Prognostic Value of miRNAs in Colorectal Cancer

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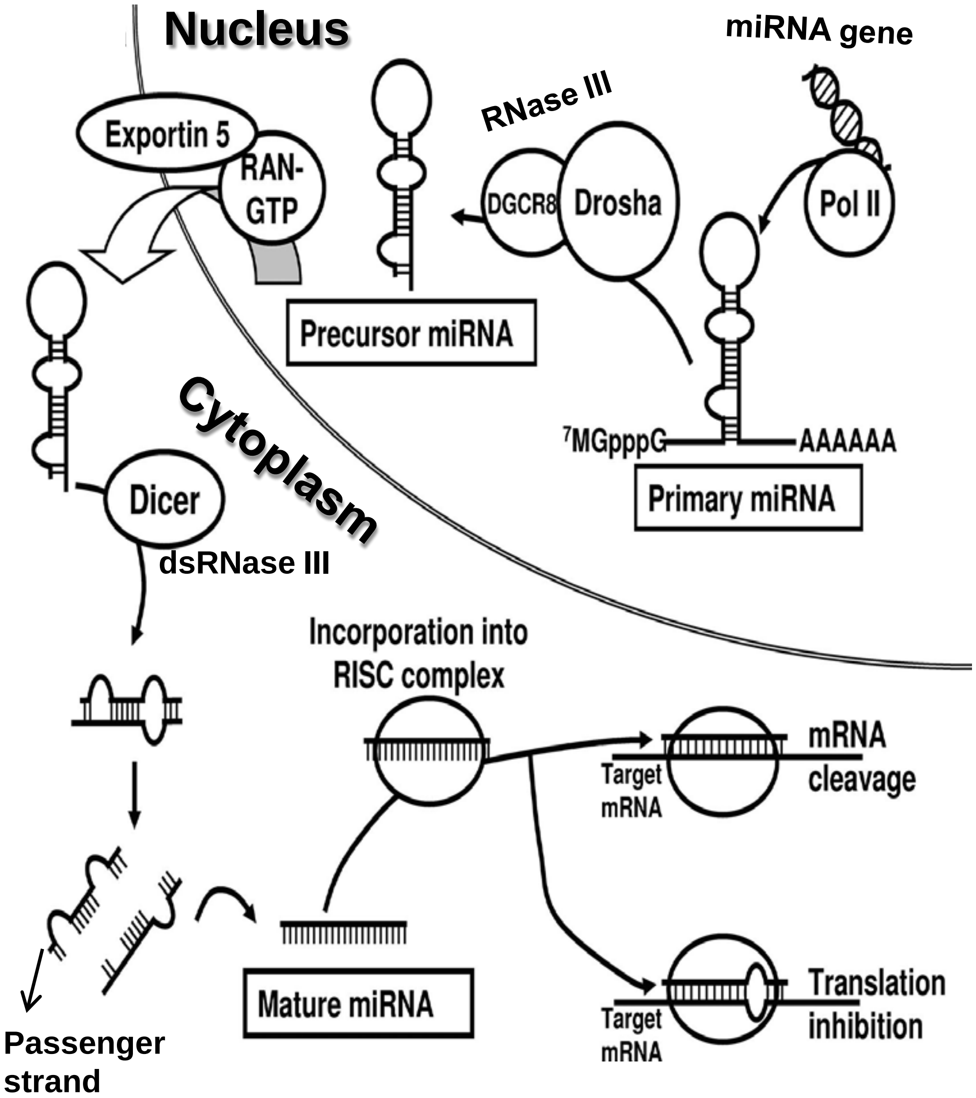
Laboratory Theme

- Our laboratory focuses on discovery and validation of cancer molecular markers (risk, early detection, prognostic, predictive, surrogate endpoint, or surveillance), understanding their underlying mechanisms, and to develop testable models (e.g. in colorectal and breast cancers) to bring them into routine clinical practice.

Objectives

- To describe the functions of miRNAs (epigenetic and gene regulation)
- To assess the prognostic/predictive value of miRNAs in colorectal cancer

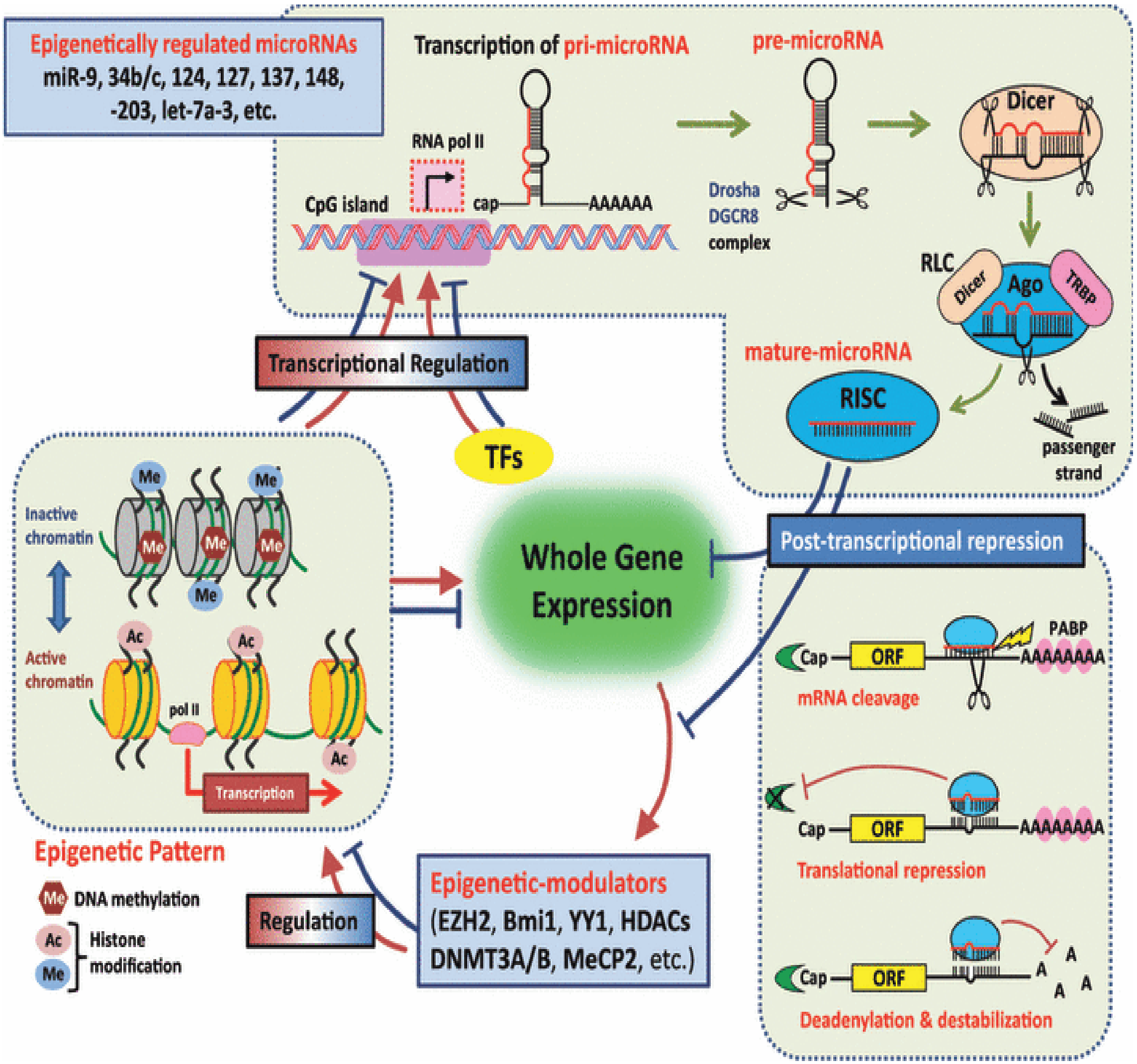
miRNAs



(modified-*Pediatr Res 61: 24R–29R, 2007)*

- MicroRNAs (miRNAs) are short non-coding RNAs (~ 18-24 nucleotide long) that regulate gene expression post-transcriptionally.
- miRNAs were first reported in *C. elegans* in 1993.
- Each miRNA is predicted to have many targets, and each mRNA may be regulated by more than one miRNA
- Currently, there are more than 1500 human miRNAs known (www.mirbase.org)

miRNAs and Epigenetics



- Epigenetics and miRNAs regulate whole gene expression pattern transcriptionally and post-transcriptionally, respectively.
- Epigenetics and miRNAs control each other to form a regulatory circuit and to maintain normal physiological functions.
- A disruption of this regulatory circuit may cause various diseases, including cardiovascular diseases and cancers.

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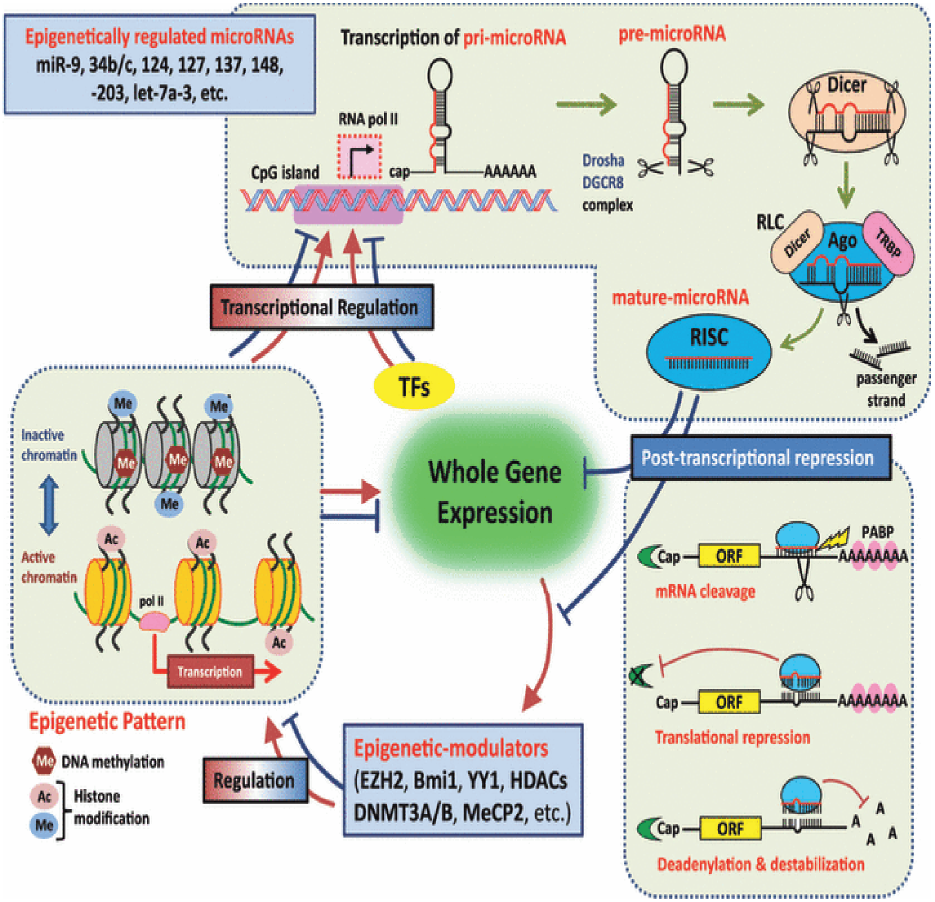
Epigenetics and miRNAs



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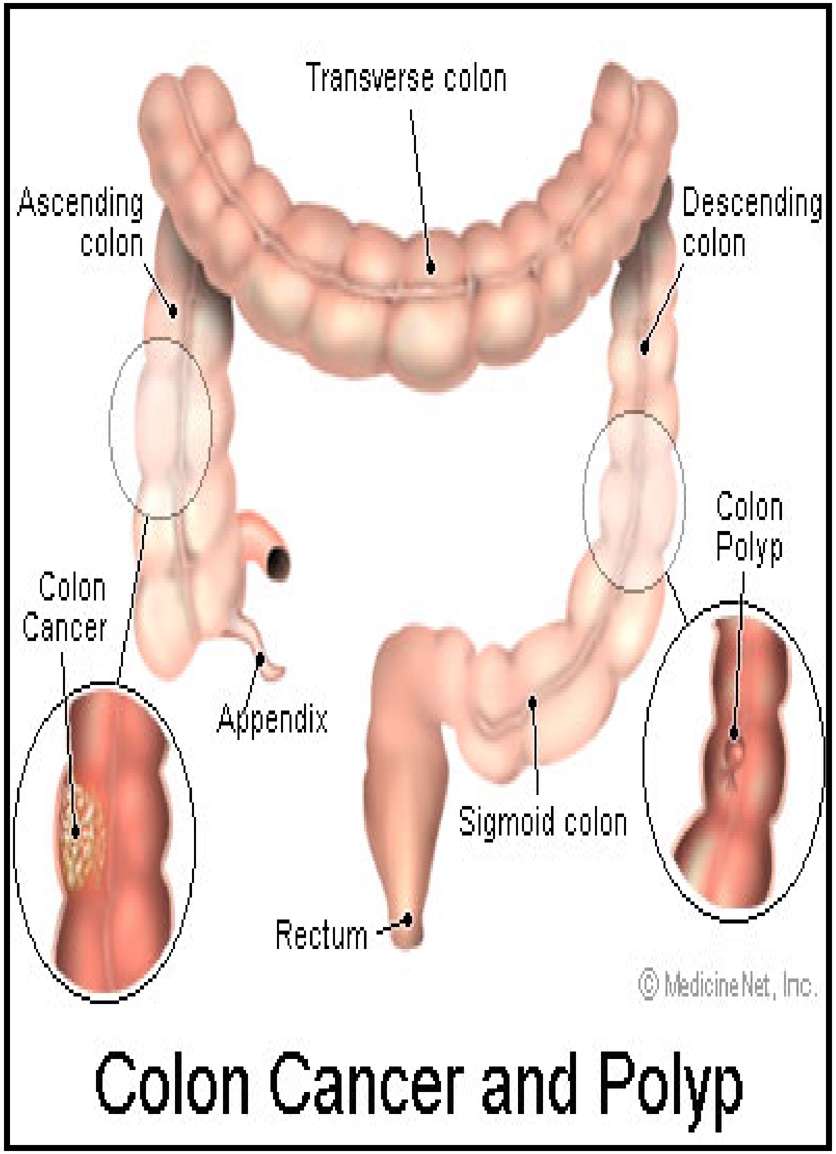
- miRNAs are regulated epigenetically.
  [E.g. miR-203 gene is epigenetically regulated. Its target genes are - SOX2, KLF4, ABL1, BCR-ABL1 (fusion), and Bmi-1.
- miRNAs regulate genes that control epigenetic pathways (E.g. miR-203 is Bmi-1, a member of the polycomb repressor complex which is histone modifier complex regulating gene expression)
- miRNAs have been shown to be definitely linked to cancer, and they can act as either oncogenes (e.g. miR21, miR146, miR155, miR372 etc.) or as tumor-suppressor genes (e.g. let7, miR127, miR145 etc.)

Evaluation of Prognostic value of miRNAs in Colorectal Cancer

Colorectal Adenocarcinoma

- Third most common cancer in the World
  - About 1,233,800 (~147,000 in US) new cases/year
- Second leading cause of cancer death in developed countries
  - About 608,600 (~56,000 in US) deaths
- Risk of developing CRC phenotypes
  - 85 - 90% Sporadic
  - 10 - 15% Familial/Hereditary



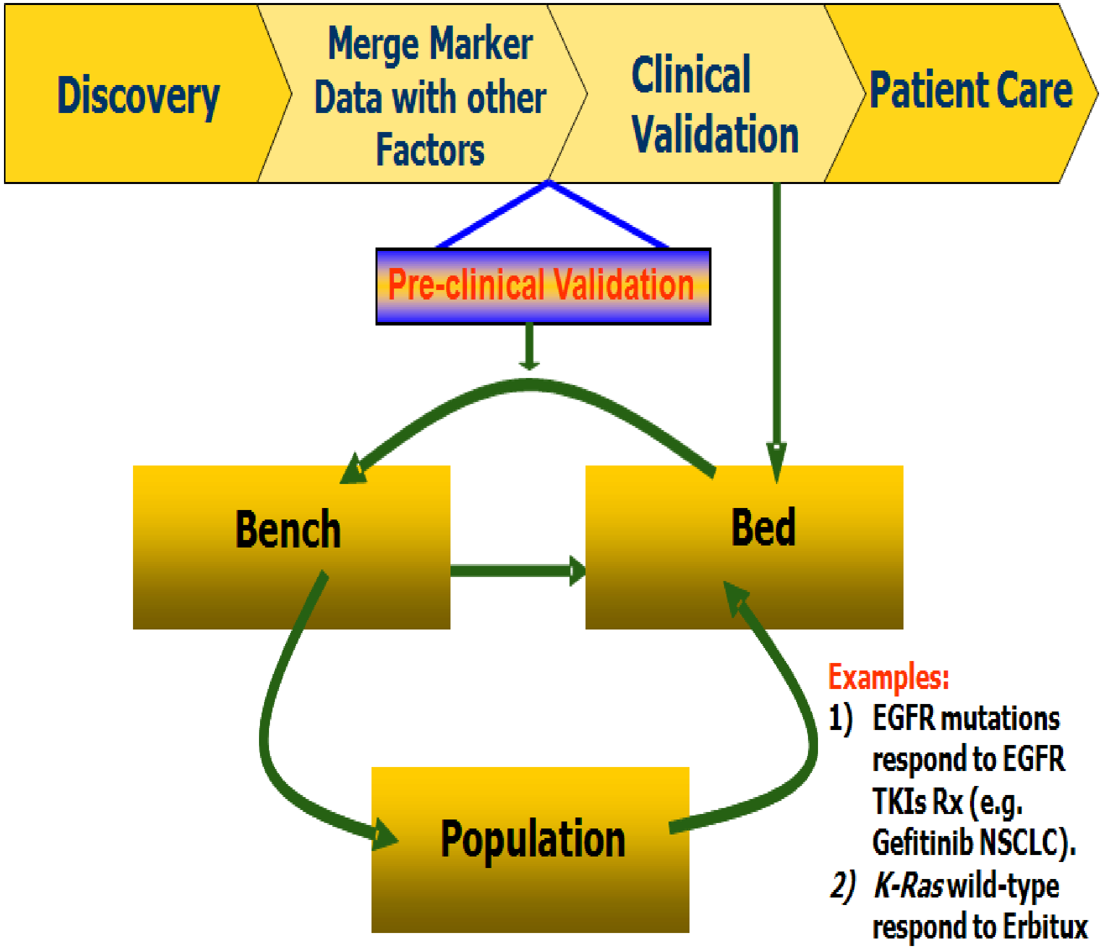
Colon Cancer and Polyp

Source: GLOBOCAN 2008.
Reported by American Cancer Society , 2012

Prognostic Value of Molecular Marker in CRCs Varies with Tumor Stage, Location, Race/Ethnicity

- p53 (IHC & DNA sequencing) – marker of poor survival when tumors are located in proximal colon particularly in non-Hispanic Caucasian patients (*Cancer 83:2456-2467, 1998*).
- Bcl-2 (IHC) - increase expression is a good prognostic markers only for Stage II CRC (*Int. J. Cancer, 74:346-358, 1997 & Cancer Biomarkers, 1; 17-27, 2006)*
- p27^{kip-1} (IHC) – increased nuclear accumulation is a good prognosticator only in Stage III CRCs (*Clin Cancer Res. 10, 1743-1752, 2004*).
- MUC1 (IHC) – increased expression is a poor prognostic indicator (*Clin Cancer Res. 6,4017, 2000*).
- MUC4 (IHC) – increased expression is a poor prognosticator only in early stage (Stages I & II) CRCs (*Cancer, 116,15:3577-86,2010*).
- Bax or Bax/Bcl-2 (IHC) - high Bax is good prognosticator and low Bax/Bcl-2 expressors are candidates for adjuvant chemotherapy (J. GI Oncol, 1(2):76-89, 2010).

Preclinical Validation Research



Discovery

Merge Marker Data with other Factors

Clinical Validation

Patient Care

Pre-clinical Validation

Bench

Bed

Population

Examples:

1) EGFR mutations respond to EGFR TKIs Rx (e.g. Gefitinib NSCLC).
2) *K-Ras* wild-type respond to Erbitux (cetuximab-CRC)

❖ To translate *scientific discoveries* arising from laboratory or clinic should be validated in large population-based studies to bring them into routine clinical practice.

Evaluation of Prognostic value of miRNAs in Colorectal Cancer

Hypothesis:

The miRNA expression profiles and their clinical consequences in CRC might vary with tumor stage and patient race/ethnicity

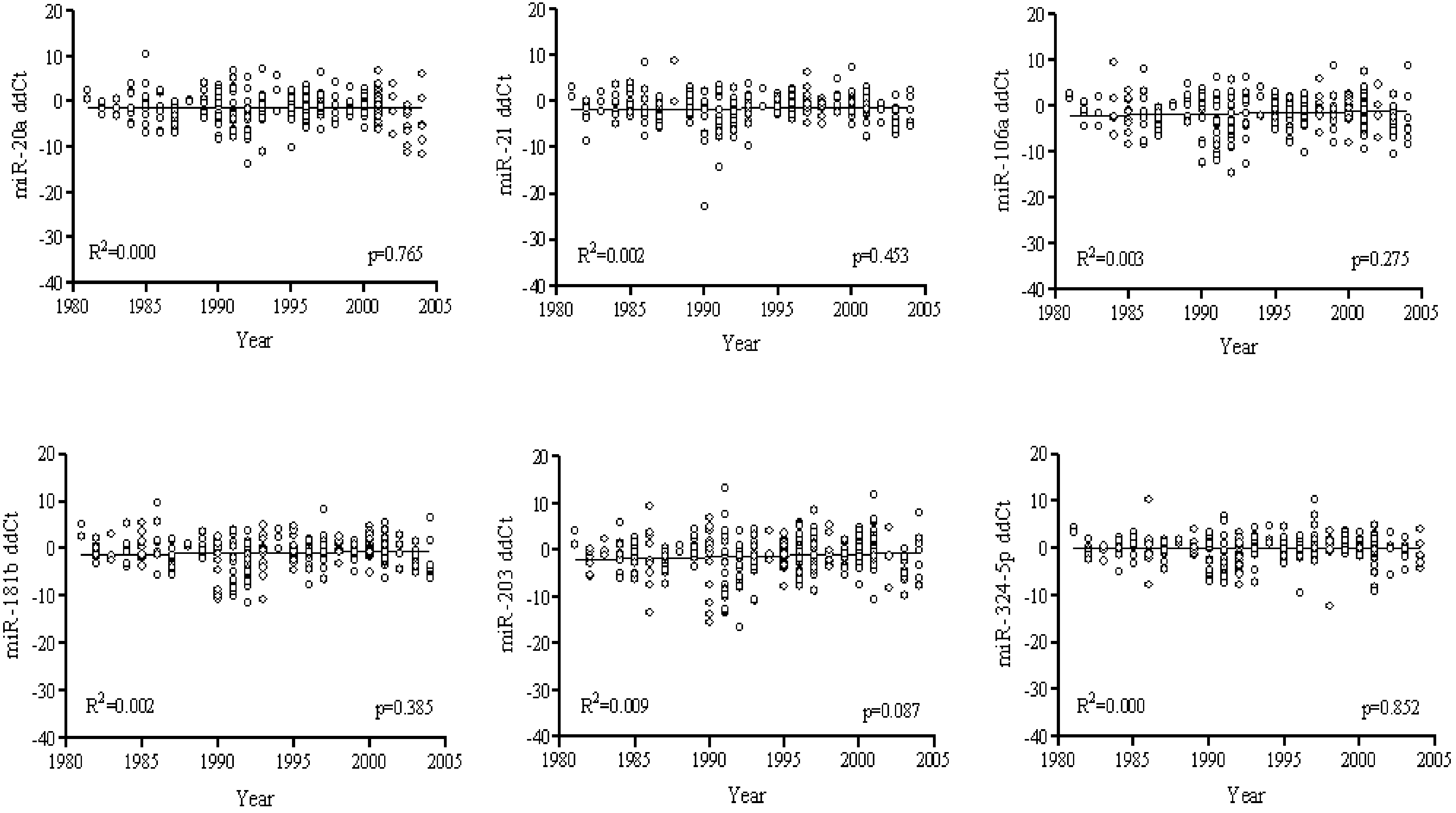
Study Design

- ❑ Top 5 up-regulated (miR-20a, miR-21, miR-106a, miR-181b, and miR-203) miRNAs were chosen from a previously published CRC study (*Schetter, A. JAMA 2008 299(4):425-436*).
- ❑ We evaluated miRNA expression profiles of 548 CRCs and their corresponding benign tissues collected from 206 African-Americans and 339 non-Hispanic Caucasians who underwent surgery at UAB hospital.
- ❑ Expression levels of mature miRNAs were estimated by RT-QPCR (TaqMan^{TM} microRNA assays) and the data were analyzed based on patient race/ethnicity.

Q: Are miRNAs Stable in Formalin-Fixed Paraffin-Embedded (FFPE) Tissues?

Stability of miRNAs in FFPE CRC Tissues

(Stored for 6-28 years, N=348)



Bovell, L et al. (Front Biosci 2012,1;4:1937-40).

Q: Are the miRNAs expression levels are different between African-American and non-Hispanic Caucasian CRC patients?

Differential expression patterns of miRNAs were observed in African American and Caucasian patients of Colorectal Cancer

(Data has been deleted because not yet published)

Prognostic and predictive value of different miRNAs varied based on tumor location, patient race/ethnicity and tumor stage

(Data has been deleted because not yet published)

Molecular underlying bases for distinct prognostic (disease recurrence/survival) and predictive (therapy efficacy) value of different miRNAs were demonstrated *in vitro*

(Data has been deleted because not yet published)

Summary of miRNAs

- Our findings suggest that in the evaluation of clinical utility of miRNAs, tumor stage and anatomic location, and patient race/ethnicity should be considered.