PLACENTAL MICRORNA EXPRESSION

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OUTLINE

- Background
- Placental miRNA expression
- Circulating placenta-specific miRNAs
- Opportunities and challenges

Background - MicroRNAs

NON-CODING RNAS

- Human genome
  - ~50-70% transcribed
  - ~1-2% protein coding
  - ~98% Noncoding
- List growing
- Groups
  - Housekeeping: tRNAs, rRNA, Telomerase RNA, etc
  - Regulatory: miRNA, long ncRNA, Antisense-RNA,etc

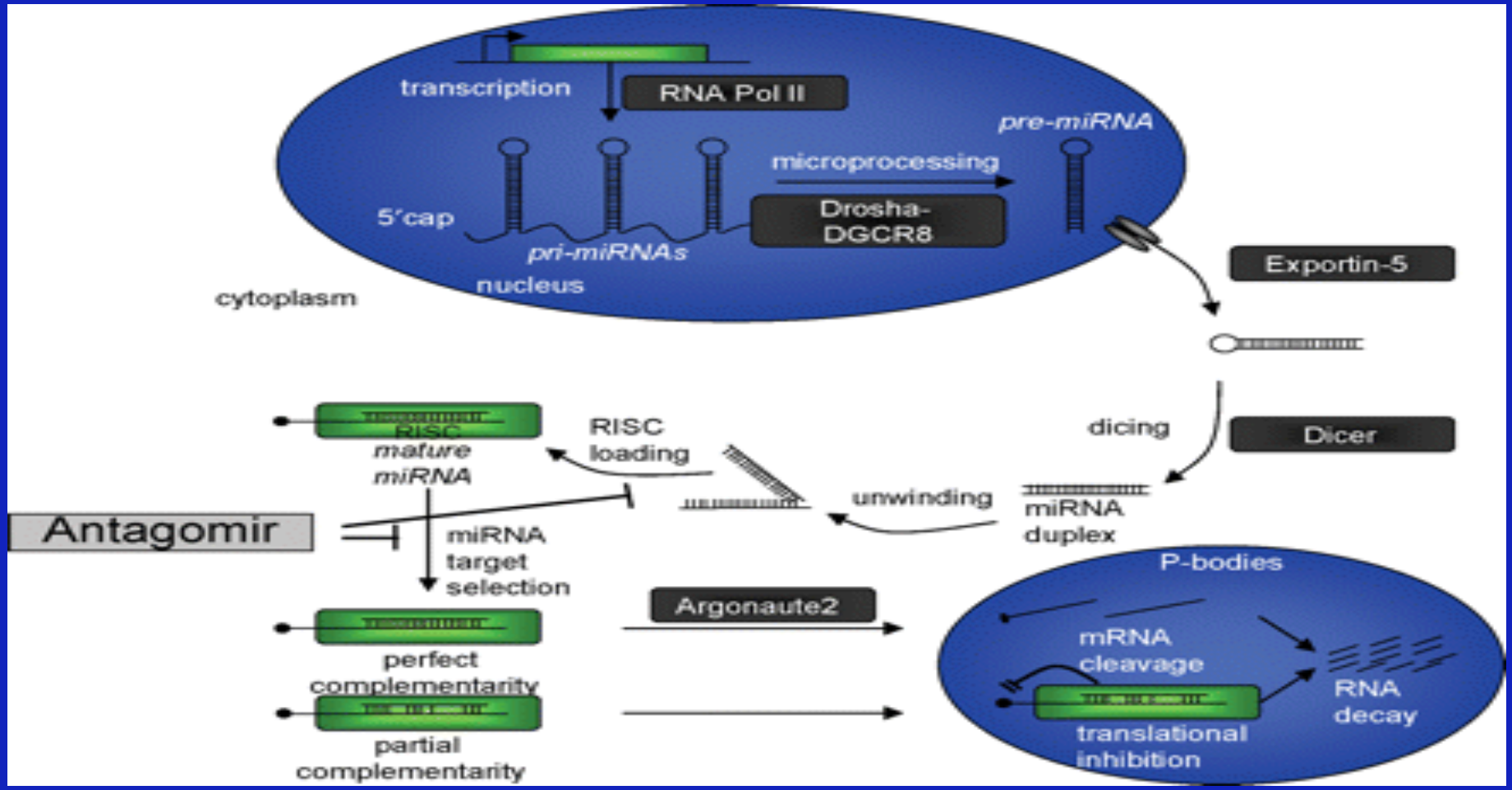
HISTORY

- Discovery: *lin-4* (1993), C. elegans
- Plants, RNAi research
- http://www.mirbase.org/
  - 28,645 entries: Release 21 (June 2014)
- ~2,500 miRNAs (human)
- Explosion of research over the past decade

BIOLOGY

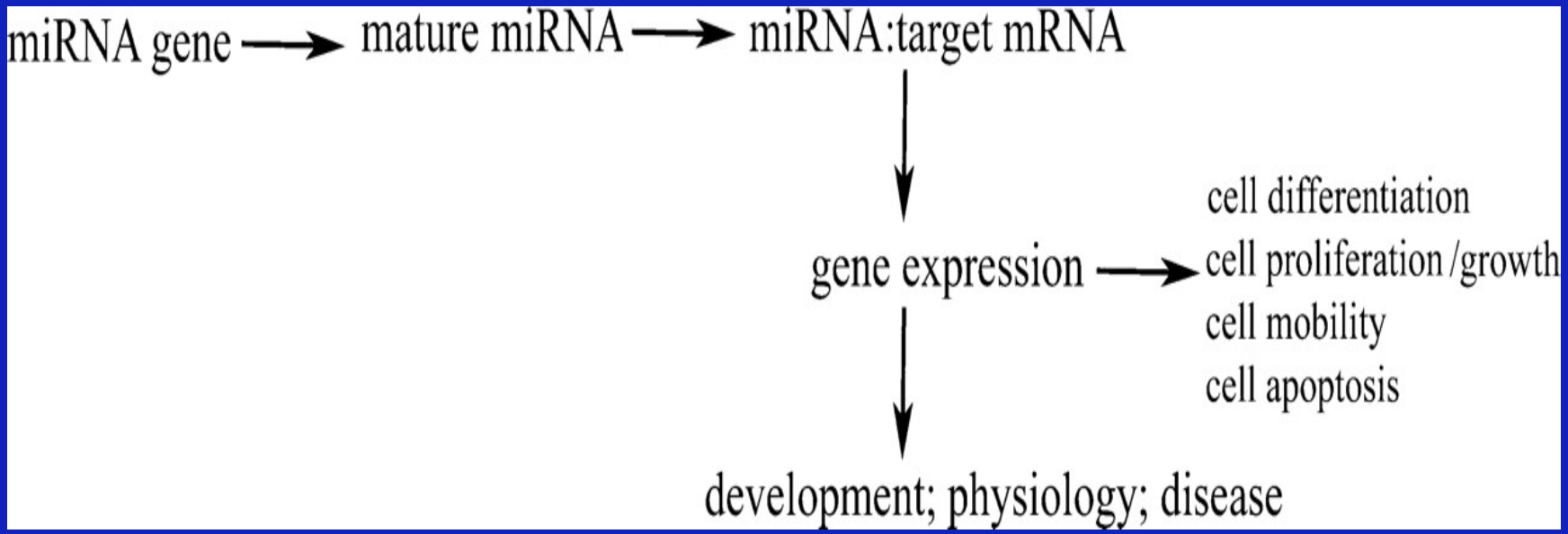
- Small (~22 nucleotides), conserved
- Production regulation: mostly unknown, tissue or developmental stage specific (1/3)
- Location
  - Introns, exon UT, intergenic
  - Single or cluster
- Post transcription regulation, though recent reports of transcription regulation

BIOLOGY



Cardiovascular Research (2008) 79, 562-570

BIOLOGY



Physiol Genomics (2008), 33:139-147

POST TRANSCRIPTION REGULATION

- Gene expression regulation (1/3 genes)
  - Target 3' UT seed regions of mRNA
  - Multiple sites on each mRNA Vs same site on many mRNA
- Mechanisms
  - Inhibit translation of mRNA (non-perfect match)
  - Promote mRNA degradation (perfect/near perfect match)
  - miRNA mediated mRNA decay

MEASUREMENT/PROFILING

Profiling method	Workflow	Sensitivity	Specificity	Throughput	Absolute quantification /accuracy	Flexibility	Can identify novel miRNAs	Data analysis	Other
qRT-PCR	Reverse transcription Optional pre-amplification PCR	+++	+++	+/++	+++	+++	N	E	Normalization
GeneChip microarrays	Biotin labeling Hybridization Staining Scanning	+	+	+++	+	+/–	N	M	Various species on the same array
Oligonucleotide microarrays	Cy3 labeling Hybridization Scanning	+/++	+/++	+++	+	++	N	M	
Exiqon miRCURY microarrays	Hy3/Hy5 labeling Hybridization Mono- or dual-wavelength scanning	++	++	+++	++	++	N	M	LNA-capture probes Allows for either mono- or dual-wavelength scanning
nCounter Analysis System	In-solution hybridization (biotinylated capture probe and a miRNA-specific fluorescent reporter probe) Complexes immobilization and alignment Scanning	++	++	++	++	++	N	M	Normalization
NextGen sequencing	3' Adaptor ligation 5' RT primer annealing 5' adapter ligation Reverse transcription PCR	++	+++	+++	++	+++	Y	D	Highest cost

J Cell Mol Med (2014), 18:371-390

DATABASES

- miRBase
- miRWalk
- miRTarBase
- Targetscan

NOMENCLATURE – CLASSIFICATION

Acronym	Meaning	Examples
3-Letter prefix	Species identification	*hsa* (*Homo sapiens*) *cel* (*Caenorhabditis elegans*)
pri-mir (lower case "r")	The primary miRNA transcript (see Figure 1)	pri-mir-1
pre-mir (lower case "r")	The precursor miRNA transcript resulting from processing of the primary transcript by the Drosha-DGCR8 complex (See Figure 1)	pre-mir-1
miR (upper case "R")	Mature miRNA	hsa-miR-1
-3p or -5p	Mature miRNA originating from the 3′ or 5′ end of the pre-miRNA, respectively	hsa-miR-10-3p hsa-miR-10-5p
a or b	Related, mature miRNA variants (i.e., differing by a nucleotide)	hsa-let 7a hsa-let 7b
-1, or -2	Identical mature miRNA sequences that originate from different genomic loci	hsa-miR-9-1 hsa-miR-9-2
miR* (miR-star)	"Passenger strand"[b] found at lower concentration, frequently degraded (retired after miRBase 16)	hsa-miR-9*
miR, miRNA, microRNA	Equivalent terms for a mature miRNA transcript used in the text of studies	

Am J Epidemiol (2014), 180, 140-152

TARGET IDENTIFICATION

- Computational approaches
  - Complementarities
    - "seed region" of 2-8 nucleotides at 5' end
  - Thermodynamic stability of complex
  - Degree of conservation of orthologues target sites in the 3' UT across species

MOTIVATION

- Understanding pathophysiology
- Generating hypotheses (e.g. Gene & Env)
- Biomarkers: prediction, prognosis, monitoring
- Therapeutic targets

Placental MiRNA Expression Preeclampsia

SWEDISH

CENTER FOR PERINATAL STUDIES

- Multiple cohort and case-control studies
  - Cohort (>6,500): Omega study, Pregnancy Migraine Study
  - Case-control: Ferritin, Abruptio etc.
- Recruitment
  - Early pregnancy (7-8 weeks, first prenatal visit)
  - Eligibility/exclusion
  - Participation (>80%), Follow up (>96%), Data (92%)
- Data collection
  - Interview-adminstered QA, FFQ,
  - Blood (16wks, on average), urine, placenta, cord blood
  - Medical records

MICROARRAY EXPERIMENT

- RNA extraction: Guanidinium TPC extraction method
- RNA QC: UV spectrophotometry and gel electrophoresis
- Platform: custom microarray @ Ocean Ridge Biosciences
- Confirmatory QRT-PCR

ANALYSIS

- Data normalized and log2 transformed
- Fold change, 1-way ANOVA, Clustering
- Targetscan (www.targetscan.com)
- Path analysis: KEGG and IPA
- Confirmatory qRT-PCR: Correlations

STUDY POPULATION CHARACTERISTICS

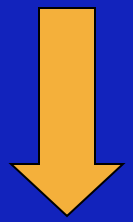
	PE Cases (N=20)	Controls (N=20)
Age*	32.8	30.4
Gestational age*	36.0	38.8
Caesarian delivery	55.0	40.0
Whites	60.0	70.0
Nullipara	65	60

*mean (otherwise %)

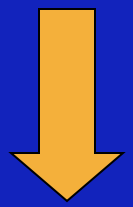
Enquobahrie et al, Am J Obstet Gynecol 2011

EXPRESSION MEASUREMENT

- 1,295 probes (human=854)



- miRNAs with ≥ 90% information (n=611)



- Differentially expressed miRNAs (n=8)
  (AFC>1.5, p-value <0.05)

Enquobahrie et al, Am J Obstet Gynecol 2011

DIFFERENTIALLY EXPRESSED MICRORNAS

MicroRNA	Location	Fold change	P-value*
hsa-miR-328	16q22.1	-1.52	0.00033
hsa-miR-584	5q33.1	-1.64	0.00188
hsa-miR-210	11p15.5	1.53	0.00276
hsa-miR-139-5p	11q13.4	-2.27	0.00354
hsa-miR-500	Xp11.23	-1.52	0.00539
hsa-miR-1247	14q32.31	-1.52	0.00581
hsa-miR-34C-5p	11q23.1	-1.54	0.01059
hsa-miR-1	20q13.33	-1.56	0.01284

**ANOVA 1-Way p-values comparing expressions among preeclampsia cases and controls.

Enquobahrie et al, Am J Obstet Gynecol 2011

PREVIOUSLY REPORTED

- miR-210
  - Hypoxia, endothelial cell response, VEGF activities
- miR-1
  - Smooth muscle calcium signaling
- miR-1247 (14q32.31 chromosomal cluster)
  - Conserved region with imprinted domain
  - Cluster (~40 miRNAs), embryonic/placental growth
  - Methylation region ~200kb upstream

Enquobahrie et al, Am J Obstet Gynecol 2011

NOVEL

- miR-584
  - Binds to conserved 3' region on lactoferrin receptor
  - Immune activation, platelet aggregation
- miR-34c-5p
  - Family of miR-34b and miR-34c
  - Mediators of p53 dependent suppression of endometrial proliferation (cell cycle)
  - Endometriosis and preeclampsia (?)

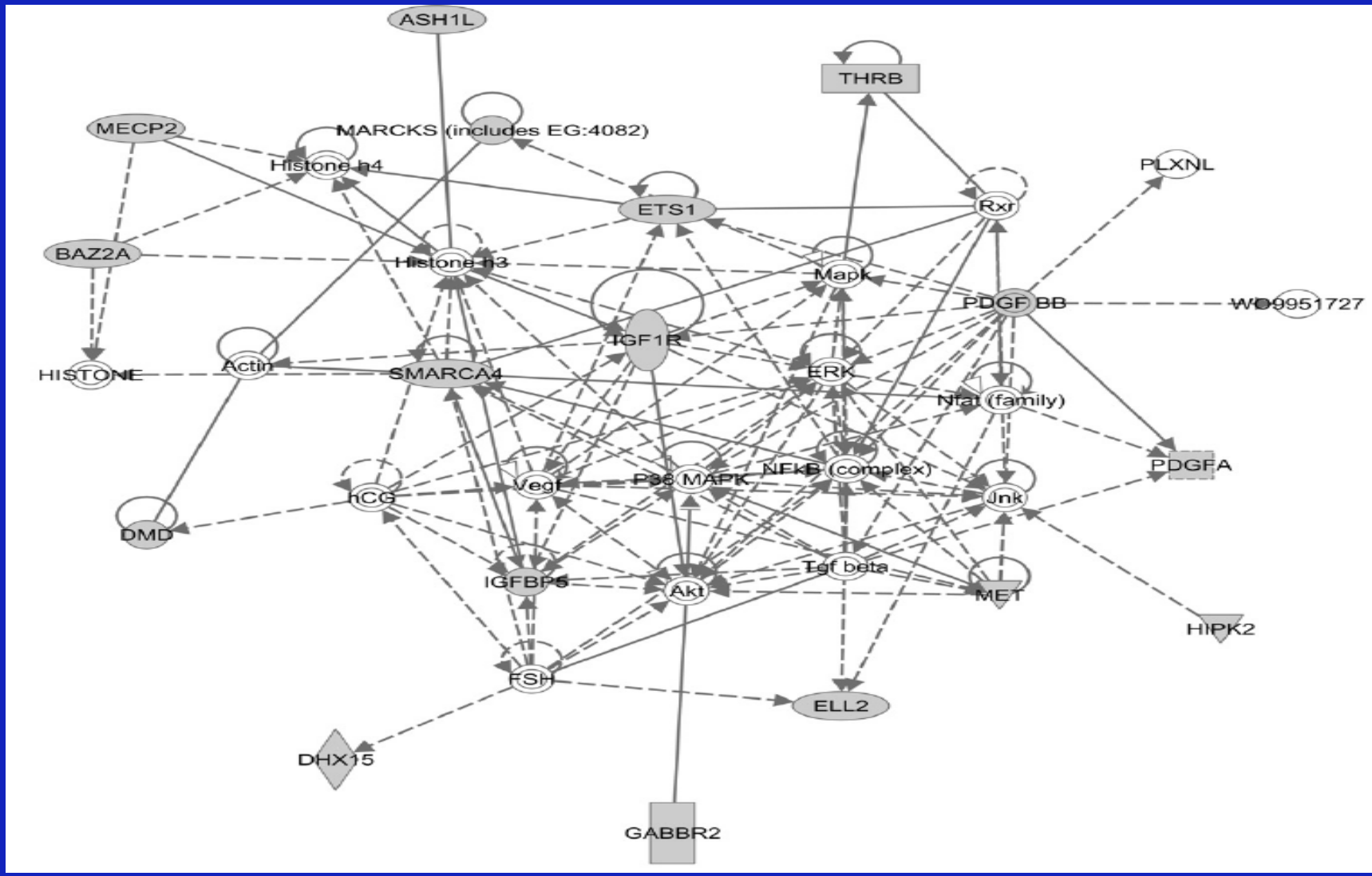
Enquobahrie et al, Am J Obstet Gynecol 2011

GENE TARGETS

- 76 genes targeted by 2 or more DE microRNAs

- Functions
  - Organ system development (CVS/RS)
  - Immune dysfunction
  - Cell cycle
  - Cell signaling

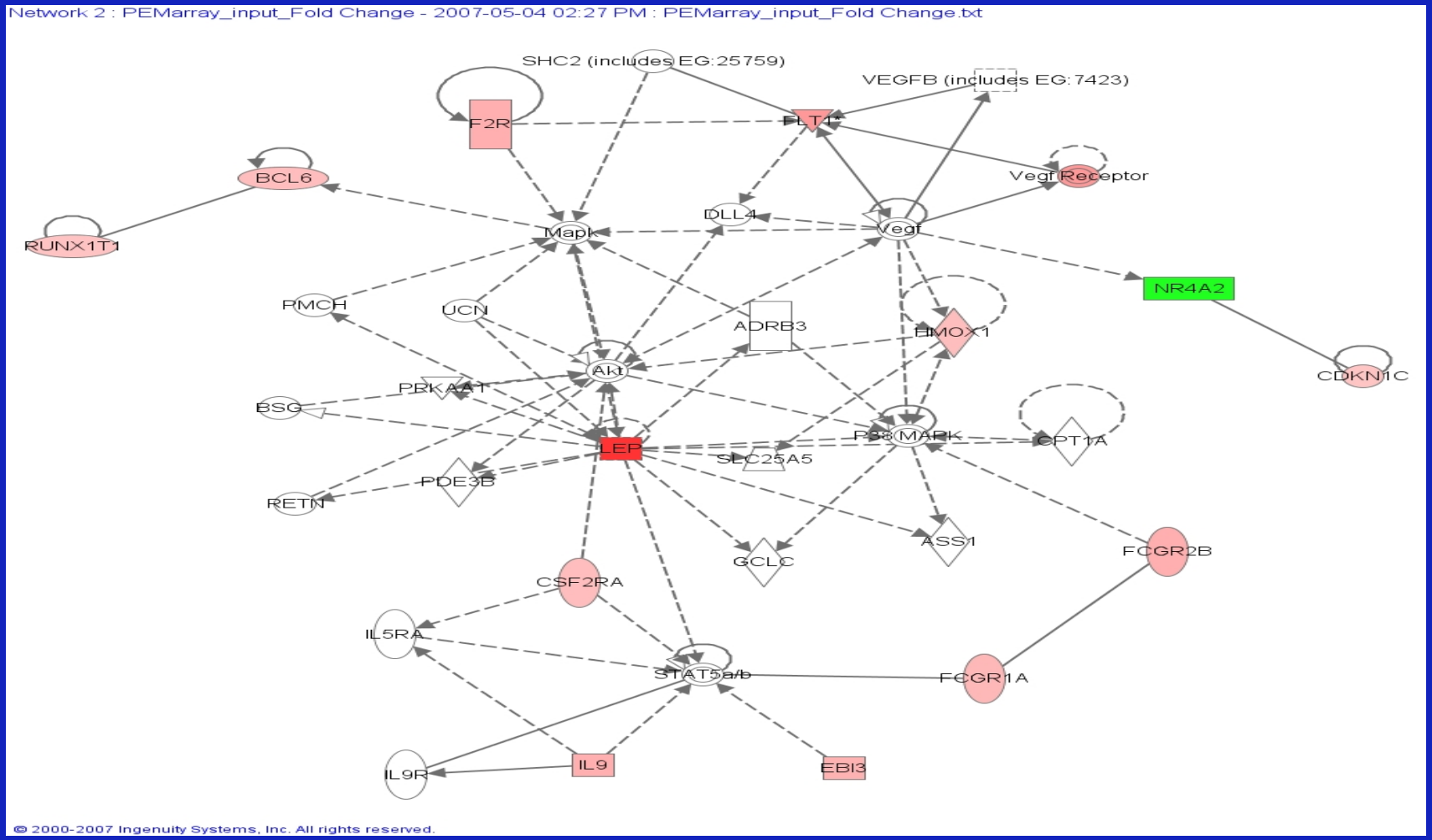
Significant network (IPA)



Genetic disorder, immunological disease, cardiovascular system development and function (score=30)

Enquobahrie et al, Am J Obstet Gynecol 2011

Network from the GE study



Cell death, cellular growth and proliferation, inflammatory disease

Am J Obstet Gynecol 2008;199:566 e1-11

SUMMARY

- Previously identified (miR-210, miR-1, and miRNA cluster at 14q32.31) and novel (miR-584 and miR-34c-5p) microRNAs differentially expressed

- Target genes participate in diverse patho-physiological processes with potential importance in preeclampsia (e.g. organ/system development, immunologic dysfunction)

Circulating Placenta-specific MicroRNAs

CIRCULATING MICRORNAS

- Passive leakage (injury, inflammation, necrosis)
  - Extracellular miRNAs regulate vascular endothelium
- Active secretion through cell-derived membrane vesicles (e.g. exosomes)
  - Adipocytes release exosomes that contain miRNAs (e.g. miR-16, -27a, 146b, -222)
- Protein-miRNA complexes (e.g. HDL-AGO2)
  - Participate in intercellular communication

PLACENTA-SPECIFIC MICRORNAS

- C19MC – 19q13.41 miRNA cluster
  - Primate specific, expressed from the paternal allele
  - Expression increases from first to third trimester
  - Member miRNAs (e.g. miR-517) regulate cell proliferation, immune function and antiviral activity
- C14MC – 14q32 miRNA cluster
  - DLK-DI03 domain, maternally imprinted
  - Expression decreases from first to third trimester
  - Member miRNAs (e.g. miR-379/410) regulate metabolic functions (glucose homeostasis and hepatic gene expression)

PLACENTA-SPECIFIC MICRORNAS

- Have been identified in plasma or serum
- Both placental expression and circulating levels has been related to preeclampsia or intrauterine growth retardation
- Most of these studies were conducted in mid/late pregnancy or post-delivery

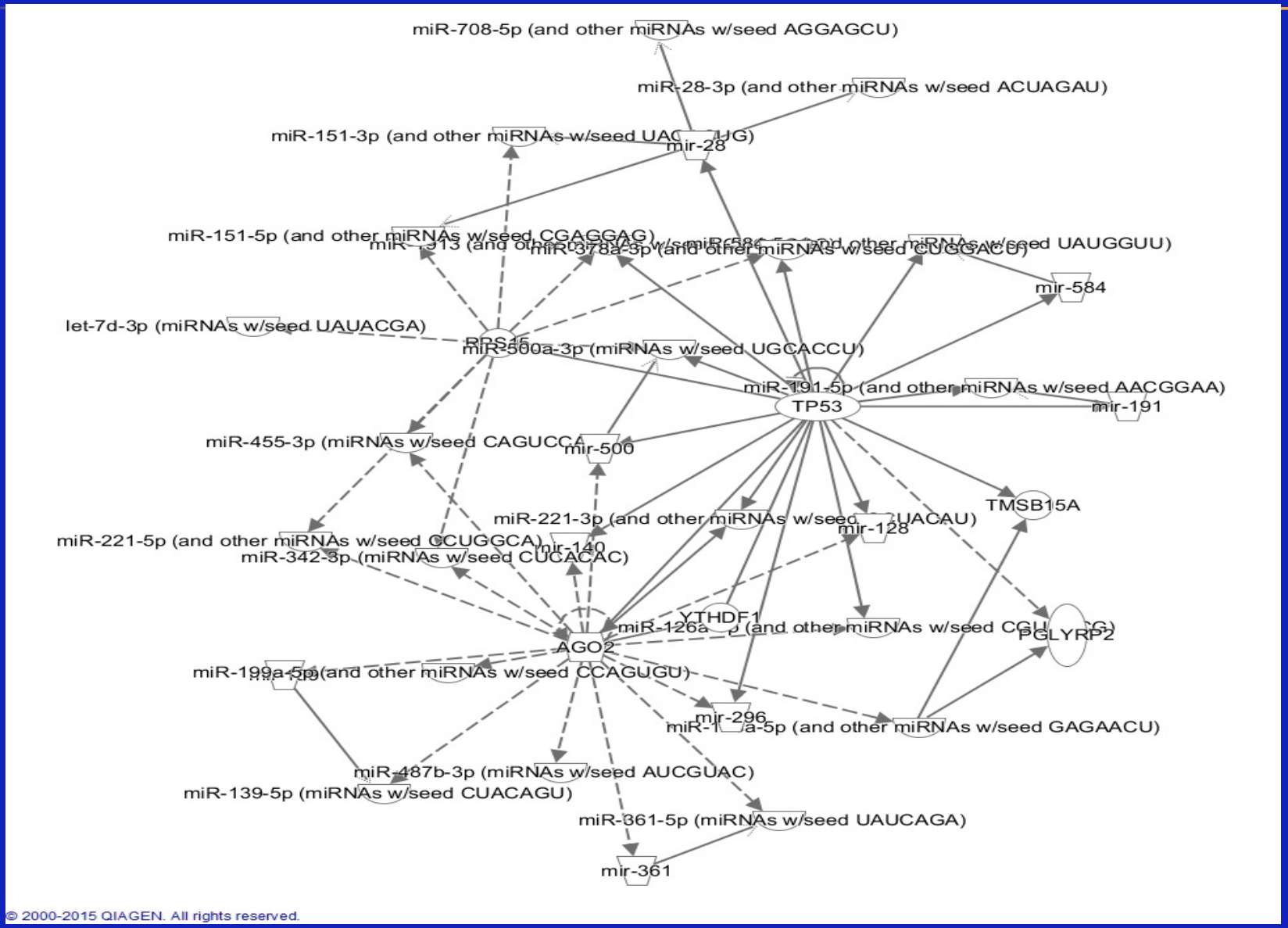
EARLY PREGNANCY CIRCULATING MICRORNAS

- Participants of the Omega Study (Seattle, WA) and POUCH (Pregnancy Outcomes and Community Health) Study (Michigan)
- Early (Omega, 8-20 weeks) to Mid (POUCH, 15-27 weeks) pregnancy blood collection
- Microarray and candidate miRNA profiling in relation to subsequent risk of preterm birth (primary outcome) and pre-pregnancy body mass index

CIRCULATING MICRORNAS AND BMI

- 27 miRNAs were differentially expressed in relation to pre-pregnancy BMI in both Omega and POUCH cohorts
- Functions in regulation of organismal injury and abnormalities, reproductive system diseases, connective tissue disorders, and cancer
- Identified miRNAs included placenta-specific miRNAs in C14MC cluster (e.g. -377)

CIRCULATING MICRORNAS AND BMI



Opportunities and Challenges

OPPORTUNITIES

- Research
  - Stability, omnipresence, smaller size, smaller # (?)
  - Uncovering gene expression regulation (DNA→RNA→Protein)
  - Role of unknown genomic regions
    - e.g. miR-15, miR-16 at 13q14.3 in CLL, chromosome 9 (MI)
  - Gene environment interactions

OPPORTUNITIES

- Therapeutics
  - + Small size practical for synthesis and delivery
  - + Effect (on regulation) can be seen quickly (in 3 wks)
  - + Antisense RNA, alternate to knock-outs

CHALLENGES - MEASUREMENT

- Limited sample volumes, low recovery, interference (e.g. anti-coagulants, platelet activation)
- Pre-processing steps, pre-analytic and analytic variables
- Lack of standardized protocols for extraction, profiling, and analyses
- Lack of good, universal control (house keeping miRNA) for normalization
- Diurnal variation and effect of fasting/non-fasting

CHALLENGES - INFERENCES

- Study population and sample differences
- Confounding/effect modification
  - lifestyle, sex (infant), race, age
- Time dependent (disease course) changes
- Target (gene) identification – functional validation
  - Complex relationships
  - Loops

CHALLENGES: THERAPEUTIC APPLICATIONS

- miRNA mimics are unstable,
- Selective targeting may be difficult, broad systemic effects
- Maintaining physiological levels of therapeutic miRNAs: over-inhibition or over-expression
- Delivery systems

CURRENT AREAS OF WORK

- Integration with other –omics (e.g. genomics, metabolomics, proteomics)
- Placenta-specific miRNAs in urine
- Histo-pathologic and/or imaging correlates of miRNA expression

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