MicroRNAs as Diagnostic and Prognostic Tools in Gastrointestinal Malignancies

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Gastrointestinal and CRC – The unmet need

- GI cancers account for ~25% of all cancer deaths in the Western world, with CRC being one of the most prevalent, resulting in 600,000 deaths worldwide each year.
- CRC affects 1.2M people globally, and remains the #2 cancer in the US, with an annual incidence of 175,000 new cases and ~58,000 deaths.
- $12+ billion spent annually on treating CRC in the US.
CRC, Importance of Early Detection and Need For Better screening methods

- CRC is a preventable disease
- Early detection of pre-cancerous lesions and cancer is the most reasonable approach for prevention.
- CRC screening is a cost-effective strategy
- Limitations of currently available screening methods:
  - Invasiveness – colonoscopy/sigmoidoscopy
  - Sensitivity – Inadequate sensitivity of FOBT/FIT (specially for advanced polyps)
  - Compliance – ~50% of the population do not follow recommended guidelines for CRC screening
Liquid Biopsy Biomarkers: The Future For CRC Screening

- There is constant shedding of tumor cells into stool from the neoplastic tissues, which provides the discovery of genetic/epigenetic “signatures”.
- The analysis of other biofluids (e.g. blood, saliva, urine etc.) for molecular biomarkers in cancer patients constitutes a promising strategy.
- Circulating nucleic acids have been identified in tumor patients.

Toiyama et al., BBRC., 2014
CRC Biomarkers- A Bench to Bedside Approach

Okugawa, Goel et al. Gastroenterology, 2015
Clinical Uses of Liquid Biopsy Biomarkers

**DIAGNOSIS**
- Urine
- Blood
- Serum
- Saliva
- Stool

**TARGETS**
- DNA
- miRNAs
- CTCs
- Exosomes

**SOURCES**
- Serum
- Blood
- Urine
- Saliva
- Stool

**LIQUID BIOPSIES**

**DISEASE MONITORING**
- Biomarkers
- Tumor size

**TREATMENT**
- Time

**PROGNOSIS**
- High risk
- Low risk

**PERSONALIZED THERAPEUTICS**
- Apoptotic cells
- miRNA-based therapy

Advantages of Liquid Biopsies over Traditional Tissue Biopsies

- Non-invasive or minimally invasive
- Less patient discomfort
- Less expensive
- Better sampling – overcomes tumor heterogeneity
- Can be implemented more frequently
- Ideal for residual disease monitoring
- Monitoring treatment response
Serum miR-21 as a Diagnostic and Prognostic Biomarker in Colorectal Cancer

Yuji Toiyama, Masanobu Takahashi, Keun Hur, Takeshi Nagasaka, Koji Tanaka, Yasuhiro Inoue, Masato Kusunoki, C. Richard Boland, Ajay Goel

Manuscript received August 1, 2012; revised November 21, 2012; accepted February 28, 2013.
High levels of serum miR-21 in patients with colorectal adenomas and cancers

Toiyama et al., Journal of National Cancer Institute, 2013
miR-21 levels are lower in Post-Surgery specimens from CRC patients

Toiyama et al., Journal of National Cancer Institute, 2013
This assay is only marginally better than FIT, and has poor sensitivity for detecting advanced polyps.
Serum miR-21 levels as a diagnostic biomarker for patients with colorectal neoplasia?

Toiyama et al., Journal of National Cancer Institute, 2013
miRNAs are frequently overexpressed in advanced CRN

miR-21

Serum miR-21 levels

P<0.0001

HC

CRN

P=0.007

HC

non-AN

AN

P=0.116

HC

non-AN

AN

P=0.003

ρ=0.250

Lesion size (mm)

Serum miR-21 levels

miR-29a

Serum miR-29a levels

P<0.0001

HC

CRN

P=0.095

HC

non-AN

AN

P=0.007

HC

non-AN

AN

P=0.003

ρ=0.253

Lesion size (mm)

Serum miR-29a levels

miR-125b

Serum miR-125b levels

P<0.0001

HC

CRN

P=0.664

HC

non-AN

AN

P=0.007

HC

non-AN

AN

P=0.689

ρ=0.035

Lesion size (mm)

Serum miR-125b levels

Yamada et al., Clinical Cancer Research, 2015
High miR-21 expression indicates poor survival in CRC

Toiyama et al., Journal of National Cancer Institute, 2012
miR-200c regulates EMT-MET switch in CRC

**Primary CRC**

- E-cadherin (High)
- Vimentin (Low)

**Circulation**

- E-cadherin (Low)
- Vimentin (High)

- ZEB1
- ETS1
- FLT1

**Liver metastasis**

- E-cadherin (High)
- Vimentin (Low)

**Epithelial Phenotype**

- Hypermethylation
- miR-200c

**EMT**

- Mesenchymal Phenotype

- E-cadherin (Low)
- Vimentin (High)

- ZEB1
- ETS1
- FLT1

**MET**

- Hypomethylation
- miR-200c

**Epithelial Phenotype**

- Proliferation
- Invasiveness
- Mobility

*Hur et al., Gut, 2012*
miR-200c regulates EMT-MET switch in CRC

Primary CRC
Circulation
Liver metastasis

miR-200c

miR-203

Hur et al., Gut, 2012
High levels of serum miR-200c and miR-203 associate with poor survival in CRC

Toiyama et al., Annals of Surgery, 2013
Identification of Metastasis-Specific miRNA Biomarkers in patients with CRC

*Hur et al., Journal of National Cancer Institute, 2015*
Expression of Specific miRNAs predict liver metastasis in patients with CRC

* * *

Hur et al., Journal of National Cancer Institute, 2015
High serum levels of oncogenic miR-885-5p predict poor OS and DFS in CRC

Hur et al., Journal of National Cancer Institute, 2015
A combined miRNA panel is a better predictor of liver metastasis in CRC

Hur et al., Journal of National Cancer Institute, 2015
Liquid Biopsy Biomarkers from Matched Tissue and Blood in CRC

1. Tissue sequencing
2. Serum/exosome sequencing
Exosomal miRNAs Have Higher Diagnostic Accuracy for Identifying Advanced Colorectal Adenomas

**Exosomal miR-21**
- AUC = 0.866
- $P < 0.001$

**Exosomal miR-29a**
- AUC = 0.851
- $P < 0.001$

**Exosomal miR-92a**
- AUC = 0.839
- $P < 0.001$

**Serum miR-21**
- AUC = 0.687
- $P = 0.158$

**Serum miR-29a**
- AUC = 0.534
- $P = 0.710$

**Serum miR-92a**
- AUC = 0.507
- $P = 0.487$
RNA-Sequencing Identified Differentially Expressed miRNAs in Pancreatic Neoplasms
Exosomal miRNAs are Promising Biomarkers for Patients with Pancreatic Cancer
Are there other classes of Non-coding RNAs involved in CRC?
SnoRNAs Are frequently up-regulated in CRC

**Screening Phase**

- **SNORD76**
- **SNORD78**
- **ACA11**
- **SNORA42**

**Validation Phase**

- **SNORD76**
- **SNORD78**
- **ACA11**
- **SNORD42**

Okugawa et al., Gut, 2015
High SNORA42 expression associates with poor OS & DFS

Overall Survival

Disease Free Survival

High SNORA42 expression associates with poor OS & DFS

Okugawa et al., Gut, 2015
PIWI Interacting RNAs (piRNAs)

- piRNAs are found in clusters throughout the genome.
- piRNAs have no clear secondary structure motifs.
- The length of a piRNA is between 26 and 31 nucleotides.
- 5’ uridine is common to piRNAs and 3’end is usually 2’-O-methylation.
- piRNAs have been found within both nuclei and cytoplasm.
- piRNAs form RNA-protein complexes through interactions with piwi proteins.
## Aberrant expression of Piwi Proteins in cancer

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Common name</th>
<th>Species</th>
<th>Known Piwi genes</th>
<th>PIWI protein expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porifera</td>
<td>Sponge</td>
<td><em>Ephydatia fluviatilis</em></td>
<td><em>EfpIwiA and EfpIwiB</em></td>
<td>Archeocytes (stem cells that differentiate into both somatic and germ cells)</td>
</tr>
<tr>
<td>Cnidaria</td>
<td>Jellyfish</td>
<td><em>Clytia hemisphaerica</em></td>
<td><em>Piwi</em></td>
<td>Somatic stem cells of the tentacle bulb (produce stinging cells characteristic of the cnidarians)</td>
</tr>
<tr>
<td></td>
<td>Jellyfish</td>
<td><em>Podocoryne carnea</em></td>
<td><em>Cniwi</em></td>
<td>Somatic stem cells of the tentacle bulb (see above); striated muscle cells capable of transdifferentiation</td>
</tr>
<tr>
<td>Ctenophora</td>
<td>Comb jellyfish</td>
<td><em>Pleurobrachia pileus</em></td>
<td><em>PpiPiwi1 and PpiPiwi2</em></td>
<td>Actively dividing adult somatic cells; germ line</td>
</tr>
<tr>
<td>Platyhelminthes</td>
<td>Planaria</td>
<td><em>Schmidtea mediterranea</em></td>
<td><em>smedwi-1, smedwi-2 and smedwi-3</em></td>
<td>Neoblasts (totipotent stem cells that can repopulate all somatic and germline lineages)</td>
</tr>
<tr>
<td></td>
<td>Saltwater flatworm</td>
<td><em>Macrostomum lignano</em></td>
<td><em>macpiwi</em></td>
<td>Neoblasts (see above)</td>
</tr>
<tr>
<td>Mollusca</td>
<td>Sea slug</td>
<td><em>Aplysia californica</em></td>
<td><em>Piwi</em></td>
<td>Nervous system, heart and germ line</td>
</tr>
<tr>
<td>Arthropoda</td>
<td>Fruitfly</td>
<td><em>Drosophila melanogaster</em></td>
<td><em>piwi, aub and AGO3</em></td>
<td>Gonad, brain, salivary gland</td>
</tr>
<tr>
<td>Chordata</td>
<td>Sea squirt</td>
<td><em>Botryllodes leachii</em> and <em>Botryllus schlosseri</em></td>
<td><em>Piwi</em></td>
<td>Stem cell population (capable of whole-body regeneration)</td>
</tr>
<tr>
<td></td>
<td>(ascidian)</td>
<td><em>Homo sapiens</em></td>
<td><em>HIWI, HILI, HIWI2 and HIWI3</em></td>
<td>Diverse cancers (breast cancer, rhabdomyosarcoma, medulloblastoma); male germ line</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td><em>Mus musculus</em></td>
<td></td>
<td>Diverse cancers (breast cancer, cervical cancer, endometrial carcinoma, seminomas, hepatocellular carcinoma, gastric cancer, pancreatic adenocarcinoma, gastrointestinal stromal tumours, colon cancer, renal cell carcinoma); haematopoietic stem cells; and male germ line</td>
</tr>
</tbody>
</table>
PiRNAs are frequently upregulated in CRC

- **piR-23619**: ns
- **piR-26525**: Fold Change -1.56
- **piR-1245**: Fold Change 7.80
- **piR-24000**: ns

Weng et al., Under Review
piR-1245 is a prognostic biomarker in CRC

Log rank test
p = 0.0265

Log rank test
p = 0.0078

Weng et al., Under Review
CIRCULAR RNAs
- THE RNA STORY IS COMING FULL CIRCLE
Circular RNAs: New Frontiers in Cancer Research

- Circular RNAs are abundant in human cells.
- CircRNAs are generated in nucleus and are very stable in cytoplasm.
ciRS-7, a Novel circRNA, and a miR-7 Sponge

- CDR1as/ciRS-7 is encoded in the genome antisense to the human CDR1 (gene) locus.
- It has over 60 miR-7 binding sites, far more than any known linear miRNA sponge.
Hypothesis and Aims

- To investigate the functional and mechanistic role of ciRS-7 in CRC
- To understand clinical significance of ciRS-7 in CRC
ciRS-7 is frequently upregulated in CRC

Testing cohort

Validation cohort

Weng et al., Under Review
High ciRS-7 levels correlate with poor survival in CRC

**Testing cohort**

*P=0.0217  
HR=2.03

**Validation cohort**

**P=0.0059  
HR=2.6349

Weng et al., Under Review
CiRS-7 activated EGFR/RAF1/MAPK pathway via suppression of miR-7 activity
A Proposed Model of ciRS-7 mediated Activation of EGFR/RAF1/MAPK pathway
Conclusions

1. Non-coding RNAs provide promising substrates for development as diagnostic, prognostic and predictive biomarkers for CRC cancer

2. The current challenge is to translate our findings into clinically viable biomarkers, that have high sensitivity and specificity for CRC, and possibly other GI cancers

3. There should be larger emphasis on translation of tissue-based biomarkers into non-invasive approaches for a better clinical management of patients with CRC.
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