate all the EAC samples (31) from normal samples across the entire set of miRNAs. They also found a large number of miRNAs that were differentially expressed between BE with HGD and the paired normal tissues and between EAC and the paired normal tissues (Table 1). These studies suggest that there are significant alterations in miRNA expression profiles across esophageal disease groups which can be used for classification purposes.

A number of more recent studies have then aimed to identify the specific miRNAs that are up-regulated or down-regulated in esophageal cancer. The methods and results from selected studies (based on their disease focus) are discussed below and summarized in Table 1. After identifying candidate miRNAs using microarray, most studies used paired samples (the tumor sample plus a normal sample adjacent to the tumor) and qRT-PCR to validate the measurement of which miRNAs are altered in expression in tumor compared to normal. qRT-PCR is appropriate because it is more quantitative and highly sensitive than other high-throughput assays requiring only nanograms of input material. Sequencing is also becoming an attractive alternative [25,26].

Feber et al. [23] found 13 miRNAs that were differentially expressed in the four histological groups that they investigated (Table 1); and this was confirmed by other studies [24]. In particular, miR-194, miR-192, and miR-200c were significantly up-regulated in EAC but not in ESCC and miR-342 was up-regulated in ESCC but not EAC. They found that other miRNAs were differentially expressed between tumor and normal tissue, but not necessarily between the two tumor types.

Kano et al. [27] identified a subset of 15 miRNAs that were down-regulated in ESCC tissue compared to adjacent normal tissue in a small sample of 10 matched pairs (Table 1). They then compared these findings to a previous study investigating the miRNA signature of bladder cancer [28] and identified four down-regulated miRNAs in common. These four miRNAs – miR-145, -30a-3p, -133a and -133b – were described as potential tumor suppressors because of their down-regulation in cancerous tissues. The authors then identified the target of the miRNAs miR-145, -133a and -133b as FSCN1 (fascin homolog 1, actin-bundling protein), and found an inverse correlation between FSCN1 expression and miR-133a and -133b levels in ESCC patients. A FSCN1 loss of function assay found significant inhibition of cell growth and invasion, indicating that it might have a role in ESCC carcinogenesis with the potential for therapeutic exploitation.

Ogawa et al. [29] examined 30 paired samples of ESCC and normal tissues and they found that 22 miRNAs were up-regulated more than 2-fold in ESCC cells if compared to normal (6 of these were up-regulated greater than 4-fold) and 4 miRNAs were down-regulated by at least 2-fold (Table 1). Three miRNAs of particular interest were noted – miR-34b and miR-139 were found to be the most consistently dysregulated and miR-129 was identified as a significant prognostic factor in ESCC. miR-34b was up-regulated in ESCC cells compared to normal and this is of particular interest, since previous studies have found that miR-34b is the direct transcriptional target of p53 and that its oncogenic action is p53 dependent [30]. The overexpression of miR-34b may itself be due to overexpression of p53 and represent a surrogate biomarker. miR-139 was also found to be down-regulated in ESCC cells compared to normal (<0.5-fold change) and was suggested to be a tumor suppressor.

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