Noninvasive Molecular Targets for Colorectal Cancer Diagnosis and Prognosis

1st Symposium on Liquid Biopsy: “The Path Towards Precision Oncology”, Santiago de Compostela, Spain

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Gastrointestinal and CRC – The unmet need

- GI cancers account for ~25% of all cancer deaths in Western world, with CRC being one 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 cancer or pre-cancerous lesions 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
Non-invasive biomarkers: The future for CRC screening

- The analysis of bodily fluids (e.g. stool, blood, etc.) for molecular biomarkers in cancer patients constitutes a promising strategy.
- There is constant shedding of tumor cells into stool from the neoplastic tissues, which provides the discovery of genetic/epigenetic “signatures”.
- 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
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

Patients without curative surgery

Patients with curative surgery

Serum miR-21 expression (normalized)

Pre-Ope (n = 15)  Post-Ope (n = 15)

Pre-Ope (n = 45)  Post-Ope (n = 45)

Toiyama et al., Journal of National Cancer Institute, 2013
FDA Approved Fecal CRC screening test

Multitarget Stool DNA Testing for Colorectal-Cancer Screening

Thomas F. Imperiale, M.D., David F. Ransohoff, M.D., Steven H. Itzkowitz, M.D., Theodore R. Levin, M.D., Philip Lavin, Ph.D., Graham P. Lidgard, Ph.D., David A. Ahlquist, M.D., and Barry M. Berger, M.D.


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?

**Normals vs CRC**

- AUC: 0.927

**Normals vs Adenomas**

- AUC: 0.803

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

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

Circulation

Liver metastasis

Epithelial Phenotype

E-cadherin (High)

Vimentin (Low)

Hyper methylation → miR-200c → ZEB1, ETS1, FLT1

EMT

Mesenchymal Phenotype

E-cadherin (Low)

Vimentin (High)

Proliferation  ▼

Invasiveness  ▲

Mobility  ▲

Met

Epithelial Phenotype

E-cadherin (High)

Vimentin (Low)

Hypo methylation → miR-200c → ZEB1, ETS1, FLT1

Proliferation  ▲

Invasiveness  ▼

Mobility  ▼

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

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
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

Okugawa et al., Gut, 2015

Overall Survival

Disease Free Survival

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High SNORA42 expression

Low SNORA42 expression

*p=0.026

Number at risk

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High SNORA42 expression

Low SNORA42 expression

*p=0.049
Exosomes: New Generation of Liquid Biopsy Biomarkers for Colorectal Neoplasia

1 Tissue sequencing

2 Serum/exosome sequencing
Multi-Vesicular Body Exocytosis

Host cell

Endosome Formation

Multi-Vesicular Body Fusion

Exosome

Target cell

Intraluminal vesicle

Endosome

Nucleus

Cytoplasm

Fusion

Endocytosis

Receptor binding

Exocytosis

CD9

CD63

CD81

Annexins

Rabs

EPCAM

EGFR

30-140 mm

Toden et al., Expert Opinions in Medical Diagnostics, 2016
Exosomal miRNAs Are Superior than Circulating miRNAs

Exosomal miR-21

Exosomal miR-29a

Exosomal miR-92a

Serum miR-21

Serum miR-29a

Serum miR-92a

Toiyama et al., OncoTarget (In review)
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
- $P = 0.487$
Conclusions

1. DNA and 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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