Levels of miRNA-215 in Advanced Gastric Lesions of African American and Caucasian Individuals

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Introduction

- Gastric cancer is one of the most common cancers worldwide and the second most common cause of cancer- related deaths in the world.
- Infection with *Helicobacter pylori* (*H. pylori*) is the main factor associated with the development of intestinal-type gastric cancer.
- The infection induces a cascade of inflammatory events leading to non-atrophic gastritis, multifocal atrophic gastritis, intestinal metaplasia, dysplasia and cancer.
- microRNA (miRNA) are small 18-23 nucleotide molecules product of the degradation of primary miRNA by Drosha and Dicer.
- Several miRNA have been associated with the inflammatory cascade induced by *H. pylori* modulating the production of cytokines and tissue damage observed in patients with gastritis.
- Preliminary data shows differential miRNA signatures in advanced stages of gastritis.
- Our previous observations show that African Americans have increased frequency of *H. pylori* infection and increased incidence of advanced gastric lesions when compared to other ethnic groups.
- **Our goals in this work were**: 1) to identify common miRNA signatures that may be used as biomarkers of disease aggressiveness; 2) to establish their role in the induction of inflammatory cytokines using cellular models.

Preliminary data

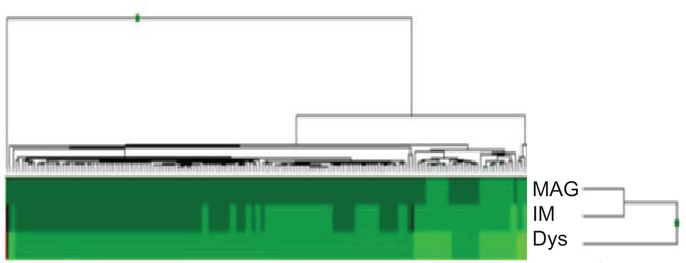

Figure 1. Microarray-based analysis suggest that patients with dysplasia exhibit a different pattern of miRNA when compared to less aggressive forms of gastritis

Materials and Method

miRNA Deep Sequencing Analysis

- Extract total RNA from gastric tissues of African American and Caucasian individuals with gastritis
- Quantify and validate the RNA samples
- Create miRNA libraries for deep sequencing
- Run miRNA sequencing using reagents and protocols from Illumina

Bioinformatics Analysis

- Each sample (output from Illumina) was mapped to mature human miRNAs (from miRBase)
- Differential expression, using the miRNA signatures from normal tissues, was determined using the R package EBSeq (v.1.5.3). The fold change between advanced gastric lesions and normal tissues was determined.

Cellular Assays

- AGS cells were transfected with miRNA-specific siRNA inhibitors and vector controls for 24 and 48 hours
- Total RNA was extracted and the levels of specific miRNA and cytokines (*IL1B, IL6, IL8, IL10, TGFB, TNFA, VEGFA*) were determined by real-time PCR. We used as housekeeping gene *GAPDH*.

Results

African American	
miRNA ID	Fold change
hsa-let-7c-5p	3.06
hsa-let-7g-5p	2.86
hsa-let-7i-5p	2.09
hsa-miR-21-5p	1.94
hsa-miR-3182	3.59
hsa-miR-486-5p	2.20
hsa-miR-6857-3p	3.08
hsa-miR-7843-5p	3.31
hsa-miR-9-5p	10.50

Table 1. miRNA signature associated to advanced lesions in AA

Caucasian	
miRNA ID	Fold change
hsa-let-7e-5p	0.49
hsa-miR-142-3p	5.11
hsa-miR-142-5p	4.41
hsa-miR-146b-5p	2.80
hsa-miR-148b-3p	0.48
hsa-miR-150-5p	4.19
hsa-miR-196a-5p	4.70
hsa-miR-196b-5p	15.95
hsa-miR-3135a	0.51
hsa-miR-320a	0.49
hsa-miR-342-3p	2.09
hsa-miR-6880-5p	2.43

Table 2. miRNA signature associated to advanced lesions in EA

	Fold Change	
miRNA ID	AA	EA
hsa-let-7b-5p	0.81	0.61
hsa-miR-1260a	1.43	1.99
hsa-miR-126-5p	1.72	1.31
hsa-miR-146a-5p	4.60	2.42
hsa-miR-155-5p	4.13	2.73
hsa-miR-215-5p	52.35	34.80

Table 3. Common miRNA signature associated to advanced lesions

Results, cont'd

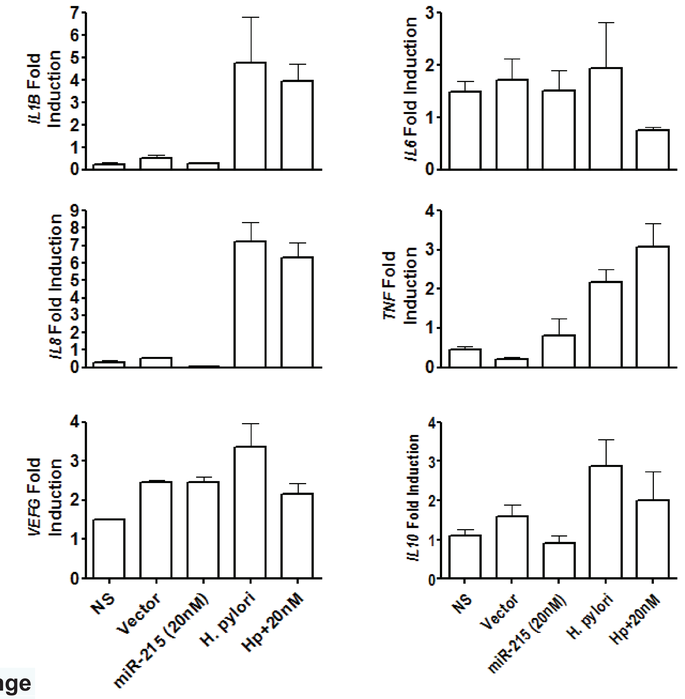

Figure 1. Differential expression of mRNA encoding pro-inflammatory cytokines (*IL1B, IL6, IL8, TNFA, VEGFA*) and anti-inflammatory *IL10* in the absence of miR-215.

Conclusions

- Specific miRNA signatures are differentially associated with advanced gastric lesions in African American and Caucasian individuals
- A common set of miRNA can be identified in advanced gastric lesions of African American and Caucasian individuals
- Opposite effects of miR-215 can be observed on the induction of *IL6* and *TNFA* mRNA
- Further experiments are required to fully understand the role played by this set of common miRNA in the development of gastritis

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