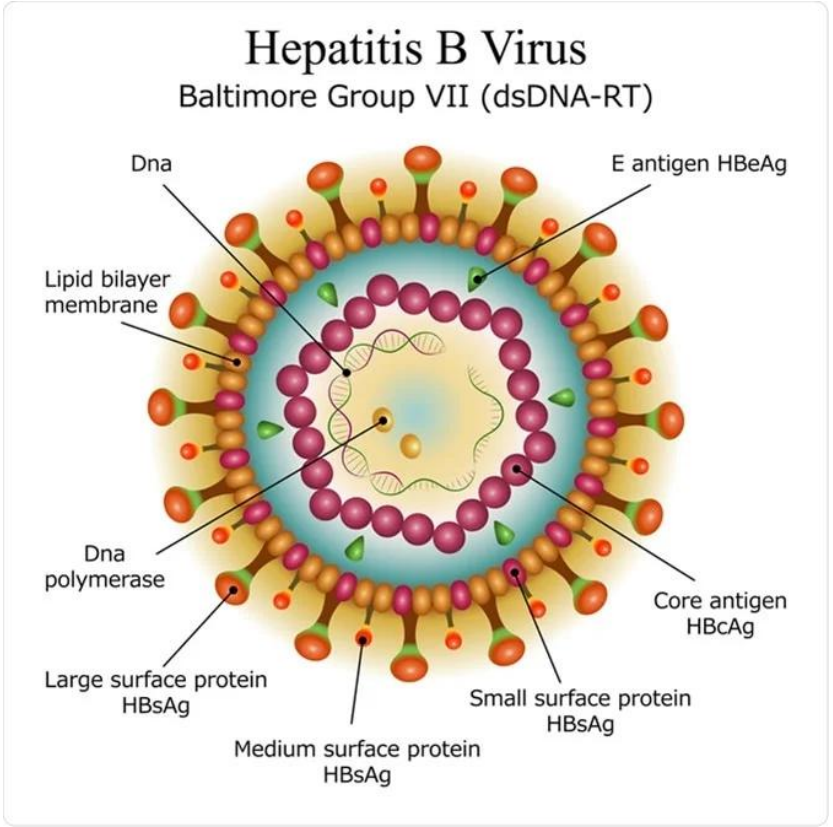
Comparisons of HBV-associated Liver fibrosis with Normal tissue

Margery (Yutian) Liu

Introduction

Liver fibrosis is the excessive accumulation of extracellular matrix proteins(EMP) including collagen that occurs in most types of chronic liver diseases.



Ref: Diagram of Hepatitis B virus particle structure. Image Credit: Moonnoon / Shutterstock

Hepatitis B virus (HBV) is a hepatotropic, enveloped, partially double-stranded DNA virus that causes chronic and acute hepatitis B infections in humans.

Types of Tissue Used

CXCL6

Front Med (Lausanne). 2021; 8: 683506.
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PMCID: PMC8317578
PMID: 34336890

Hepatic BRD4 Is Upregulated in Liver Fibrosis of Various Etiologies and Positively Correlated to Fibrotic Severity

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Liver Tissues :

Eight patients who underwent surgical resection for the treatment of **HBV-associated** liver cancer in the liver fibrosis group (n = 4) or **hemangioma** in the control group (n = 4)

Ref: Wu C, Cheng D, Peng Y, et al. Hepatic BRD4 Is Upregulated in Liver Fibrosis of Various Etiologies and Positively Correlated to Fibrotic Severity. Front Med (Lausanne). 2021;8:683506. Published 2021 Jul 14. doi:10.3389/fmed.2021.683506

Priori Hypothesis

- Inflammation is a major contributor to the pathogenesis of almost all liver diseases.
- Low-molecular-weight proteins called chemokines are the main drivers of liver infiltration by immune cells such as macrophages, neutrophils and others during an inflammatory response.
- CXC- motifs are expected to be upregulated in the liver fibrosis group.

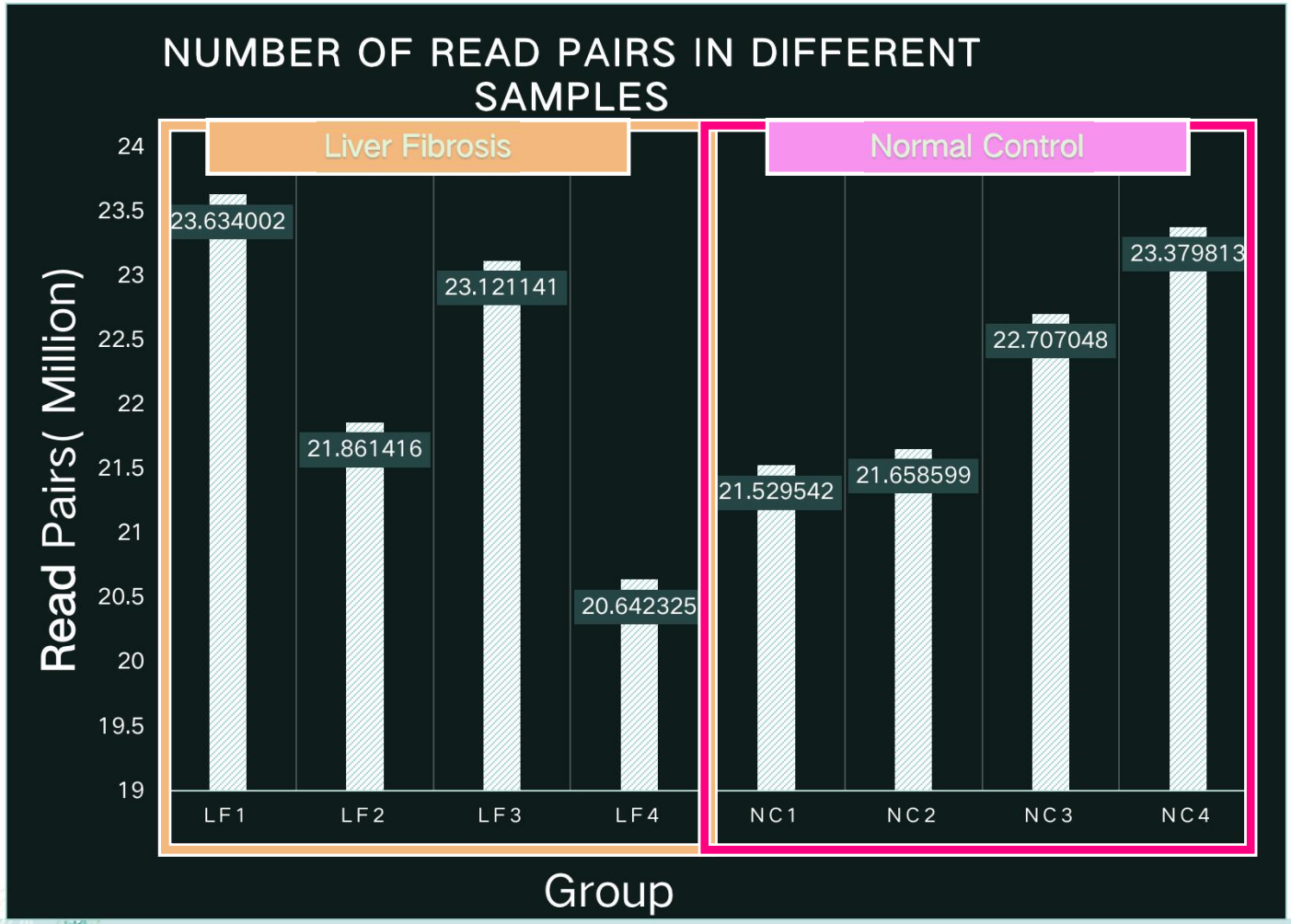
Sequencing Source

- 8 patients
- Liver Fibrosis Group(n=4): HBV-associated
- Control Group(n=4)
- Sequencing data source: (GEO: GSE171294)
- Sequencing reads: Paired end

- Total RNA Extracted -- the RNeasy Mini Kit
- Generate Sequencing Libraries -- UltraTM RNA Library Prep Kit for Illumina
- 150 bp paired-end reads were generated
- 2 FASTQ files for each sample

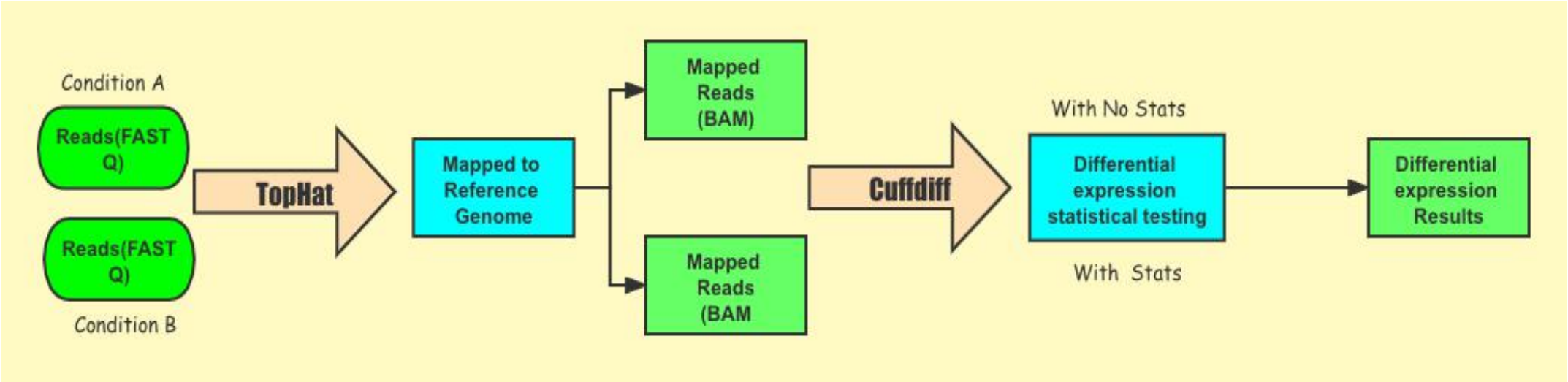
Samples	Group	Single or Paired End	SRA
GSM5222239	Normal Control 1	Paried End	SRX10498444
GSM5222240	Normal Control 2	Paried End	SRX10498445
GSM5222241	Normal Control 3	Paried End	SRX10498446
GSM5222242	Normal Control 4	Paried End	SRX10498447
GSM5222243	LIver Fibrosis 1	Paried End	SRX10498448
GSM5222244	LIver Fibrosis 2	Paried End	SRX10498449
GSM5222245	LIver Fibrosis 3	Paried End	SRX10498450
GSM5222246	LIver Fibrosis 4	Paried End	SRX10498451

Pre-alignment sequencing metrics



Maximum (M)	LF1	23.634002
Minimum (M)	LF4	20.642325
Average (M)		22.31673575

Processing Pipeline



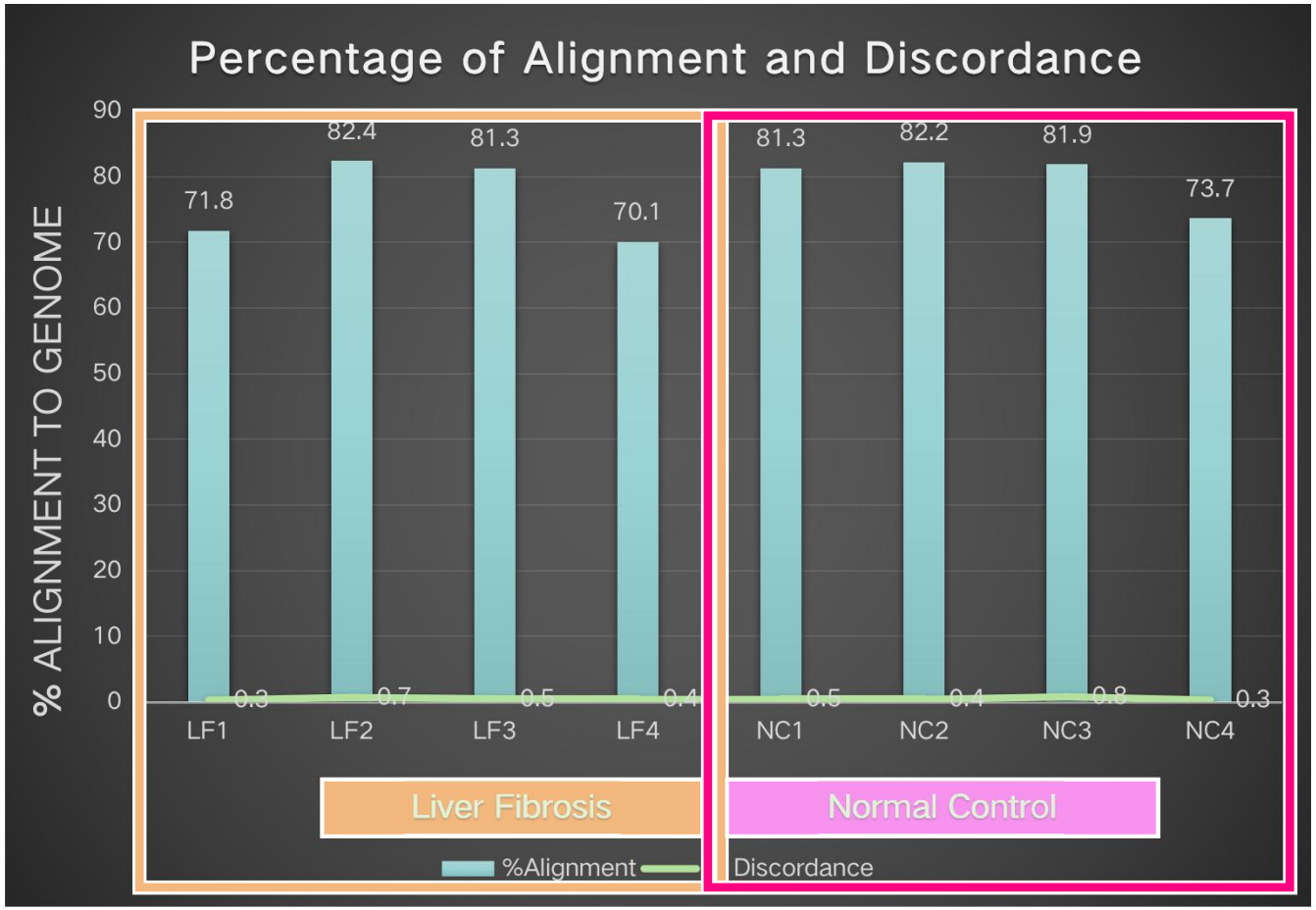
Ref:Homo_sapiens.GRCh38.104.chr.gtf
ensembl.GRCh38.104.fa

Processing Pipeline

	Alignment	Counting	Differential Gene Expression Analysis
Tool	TopHat 2	Cuffdiff ((No Stats)	Cuffdiff(With Stats)
Input	FASTQ files	BAM from alignment	BAM from alignment
Output	BAM files	genes.fpkm_tracking	gene.exp_diff

- genes.fpkm_tracking : FPKM values for ALL the samples and columns reflect the labels (-L)
- gene.exp_diff : statistical tests for two groups and columns reflect the group labels (-L)

Alignment Metrics and Statistics



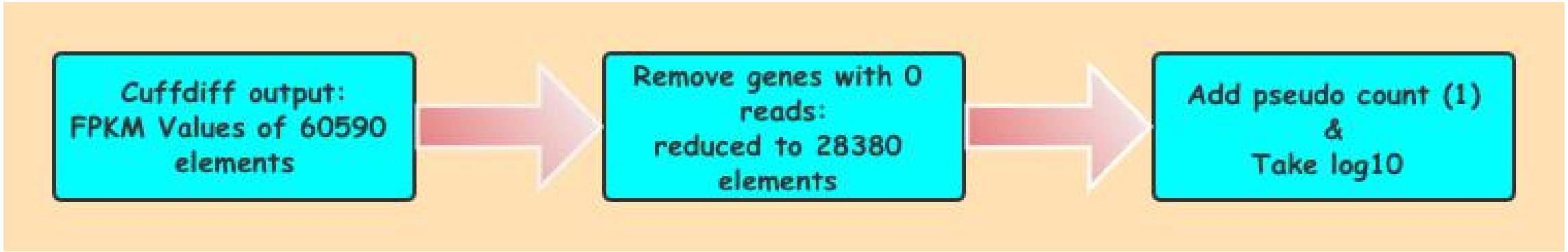
% Alignment

Maximum (%)	LF2	82.4
Minimum (%)	LF4	70.1
Average(%)	78.0875	

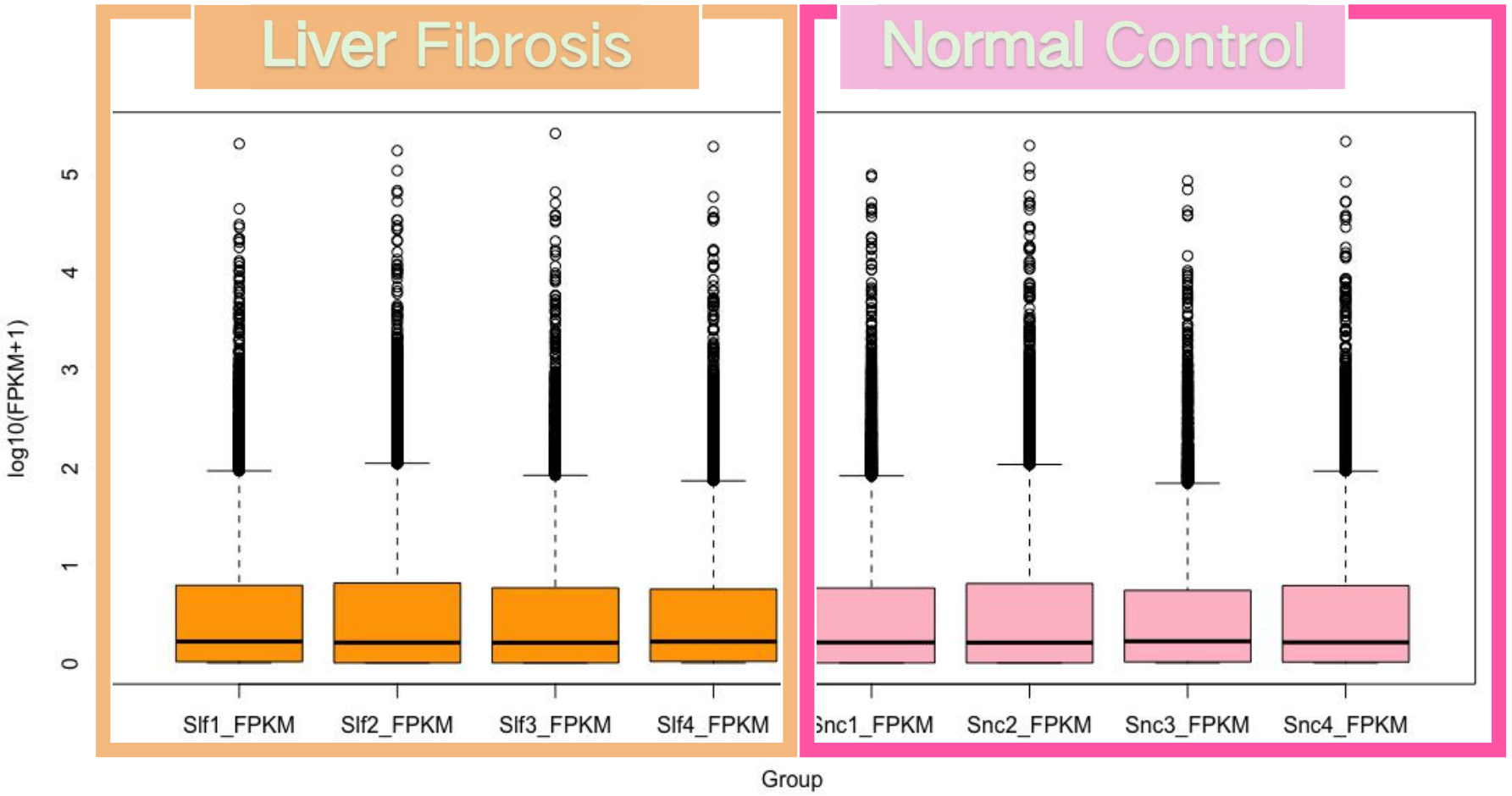
% Discordance

Maximum %	NC3	0.8
Minimum %	LF1/NC4	0.3
Average	0.4875	

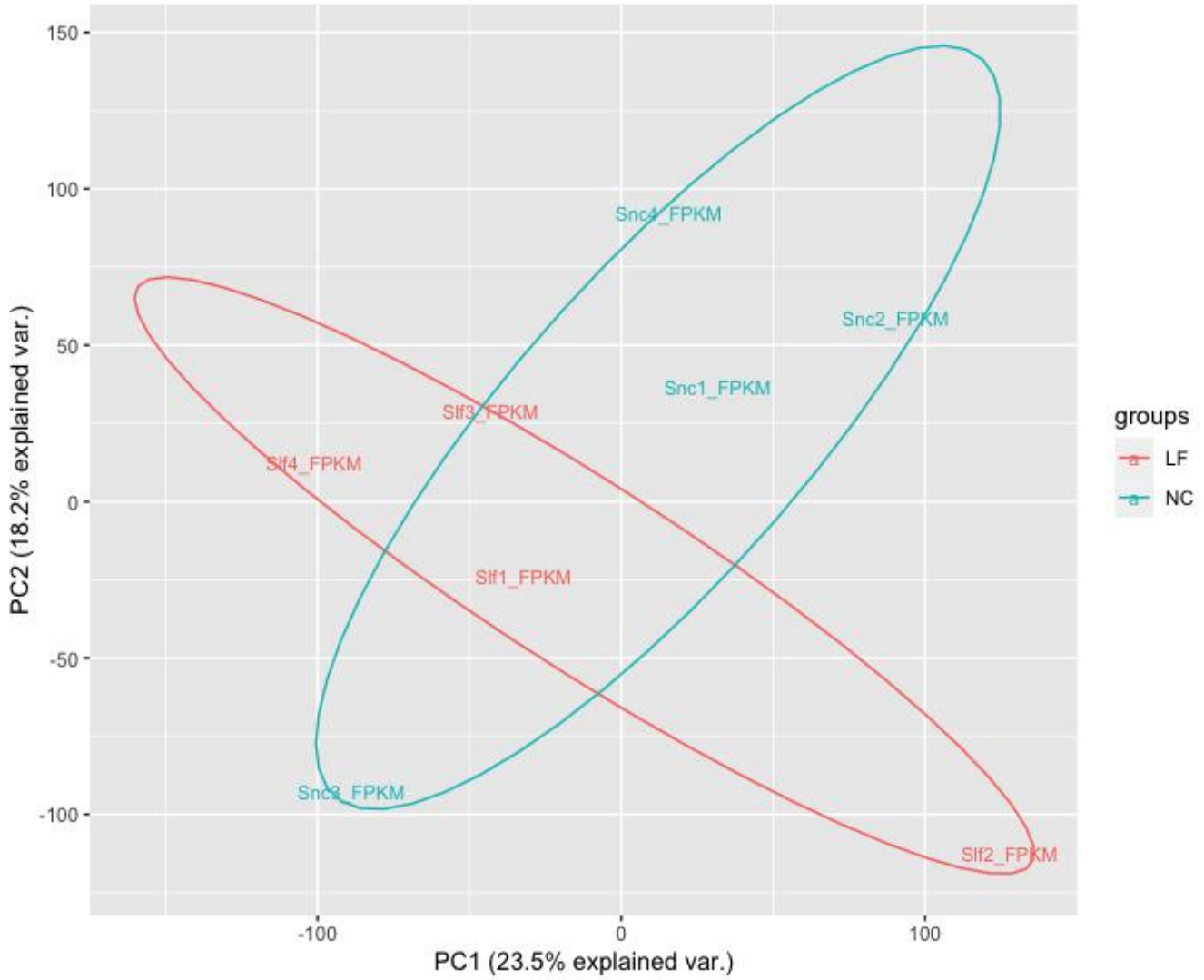
Data Pre-Processing



Box Plot : Filter Samples(28380 elements)



PCA Plot：Filter Samples(28380 elements)



DIFFERENTIAL EXPRESSION GENES SELETION

ABS(Log2Fold Difference) >= 1.5

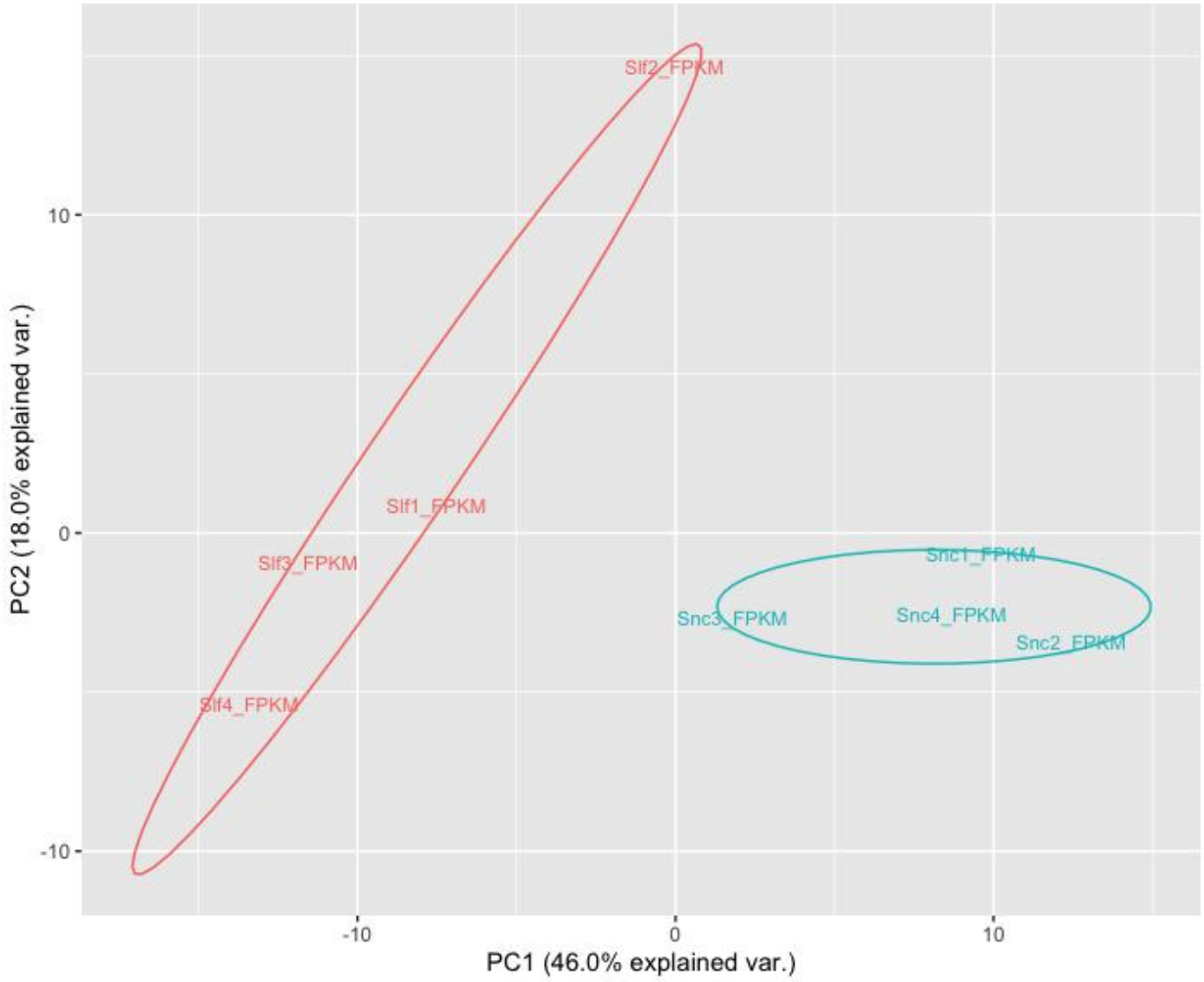
P-Value <= 0.01

Result: 216 filtered genes

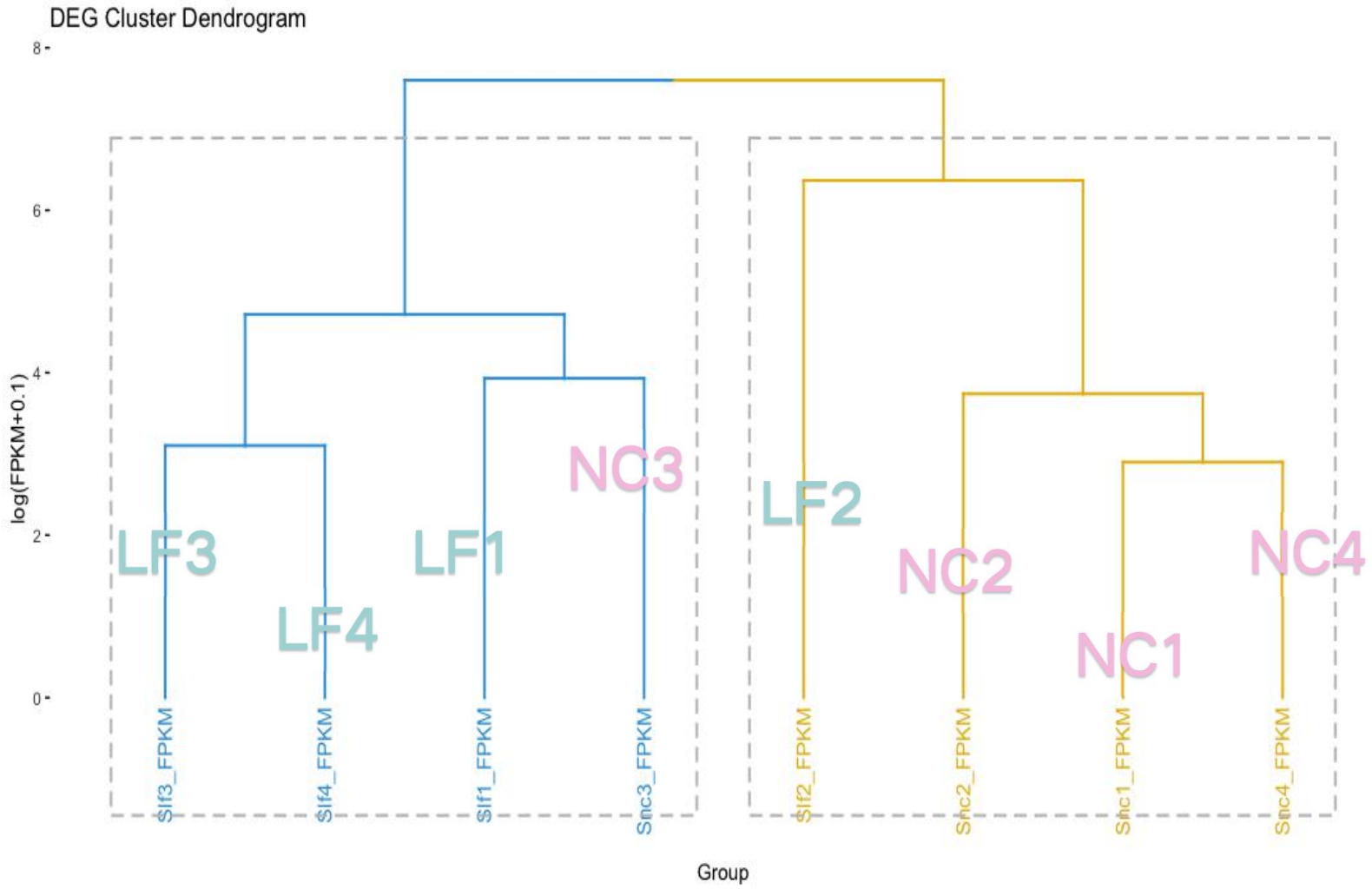
- 125 upregulated, 91 downregulated

PCA Plot :

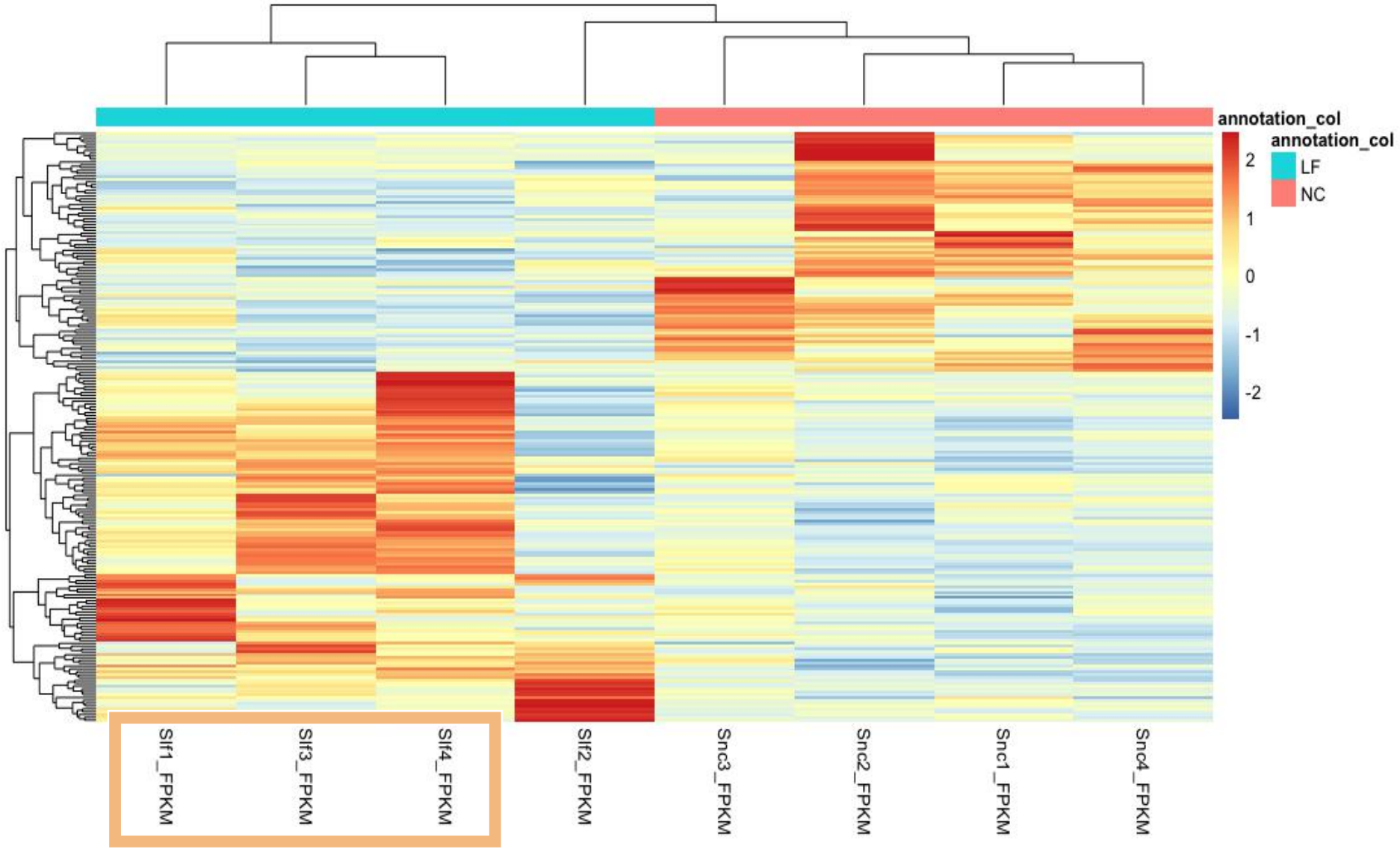
Differential Expressed Genes (DEG : 216 filted genes)



Cluster Dendrogram: Differential Expressed Genes
(DEG : 216 filted genes)



HeatMap : Differential Expressed Genes
(DEG : 216 filted genes)



IPA PATHWAY ANALYSIS

Differential Expressed Genes(DEGs):

pvalue <= 0.5 & abs(log2foldchange >=1.5)

216DEGs: 125 upregulated,
91downregulated

Upregulated Pathway

Disease of Functions Annotation	P-Value	Molecules
Inflammation of Organ	0.00037	CD79A,CRP,CXCL10,CXCL13,CXCL6,CXCL9,CXCR3,CXCR4,DUSP2,FAM153A/FAM153B,IGHG1,IGKC,IL32,PDCD1,PLA2G2A,SOCS1,UBD

Consistent with the priori hypothesis CXC- motifs are expected to be upregulated in the liver fibrosis group

CXCR3 is the associated Receptor of CXCL9 & CXCL10&CXCL11

The anti-fibrotic and anti-angiogenic CXCR3 is the key receptor on T cells, NK cells and possibly HSCs, with CXCL9 , CXCL10, CXCL11 as its ligands in the liver

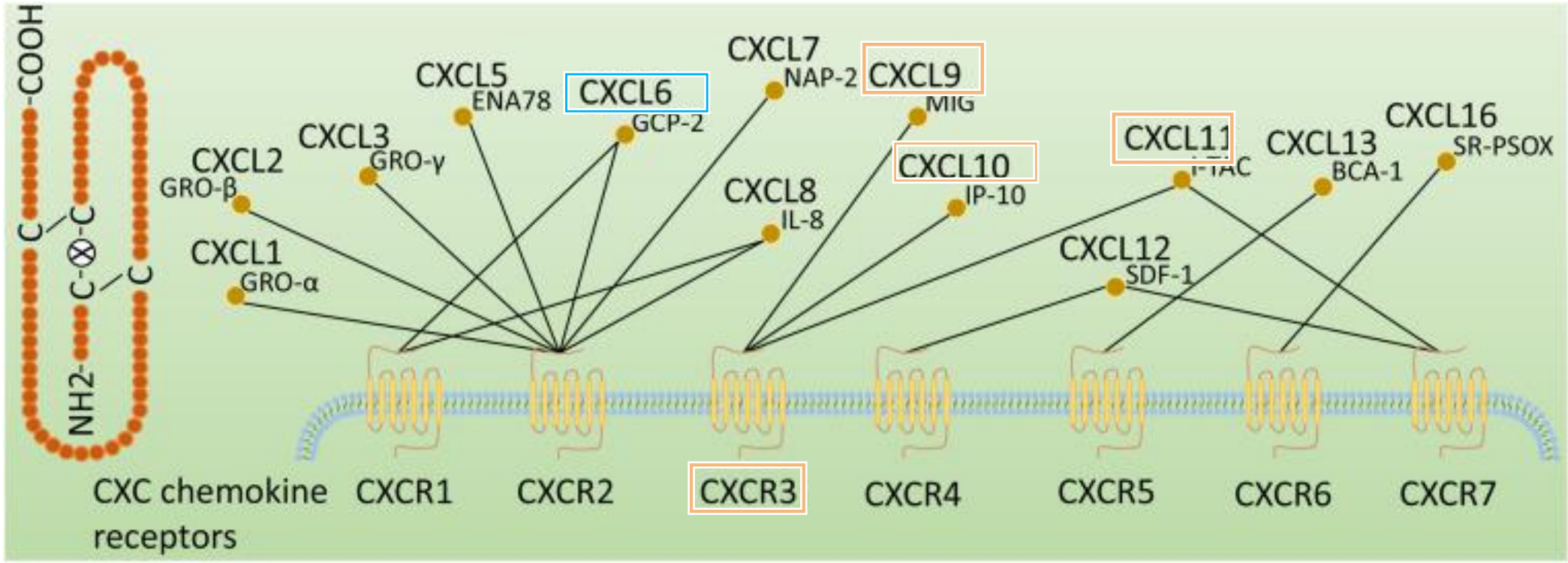
Table 1 List of chemokines and their receptors in the liver

From: Regulation and functional roles of chemokines in liver diseases

Standard name of chemokine ligand	Alternate name	Associated receptor	Key immunoregulatory functions
CXC			
CXCL1	Human: GROa, MGSA Mouse: KC	CXCR2	Neutrophil trafficking
CXCL2	Human: GROb, MIP2α Mouse: MIP2	CXCR2	Neutrophil trafficking and transendothelial migration
CXCL5	Human: ENA78 Mouse: LIX	CXCR2	Neutrophil trafficking
CXCL6	GCP2	CXCR1 and CXCR2	Neutrophil–monocyte trafficking
CXCL8	IL-8	CXCR1 and CXCR2	Neutrophil–monocyte trafficking
CXCL9	MIG	CXCR3	T_H1 immune response
CXCL10	IP-10	CXCR3	T_H1 immune response
CXCL11	ITAC	CXCR3	T_H1 immune response
CXCL12	SDF1	CXCR4 and CXCR7	Hepatic stellate cells, liver sinusoidal endothelial cells, hepatocellular carcinomas
CXCL13	BLC, BCA1	CXCR5	B cells
CXCL16	SRPSOX	CXCR6	Natural killer T cells

Ref: Cao, S., Liu, M., Sehrawat, T.S. et al. Regulation and functional roles of chemokines in liver diseases. Nat Rev Gastroenterol Hepatol 18, 630 – 647 (2021). https://doi.org/10.1038/s41575-021-00444-2

Classification of CXC chemokine receptors



Ref:Wang S, Gao S, Li Y, Qian X, Luan J, Lv X. Emerging Importance of Chemokine Receptor CXCR4 and Its Ligand in Liver Disease. Front Cell Dev Biol. 2021;9:716842. Published 2021 Jul 27. doi:10.3389/fcell.2021.716842

Cuffdiff Results of CXCL11,CXCL10, CXCL9&CXCR3

Gene	Sample 1	Sample 2	Status	Value1	Value2	log2(FoldChange)	P-Value
CXCL10	Liver Fibrosis	Normal Control	OK	68.3166	7.70311	-3.14872	0.00005
CXCL9	Liver Fibrosis	Normal Control	OK	56.4557	5.66805	-3.31619	0.00115
CXCR3	Liver Fibrosis	Normal Control	OK	1.91006	0.543254	-1.81392	0.00845
CXCL11	Liver Fibrosis	Normal Control	OK	2.55493	0.584771	-2.12734	0.2626

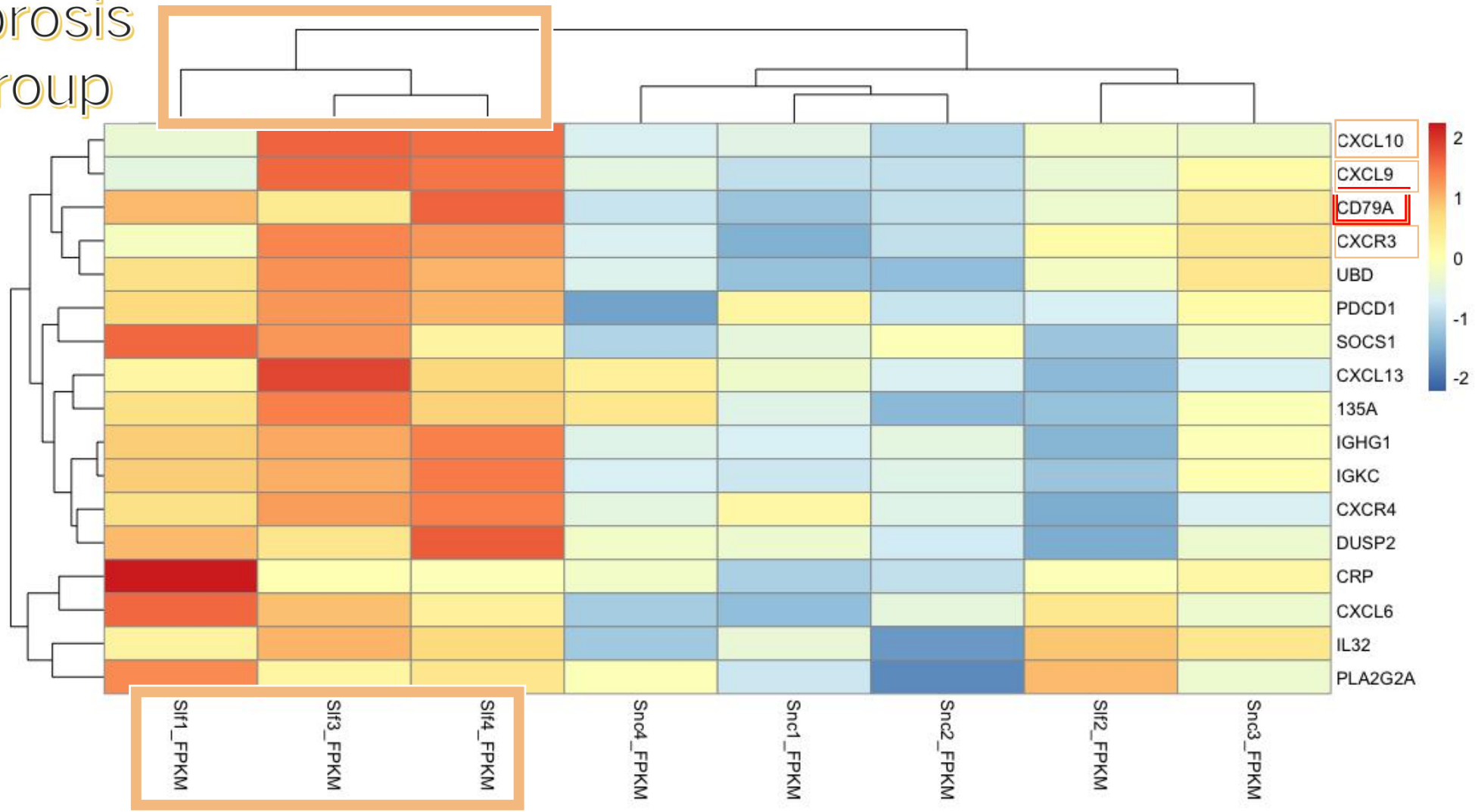
Funtion of CXCL9/10/11 in HBV

- CXCL9, CXCL10, and CXCL11 are induced by HBV-positive hepatocytes with coexisting NK cells and pDCs, which synergistically produce IFN-α and IFN-γ in response to HBV.
- CXCL9 is induced by IFN-γ but not by IFN-α/β. CXCL10 is strongly induced by IFN-γ as well as IFN-α/β . CXCL11 is induced by IFN-γ and IFN-β, and weakly by IFN-α.
- CXCL9, CXCL10, and CXCL11 Chemokines can be regarded as Predictors of Liver Fibrosis.

Ref : Yoshio S, Mano Y, Doi H, et al. Cytokine and chemokine signatures associated with hepatitis B surface antigen loss in hepatitis B patients. JCI Insight. 2018;3(20):e122268. Published 2018 Oct 18. doi:10.1172/jci.insight.122268

HeatMap of Pathway : Inflammation of Organ

Liver Fibrosis Group



Cuffdiff and FPKM Results of CD79A

Gene	LF1_FPKM	LF2_FPKM	LF3_FPKM	LF4_FPKM	NC1_FPKM	NC2_FPKM	NC3_FPKM	NC4_FPKM
CD79A	10.423	2.98631	6.06395	19.2448	1.29646	1.72012	5.60079	1.76409

Gene	Sample1	Sample2	Status	Value1	Value2	log2(FoldChange)	P-Value
CD79A	Liver Fibrosis	Normal Control	OK	10.0787	2.96656	-1.76445	0.00025

CD79A

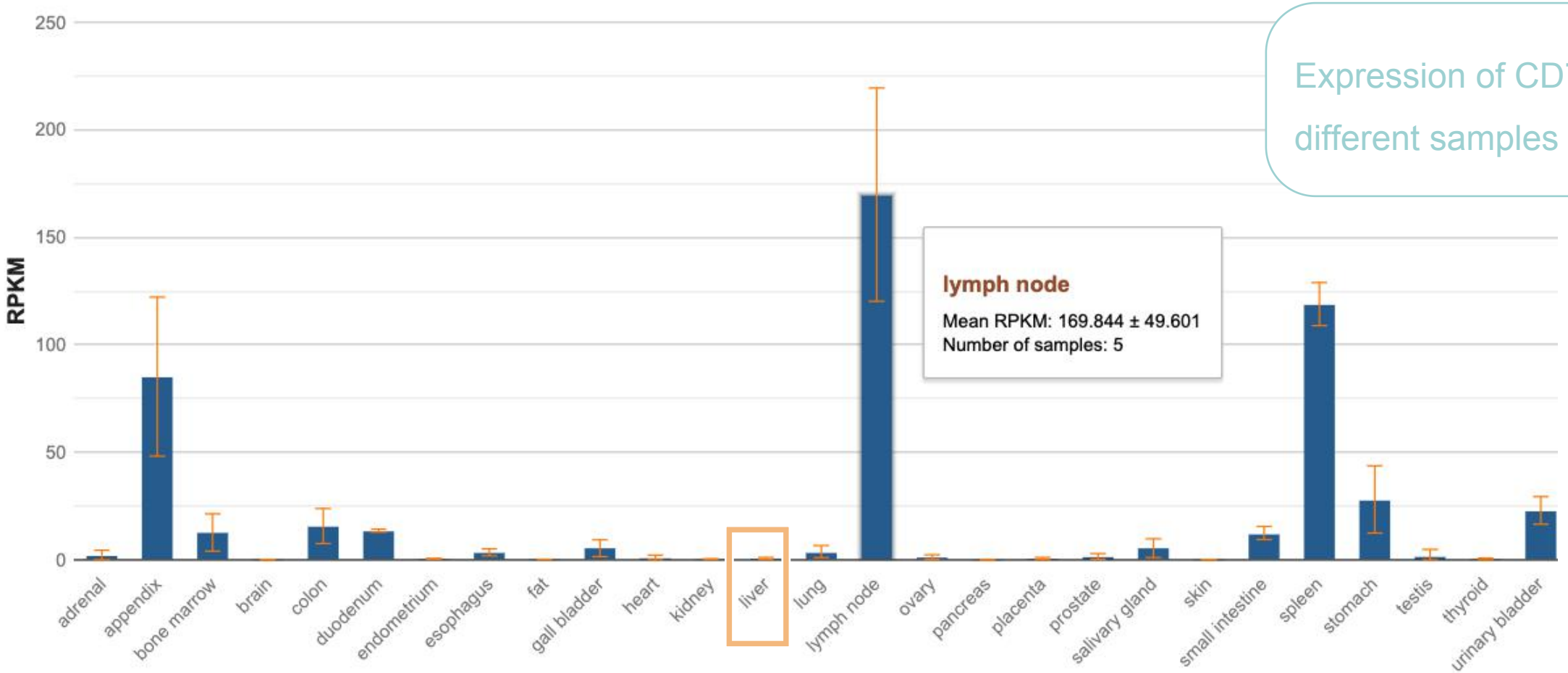
The B lymphocyte antigen receptor is a multimeric complex that includes the antigen-specific component, surface immunoglobulin (Ig). Surface Ig non-covalently associates with two other proteins, Ig-alpha and Ig-beta, which are necessary for expression and function of the B-cell antigen receptor. This gene encodes the Ig-alpha protein of the B-cell antigen component. [provided by RefSeq, Jul 2008]

New Hypothesis

CD79A maybe also correlated with liver fibrosis and can possibly be as predictors for HBV

HPA RNA-seq normal tissues

- Project title: HPA RNA-seq normal tissues
- Description: RNA-seq was performed of tissue samples from 95 human individuals representing 27 different tissues in order to determine tissue-specificity of all protein-coding genes
- BioProject: PRJEB4337
- Publication: PMID 24309898
- Analysis date: Wed Apr 4 07:08:55 2018



Expression of CD79A in different samples

Ref:https://www.ncbi.nlm.nih.gov/gene/973#gene-expression

Functions of Lymph nodes in Liver Fibrosis

- Lymph nodes are well known to exist in the hepatoduodenal ligament.
- In ultrasound study, enlarged lymph nodes could be demonstrated in the hilus hepatis of almost all patients with CHB or CHC.
- Enlarged lymph nodes can be a good indicator of inflammatory activity by the liver in CHB,especially those wider than 5 mm, suggested chronic HBV or HCV infection.
- Several effective medications are available to inhibit HBV replication with liver fibrosis regression by reducing liver inflammation and cellular damage in most patients with chronic hepatitis B (CHB).
- The assessment of liver necroinflammatory activity and fibrosis for patients with CHB is helpful for determining prognosis and treatment strategy.

The expression of CD79A might used to evaluated the degree of liver fibrosis

Ref:Shu J, Zhao JN, Han FG, et al. Chronic hepatitis B: Enlarged perihepatic lymph nodes correlated with hepatic histopathology. World J Radiol. 2013;5(5):208-214. doi:10.4329/wjr.v5.i5.208

Summary

- The IPA anlysis results show that many pathways related to inflammation are up-regulated.
- In the Inflammation of Organ pathway , CXCL9, CXCL10, CXCL11 and CXCR3 are up-regulated which is consistent with previous studies.
- In the Inflammation of Organ pathway , CXCL6,PLA2G2A and IL32 are up-regulated which is consistent with previous studies[1][2][3].
- CD79A in the in the Inflammation of Organ pathway is up-regulated similarly as CXCL9, CXCL10, CXCL11 and CXCR3 . It maybe also correlated with liver fibrosis which could be useful for evaluating the degree of patients with liver fibrosis.

Protein anaysis CD79A
Ig-alpha protein of the B-cell antigen component
Immunoassay

[1]Wu C, Cheng D, Peng Y, et al. Hepatic BRD4 Is Upregulated in Liver Fibrosis of Various Etiologies and Positively Correlated to Fibrotic Severity. Front Med (Lausanne). 2021;8:683506. Published 2021 Jul 14. doi:10.3389/fmed.2021.683506

[2]Zhu C, Song H, Shen B, Wu L, Liu F, Liu X. Promoting effect of hepatitis B virus on the expressoin of phospholipase A2 group IIA. Lipids Health Dis. 2017 Jan 11;16(1):5. doi: 10.1186/s12944-016-0400-7. PMID: 28077172; PMCID: PMC5225502.

[3]Xu Q, Pan X, Shu X, Cao H, Li X, Zhang K, Lu J, Zou Y, Li X, Liu H, Zhang Y, Yang D, Ning Q, Shen G, Li G. Increased interleukin-32 expression in chronic hepatitis B virus-infected liver. J Infect. 2012 Oct;65(4):336-42. doi: 10.1016/j.jinf.2012.05.009. Epub 2012 Jun 8. PMID: 22687868.

THANK YOU