Clinical Chemistry
Trainee Council

PEARLS OF LABORATORY MEDICINE

Introduction to MicroRNA

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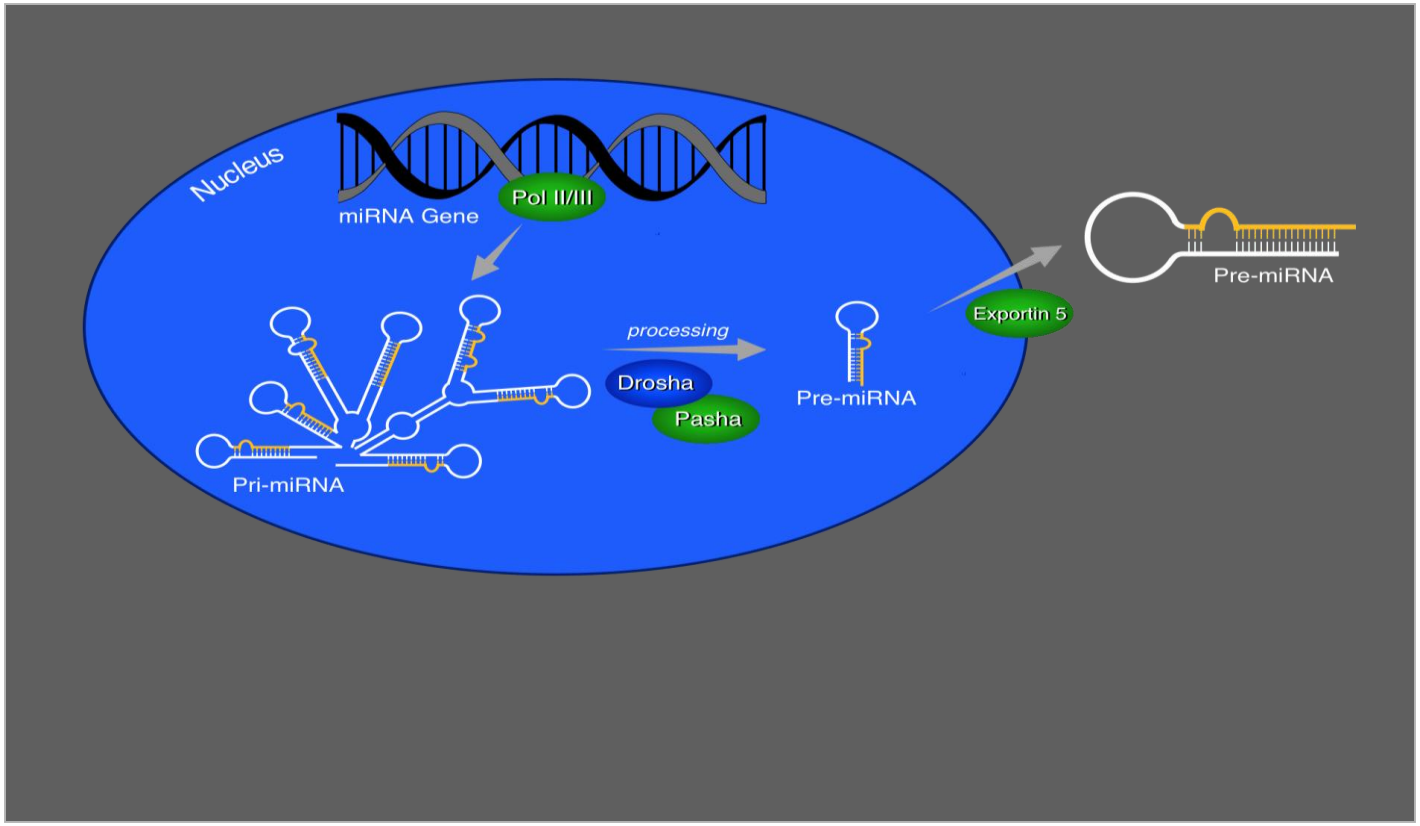
What are miRNAs?

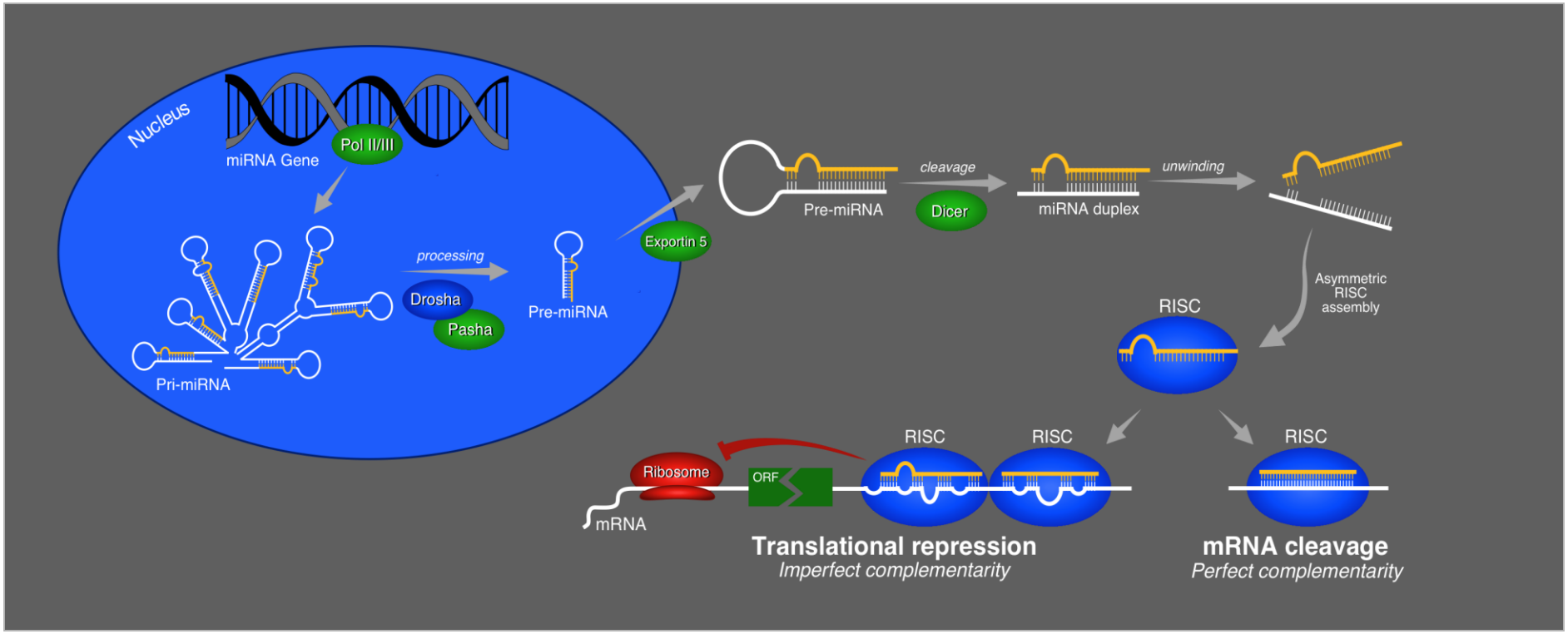
- Small non-protein coding RNA sequences (18-24 nt)
- Regulate gene expression thereby controlling many cellular processes
  - A single miRNA can target 100s of genes
- Alterations in miRNA expression have been demonstrated in a number of diseases
  - Cancer
  - Cardiovascular disease
  - Alzheimer's disease

miRNA Structure and Synthesis



- Generated via Pol II or III as poly-stem structure and commonly form stem-loop structures after cleavage.

miRNA Structure and Synthesis



- miRNAs modulate gene expression by binding and repressing mRNAs.
- A third of all human genes are regulated in this manner.

Analysis of miRNA

- Advantages
  - Stable
  - Present in multiple tissues and fluids
  - Limited number of miRNAs
- Disadvantages
  - Low concentration
  - Lack of analytical and clinical validation
  - Pre-analytical and analytical variations due to the use of different technologies

miRNA profiling approaches

	Technologies		
Feature	***Sequencing***	***Microarray Assays***	***qPCR Assays***
Discovery	Yes	No	No
SNPs	Yes	No	No
Template Requirement	Low	High	Low
Reproducibility	Ultrahigh	Low	High
Cost	High	Medium	Medium
Data Complexity	Ultrahigh	High	High

Circulating miRNAs

- miRNAs can be detected in the bloodstream
  - Mitchell et al., 2008
    - miRNAs are present in plasma or serum
    - Stable after prolonged incubation of the specimens at room temperature and resistant to multiple freeze-thaw cycles
    - Serum miR-141 was frequently overexpressed in prostate cancer patients when compared to healthy controls
  - Chen et al., 2008
    - Evaluated serum miRNAs in healthy individuals and patients with lung cancer, colorectal cancer, and diabetes
    - Identified disease-specific miRNAs and suggested that human serum contains miRNA fingerprints of ongoing diseases

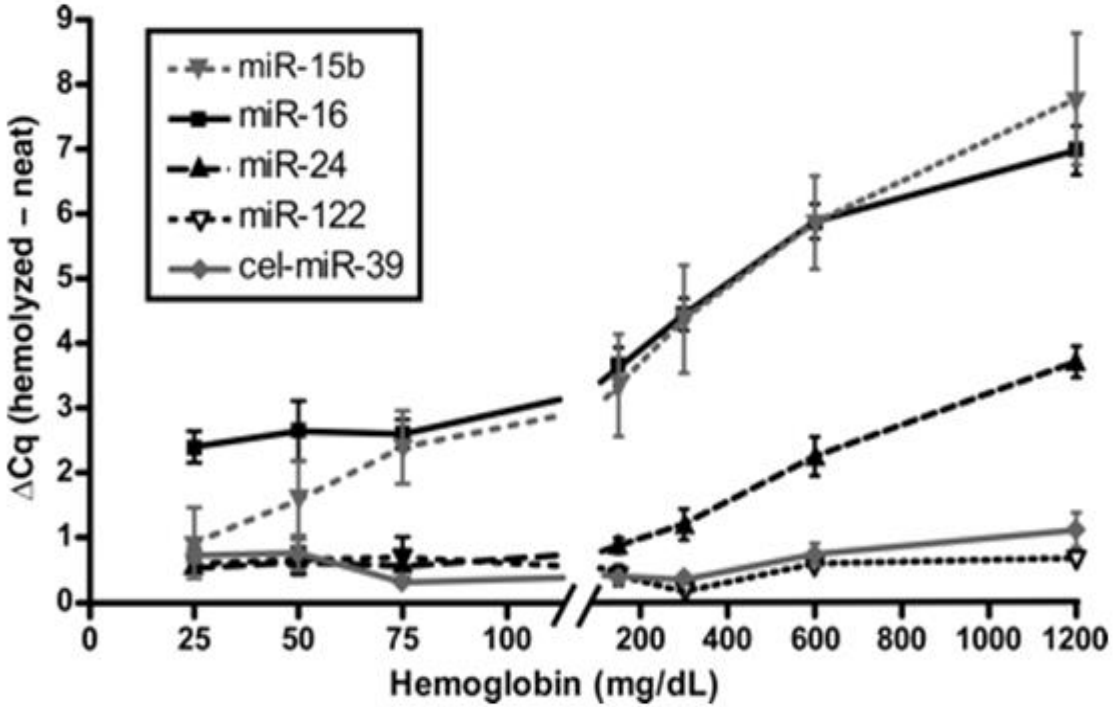
Circulating miRNAs

- How are miRNAs released into the bloodstream?
  - miRNAs are protected from endogenous RNAse activity
    - Unlikely that circulating miRNAs are passively leaked from dying tumor cells as naked RNA molecules
    - Circulating miRNAs have been found to be packaged into various membrane-bound vesicles
      - Exosomes
      - Microvesicles
      - Apoptotic bodies
    - Circulate bound to RNA-binding proteins and link to high-density lipoproteins

Circulating miRNAs

- What variables might affect interpretation of circulating miRNAs?
  - Hemolysis
    - Might falsely increase miRNAs levels
  - Specimen source
    - Cellular contaminants might falsely increase miRNAs levels
  - Template
    - Total RNA versus fixed volume

Effect of hemolysis on miRNA quantification



- miRNAs present at higher concentrations in erythrocytes will be more impacted by hemolysis

McDonald JS et al. Analysis of circulating microRNA: preanalytical and analytical challenges. Clin Chem. 2011; 57:833-40.

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Specimen type: serum or plasma?

- Lower concentrations of miRNA in serum compared to plasma
  - Due to the presence of cellular contaminants derived from platelets
  - Cells have higher concentrations of miRNA
  - Use of serum samples will minimize variable amounts of platelet contamination

Potential Applications of miRNAs

- Disease Biomarkers
  - Diagnosis
  - Prognosis
    - ↑ miR-196a-2 predicts poor survival in patients with pancreatic cancer (Bloomston M. et al., 2007).
  - Response to therapy
    - ↑ miR-221 and mir-222 in tamoxifen-resistant breast cancer (Miller TE, et al.,2008).
- Therapeutics
  - Re-establish expression of silenced miRNA
  - Silencing the expression of miRNA
    - Example: Miravirsen (Santaris Pharma) an inhibitor of miRNA-122 for the treatment of HCV infection (Janssen HL, et al.,2013).

miRNAs in Cancer

Cancer	Up-regulated miRNA	Down-regulated miRNA
Breast	**miR-21**, miR-155, miR-29b-2	miR-143, **miR-145**, miR-155, miR-200
Lung	**miR-21**, miR-189, miR-200b, miR-17-92 cluster	let-7 family, miR-126, miR-30a, miR-143, **miR-145**, miR-188, miR-331, miR-34s
Colon	miR-223, **miR-21**, miR-17, miR 106m, miR-34s	miR-143, **miR-145**, miR-195, miR-130a, miR-331
Prostate	Let-7d, miR-195, miR-203, miR-125b, miR-20a, miR- miR-221, miR-222	miR-143, **miR-145**, miR-128a, miR-146a, miR-126
Brain	**miR-21**, miR-221	
HCC	miR-34s, miR-224, miR-18, **miR-21**	miR-17-19b cluster, miR-200a, miR- 125a, miR-199a, miR-195
CLL	miR-15, miR-16	
Ovarian	miR-200a,b,c, miR-141	miR-199a, miR-140, **miR-145**, miR-125b
Pancreatic	miR-221, miR-181a, **miR-21**	miR148a,b
Gastric	**miR-21**, miR-103, miR223	miR-218

miRNA in practice

- *miRInform*® Pancreas
  - Expression levels of the seven miRNAs
  - Aid in diagnosis of pancreatic ductal adenocarcinoma
  - Score < 0.5 = benign and score 0.5 -1 = PDAC

- Rosetta Lung Cancer Test™
  - Expression level of eight miRNAs
  - Discriminate between four main types of primary lung cancer: squamous cell carcinoma, non squamous cell carcinoma, small cell carcinoma, and lung carcinoid tumors.

Points to Remember

- miRNAs are small nucleotide single-stranded non-coding RNAs that act as post-transcriptional regulators of gene expression.
- miRNAs are emerging biomarkers with a wide range of potential clinical applications.
- Proof-of-principle on the utility of miRNAs has been established but additional work is needed to validate the miRNAs/disease association in independent studies.
- Analysis of tissue miRNAs has reach clinical diagnosis; however, application of these biomarkers in serum or plasma represents bigger challenges.

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Disclosures/Potential Conflicts of Interest

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