Mapping posttranscriptional regulation of the human glycome uncovers microRNA defining the glycocode

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Backgroud

糖组学是继基因组学和蛋白质组学后的新兴研究领域，是专门研究糖链表达、调控和生理功能的一门学科，主要研究糖蛋白的编码基因，分析和探明蛋白质糖基化的内部机制。

蛋白质糖基化是蛋白质翻译后的一种重要的加工过程，蛋白质上连接的聚糖可以影响到蛋白质的结构和活性。虽然糖不是基因的直接表达产物，但是糖由基因编码的糖基转移酶和糖合成酶特异反应合成的。

Backgroud

糖蛋白普遍存在于动物、植物及微生物中，种类繁多，功能广泛。糖蛋白可分为三类：①可溶性糖蛋白，包括酶、肽类激素、抗体以及某些生长因子、干扰素等。②膜结合蛋白，常参与细胞识别，并可作为特定细胞或细胞在特定阶段的表面标志或表面抗原。③结构糖蛋白，它们的功能不仅仅是作为细胞外基质的结构成分起支持、连接及缓冲作用，更重要的是参与细胞识别、粘着及迁移，并调控细胞的增殖及分化。

Backgroud

Glycans control multiple aspects of cell biology, including cell-cell communication, cancer metastasis, and inflammation. Little is known about the regulatory networks controlling this complex biosynthetic process. This article take a unique systems-based approach to identify connections between miRNA and the glycome.

Materials

Cell Lines. The NCI-60 is a cancer cell line panel containing 59 cell lines from nine tissue types. The NCI-60 cell set was from the Division of Cancer Treatment and Diagnosis Tumor Repository (National Cancer Institute, Frederick, MD).

Methods

- Lectin Microarray
- SVD and Multidimensional Analysis
- Mapping miRNA Onto Glycosylation Pathway
- Fluorescence Microscopy
- Transfection of miRNA Mimics and Inhibitors
- RNA Extraction and Real-Time qPCR
- Western Blotting
- Luciferase Reporter Assay

Methods

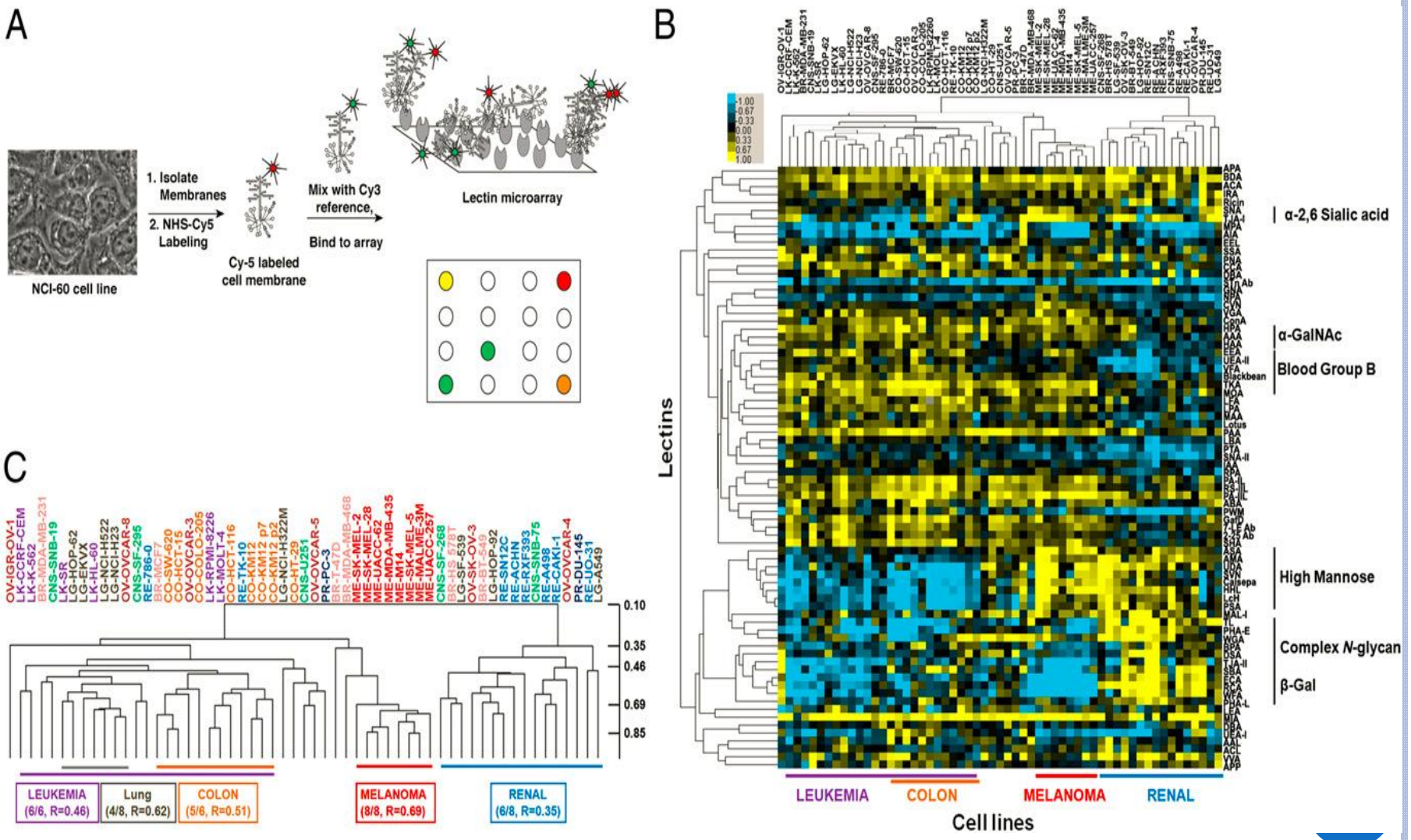
凝集素是可与特定糖链结构专一性结合的蛋白质，凝集素芯片技术是将凝集素固定在芯片上，再用荧光标记的样本去检测芯片，样本通过自身糖链与固定凝集素特异识别，通过芯片扫描仪分析荧光信号，能简便、快速、高通量的检测分析糖蛋白。

Results

- **Glycomic Analysis of the NCI-60 Reveals Tissue-Specific Glycan Signature.** Discrete glycan signatues were observed for four tissue types: colon, leukemia, melanoma, and renal.

  Melanoma and renal cell lines showed enrichment in high mannose but were distinguished by differing levers of complex multiantenarary N-glycans. In contrast, leukemia and colon had lower levers of high mannose and divergent levers of complex multiantennary glycans and β-GalNAc epitopes.

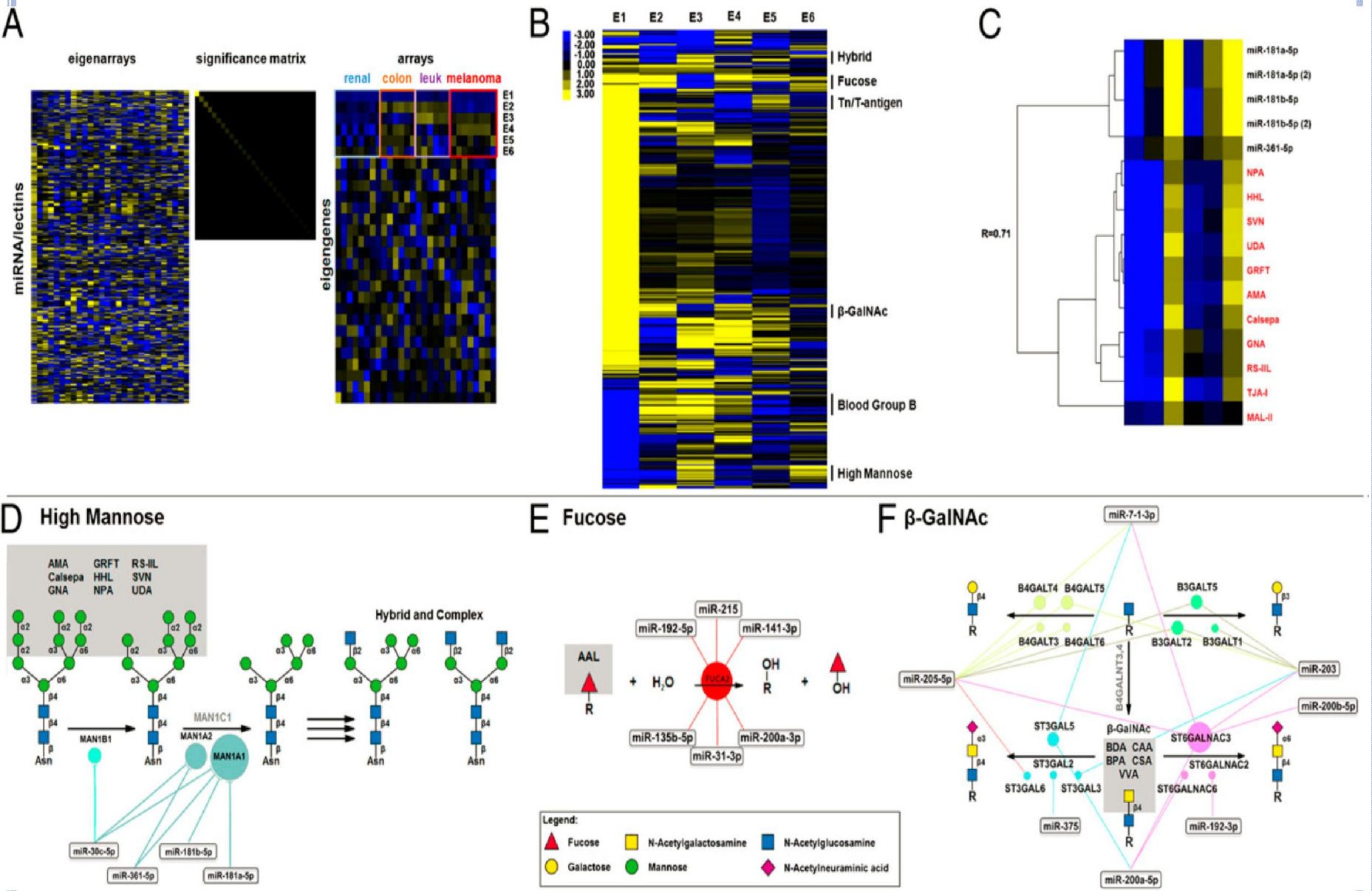
Ratiometric comparision of NCI-60 cell lines



Results

- **Multidimensial Singular Value Decomposition Analysis Identifies Regulatory miRNA/Glycosylation Networks.**We identified regulatory networks for high mannose, fucose, terminal β-GalNAc, Tn and T-antigens, hybrid-N-glycans, blood group B, suggesting widespread control of glycan biosynthetic pathways by miRNA.

Mapping of miRNA/glycosylation network



High Mannose Network

MicroRNA: MiR-30c, MiR-181b-5p, MiR-361-5p, MiR-181a-5p

Transfect HT-29 with miRNA mimics, increasing binding of the high-mannose lectins by two fold.

target: *MAN1* family of enzyme

Treat HT-29 with mimics and inhibitors of miRNA and examine *MAN1A1* expression level . No change was observed.

Analysis of *MAN1* transcript level in 4 NCI-60 cell lines identified *MAN1A2* as the predomident mRNA.

High Mannose Network

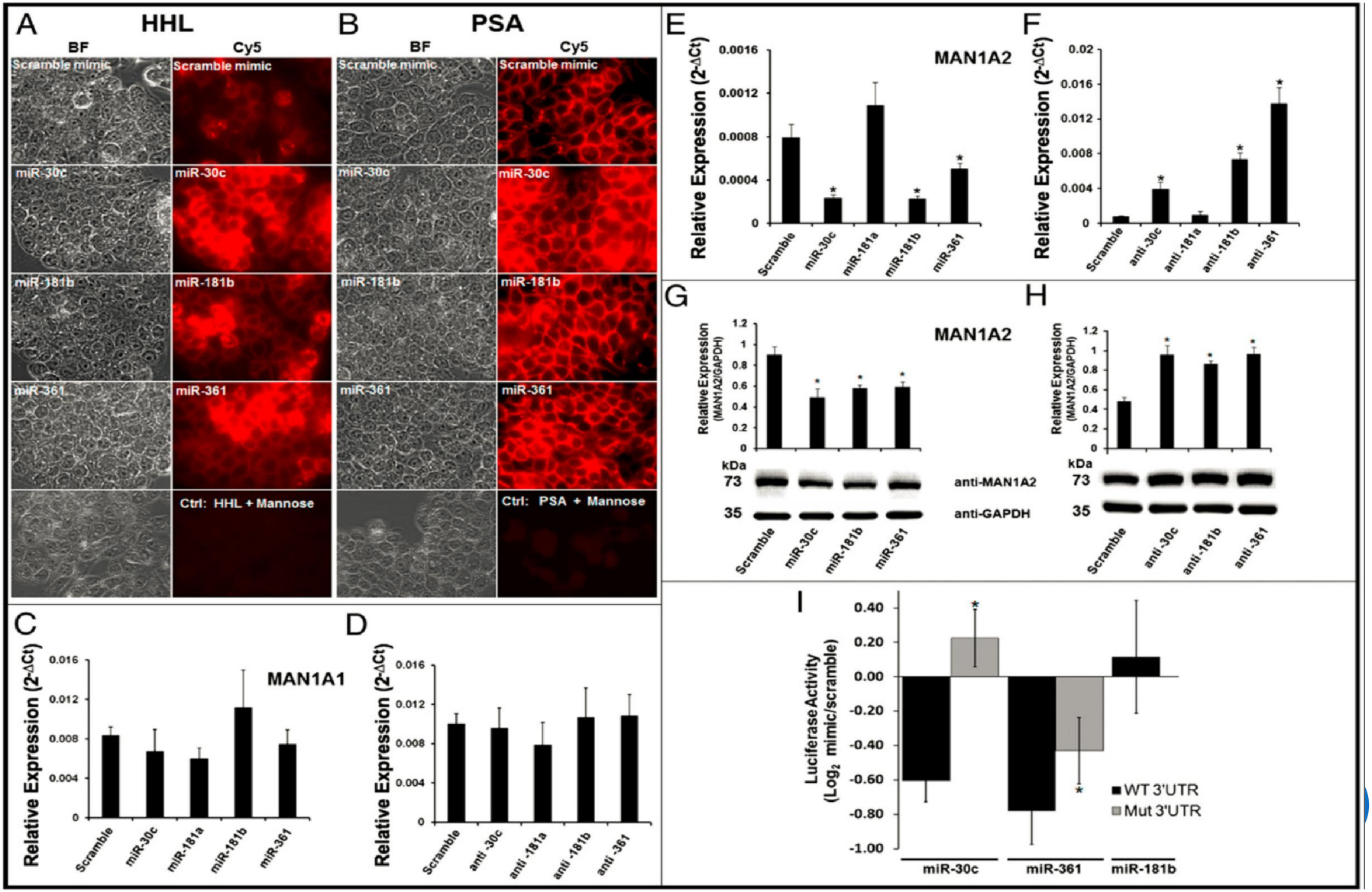
We observe strong down-regulation at transcript and protein levels for *MAN1A2* in respsonse to miR-30c, -181b-5p, -361-5p mimics, but no response for miR-181a-5p.

Luciferase-*MAN1A2*- 3′-UTR reporter assay: Micmics of miR-30c and -361-5p inhibited lusiferase expression. miR-181b-5P did not affect luciferase levels.

Binding site: MiR-30c, MiR-361-5p是3′-UTR, MiR-181b-5p不确定

Mutation of the biding site of miR-30c or -361-5p abrogated the effect of these miRNA on MAN1A2- 3′-UTR reporter .

Validation of the high mannose network



Results

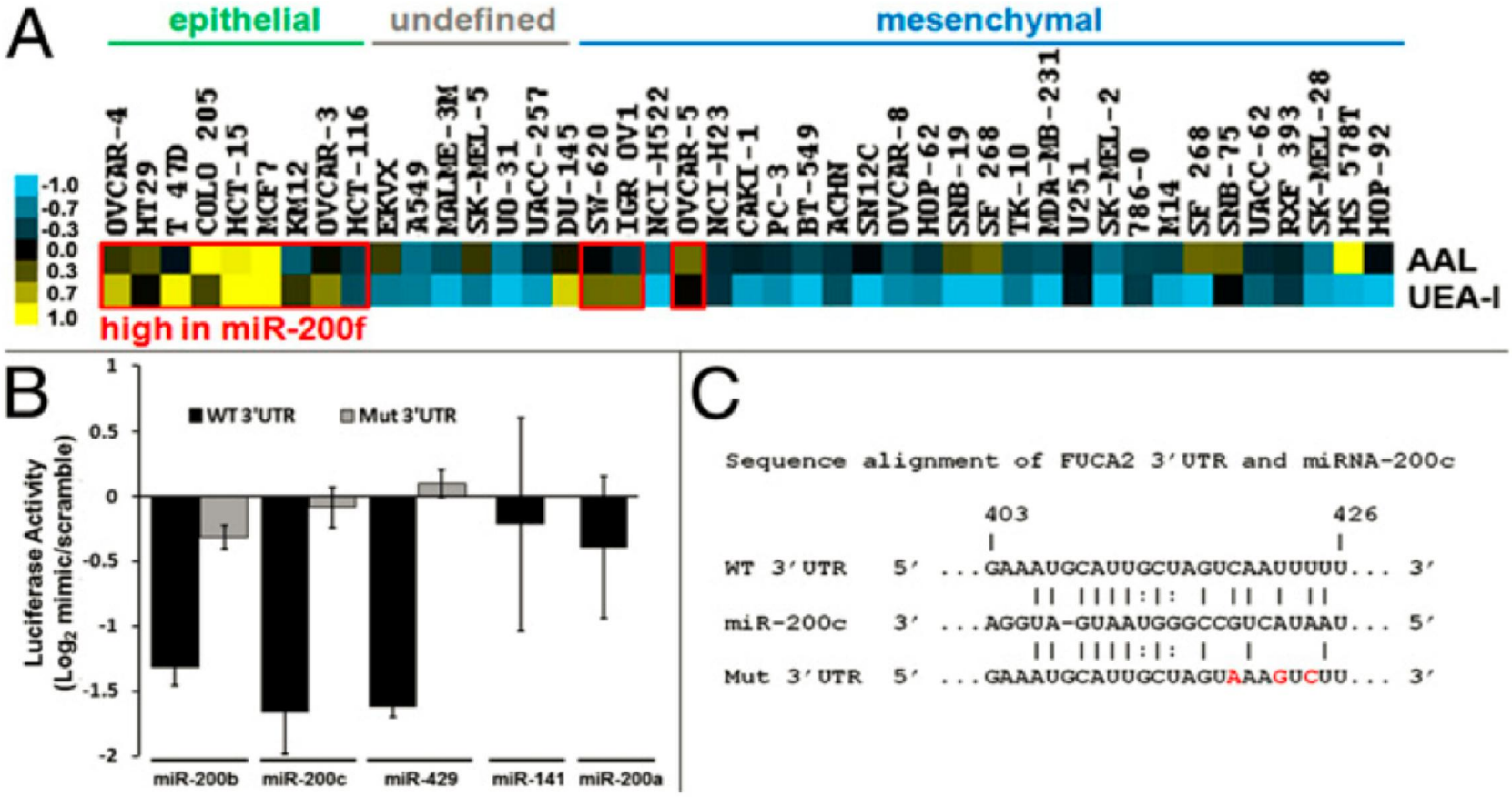
- **Fucose Network.**

  target: *FUCA2*

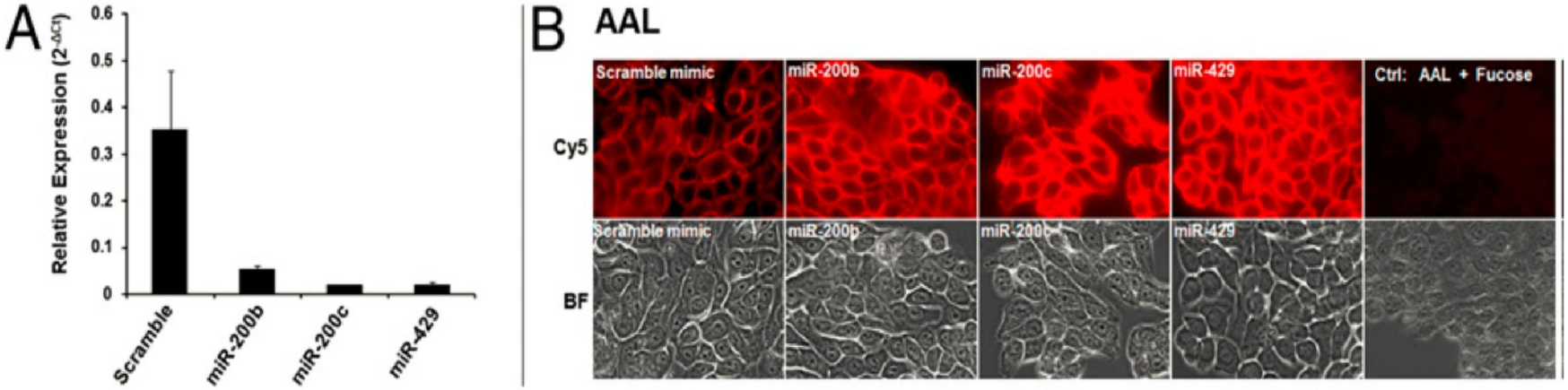
  MicroRNA: MiR-200b, -200c, -429, -141,-200a

  Binding site:都是3′-UTR

Validation of the fucose network



MiR-200f members down-regulate *FUCA2* expression and increase fucosylation in HT-29



Results

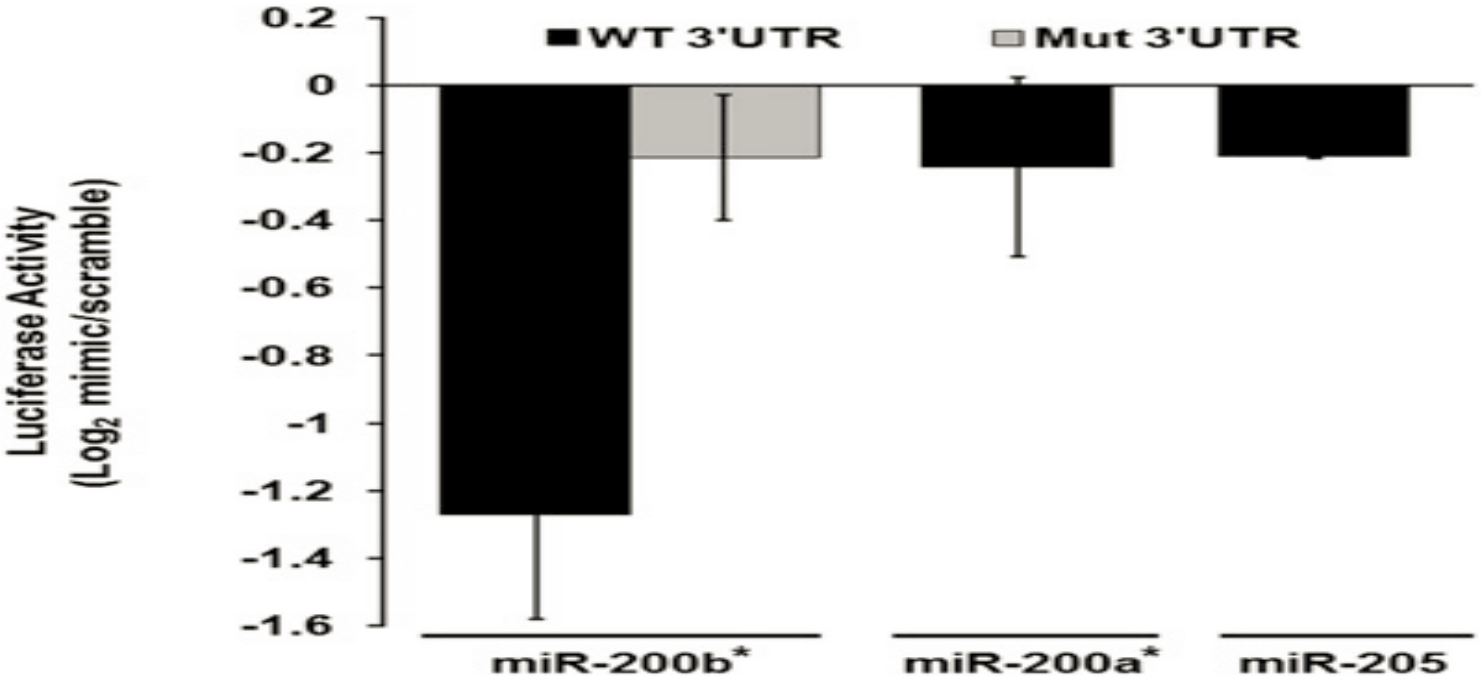
- **β-GalNAc Network.**

  target: *ST6FALNAC3*

  MicroRNA: MiR-200b-5P, -200a-5p, -205-5p

  Binding site: 3′-UTR

Validation of β-GalNAc Network



Conclusions

miRNAs are master regulators and control the glycan biosynthetic network through direct interation with glycogene transcripts .（大部分通过结合在mRNA的3′非编码区起作用）

Our work begins to map glycans onto critical regulatory networks controlling cell phenotype.

End

创新点：将生物信息学方法与实验方法有机结合起来　　研究生物学问题。

启发：生物信息学的预测算法能够为我们研究生物学问题起导向作用，使我们的研究具有针对性。

改进之处：　MicroRNA flanking bases 与target specificity的关系有待于进一步研究。

MicoRNA与target的结合位点是否局限于3′-UTR。

MiRNA target 预测算法需要进一步改进，发掘潜在结合位点。

本文只验证了翻译水平上调控机制，转录水平的调控机制并不清楚。

Thank you for your attention!