World Journal of Gastroenterology

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World J Gastroenterol 2017 November 21; 23(43): 7653-7656

DOI: 10.3748/wjg.v23.i43.7653

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Noncoding RNAs as drivers of the phenotypic plasticity of oesophageal mucosa

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Author contributions: Facchin S, Munari G, Fanelli GN, Lorenzon G reviewed the literature and wrote the first draft of the paper; Fassan M and Savarino E conceived the idea, reviewed the literature and contributed to writing the paper and edited it extensively.

Conflict-of-interest statement: No conflict of interest related to this publication to be declared.

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Manuscript source: Invited manuscript

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Received: August 22, 2017
Peer-review started: August 23, 2017
First decision: September 28, 2017
Revised: October 18, 2017
Accepted: October 26, 2017
Article in press: October 26, 2017
Published online: November 21, 2017

Abstract

The histological commitment of the lower oesophageal mucosa largely depends on a complex molecular landscape. After extended inflammatory insult due to gastroesophageal reflux disease, squamous oesophageal mucosa may differentiate into columnar metaplastic mucosa. In this setting, the presence of intestinal metaplasia is considered the starting point of Barrett's carcinogenetic cascade. Aside from secondary prevention strategies for Barrett's mucosa (BM) patients, there are multiple endoscopic ablative therapies available for BM eradication and for the replacement of metaplastic epithelia with a neosquamous mucosa. However, BM frequently recurs in a few years, which supports the notable phenotypic plasticity of the oesophageal mucosa. In recent years, several reports pinpointed a class of small noncoding RNAs, the microRNAs (miRNAs), as principal effectors and regulators of oesophageal mucosa metaplastic (and neoplastic) transformation. Because of miRNAs notable stability in fixed archival diagnostic specimens, expression profiling of miRNAs represent an innovative diagnostic, prognostic and predictive tool in the stratification of phenotypic alterations in the oesophageal mucosa.

Key words: Barrett's mucosa; Biomarkers; Noncoding RNAs; MicroRNAs; Metaplasia

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Core tip: Recent advances in understanding the molecular role of noncoding RNAs in Barrett's carcinogenesis have significantly contributed to the identification of novel and alternative molecular pathways involved in this carcinogenetic setting. In the future, these data may significantly influence the planning of secondary prevention strategies for Barrett's mucosa patients and help to select new therapies.

Fassan M, Facchin S, Munari G, Fanelli GN, Lorenzon G, Savarino E. Noncoding RNAs as drivers of the phenotypic plasticity of oesophageal mucosa. *World J Gastroenterol* 2017; 23(43): 7653-7656 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i43/7653.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i43.7653

INTRODUCTION

The phenotypic commitment of gastro-oesophageal junction mucosa suffers from the "original sin" of its extreme morphological plasticity during intrauterine development. Specifically, it corresponds to a hybrid epithelium that can differentiate towards divergent mucosal phenotypes[1].

In the adult, the squamous-differentiated mucosa conserves the ability to metaplasticly reverse its phenotype under the stimulus of long-lasting inflammatory insults, resulting in differentiation into columnar metaplastic mucosa[2]. Among the different columnar phenotypes, however, only the histological finding of intestinal metaplasia is considered by most gastroenterology societies to be a prerequisite for a diagnosis of Barrett's mucosa (BM), the cancerization field in which a significant number of oesophageal adenocarcinomas develop[2]. In fact, BM represents the initial phenotypic shift of a multistep carcinogenetic process known as Barrett's carcinogenesis[3].

Aside from secondary prevention strategies mainly based on endoscopic (and bioptic) surveillance protocols, there are multiple endoscopic therapies available for BM eradication. These include radiofrequency ablation, cryoablation and photodynamic therapy[2]. These eradicative therapies rely of the replacement of BM with neosquamous epithelium, which further supports the extraordinary phenotypic plasticity of the oesophageal mucosa. However, both the stability and functional characteristics of this neosquamous mucosa have not yet completely comprehended. More importantly, it has been shown that BM frequently recurs in a few years, which suggests that the transforming characteristics of the neosquamous oesophageal mucosa do not change after treatment.

Only fragmentary information is available on the molecular changes driving the phenotypic shift from native squamous oesophageal epithelium to metaplastic Barrett's. Most studies have focused on the dysregulation of the Homeobox gene family, which is involved in keeping the squamous commitment of the oesophageal mucosa. In recent years, several reports pinpointed a class of small noncoding RNAs (ncRNAs), the microRNAs (miRNAs), as principal effectors and regulators of oesophageal mucosa plasticity[4].

AFFINITY OF MIRNA ANALYSIS FOR GASTROINTESTINAL BIOPSIES

The great dichotomy observed between the well-established histopathological characterization and classification of the phenotypic lesions occurring in the gastrointestinal tract (both inflammatory and neoplastic) in comparison to their poor molecular typing, is mainly due to the incompatibility of comprehensive molecular testing on formalin-fixed paraffin-embedded (FFPE) tissues. Notably, FFPE specimens currently represent the largest proportion of routine diagnostic gastrointestinal samples.

The introduction of innovative technologies such as targeted next-generation sequencing (NGS) has allowed for feasible, accurate and comprehensive molecular characterization of FFPE neoplastic specimens. However, the molecular landscape of early preneoplastic lesions, such as oesophageal mucosa metaplasia, is mainly characterized by the dysregulation of complex epigenetic pathways rather than the accumulation of genetic alterations. This significantly downgrades the efficacy of NGS applications in the study of (oesophageal) preneoplastic lesions.

Among the different tested biomarkers, miRNAs emerged because of their notable in structural stability, which allows them to be assayed in FFPE tissue samples. The excellent reproducibility and accuracy of miRNA expression profiling in archived specimens has been broadly demonstrated, and the introduction of FFPE-compatible high-throughput miRNA detection technologies, such as microarray profiling, allowed the extensive study of miRNA dysregulation in many gastrointestinal settings. Another important miRNA-related tool is the visualization of miRNA expression at cellular/subcellular level by *in situ* hybridization (ISH), which enabled the discovery of the cellular source of the miRNA's dysregulation (*i.e.*, epithelial *vs* inflammatory commitment).

The expression of miRNAs can be either up-regulated or down-regulated in pathological tissue samples. They can also act as tumour-suppressor genes or oncogenes based on their miRNA-specific downstream target or targets. Notably, different molecular mechanisms, including chromosomal alterations of the miRNA genes, point mutations, epigenetics mechanisms or alterations in the machinery responsible for miRNA production, have been described.

Most miRNA studies in gastrointestinal pathology have consisted of high-throughput profiling to investigate global patterns of miRNA dysregulation.

The so-called "miRNA fingerprints" have been largely demonstrated to discriminate among pre-neoplastic, inflammatory conditions and malignancies, which is an important attribute in the gastrointestinal diagnostic setting.

NONCODING RNA DYSREGULATION DURING BARRETT'S CARCINOGENESIS

Several reports have used comprehensive miRNA expression profiling to demonstrate a clear involvement of miRNA dysregulation in oesophageal Barrett's carcinogenesis[5]. Oesophageal epithelial miRNAs may be used to diagnose BM and possibly monitor its progression to adenocarcinoma.

A recent meta-analysis on this topic revealed that, compared to normal squamous mucosa, BM is characterized by up-regulated miR-192, miR-194 and miR-215 and down-regulated miR-203 and miR-205[6]. In the same analysis, the authors demonstrated that, compared to normal squamous mucosa, Barrett's adenocarcinoma had a higher expression of miR-21, miR-192, miR-194 and miR-215 and a reduced expression of let-7c, miR-203, miR-205 and miR-944[6].

Among the most dysregulated miRNAs, the "oncomiR" miR-21 emerged as one of the most highly up-regulated during Barrett's carcinogenesis, being up-regulated in both high-grade dysplastic and adenocarcinoma samples. Notably, this miRNA is exerting its oncogenic function by targeting of several tumour-suppressor genes, such as *PTEN*, *PDCD4*, *RECK* and *TPM1*[5].

Another significantly up-regulated miRNA that did not emerge from the meta-analytic study, is the miR-196a, which targets *ANXA1*, *SPRR2C*, and *S100A9*[5]. Interestingly, the expression of the related proteins of these three targeted genes is characteristically decreased or lost during the neoplastic transformation of oesophageal tissue. Moreover, miR-196a expression, in combination with three other miRNAs profiles (*i.e.*, miR-192, miR-194, and miR-196b), can adequately stratify BM patients according to their risk of disease progression over a course of 5 years[5].

Some miRNAs are organized as a cluster of genes expressed by a single transcription unit, called a polycistron. The miR-106b-25 polycistron on chromosome 7q22.1, which contains miR-25, miR-93 and miR-106b, has been found to be increasingly activated in successive stages of Barrett's carcinogenesis, with potential *in vitro* proliferative, antiapoptotic, and cell cycle promoting effects and *in vivo* tumourigenic effects by targeting p21 and Bim *et al*[5].

Aside from oncogenic miRNAs, other important down-regulated miRNAs during Barrett's carcinogenesis are miR-31 and miR-375, which have been proposed to be specifically associated with early- and late-stage malignant progression, respectively[5].

As stated above, one of the most important goals in the study of Barrett's pathology is to find adequate predictive biomarkers of BM recurrence after endoscopic ablative therapy and formation of the neosquamous epithelium. Dijckmeester and colleagues found that miR-143 expression was significantly higher in neosquamous and normal squamous epithelium from BM patients before and after ablative therapy compared to normal squamous epithelium from control subjects[7]. It is worth adding that miR-143 is highly expressed in normal colon tissues, and it has a significant role in suppressing colorectal cancer cell growth by inhibiting *KRAS* translation. Overall, these data suggest that neosquamous epithelium is an unsteady "flexible" phenotype prone to reversion to BM.

In the current issue of World Journal of Gastroenterology, Sreedharan and colleagues supported the phenotypical fragility of the neosquamous epithelium investigating miRNA expression profiles using high-throughput screening. They found that neosquamous mucosa arising after ablation of BM is characterized by miRNA dysregulation that may contribute to a decreased barrier function that leads to an increased susceptibility to reflux-induced disease (and therefore a faster metaplastic transformation)[8]. Notably, these data may have clinical implications since they open the field to a more tailored medical management of BM patients. Specifically, they suggest the need for more aggressive therapy in ablated patients with a specific miRNA dysregulation who are at higher risk of intestinal metaplasia recurrence.

The recent characterization of the functional relevance of the "noncoding genome" has demonstrated that miRNAs represent just the tip of an iceberg of ncRNA families. This iceberg includes transcribed ultraconserved regions (T-UCRs), small nucleolar RNAs (snoRNAs), PIWI-interacting RNAs (piRNAs), large intergenic non-coding RNAs (lincRNAs) and, overall, the heterogeneous group of long non-coding RNAs (lncRNAs)[9].

Among the others, the actin filament associated protein 1-antisense RNA 1 (AFAP1-AS1) lncRNA is overexpressed in both BM and adenocarcinoma compared to matched normal samples. This finding supports an oncogenic function during oesophageal mucosa transformation[9].

Our group investigated the expression profiles of T-UCRs during Barrett's carcinogenesis using microarray analysis. We found that a 9 T-UCR signature was associated with BM but not with normal squamous mucosa[10]. T-UCRs were discovered in 2004 after bioinformatic comparisons drawn between mouse, rat, and human genomes. They are absolutely conserved (100% identity with no insertions or deletions) between the three vertebrate species. Interestingly, we observed that a peculiar T-UCRs expression profile was

associated with similar histological lesions in humans and in two murine models of Barrett's carcinogenesis, which supports T-UCRs as novel diagnostic tools for the biological profiling of BM-associated lesions.

CONCLUSION

As in other carcinogenetic settings, advances in the understanding of the molecular role of ncRNAs in Barrett's carcinogenesis are significantly contributing to the identification of novel and alternative molecular pathways. These data are starting to influence the planning of secondary prevention strategies and the selection of new therapies[6,9]. In fact, dysregulated expression of miRNAs has been readily detected in a variety of biological fluids obtained from patients with gastrointestinal cancer, highlighting the high molecular stability of miRNAs in these biofluids and providing a biological rationale for developing them as liquid biopsy biomarkers[11]. In comparison to traditional secondary prevention strategies, the liquid biopsy approach is mini-invasive and allows an overall molecular comprehension of the disease, not suffering from the presence of intratumoral molecular heterogeneity.

From a therapeutic perspective, preclinical models have consistently underlined the feasibility and efficacy of ncRNA-based therapies, which have been successfully translated into clinical trials. Of note, in just the past 5 years, over 100 miRNAs antisense oligonucleotide-based therapies have been tested in phase Ⅰ clinical trials, a quarter of which have reached phase Ⅱ/Ⅲ [12]. Overall, these data are highlighting the clinical impact of miRNAs' dysregulation during esophageal carcinogenesis. The next step will be the definitive introduction of the ncRNA world into clinical practice.

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P- Reviewer: Mavridis K, Yu XJ **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Ma YJ

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ISSN 1007-9327

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