A Bayesian two-step integrative procedure incorporating prior knowledge for the identification of miRNA-mRNAs involved in hepatocellular carcinoma.

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Outline

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Biological context - HCC context

Hepatocellular carcinoma (HCC) is the **most common** type of liver cancer and the **third cause** of cancer deaths worldwide.

An **aggressive** cancer because:

- diagnosed at advanced stages (in many cases, HCC detected in people with liver cirrhosis (CIRR)),
- known diagnostic markers have low sensitivity for early detection.

The identification of **novel** diagnostic biomarkers for early detection of HCC is **crucial** and is still an active research area.

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Biological context - General context

MicroRNAs (miRNAs) are small single-stranded non-coding RNAs that regulate target gene expression [1]

Role of miRNAs is **crucial** in many biological processes, in particular those underlying diseases.

↬ The study of their effects may be achieved by linking miRNAs to target genes.

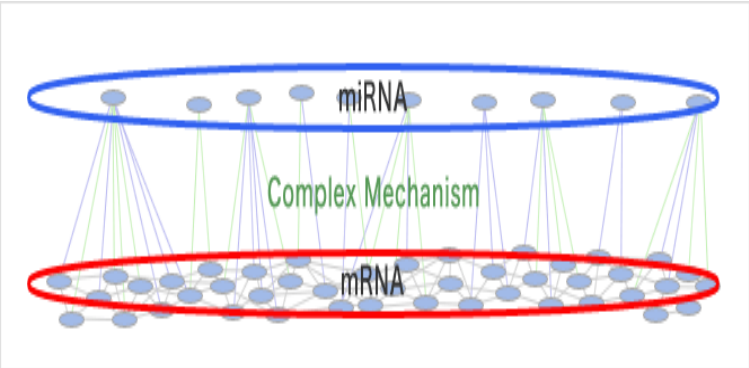

Figure 1: Possible relationships between miRNAs and mRNAs: one miRNA may have multiple mRNA targets and vice-versa.

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Biological context - Coming back to HCC

Goal

- A better understanding of the biological mechanisms involved in HCC through the identification of miRNA-mRNA disease-associated pairs.

Why ?

- Previous studies: miRNA-mRNA pairs play a **crucial role** in the activation of oncogenic or carcinogenesis pathways in liver diseases or HCC.

But

- The characterization of the relationships between miRNAs and mRNAs is still a **challenge**.

Need to use/develop appropriate statistical methods for a better understanding of links between miRNAs and mRNAs and for identifying relevant pairs.

Statistical context

Objectives

- to improve the understanding of associations / to discover new ones between miRNAs and mRNAs,
- to improve the understanding of associations among mRNAs after considering effects of miRNAs,
- to identify relevant disease-associated miRNA-mRNA pairs.

Many works have shown that:

- the integration of data from various molecular levels leads to better results than analyses considering only one dataset,
- the integration of prior knowledge into statistical models leads to promising results.

We propose a Bayesian two-step **integrative** procedure for analyzing miRNA-seq and mRNA-seq data from patients with HCC or CIRR while integrating **prior knowledge** accumulated from biological experiments or statistical analyses.

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Samples

Human liver tissues from 64 adult patients recruited at MedStar Georgetown University Hospital.

Table 1: Characteristics of patient-derived samples

		HCC (N=39)	CIRR (N=25)	p-value
Age	Mean(SD)	62.02 (11.46)	50.05 (12.1)	**0.0013**
Gender	Male	77%	72%	0.7683
Race	EA	41%	64%	**0.0202**
	AA	33%	32%	
	Asian	26%	0%	
	other	0%	4%	

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miRNA-seq and mRNA-seq data

Samples

- RNA samples extracted from the 64 liver tissues and analyzed by Illumina Hiseq 4000 [2],
- Gaussianization of data with the R package *huge* [3],
- Selection of 106 mRNAs and 261 miRNAs selected by using Student t-tests and a p-value cut-off of 0.05 after false discovery rate correction.

Prior knowledge between miRNAs and mRNAs

Scores measuring **the belief in the association** between mRNAs and miRNAs (Ingenuity Pathway Analysis (IPA) Target filter analysis tool [4]):

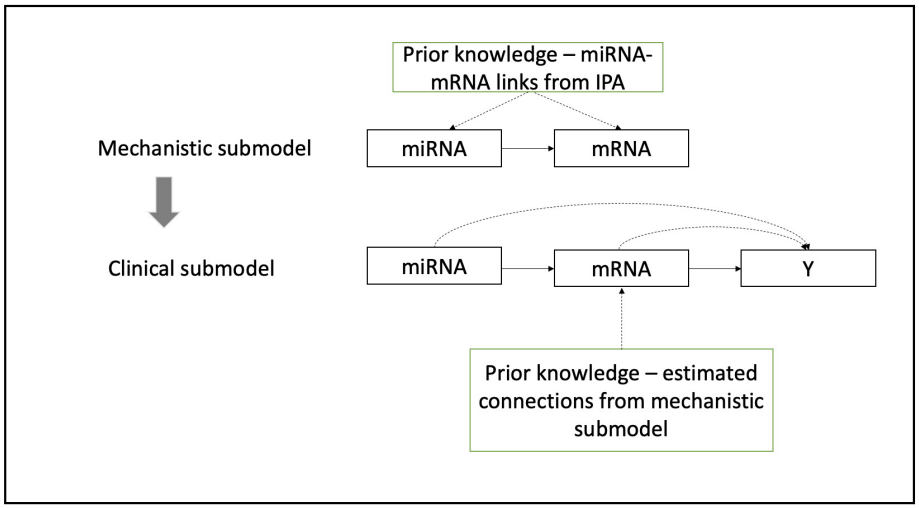
- Experimentally verified associations (score = 1),
- Predicted associations (scores = 0.75 for highly predicted, score = 0.5 for moderate predicted, and score = 0 otherwise).

Statistical model

Bayesian two-step intgrative procedure

Extension of the approach proposed by [5] and consists of two submodels:

- a mechanistic submodel: relating miRNAs and mRNAs,
- a clinical submodel: relating the phenotypic outcome to mRNA and miRNA expression levels



Bayesian two-step integrative procedure

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Mechanistic submodel - Variable selection

Bayesian variable selection using spike-and-slab prior integrating prior knowledge from biological experiments

The expression level of gene j ($j = 1, \ldots, q$) is modeled by:

$$G_j = \text{miRNA}_1\beta_1 + \text{miRNA}_2\beta_2 + \cdots + \text{miRNA}_m\beta_m + \cdots + \text{miRNA}_p\beta_p + \varepsilon_j$$

- Prior inclusion probability of miRNA k: $\boldsymbol{p(\gamma_{jk}|\tau)} = \frac{\exp(\eta+\tau s_{jk})}{1+\exp(\eta+\tau s_{jk})}$, s_{jk} score between gene j and miRNA k, $\tau \sim \mathcal{G}(a, b)$ and η fixed

↬ **Selection** of the relevant miRNAs ($\beta_k \neq 0$ for $k = 1, \ldots, p$) while **integrating prior knowledge**

Decomposition of the mRNA expression level into two parts:

$$G_j = \underbrace{G_{miRNA}}_{\text{modulation via miRNAs}} + \underbrace{G_{\overline{miRNA}}}_{\text{modulation via other factors than miRNAs}}$$

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Mechanistic submodel - Conditional Gaussian graph

Estimated graph

Gaussian graphical model (GGM) [6] used to estimate a graph structure for gene expressions adjusted for miRNAs ($G_{\overline{miRNA}}$):
$\hookrightarrow$ Covariate-adjusted Gaussian graph or conditional Gaussian graph or **adjusted** graph[7].

Note that we have also estimated an **undajusted** graph based on the raw gene expressions.

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Clinical submodel

Bayesian variable using spike-and-slab prior integrating prior knowledge from statistical analysis

Probit model where the linear predictor is given by:

$$\text{probit } P(Y=1) \sim G_{miRNA} + \underbrace{G_{\overline{miRNA}}}_{\text{Adjusted graph}} + \overline{\text{mRNA}} + \overline{\text{miRNA}} \quad (1)$$

with

- $\overline{\text{mRNA}}$: the set of mRNAs with no related miRNA in the mechanistic submodel,
- $\overline{\text{miRNA}}$: the set of miRNAs with no association to any of the mRNAs in the mechanistic submodel.

↬ **Selection** of the relevant mRNAs, miRNAs, and miRNA-mRNA pairs associated with HCC status while **integrating dependence structure**

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Mechanistic submodel

Spike-and-slab variable selection approach:

- 371 miRNA-mRNA pairs identified, 22 of which are experimentally verified.

Estimated graphs:

- before adjusting for miRNAs: 497 edges,
- after adjusting for miRNAS: 101 edges.

↬ Majority of gene-gene interactions not maintained after accounting for the miRNA regulation of these genes.

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Mechanistic submodel

Focus on 5 genes connected in the unadjusted graph and conditionnaly independent in the adjusted graph.

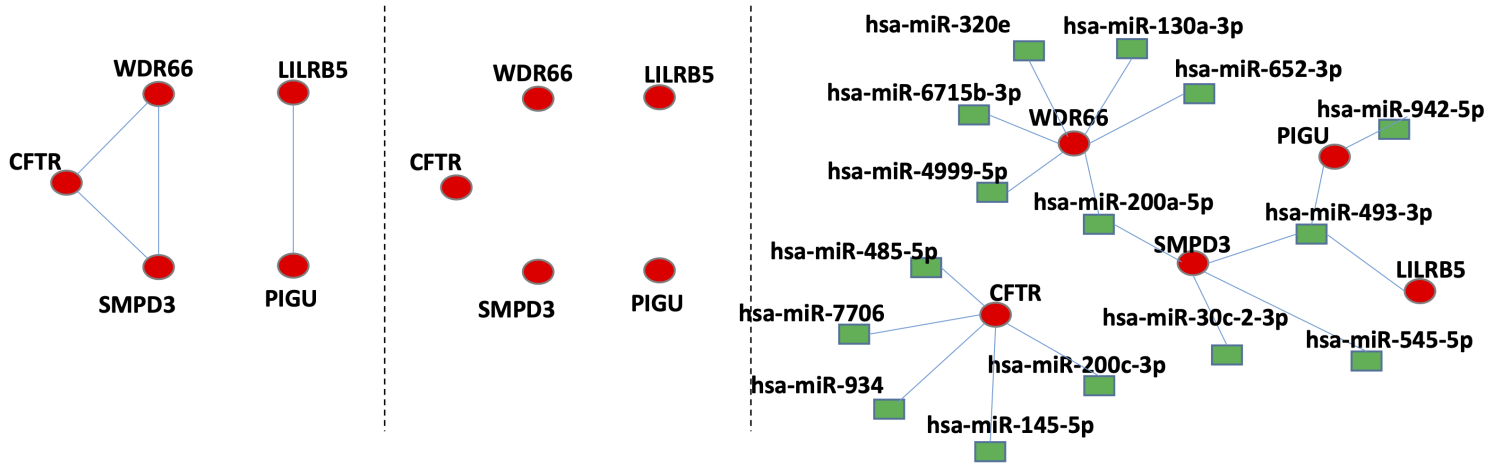

Figure 2: Unadjusted graph (left), adjusted graph (middle), and adjusted graph with associated miRNAs (right).

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Clinical submodel

Clinical submodel selects: 21 mRNAs, 5 miRNAs, and 66 miRNA-mRNA pairs

- 3 of the 66 pairs are experimentally verified.

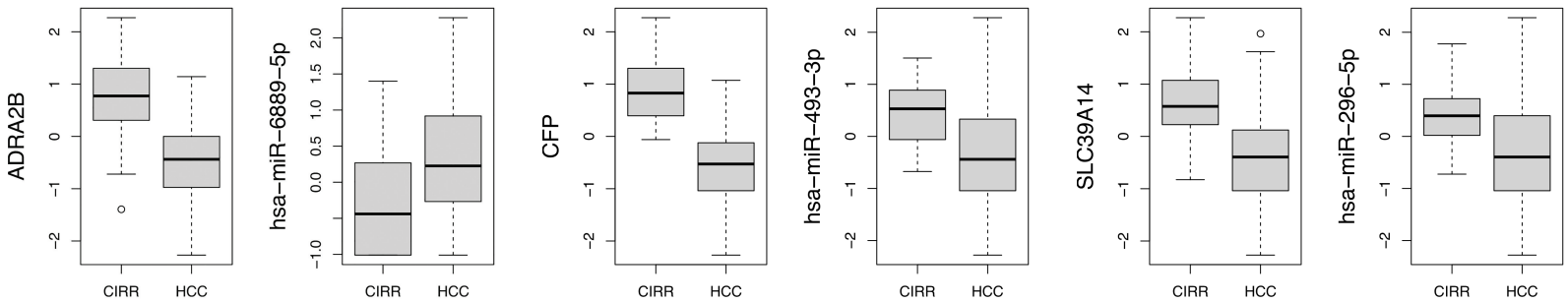

Figure 3: Boxplots of miRNA and mRNA expressions across disease status for three experimentally verified pairs.

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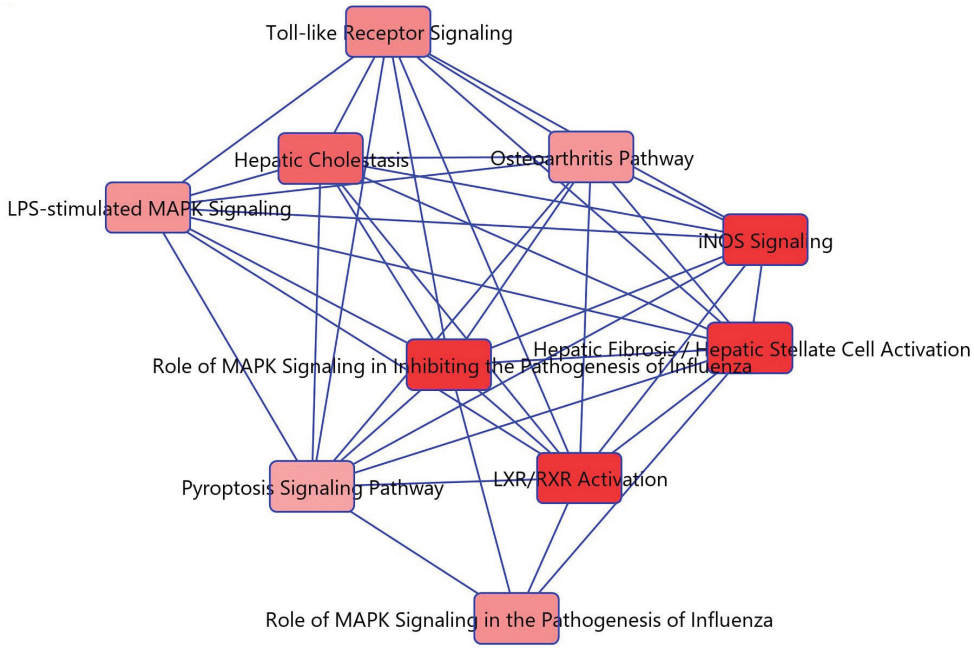
Clinical submodel: Pathway analysis using the IPA tool

Top 10 pathways represented by the molecules selected from the clinical model. Darker red color for pathways with higher significance.



↬ The three highest significant pathways are known to be significantly enriched in HCC

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Conclusion/Perspectives

New Bayesian integrative approach extending [5] and incorporating knowledge from various sources at the different stages of the modeling for studying diseases.

Conclusion

- Adjusted/unadjusted graphs help improve the understanding of relationships between genes,
- The proposed approach helps narrow down to the most important mRNAs and miRNAs as well as miRNA-mRNA pairs,
- Key pathways identified.

↬ Biological relevance of studying molecular interactions and of integrating prior knowledge when analyzing mRNA-seq and miRNA-seq.

Perspectives

- Computational improvement for analyzing high-dimensional dataset,
- Findings need to be experimentally validated on larger dataset to confirm their potential as diagnostic or prognostic biomarkers,.

Thanks for your attention!

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