Biomarker discovery in sarcoidosis: the emerging role of microRNA

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Thinking broadly about biomarker discovery

• Biomarker definition: “anything that describes the biologic process of the disease”
  • Great examples of a wide definition of biomarker at this conference!
  • Molecular and clinical data-derived biomarkers inform each other, fill in gaps in knowledge
  • MicroRNA can be particularly useful to discover new biological pathways in idiopathic disease
What makes a great biomarker?

- Intentional study design
  - Recruitment of cohorts that can answer the desired question

- What type of information does a clinician want to obtain in a particular setting?

- How reliable is that information?

- What value does the information provide to the clinician/patient?

- Great biomarkers provide actionable information.
Consideration of the Biomarker Type Improves Translation to the Clinic

- Diagnostic
- Disease susceptibility
- Prognostic
- Disease activity – different than predictive/prognostic
- Drug efficacy

A small but growing body of sarcoidosis literature touches on each of these through microRNA studies
MicroRNA (miRNA) basics

• 18-25 nucleotides
• Non-coding RNA
• Bind to target mRNAs in a sequence-specific manner
  • cleavage and degradation of the target mRNA
  • inhibition of translation of the target mRNA into protein.
• “master regulators” - silence or modulate multiple target sets of genes
1. A protein called exportin-5 transports a hairpin primary microRNA (pri-miRNA) out of the nucleus.

2. An enzyme called dicer (not shown) trims the pri-miRNA and removes the hairpin loop, leaving a double stranded microRNA duplex molecule.

3. In plant cells, the microRNA is usually perfectly complementary to its target mRNA molecule. The microRNA will bond with it and cause the mRNA to break down.

2½. Meanwhile, one of the strands joins a group of proteins, forming an microRNA-protein complex. The other strand, known as a passenger strand is usually discarded. How this all happens is still not very well understood.

4. In animal cells, the microRNA nucleotides typically don't pair up with the mRNA nucleotides as well. Their base pairing often follows a pattern though.

5. The microRNA-protein complex's presence blocks translation as well as speeding up deadenylation (breakdown of the Poly-A tail), which causes the mRNA to be degraded sooner and translated less.
Attractiveness of microRNA as a biomarker

• Minimal invasiveness
  • Detectable microRNA in all human biofluids (blood, urine, sputum) despite hostile environments promoting RNA degradation

• Remarkable stability over time in biobanked samples
  • Nearly 20 years demonstrated by ACCESS samples

• Nucleic acid assays, such as miRNA assays, enjoy favorable Food and Drug Administration (FDA) regulatory guidelines for multiplexing compared with protein assays. This could make miRNA profiling more accurate and far more cost effective than protein assays\(^1\).

1. Vencken et al Thorax 2015
Non coding RNA pathway overlap is seen in chronic lung disease, particularly in organ development and safeguarding of tissue integrity.

**Example:** miR-17-92 downregulated after embryonic lung development; when it is not, it associates with tumorigenesis.
microRNA in sarcoidosis

• Crouser et al 2014 – first to study tissue samples
• miRNA in lung tissue and blood (PBMCs) from pulmonary sarcoidosis patients
• Microarray chip followed by qRT-PCR validation of differentially expressed miRNA
MicroRNA expression is tissue specific

• Key findings from early study (Crouser 2014):
  • expression in lung, blood, and lymph node tissues not identical.
  • miR-20a and miR-302c were reduced in lung and elevated in lymph node tissues relative to matching controls
  • miR-92b and miR-206 were elevated in both lung and lymph node tissues of sarcoidosis patients.
  • fixed and frozen tissues yielded similar miRNA expression results
  • Pathway analysis in both blood and tissue identified TGFbeta/WNT molecular pathway
Comparison of miRNA in BAL vs PBMC samples also yields mixed pathway overlap.
TGFbeta pathway importance is re-enforced by analysis of serum miRNA from Lofgren’s (LS) versus non Lofgren’s patient

- Novosadova et al: Three miRNAs (miR-21-5p, miR-340-5p, and miR-212-3p) differed between patients with LS and those without LS (n=12 in each group)

- Of Non-LS patients, 2 were stage IV on CXR and 10 were stage III
  - Is TGFbeta driving early pro-fibrotic change in stage III patients?
IFNgamma pathway is also implicated by miRNA PBMCs

• Jazwa et al 2015:
  • increased level of miR-34a in sarcoidosis PBMCs
  • upregulated levels of interferon gamma, IFN gamma inducible protein (IP-10) and vascular endothelial growth factor were detected in sera of patients when compared to healthy subjects.
Can miRNA expression and miRNA-gene interactions be developed into a sarcoidosis gene signature for prognosis or diagnosis?

• 17-gene signature developed by Zhou et al 2016: differentiates sarcoidosis from healthy controls
  • enriched Gene Ontology (GO) biological process terms and KEGG pathways implicated JAK-STAT
Can miRNA expression and miRNA-gene interactions be developed into a sarcoidosis gene signature for prognosis or diagnosis?

- Ascoli et al 2017:
  - Machine learning algorithm selected 54 feature miRNAs that discriminated between sarcoidosis and controls.
  - Subsequently, logistic regression models adjusted for sex, age, smoking history, and use of immunomodulatory therapy further selected eight signature miRNAs for prognostic implication.
Summary / Key Points

- microRNA shows promise as a minimally invasive, stable, and potentially cost effective biomarker
- microRNA in sarcoidosis patients has potential for further clinically development as a biomarker, but the current data is diffuse and needs validation
  - Honing of cohorts for specific biomarker intention is key; heterogeneity of sarcoidosis clinical phenotypes must be addressed
  - Number of patients and diversity are key pitfalls
  - Opportunity to address tissue specificity, type of sample (serum vs plasma), and standardization of lab protocols in growing biobanking efforts
Thank you!

• questions