Liver Tumor Survival Analysis

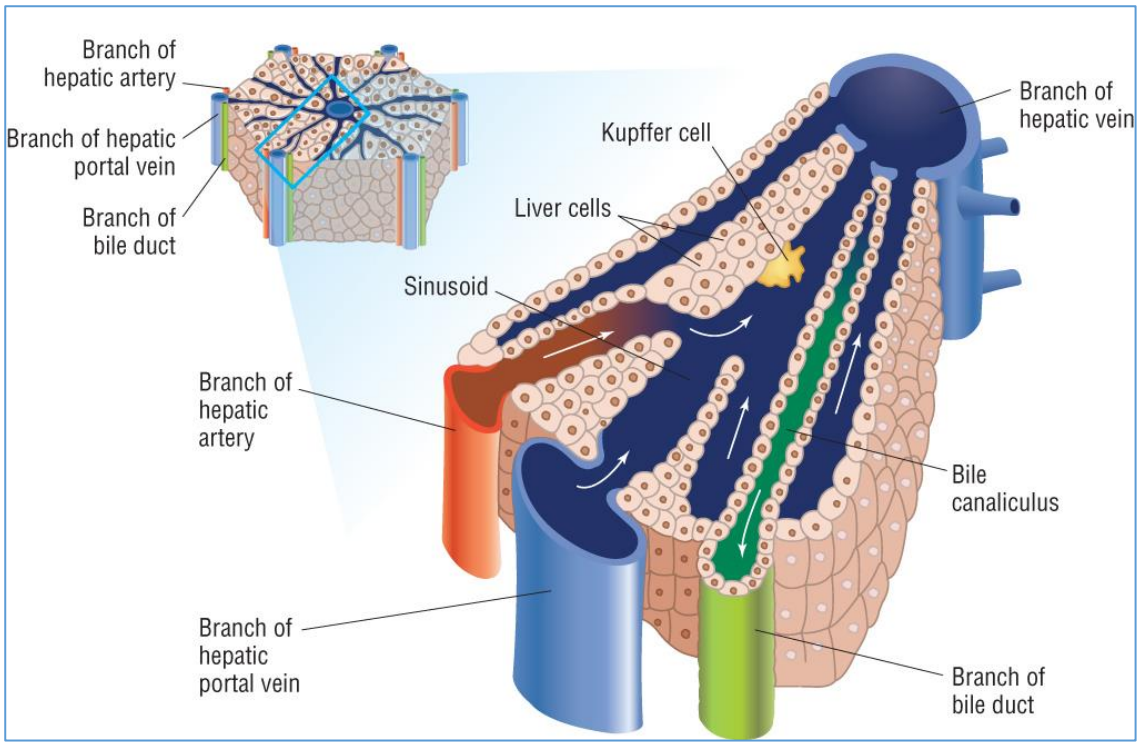
Dina Mansour Aly

Introduction

Liver represents a vital organ in the human body. Hepatocytes (liver cells) play a key role in many enzymatic metabolic pathways; carbohydrate metabolism especially blood glucose homeostasis by monitoring gluconeogensis and glycogenolysis according to the blood sugar concentration, fat metabolism; beta oxidation of fatty acid, de novo cholesterol synthesis, de novo fat synthesis (phospholipids), protein metabolism; non-essential amino acid synthesis, deamination of aminoacids, protein synthesis (serum albumin, fibrinogen, clotting factors) and protein storage as well as urea and bile metabolism and detoxification of foreign chemicals (1-5).

Liver Architecture

Liver has a fascinating architecture [Figure 1]. Hepatocytes form 80% of the liver parenchymal tissue, polygonal cells characterized by high amount of rough endoplasmic reticulum (has ribosomes; protein factories), smooth endoplasmic reticulum, golgi apparatus, and mitochondria that makes them efficient and professional metabolics. Hepatocytes connect as anastomosing plates with borders that are in contact with other hepatocytes or with fenestrated endothelial cells lined sinisoids. which are vascular channels and have phagocytic Kupffer cells, Space of Disse, a lympahatic collecting space which collects lymph for delivery to lymphatic capillaries. Liver connective tissue divide the liver into unique hexagonal shaped lobules with portal triads at the vertics and central vein. Lobule is the structural unit of the liver.



Figur 1: Liver Architecture (6)

Liver cells has unique cell division and repair. Mutations in the liver DNA has a dramatic effect on the liver function and hence the overall health of the body. Most common liver disease are viral hepatitis (A,B,C,D,E), cirrhosis, nonalcoholic fatty liver, liver failure. Liver cancers are the third common cancers in developing countries and the sixth in developed countries.

Liver cancer is the third life-threatening disease due to its very poor prognosis. Next generation sequencing technologies has enabled the DNA analysis of patients with liver tumors. Differential transcript expression analysis helps to determine which proteins are affected in different liver tumor subtypes and can be used for furthur analysis to determine the pathways affected or that cause liver tumors. Understanding the genes, proteins and the metabolic pathways that are affected in liver tumors makes classifying of liver tumors based on genetic makeup and gives a driving force for the development of new target agents to save the liver (7).

Liver cancer classification

Liver tumors are classified to primary liver cancer (PLC), mesenchymal cancers of the liver and secondary liver cancers. Hepatocellular carcinoma (HCC) being the most common tumor of PLC, followed by cholangiocarcinoma. Fibrolamellar carcinoma (FLC) is an uncommon primary liver tumor affecting young patients. Hepatoblastoma (HB) is the most common primary liver tumor in childhood. Liver Angiosarcoma (ASL) and Epitheloid hemangioendothelioma are mesenchymal cancers of the liver. Breast cancer, lung cancer and colorectal cancers are the common primary tumors for secondary liver cancers by metastasis (8).

Risk factors

Viral hepatitis (B,C), alcohol consumption, nonalcoholic fatty liver, hemochromatosis, aflatoxin, diabetes mellitus, obesity and smoking are the most common risk factors for hepatocellular carcinoma. Hepatitis C, hepatolithiasis, liver fluke infection, primary sclerosing cholangitis, and thorotrast are known risk factors for cholangiocarcinoma. . Fibrolamellar carcinoma is not associated with the common HCC risk factors (9).

Liver Tumor Genetic Data

RNAseq Dataset

Pathology atlas data TCGA is downloaded for 336 individuals and 816 transcripts expression in each individual. The liver tumor individuals' demographic data are shown in Table 1.

Table 1. Liver tumor dataset demographics.

Parameter	Frequency
Gender - Males	- 246
- Females	- 119
Age - Youth-group (< 30 years)	- 13
- Adult-group (30-60 years)	- 362
- Old-group (>60 years)	- 197
Race - American indian or alaska native	- 1
-Asian	- 155
-White	- 182
-Black or african American	- 17
- not reported	- 10
Stage -Stage I	- 170
-Stage II	- 84
-Stage III	- 83
-Stage IV	- 4
-Not reported	- 24
Transcripts	- 816

Methods

Liver tumor RNAseq data was analysed in R using packages “survival” and “survminer”(10,11). Kaplan-Meier survival model was applied to the whole dataset, geneder, stage and race to calculate the median survival. Cox regression analysis was done in R for the whole dataset using all four covariates; gender, age, stage and race. Score_test p-values were corrected for multiple comparisons by FDR. The transcripts were sorted by p-values and q-values. Top differentially expressed transcripts were annotated by NCBI_Gene database (12). Pathway analysis using the top transcripts was run in Reactome (13). The dataset was then subset based on age to three sets: youth, adult and old. All the above analysis was run for each age group and the top differentially expressed transcripts were annotated and used for pathway analysis. Races in liver dataset were Afro_American, White and Asian; Black and non_reported were excluded from further analysis due to small sample size. The liver data was subset based on the races to three groups; Asian, Afro_american and White. The analysis was rerun as before for each race_group. Staging in liver tumor is very important parameter to determine prognosis of the disease. Three stage groups were formed; Stage_I, Stage_II and Stage_III (including all stage III; stage iiia, iiib and iiic). Stage_IV were excluded from further analysis (sample size).

Results

Kaplan Meier Survival analysis

1- Kaplan Meier analysis of all Liver_tumor_data:

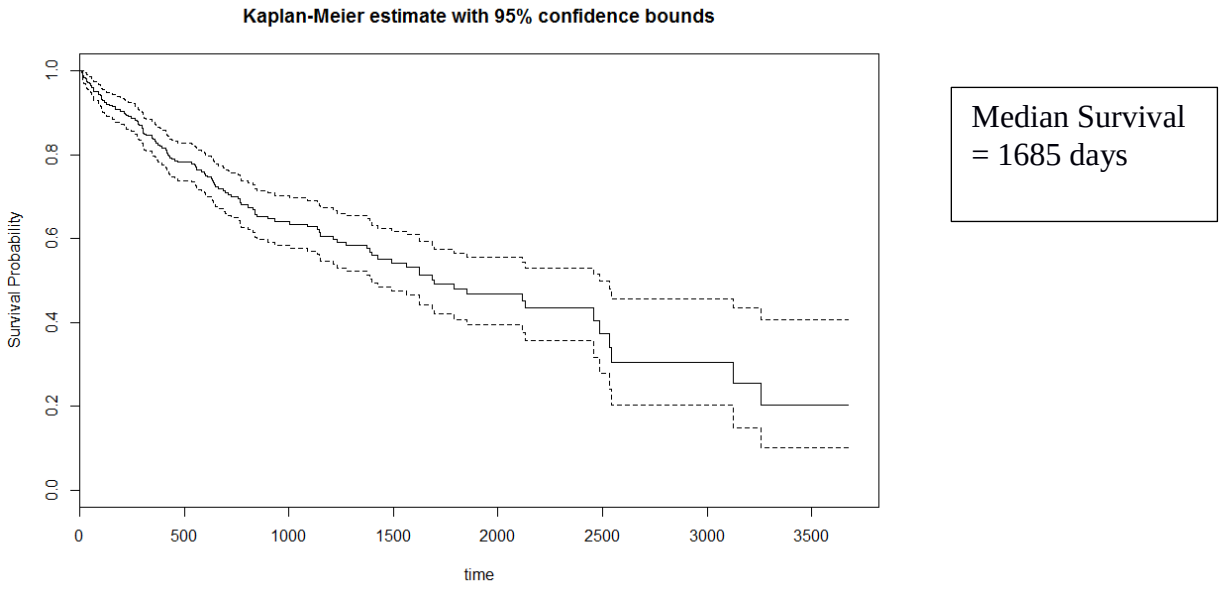


Figure 2: Kaplan Meier Survival fit of Liver Tumor dataset.

Median survival in all liver samples without any categorization was 1685 days.

2- Kaplan Meier analysis of liver tumor data in different gender:

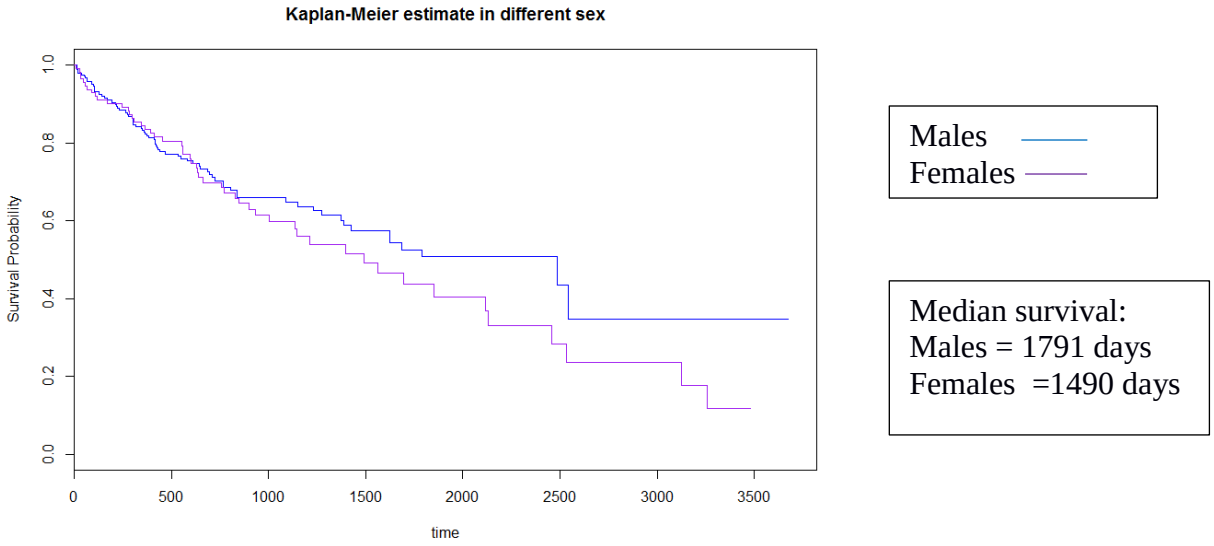


Figure 3: Kaplan Meier Survival fit of Liver Tumor dataset in different gender.

Median survival in males and females was 1791 days and 1490 days.

3- Kaplan Meier analysis in different stages:

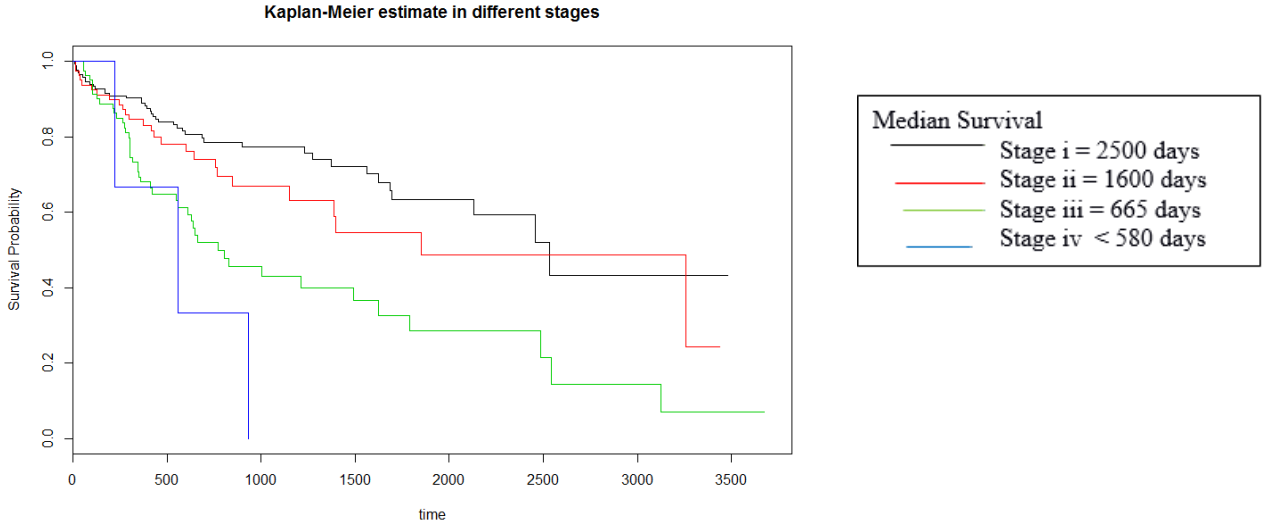


Figure 4: Kaplan Meier Survival fit of Liver Tumor dataset in different stages.

Median survival in Stage I is 2500 days, Stage II 1600 days, Stage III 665 days and Stage IV less than 580 days.

4- Kaplan Meier in different races:

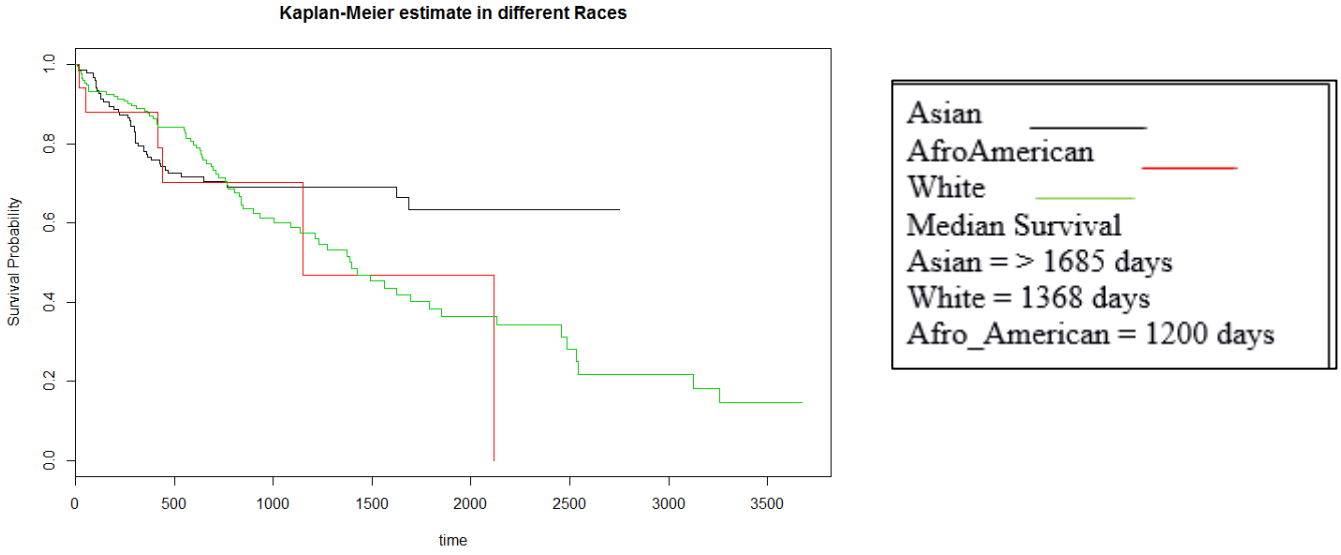


Figure 5: Kaplan Meier Survival fit of Liver Tumor dataset in different races.

Median survival in Asian population is more than 1685 days, White is about 1368 days and in Afro_americans is about 1200 days**.**

5- Kaplan Meier in different age-groups:

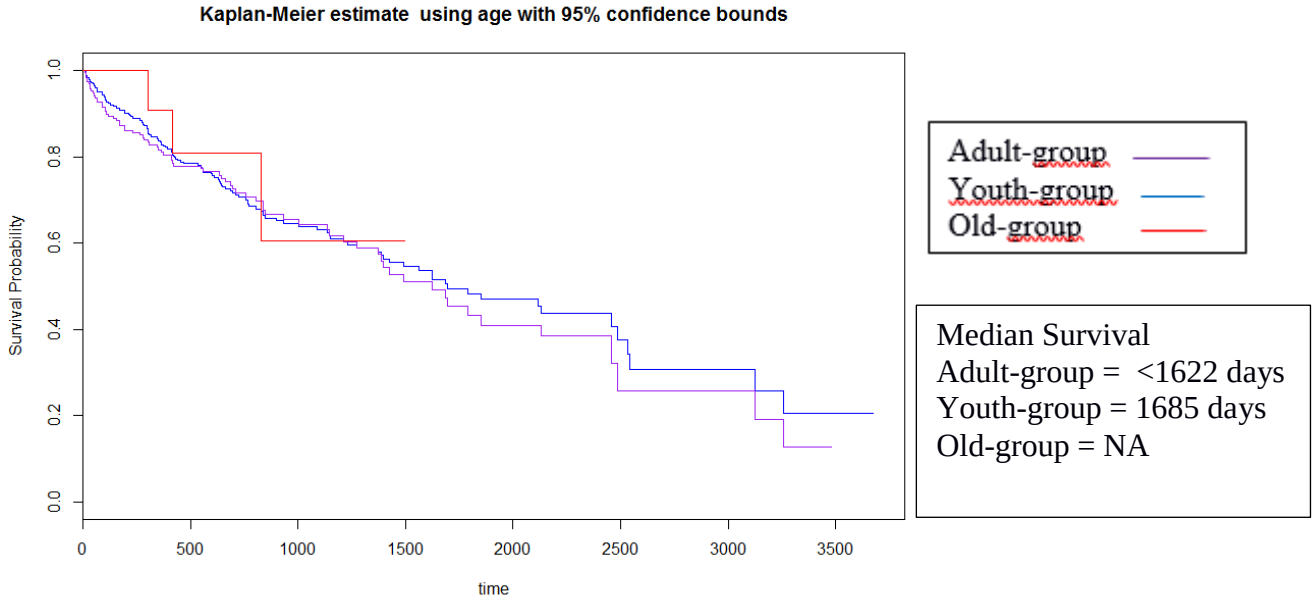


Figure 6: Kaplan Meier Survival fit of Liver Tumor dataset in different age groups.

Median survival in Youth-group population is more than 827 days, Adult-group and in Old-group.

Table 2. Median Survival of Liver tumor individuals

Group	Median Survival (days)	SE	CI (95%)
ALL	1685	0.03869	0.431 0.583
Males	1791	0.04997	0.418 0.616
Females	1490	0.06218	0.38290.629
Youth-group	1685	0.1976	0.320 1
Adult-group	< 1622	0.03887	0.434 0.587
Old-group	NA	NA	NA
Stage I	< 2532	0.10902	0.264 0.709
Stage II	< 1852	0.0929	0.3338 0.706
Stage III	660	0.0725	0.3803 0.668
Stage IV	NA	NA	NA
White	1386	0.05128	0.4091 0.612
Asian	NA	NA	NA
Afro_American	NA	NA	NA 1

Cox Regression Analysis

1- Cox Regression of Survival using age, gender, stage and race as covariates:

Top GWAS significant differentially expressed transcripts were on the genes; ***NCAPD2, ANLN, NOP58, FUCA2, DBF4, VRK2 and FAM136A*** ($p < 5e-08$) [Table 3]. Top pathways affected in liver tumor; SUMOylation of RNA binding proteins, Plasmalogen biosynthesis, nuclear envelop breakdown, HDACs deacetylate histones, regulation of PTEN gene transcription, negative regulation of TCF-dependent signaling by DVL-interacting proteins and cell cycle mitosis [Figure 7]

Table 3. Top significant Differentially expressed transcripts in liver tumor samples.

Transcript_ID	Gene_coding	mean_expression	sc_all_cov_P	sc_FDR
ENSG00000010292	***NCAPD2***	2.4760328105841	3.9387848538297e-10	1.06741069538785e-07
ENSG00000011426	***ANLN***	3.98177489501828	9.55974743810373e-10	1.94301866679458e-07
ENSG00000055044	***NOP58***	13.1395426473703	1.5278419640552e-09	2.48427103355376e-07
ENSG00000001036	***FUCA2***	4.3691496147043	2.53059118104915e-09	3.4289510503216e-07
ENSG00000006634	***DBF4***	4.53422080012747	2.96812863176399e-09	3.44726939660589e-07
ENSG00000028116	***VRK2***	1.59638074915355	5.52628931504984e-09	5.6160915164194e-07
ENSG00000035141	***FAM136A***	18.9408532553226	7.43444894535372e-09	6.71578554730286e-07
ENSG00000057608	***GDI2***	2.9933462787894	1.33060937823615e-08	1.08178542450599e-06
ENSG00000023909	***GCLM***	2.76541465545887	1.78266791328596e-08	1.31755364863771e-06
ENSG00000058804	***NDC1***	4.24041585547021	1.9978824261635e-08	1.35356534372577e-06
ENSG00000004487	***KDM1A***	34.7931889120134	2.97065685384013e-08	1.45731176349946e-06
ENSG00000009844	***VTA1***	1.25381663644389	2.34891205375121e-08	1.45731176349946e-06
ENSG00000018699	***TTC27***	1.91420168041969	2.70119481227127e-08	1.45731176349946e-06
ENSG00000025156	***HSF2***	11.1142505760055	3.04726937017108e-08	1.45731176349946e-06
ENSG00000033050	***ABCF2***	1.81746063109551	2.9044138316614e-08	1.45731176349946e-06
ENSG00000057935	***MTA3***	0.347566773165742	6.57435664797035e-08	2.96941775266661e-06
ENSG00000004975	***DVL2***	32.5565700495215	7.78168753878816e-08	3.01262474715942e-06
ENSG00000018510	***AGPS***	1.86810384596852	7.67337593465811e-08	3.01262474715942e-06

Description | Molecules | Structures | Expression | Analysis 135 | Downloads

Results for: UNIPROT (135) ▾ | Type: Overrepresentation | [Data: Gene_coding all liver_tumor_group] | Results | Identifiers not four

Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
SUMOylation of RNA binding proteins	2	49	0.004	2.88E-3	1.13E-1	3	4	0	Homo sapiens
Plasmalogen biosynthesis	1	2	0	3.26E-3	1.13E-1	2	2	0	Homo sapiens
Nuclear Envelope Breakdown	2	56	0.005	3.73E-3	1.13E-1	3	13	0.001	Homo sapiens
HDACs deacetylate histones	2	60	0.005	4.27E-3	1.13E-1	1	4	0	Homo sapiens
Regulation of PTEN gene transcription	2	60	0.005	4.27E-3	1.13E-1	3	15	0.001	Homo sapiens
Negative regulation of TCF-dependent signaling by DVL-interacting proteins	1	5	0	8.12E-3	1.13E-1	2	2	0	Homo sapiens
Cell Cycle, Mitotic	4	513	0.046	8.46E-3	1.13E-1	14	334	0.03	Homo sapiens
WNT mediated activation of DVL	1	8	0.001	1.3E-2	1.13E-1	4	4	0	Homo sapiens
SUMO E3 ligases SUMOylate target proteins	2	116	0.011	1.51E-2	1.13E-1	11	62	0.006	Homo sapiens
Mitotic Prophase	2	117	0.011	1.54E-2	1.13E-1	3	34	0.003	Homo sapiens
Cell Cycle	4	620	0.056	1.62E-2	1.13E-1	16	422	0.038	Homo sapiens
Clearance of Nuclear Envelope Membranes from Chromatin	1	10	0.001	1.62E-2	1.13E-1	1	1	0	Homo sapiens
SUMOylation	2	122	0.011	1.66E-2	1.13E-1	11	71	0.006	Homo sapiens
Condensation of Prometaphase Chromosomes	1	11	0.001	1.78E-2	1.13E-1	4	4	0	Homo sapiens
Glutathione synthesis and recycling	1	13	0.001	2.1E-2	1.13E-1	1	7	0.001	Homo sapiens
PTEN Regulation	2	139	0.013	2.12E-2	1.13E-1	3	56	0.005	Homo sapiens
M Phase	3	371	0.034	2.12E-2	1.13E-1	8	66	0.006	Homo sapiens
Nuclear Envelope Reassembly	1	14	0.001	2.26E-2	1.13E-1	1	3	0	Homo sapiens
Initiation of Nuclear Envelope Reformation	1	14	0.001	2.26E-2	1.13E-1	1	3	0	Homo sapiens
WNT5A-dependent internalization of FZD4	1	15	0.001	2.42E-2	1.13E-1	4	5	0	Homo sapiens

Figure 7. Reactome pathway analysis showing top affected pathways in liver tumor samples.

2- Cox Regression of Survival and liver tumor individual's age

2a- Cox Regression of Survival in all liver tumor individuals with different age:

Top differentially expressed transcripts suggestive-associated with the age of liver tumor individuals were on the genes; ***NCAPD2, ANLN, DBF4, NDC1, VRK2 and NOP58*** ($p < 5e-05$) [Table 4]. Top pathways affected in liver tumor; SUMOylation of RNA binding proteins, Nuclear envelop breakdown, cell cycle mitotic and SUMO E3 ligases SUMOylate target proteins [Figure 8].

Table 3. Top differentially expressed transcripts associated with Age in liver tumor samples

Transcript_ID	Gene_coding	mean_expression	sc_age_P_value	sc_age_FDR
ENSG00000010292	***NCAPD2***	2.4760328105841	3.80885190054414e-08	7.74149148785596e-06
ENSG00000011426	***ANLN***	3.98177489501828	3.19581188179541e-08	7.74149148785596e-06
ENSG00000006634	***DBF4***	4.53422080012747	8.42061907979641e-08	1.3691926623749e-05
ENSG00000058804	***NDC1***	4.24041585547021	3.08695377482238e-07	4.18282236488432e-05
ENSG00000028116	***VRK2***	1.59638074915355	1.17738782967614e-06	0.000136745186503815
ENSG00000055044	***NOP58***	13.1395426473703	1.35766770292811e-06	0.000137972980310069
ENSG00000033050	***ABCF2***	1.81746063109551	2.46697517913841e-06	0.000220336158679868
ENSG00000057608	***GDI2***	2.9933462787894	2.71016185338091e-06	0.000220336158679868
ENSG00000001036	***FUCA2***	4.3691496147043	4.58283550275329e-06	0.000338713205794402
ENSG00000018699	***TTC27***	1.91420168041969	5.20926933123e-06	0.000352927997190833
ENSG00000035141	***FAM136A***	18.9408532553226	5.87152946374125e-06	0.000367196419540126
ENSG00000004487	***KDM1A***	34.7931889120134	6.96516551756954e-06	0.000404477111841717
ENSG00000023909	***GCLM***	2.76541465545887	8.85294741337361e-06	0.00047982974980485

Description | Molecules | Structures | Expression | Analysis 111 | Downloads

Results for: UNIPROT (111) ▾ | Type: Overrepresentation | [Data: Gene_coding for transcripts associated with Age:] | Results | Identifiers not found:

Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
SUMOylation of RNA binding proteins	2	49	0.004	1.49E-3	9.04E-2	3	4	0	Homo sapiens
Nuclear Envelope Breakdown	2	56	0.005	1.94E-3	9.04E-2	3	13	0.001	Homo sapiens
Cell Cycle, Mitotic	4	513	0.046	2.38E-3	9.04E-2	14	334	0.03	Homo sapiens
Cell Cycle	4	620	0.056	4.73E-3	9.56E-2	16	422	0.038	Homo sapiens
SUMO E3 ligases SUMOylate target proteins	2	116	0.011	7.98E-3	9.56E-2	11	62	0.006	Homo sapiens
Mitotic Prophase	2	117	0.011	8.11E-3	9.56E-2	3	34	0.003	Homo sapiens
M Phase	3	371	0.034	8.44E-3	9.56E-2	8	66	0.006	Homo sapiens
SUMOylation	2	122	0.011	8.79E-3	9.56E-2	11	71	0.006	Homo sapiens
Clearance of Nuclear Envelope Membranes from Chromatin	1	10	0.001	1.17E-2	9.56E-2	1	1	0	Homo sapiens
Condensation of Prometaphase Chromosomes	1	11	0.001	1.29E-2	9.56E-2	4	4	0	Homo sapiens
Glutathione synthesis and recycling	1	13	0.001	1.52E-2	9.56E-2	1	7	0.001	Homo sapiens
Nuclear Envelope Reassembly	1	14	0.001	1.64E-2	9.56E-2	1	3	0	Homo sapiens
Initiation of Nuclear Envelope Reformation	1	14	0.001	1.64E-2	9.56E-2	1	3	0	Homo sapiens
HDMs demethylate histones	1	27	0.002	3.13E-2	9.56E-2	2	17	0.002	Homo sapiens
Activation of the pre-replicative complex	1	32	0.003	3.71E-2	9.56E-2	6	8	0.001	Homo sapiens
Regulation of Glucokinase by Glucokinase Regulatory Protein	1	34	0.003	3.93E-2	9.56E-2	1	5	0	Homo sapiens
Activated PKN1 stimulates transcription of AR (androgen receptor) regulated genes KLK2 and KLK3	1	36	0.003	4.16E-2	9.56E-2	3	11	0.001	Homo sapiens
Transport of the SLBP independent Mature mRNA	1	37	0.003	4.27E-2	9.56E-2	3	3	0	Homo sapiens
Nuclear import of Rev protein	1	37	0.003	4.27E-2	9.56E-2	2	7	0.001	Homo sapiens
Activation of ATR in response to replication stress	1	37	0.003	4.27E-2	9.56E-2	2	9	0.001	Homo sapiens

Figure 8. Reactome pathway analysis representing top affected pathways associated with the age of the individuals having liver tumors.

2b. Cox Regression of Survival in Different age groups of liver tumor individuals:

The liver tumor individuals were categorized based on their age to three groups; Youth-group (age < 30 years), adult-group (age 30-60 years) and Old-group (age > 60 years).

2b)i. Cox Regression of Survival in the Youth-group (age < 30 years) having liver tumor:

ABCC8, STMN4, EPN3 and LGALS14 were the genes coding for the top differentially expressed transcripts in the youth-group of liver tumors [Table 5]. These transcripts were not GWAS significant, however, Reactome pathway analysis reflected the main pathways affected in young individuals with liver tumors. The main pathways affected in this age group are defective ABCC8 that can cause hypoglycemias and hyperglycemias and defect in ATP sensitive potassium channels, inward rectifying potassium channels as well as defect in ABC transporters [Figure 9].

Table 5. Top differentially expressed transcripts associated with Young liver tumor samples

Transcript_ID	Gene_coding	mean_expression	sc_youth_P_value	sc_youth_FDR
ENSG00000006071	***ABCC8***	10.6478641441293	0.000297228671044092	0.119672954110092
ENSG00000015592	***STMN4***	13.5500413397484	0.000348843249984188	0.119672954110092
ENSG00000049283	***EPN3***	3.6917573759521	0.000443781041199354	0.119672954110092
ENSG00000006659	***LGALS14***	0.0976655002882767	0.00160982848804059	0.127598747330998

Description | Molecules | Structures | Expression | Analysis 13 | Downloads

Results for: UNIPROT (13) ▾ | Type: Overrepresentation | [Data: Gene_coding for transcripts in Youth:] | Results | Identifiers not found:

Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
Defective ABCC8 can cause hypoglycemias and hyperglycemias	1	2	0	7.25E-4	8.69E-3	2	2	0	Homo sapiens
ATP sensitive Potassium channels	1	4	0	1.45E-3	8.69E-3	1	2	0	Homo sapiens
Inwardly rectifying K+ channels	1	31	0.003	1.12E-2	3.72E-2	1	5	0	Homo sapiens
ABC transporter disorders	1	81	0.007	2.9E-2	3.72E-2	2	22	0.002	Homo sapiens
Regulation of insulin secretion	1	81	0.007	2.9E-2	3.72E-2	1	37	0.003	Homo sapiens
Potassium Channels	1	99	0.009	3.54E-2	3.72E-2	1	17	0.002	Homo sapiens
ABC-family proteins mediated transport	1	104	0.009	3.72E-2	3.72E-2	1	28	0.003	Homo sapiens
Integration of energy metabolism	1	111	0.01	3.96E-2	3.96E-2	1	65	0.006	Homo sapiens
Disorders of transmembrane transporters	1	149	0.014	5.29E-2	5.29E-2	2	87	0.008	Homo sapiens
Neuronal System	1	370	0.034	1.28E-1	1.28E-1	1	156	0.014	Homo sapiens
Transport of small molecules	1	727	0.066	2.39E-1	2.39E-1	1	428	0.039	Homo sapiens
Disease	1	1,147	0.104	3.55E-1	3.55E-1	2	891	0.081	Homo sapiens
Metabolism	1	2,110	0.191	5.72E-1	5.72E-1	1	1,868	0.17	Homo sapiens

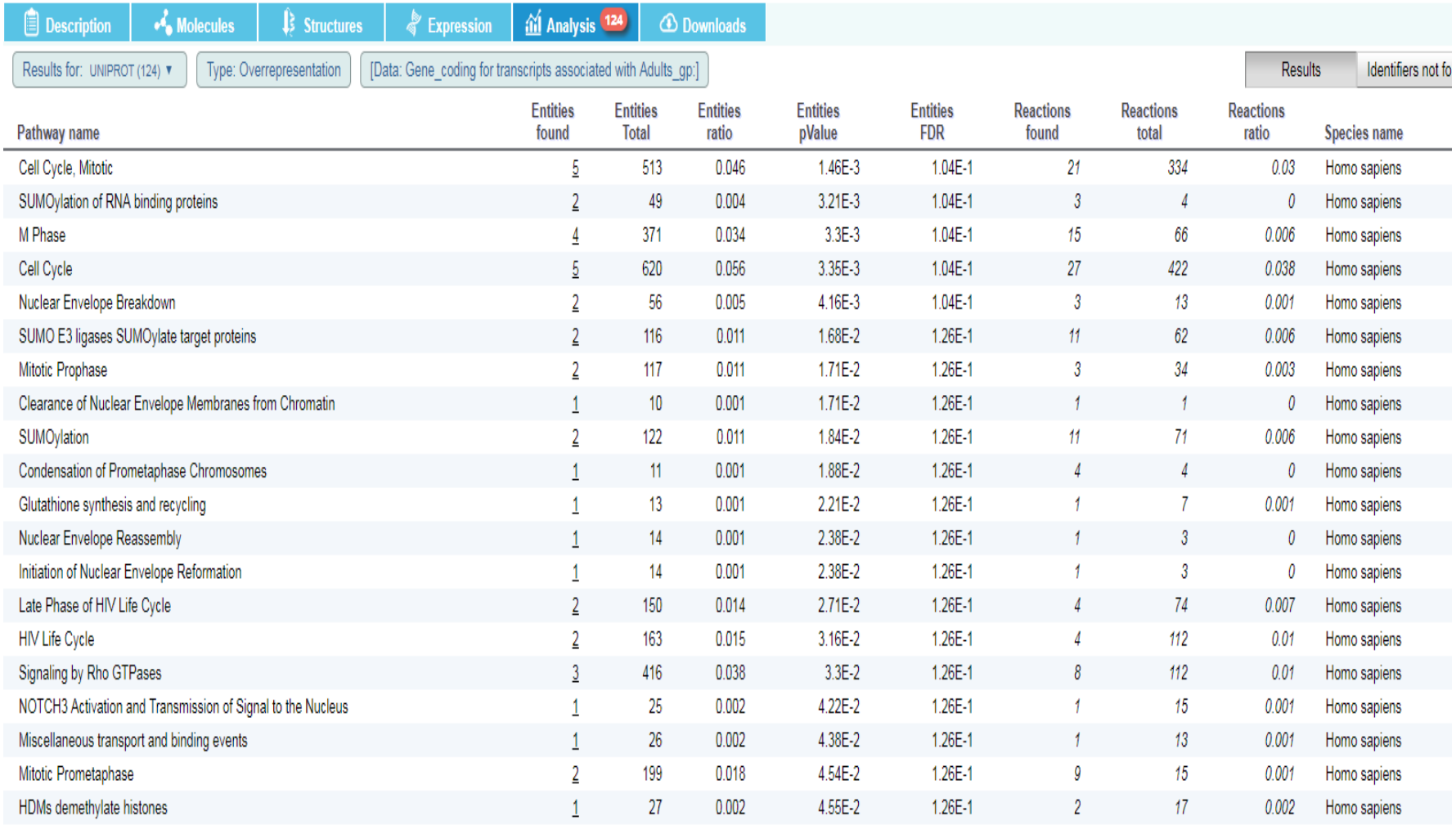
Figure 9. Reactome pathway analysis representing top affected pathways in Youth-group of liver tumor individuals.

2b) ii. Cox Regression of Survival in the adult-group (age 30-60 years) having liver tumor:

ANLN, NCAPD2, DBF4, NDC1, VRK2 were the genes coding for the top GWAS significant differentially expressed transcripts in the adult-group of liver tumors [Table 6]. Reactome pathway analysis showed the main pathways affected in adult individuals with liver tumors. The main pathways affected in adult age group are cell cycle mitosis, SUMOylation of RNA binding proteins, M phase, Nuclear envelop breakdown and SUMO E3 ligases SUMOylate target proteins [Figure 10].

Table 6. Top differentially expressed transcripts associated with Adult liver tumor samples

Transcript_ID	Gene_coding	mean_expression	sc_adults_P_value	sc_adults_FDR
ENSG00000011426	***ANLN***	3.98177489501828	5.25815280205677e-10	4.25384561686393e-07
ENSG00000010292	***NCAPD2***	2.4760328105841	1.15920018028959e-09	4.68896472927139e-07
ENSG00000006634	***DBF4***	4.53422080012747	4.87653684100309e-09	1.3150394347905e-06
ENSG00000058804	***NDC1***	4.24041585547021	6.8631726968249e-09	1.38807667793284e-06
ENSG00000028116	***VRK2***	1.59638074915355	2.58738304115269e-08	4.18638576058505e-06
ENSG00000055044	***NOP58***	13.1395426473703	4.09022065106512e-08	5.51498084451947e-06
ENSG00000033050	***ABCF2***	1.81746063109551	1.12507946181672e-07	1.21131181032713e-05
ENSG00000057608	***GDI2***	2.9933462787894	1.19783615359914e-07	1.21131181032713e-05
ENSG00000023909	***GCLM***	2.76541465545887	2.33535984106581e-07	2.09922901269138e-05
ENSG00000001036	***FUCA2***	4.3691496147043	2.83983955795541e-07	2.29743020238593e-05
ENSG00000035141	***FAM136A***	18.9408532553226	3.51879791526954e-07	2.58791592132096e-05
ENSG00000004487	***KDM1A***	34.7931889120134	4.25102467671579e-07	2.86589913621923e-05
ENSG00000040487	***PQLC2***	14.526932697851	5.55667995727482e-07	3.4579646811041e-05
ENSG00000040275	***SPDL1***	0.931267456282186	7.46265198636742e-07	4.31234675497946e-05
ENSG00000018699	***TTC27***	1.91420168041969	9.05816038287988e-07	4.88536783316655e-05
ENSG00000013810	***TACC3***	5.27965029048592	1.10777573381071e-06	5.6011910540804e-05
ENSG00000053372	***MRTO4***	2.20101293347763	1.35376356691363e-06	6.44232191548898e-05
ENSG00000009844	***VTA1***	1.25381663644389	1.98289010333585e-06	8.91198940888168e-05
ENSG00000020256	***ZFP64***	1.43324891461347	2.09727257960424e-06	8.92996587842016e-05



Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
Cell Cycle, Mitotic	5	513	0.046	1.46E-3	1.04E-1	21	334	0.03	Homo sapiens
SUMOylation of RNA binding proteins	2	49	0.004	3.21E-3	1.04E-1	3	4	0	Homo sapiens
M Phase	4	371	0.034	3.3E-3	1.04E-1	15	66	0.006	Homo sapiens
Cell Cycle	5	620	0.056	3.35E-3	1.04E-1	27	422	0.038	Homo sapiens
Nuclear Envelope Breakdown	2	56	0.005	4.16E-3	1.04E-1	3	13	0.001	Homo sapiens
SUMO E3 ligases SUMOylate target proteins	2	116	0.011	1.68E-2	1.26E-1	11	62	0.006	Homo sapiens
Mitotic Prophase	2	117	0.011	1.71E-2	1.26E-1	3	34	0.003	Homo sapiens
Clearance of Nuclear Envelope Membranes from Chromatin	1	10	0.001	1.71E-2	1.26E-1	1	1	0	Homo sapiens
SUMOylation	2	122	0.011	1.84E-2	1.26E-1	11	71	0.006	Homo sapiens
Condensation of Prometaphase Chromosomes	1	11	0.001	1.88E-2	1.26E-1	4	4	0	Homo sapiens
Glutathione synthesis and recycling	1	13	0.001	2.21E-2	1.26E-1	1	7	0.001	Homo sapiens
Nuclear Envelope Reassembly	1	14	0.001	2.38E-2	1.26E-1	1	3	0	Homo sapiens
Initiation of Nuclear Envelope Reformation	1	14	0.001	2.38E-2	1.26E-1	1	3	0	Homo sapiens
Late Phase of HIV Life Cycle	2	150	0.014	2.71E-2	1.26E-1	4	74	0.007	Homo sapiens
HIV Life Cycle	2	163	0.015	3.16E-2	1.26E-1	4	112	0.01	Homo sapiens
Signaling by Rho GTPases	3	416	0.038	3.3E-2	1.26E-1	8	112	0.01	Homo sapiens
NOTCH3 Activation and Transmission of Signal to the Nucleus	1	25	0.002	4.22E-2	1.26E-1	1	15	0.001	Homo sapiens
Miscellaneous transport and binding events	1	26	0.002	4.38E-2	1.26E-1	1	13	0.001	Homo sapiens
Mitotic Prometaphase	2	199	0.018	4.54E-2	1.26E-1	9	15	0.001	Homo sapiens
HDMs demethylate histones	1	27	0.002	4.55E-2	1.26E-1	2	17	0.002	Homo sapiens

Figure 10. Reactome pathway analysis representing top affected pathways in Adult-group of liver tumor individuals.

2b) iii. Cox Regression of Survival in the Old-group (age > 60 years) having liver tumor:

DBF4, ANLN, HEXB, STARD3NL and ***ABCF2*** were the genes coding for the top differentially expressed transcripts in the Old-group of liver tumors [Table 7]. Reactome pathway analysis showed the main pathways affected in adult individuals with liver tumors. The main pathways affected in old age group are hyaluronan uptake and degradation, pregnenolone biosynthesis, keratan sulfate degradation, Glutathione synthesis and recycling, CS/DS degradation, HDMs demethylation of histones, activated ***PKN1*** stimulates transcription of AR (Androgen receptor) regulated genes ***KLK2*** and ***KLK3,*** and activation of ATR in response to replication stress [Figure 11].

Table 7. Top differentially expressed transcripts associated with Old liver tumor samples

Transcript_ID	Gene_coding	mean_expression	sc_old_P_value	sc_old_FDR
ENSG00000006634	***DBF4***	4.53422080012747	5.8088534315992e-08	4.69936242616375e-05
ENSG00000011426	***ANLN***	3.98177489501828	3.15911294901383e-07	8.51907458584063e-05
ENSG00000049860	***HEXB***	6.12892506817414	3.04371959636285e-07	8.51907458584063e-05
ENSG00000010270	***STARD3NL***	2.11870151720299	1.10272583375703e-06	0.000178421039901887
ENSG00000033050	***ABCF2***	1.81746063109551	1.03537127682518e-06	0.000178421039901887
ENSG00000040487	***PQLC2***	14.526932697851	4.02780118535784e-06	0.000543081859825749
ENSG00000023909	***GCLM***	2.76541465545887	4.72248732086289e-06	0.000545784606082583
ENSG00000004487	***KDM1A***	34.7931889120134	8.27030462902112e-06	0.000836334555609761

Description | Molecules | Structures | Expression | Analysis 50 | Downloads

Results for: UNIPROT (50) ▾ | Type: Overrepresentation | [Data: Gene_coding for transcripts associated with Old_gp:] | Results | Identifiers not found

Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
Hyaluronan uptake and degradation	1	12	0.001	8.67E-3	7.7E-2	1	7	0.001	Homo sapiens
Pregnenolone biosynthesis	1	12	0.001	8.67E-3	7.7E-2	1	7	0.001	Homo sapiens
Keratan sulfate degradation	1	13	0.001	9.39E-3	7.7E-2	1	6	0.001	Homo sapiens
Glutathione synthesis and recycling	1	13	0.001	9.39E-3	7.7E-2	1	7	0.001	Homo sapiens
CS/DS degradation	1	14	0.001	1.01E-2	7.7E-2	1	9	0.001	Homo sapiens
Hyaluronan metabolism	1	17	0.002	1.23E-2	7.7E-2	1	10	0.001	Homo sapiens
Miscellaneous transport and binding events	1	26	0.002	1.87E-2	7.7E-2	1	13	0.001	Homo sapiens
HDMs demethylate histones	1	27	0.002	1.94E-2	7.7E-2	2	17	0.002	Homo sapiens
Activation of the pre-replicative complex	1	32	0.003	2.3E-2	7.7E-2	6	8	0.001	Homo sapiens
Keratan sulfate/keratin metabolism	1	34	0.003	2.44E-2	7.7E-2	1	15	0.001	Homo sapiens
Metabolism of steroid hormones	1	34	0.003	2.44E-2	7.7E-2	1	32	0.003	Homo sapiens
Activated PKN1 stimulates transcription of AR (androgen receptor) regulated genes KLK2 and KLK3	1	36	0.003	2.58E-2	7.7E-2	3	11	0.001	Homo sapiens
Activation of ATR in response to replication stress	1	37	0.003	2.65E-2	7.7E-2	2	9	0.001	Homo sapiens
Glutathione conjugation	1	37	0.003	2.65E-2	7.7E-2	1	12	0.001	Homo sapiens
Glycosphingolipid metabolism	1	46	0.004	3.29E-2	7.7E-2	2	31	0.003	Homo sapiens
Chondroitin sulfate/dermatan sulfate metabolism	1	50	0.005	3.57E-2	7.7E-2	1	24	0.002	Homo sapiens
HDACs deacetylate histones	1	60	0.005	4.27E-2	7.7E-2	1	4	0	Homo sapiens
Regulation of PTEN gene transcription	1	60	0.005	4.27E-2	7.7E-2	2	15	0.001	Homo sapiens
RHO GTPases activate PKNs	1	63	0.006	4.48E-2	7.7E-2	3	20	0.002	Homo sapiens
DNA Replication Pre-Initiation	1	85	0.008	6E-2	7.7E-2	6	20	0.002	Homo sapiens

Figure 11. Reactome pathway analysis representing top affected pathways in Old-group of liver tumor individuals.

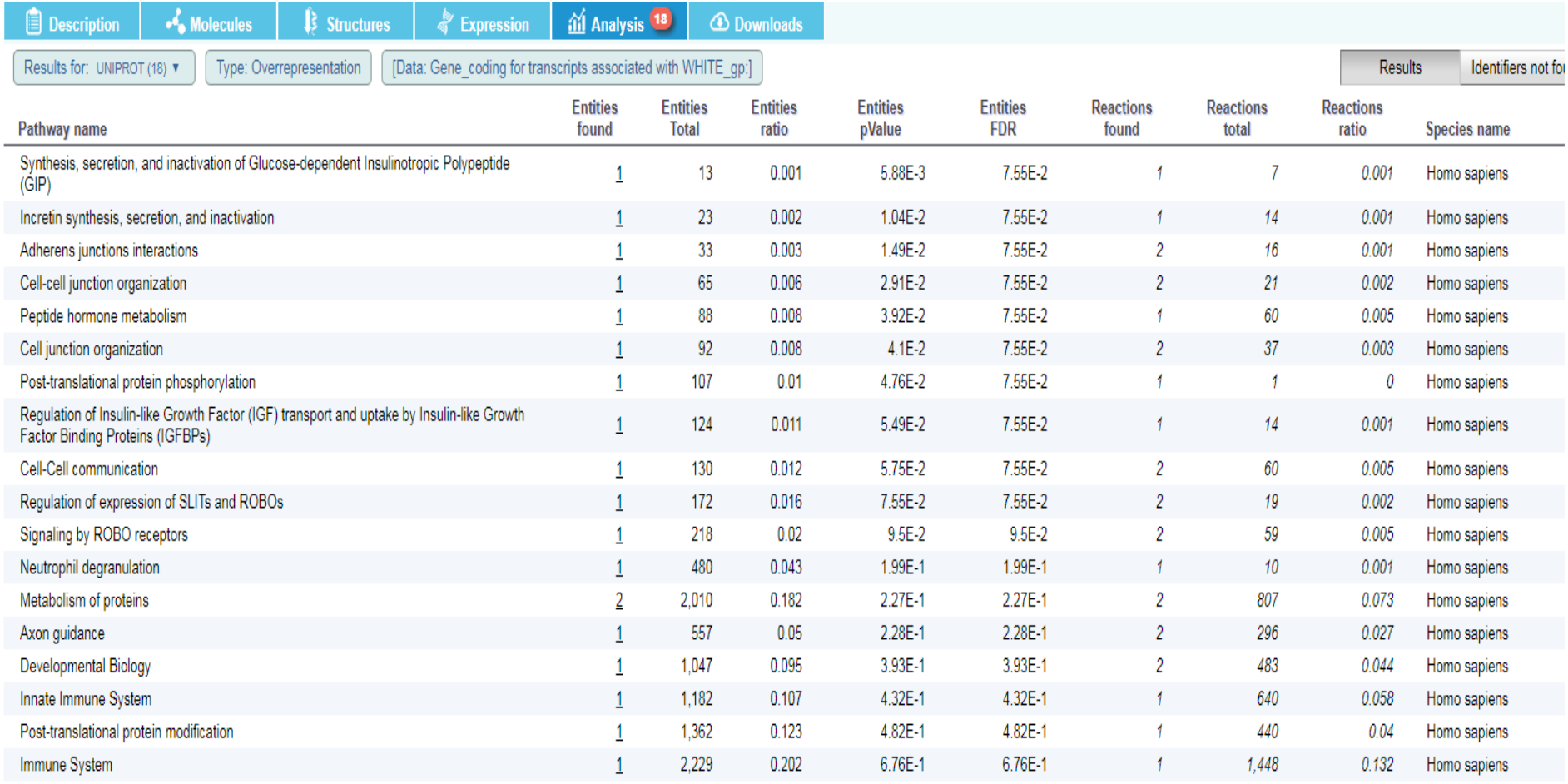
3. Cox Regression of Survival in different races liver tumor individuals

3a. Cox Regression of Survival in White liver tumor individuals

TKTL1, CDH10, ISL1, FUCA2 and ***PHF21B*** were the genes coding for the top differentially expressed transcripts in the White individuals of liver tumors [Table 8]. Reactome pathway analysis showed the main pathways affected in White individuals with liver tumors. The main pathways affected in the White individuals are synthesis secretin and inactivation of Glucose-dependant Insulinotropic polypeptide (***GIP***), adherens and cell junction, peptide hormone metabolism and regulation of Insulin-like Growth Factor (***IGF***) transport and uptake by Insulin-like Growth Factor Binding Proteins (***IGFBPs***) [Figure 12].

Table 8. Top differentially expressed transcripts associated with White liver tumor samples

Transcript_ID	Gene_coding	mean_expression	sc_white_P_value	sc_white_FDR
ENSG00000007350	***TKTL1***	35.9357215848277	1.33060733564783e-06	0.00107646133453909
ENSG00000040731	***CDH10***	2.73778785544828	2.77654805851402e-05	0.0112311368966892
ENSG00000016082	***ISL1***	0.9816542629418	5.59109558527782e-05	0.0150773210949659
ENSG00000001036	***FUCA2***	4.3691496147043	0.000108057840141895	0.0174837585349586
ENSG00000056487	***PHF21B***	0.370759733664315	9.54387798969281e-05	0.0174837585349586



Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
Synthesis, secretion, and inactivation of Glucose-dependent Insulinotropic Polypeptide (GIP)	1	13	0.001	5.88E-3	7.55E-2	1	7	0.001	Homo sapiens
Incretin synthesis, secretion, and inactivation	1	23	0.002	1.04E-2	7.55E-2	1	14	0.001	Homo sapiens
Adherens junctions interactions	1	33	0.003	1.49E-2	7.55E-2	2	16	0.001	Homo sapiens
Cell-cell junction organization	1	65	0.006	2.91E-2	7.55E-2	2	21	0.002	Homo sapiens
Peptide hormone metabolism	1	88	0.008	3.92E-2	7.55E-2	1	60	0.005	Homo sapiens
Cell junction organization	1	92	0.008	4.1E-2	7.55E-2	2	37	0.003	Homo sapiens
Post-translational protein phosphorylation	1	107	0.01	4.76E-2	7.55E-2	1	1	0	Homo sapiens
Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)	1	124	0.011	5.49E-2	7.55E-2	1	14	0.001	Homo sapiens
Cell-Cell communication	1	130	0.012	5.75E-2	7.55E-2	2	60	0.005	Homo sapiens
Regulation of expression of SLITs and ROBOs	1	172	0.016	7.55E-2	7.55E-2	2	19	0.002	Homo sapiens
Signaling by ROBO receptors	1	218	0.02	9.5E-2	9.5E-2	2	59	0.005	Homo sapiens
Neutrophil degranulation	1	480	0.043	1.99E-1	1.99E-1	1	10	0.001	Homo sapiens
Metabolism of proteins	2	2,010	0.182	2.27E-1	2.27E-1	2	807	0.073	Homo sapiens
Axon guidance	1	557	0.05	2.28E-1	2.28E-1	2	296	0.027	Homo sapiens
Developmental Biology	1	1,047	0.095	3.93E-1	3.93E-1	2	483	0.044	Homo sapiens
Innate Immune System	1	1,182	0.107	4.32E-1	4.32E-1	1	640	0.058	Homo sapiens
Post-translational protein modification	1	1,362	0.123	4.82E-1	4.82E-1	1	440	0.04	Homo sapiens
Immune System	1	2,229	0.202	6.76E-1	6.76E-1	1	1,448	0.132	Homo sapiens

Figure 12. Reactome pathway analysis representing top affected pathways in White liver tumor individuals.

3b. Cox Regression of Survival in Asian liver tumor individuals:

NDC1, DSG2, DEPDC1, LAMC2, ZNF207, VRK2, NCAPD2, DEPDC1B, ANLN, UBA6, SCMH1, CDC27, E2F2 and ***ZZZ3*** are the genes coding for the top GWAS significant differentially expressed transcripts. ***SPDL1, MAP3K9, ARNTL2, GCLM and DBF4*** are the genes coding for differentially expressed transcripts that are not GWAS significant in the Asian individuals of liver tumors [Table 9]. The main affected pathways in Asian individuals with liver tumors are cell cycle mitosis, SUMOylation of RNA binding proteins and chromatin organization proteins and DNA damage and repair proteins, M phase, Nuclear envelop breakdown and SUMO E3 ligases SUMOylate target proteins [Figure 13].

Table 9. Top differentially expressed transcripts associated with Asian liver tumor samples

Transcript_ID	Gene_coding	mean_expression	sc_asian_P_value	sc_asian_FDR
ENSG00000058804	***NDC1***	4.24041585547021	3.63042929052426e-14	2.93701729603413e-11
ENSG00000046604	***DSG2***	2.1454129923765	7.86148923737073e-13	3.17997239651646e-10
ENSG00000024526	***DEPDC1***	32.0789048076069	2.96951352396491e-12	8.00778813629204e-10
ENSG00000058085	***LAMC2***	2.81347995494975	1.59069424299219e-11	3.2171791064517e-09
ENSG00000010244	***ZNF207***	0.335961727444148	5.88954440772227e-11	9.52928285169463e-09
ENSG00000028116	***VRK2***	1.59638074915355	2.17987849993051e-10	2.93920284407297e-08
ENSG00000010292	***NCAPD2***	2.4760328105841	3.74911657274879e-10	3.79129413419221e-08
ENSG00000035499	***DEPDC1B***	4.57887066858399	3.60739882410144e-10	3.79129413419221e-08
ENSG00000011426	***ANLN***	3.98177489501828	1.6054060303361e-09	1.44308164282434e-07
ENSG00000033178	***UBA6***	0.0524387649790027	4.77382455787989e-09	3.86202406732483e-07
ENSG00000010803	***SCMH1***	2.78259105652028	1.18844019070252e-08	8.74043740253035e-07
ENSG00000004897	***CDC27***	3.53178524521041	1.36716582499474e-08	9.21697627017287e-07
ENSG00000007968	***E2F2***	12.099980190359	1.85930352225583e-08	1.15705888423459e-06
ENSG00000036549	***ZZZ3***	10.417759884465	2.28425146575617e-08	1.3199710255691e-06
ENSG00000040275	***SPDL1***	0.931267456282186	3.09926410091421e-08	1.6715364384264e-06
ENSG00000006432	***MAP3K9***	1.36797742745194	8.31467071860104e-08	4.20410538209265e-06
ENSG00000029153	***ARNTL2***	15.5763532562662	1.04205404194424e-07	4.95895129372288e-06
ENSG00000023909	***GCLM***	2.76541465545887	1.29339946974838e-07	5.813112061258e-06
ENSG00000006634	***DBF4***	4.53422080012747	1.46460391037273e-07	5.92432281745769e-06

Description | Molecules | Structures | Expression | Analysis 159 | Downloads

Results for: UNIPROT (159) ▾ | Type: Overrepresentation | [Data: Gene_coding for ASIAN] | Results | Identifiers not

Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
Cell Cycle, Mitotic	7	513	0.046	1.44E-5	2.45E-3	50	334	0.03	Homo sapiens
Cell Cycle	7	620	0.056	4.88E-5	4.15E-3	56	422	0.038	Homo sapiens
M Phase	5	371	0.034	3.36E-4	1.88E-2	18	66	0.006	Homo sapiens
Cellular Senescence	3	164	0.015	2.66E-3	8.34E-2	17	88	0.008	Homo sapiens
SUMOylation of RNA binding proteins	2	49	0.004	3.21E-3	8.34E-2	2	4	0	Homo sapiens
Nuclear Envelope Breakdown	2	56	0.005	4.16E-3	8.34E-2	3	13	0.001	Homo sapiens
Cellular responses to stress	4	405	0.037	4.52E-3	8.34E-2	18	181	0.016	Homo sapiens
SUMOylation of chromatin organization proteins	2	60	0.005	4.75E-3	8.34E-2	4	15	0.001	Homo sapiens
Mitotic Anaphase	3	203	0.018	4.84E-3	8.34E-2	6	11	0.001	Homo sapiens
Mitotic Metaphase and Anaphase	3	204	0.018	4.9E-3	8.34E-2	6	12	0.001	Homo sapiens
SUMOylation of DNA damage response and repair proteins	2	79	0.007	8.08E-3	9.3E-2	5	24	0.002	Homo sapiens
Cellular responses to external stimuli	4	482	0.044	8.32E-3	9.3E-2	18	251	0.023	Homo sapiens
DNA Replication Pre-Initiation	2	85	0.008	9.3E-3	9.3E-2	7	20	0.002	Homo sapiens
M/G1 Transition	2	85	0.008	9.3E-3	9.3E-2	7	20	0.002	Homo sapiens
Cell Cycle Checkpoints	3	274	0.025	1.1E-2	9.39E-2	7	56	0.005	Homo sapiens
Oxidative Stress Induced Senescence	2	94	0.009	1.13E-2	9.39E-2	9	39	0.004	Homo sapiens
DNA Replication	2	108	0.01	1.47E-2	9.39E-2	7	47	0.004	Homo sapiens
Mitotic Spindle Checkpoint	2	110	0.01	1.52E-2	9.39E-2	5	7	0.001	Homo sapiens
SUMO E3 ligases SUMOylate target proteins	2	116	0.011	1.68E-2	9.39E-2	13	62	0.006	Homo sapiens
Mitotic Prophase	2	117	0.011	1.71E-2	9.39E-2	3	34	0.003	Homo sapiens

Figure 13. Reactome pathway analysis representing top affected pathways in Asian liver tumor individuals.

3c. Cox Regression of Survival in Afro-American liver tumor individuals

MTMR11, FUCA2, GRAMD1B, SEMA3F, HECW1, TMEM176A, USH1C, BID and ***DNAH5*** are the genes coding for differentially expressed transcripts that are not GWAS significant in the Afro-American individuals with liver tumors [Table 10]. The main affected pathways in Afro-American individuals with liver tumors TP53 regulation of genes involved in Cytochrome C release and cell death genes, activation and oligomerization of BAK and BAX, activation and mitochondrial translocation of BID, programmed cell death, TCF signaling in response to Wnt signaling and regulation of Insulin-like Growth Factor (***IGF***) transport and uptake by Insulin-like Growth Factor Binding Proteins (***IGFBPs***) [Figure 14].

Table 10. Top differentially expressed transcripts in Afro_american liver tumor individuals

Transcript_ID	Gene_coding	mean_expression	sc_AFRO_P_value	sc_AFRO_FDR
ENSG00000014914	***MTMR11***	10.7025557949761	6.8712470825627e-05	0.0555883888979322
ENSG00000001036	***FUCA2***	4.3691496147043	0.00152324885661803	0.481349547809064
ENSG00000023171	***GRAMD1B***	2.11704265013741	0.00178497978173942	0.481349547809064
ENSG00000001617	***SEMA3F***	30.6793336991256	0.00507904257513281	0.488925385731915
ENSG00000002746	***HECW1***	0.689809027168964	0.00545672087852789	0.488925385731915
ENSG00000002933	***TMEM176A***	1.72164825574494	0.00573891655863024	0.488925385731915
ENSG00000006611	***USH1C***	2.21752843966766	0.00583077475721938	0.488925385731915
ENSG00000015475	***BID***	0.0307121370057753	0.00599392466693816	0.488925385731915
ENSG00000039139	***DNAH5***	0.00789408099784931	0.00664793478745496	0.488925385731915

Description | Molecules | Structures | Expression | Analysis 26 | Downloads

Results for: TOTAL (26) ▾ | Type: Overrepresentation | [Data: Gene_coding for transcripts associated with Afro_americans:] | Results | Identifiers

Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
TP53 Regulates Transcription of Genes Involved in Cytochrome C Release	2	33	0.002	2.63E-4	6.85E-3	2	25	0.002	Homo sapiens
Activation and oligomerization of BAK protein	1	2	0	1.47E-3	9.8E-3	2	3	0	Homo sapiens
Activation, translocation and oligomerization of BAX	1	2	0	1.47E-3	9.8E-3	2	4	0	Homo sapiens
TP53 Regulates Transcription of Cell Death Genes	2	83	0.006	1.63E-3	9.8E-3	2	68	0.006	Homo sapiens
Activation, myristolyation of BID and translocation to mitochondria	1	4	0	2.95E-3	1.47E-2	4	4	0	Homo sapiens
BH3-only proteins associate with and inactivate anti-apoptotic BCL-2 members	1	8	0.001	5.89E-3	2.35E-2	3	3	0	Homo sapiens
Activation of BAD and translocation to mitochondria	1	19	0.001	1.39E-2	4.18E-2	1	5	0	Homo sapiens
Activation of BH3-only proteins	1	36	0.003	2.62E-2	7.11E-2	1	19	0.002	Homo sapiens
Intrinsic Pathway for Apoptosis	1	49	0.004	3.56E-2	7.11E-2	12	46	0.004	Homo sapiens
Degradation of DVL	1	57	0.004	4.13E-2	8.25E-2	3	7	0.001	Homo sapiens
Transcriptional Regulation by TP53	2	486	0.036	4.78E-2	9.56E-2	2	259	0.023	Homo sapiens
Post-translational protein phosphorylation	1	109	0.008	7.76E-2	1.23E-1	1	1	0	Homo sapiens
Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)	1	127	0.009	8.98E-2	1.23E-1	1	14	0.001	Homo sapiens
Apoptosis	1	177	0.013	1.23E-1	1.23E-1	12	122	0.011	Homo sapiens
Programmed Cell Death	1	185	0.014	1.28E-1	1.28E-1	12	135	0.012	Homo sapiens
TCF dependent signaling in response to WNT	1	216	0.016	1.48E-1	1.48E-1	3	71	0.006	Homo sapiens
Signaling by WNT	1	330	0.024	2.18E-1	2.18E-1	3	156	0.014	Homo sapiens
Generic Transcription Pathway	2	1,389	0.102	2.74E-1	2.74E-1	2	649	0.058	Homo sapiens
Neutrophil degranulation	1	480	0.035	3.03E-1	3.03E-1	1	10	0.001	Homo sapiens
RNA Polymerase II Transcription	2	1,528	0.113	3.13E-1	3.13E-1	2	708	0.063	Homo sapiens

Figure 14. Reactome pathway analysis representing top affected pathways in Afro-American liver tumor individuals.

4. Cox Regression of Survival in Male and Female liver tumor individuals

4a. Cox Regression of Survival in Male liver tumor individuals

NDC1, ANLN, VRK2, DEPDC1, NCDN, MAP3K9, NCAPD2 and NOP58 are the genes coding for top GWAS significant differentially expressed transcripts in Male individuals with liver tumors [Table 11]. The main affected pathways in Male individuals with liver tumors are cell cycle mitosis, SUMOylation of RNA binding proteins and chromatin organization proteins and DNA damage and repair proteins, M phase, Nuclear envelop breakdown and SUMO E3 ligases SUMOylate target proteins [Figure 15]

Table 11. Top differentially expressed transcripts in Male liver tumor individuals

Transcript_ID	Gene_coding	mean_expression	sc_MALES_P_value	sc_MALES_FDR
ENSG00000058804	***NDC1***	4.24041585547021	3.21587201312923e-12	2.60164045862155e-09
ENSG00000011426	***ANLN***	3.98177489501828	3.32264438185348e-10	1.34400965245973e-07
ENSG00000028116	***VRK2***	1.59638074915355	5.55241741473367e-10	1.49730189617318e-07
ENSG00000024526	***DEPDC1***	32.0789048076069	3.13900883064377e-09	6.34864535997702e-07
ENSG00000020129	***NCDN***	1.60904782244513	4.74507677594715e-09	7.67753422348249e-07
ENSG00000006432	***MAP3K9***	1.36797742745194	6.89614154669727e-09	9.29829751879682e-07
ENSG00000010292	***NCAPD2***	2.4760328105841	1.3280181287989e-08	1.53480952314044e-06
ENSG00000055044	***NOP58***	13.1395426473703	2.87691019895675e-08	2.6973446132672e-06
ENSG00000006747	***SCIN***	0.462995146469433	3.32583067752523e-08	2.6973446132672e-06
ENSG00000023909	***GCLM***	2.76541465545887	3.33417133902003e-08	2.6973446132672e-06
ENSG00000022556	***NLRP2***	8.75203865515192	1.28052311976212e-07	9.41766548988686e-06
ENSG00000007341	***ST7L***	2.02812950366937	1.53903203847783e-07	1.0375640992738e-05
ENSG00000033050	***ABCF2***	1.81746063109551	1.82795190650786e-07	1.13754853258835e-05
ENSG00000040275	***SPDL1***	0.931267456282186	2.37381231671385e-07	1.37172440301536e-05
ENSG00000051341	***PQLQ***	7.62104128298136	3.66863623812996e-07	1.71529747569744e-05
ENSG00000046604	***DSG2***	2.1454129923765	3.67240256538004e-07	1.71529747569744e-05
ENSG00000006634	***DBF4***	4.53422080012747	3.75864925850067e-07	1.71529747569744e-05
ENSG00000048545	***GUCA1A***	1.37964682506708	3.8164838767063e-07	1.71529747569744e-05
ENSG00000036549	***ZZZ3***	10.417759884465	7.33924695239807e-07	3.05120234549872e-05

Results for: UNIPROT (113) ▾ | Type: Overrepresentation | [Data: Gene_coding for transcripts associated with Males liver tumors:] | Results | Identifiers not

Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
Cell Cycle, Mitotic	5	513	0.046	1.46E-3	9.56E-2	21	334	0.03	Homo sapiens
SUMOylation of RNA binding proteins	2	49	0.004	3.21E-3	9.56E-2	3	4	0	Homo sapiens
M Phase	4	371	0.034	3.3E-3	9.56E-2	15	66	0.006	Homo sapiens
Cell Cycle	5	620	0.056	3.35E-3	9.56E-2	27	422	0.038	Homo sapiens
Nuclear Envelope Breakdown	2	56	0.005	4.16E-3	9.56E-2	3	13	0.001	Homo sapiens
SUMO E3 ligases SUMOylate target proteins	2	116	0.011	1.68E-2	1.37E-1	11	62	0.006	Homo sapiens
Mitotic Prophase	2	117	0.011	1.71E-2	1.37E-1	3	34	0.003	Homo sapiens
Clearance of Nuclear Envelope Membranes from Chromatin	1	10	0.001	1.71E-2	1.37E-1	1	1	0	Homo sapiens
SUMOylation	2	122	0.011	1.84E-2	1.37E-1	11	71	0.006	Homo sapiens
Condensation of Prometaphase Chromosomes	1	11	0.001	1.88E-2	1.37E-1	4	4	0	Homo sapiens
Apoptotic cleavage of cell adhesion proteins	1	11	0.001	1.88E-2	1.37E-1	1	10	0.001	Homo sapiens
Glutathione synthesis and recycling	1	13	0.001	2.21E-2	1.37E-1	1	7	0.001	Homo sapiens
Initiation of Nuclear Envelope Reformation	1	14	0.001	2.38E-2	1.37E-1	1	3	0	Homo sapiens
Nuclear Envelope Reassembly	1	14	0.001	2.38E-2	1.37E-1	1	3	0	Homo sapiens
Mitotic Prometaphase	2	199	0.018	4.54E-2	1.37E-1	9	15	0.001	Homo sapiens
Mitotic Anaphase	2	203	0.018	4.7E-2	1.37E-1	3	11	0.001	Homo sapiens
Mitotic Metaphase and Anaphase	2	204	0.018	4.75E-2	1.37E-1	3	12	0.001	Homo sapiens
Activation of the pre-replicative complex	1	32	0.003	5.37E-2	1.37E-1	6	8	0.001	Homo sapiens
Inactivation, recovery and regulation of the phototransduction cascade	1	33	0.003	5.53E-2	1.37E-1	2	19	0.002	Homo sapiens
Regulation of Glucokinase by Glucokinase Regulatory Protein	1	34	0.003	5.69E-2	1.37E-1	1	5	0	Homo sapiens

Figure 15. Reactome pathway analysis representing top affected pathways in Male liver tumor individuals.

4b. Cox Regression of Survival in Female liver tumor individuals

SLC7A14 is the gene coding for the GWAS significant differentially expressed transcript in Female liver tumor individuals. ***ENTPD2, PHKA2, AOC1, PQLC2, DBF4, FUCA2, RETSAT and AGPS*** are the genes coding for the top differentially expressed transcripts that are not GWAS significant [Table 12]. The main affected pathways in Female liver tumor individuals are plasmalogen biosynthesis, NTPDase protein hydrolyse the phosphate bond, glycogen metabolism, activation of pre-replicative complex and activation of ATR in response to replication stress [Figure 16].

Table 12. Top differentially expressed transcripts in Female liver tumor individuals

Transcript_ID	Gene coding	mean_expression	sc_FEMALES_P_value	sc_FEMALES_FDR
ENSG00000013293	***SLC7A14***	8.30393388969457	4.09421931912846e-08	3.31222342917492e-05
ENSG00000054179	***ENTPD2***	2.41803151159627	8.59438662135226e-05	0.0347642938833699
ENSG00000044446	***PHKA2***	0.124605628576726	0.000332902220052222	0.0699031035557166
ENSG00000002726	***AOC1***	8.23237182930573	0.000345627211647548	0.0699031035557166
ENSG00000040487	***PQLC2***	14.526932697851	0.000657266284508573	0.105815597932171
ENSG00000006634	***DBF4***	4.53422080012747	0.000784788118161961	0.105815597932171
ENSG00000001036	***FUCA2***	4.3691496147043	0.00108912310982223	0.125871513692312
ENSG00000042445	***RETSAT***	0.0764464076874384	0.00178199963778469	0.178216276111118
ENSG00000018510	***AGPS***	1.86810384596852	0.00219705109133861	0.178216276111118

Description | Molecules | Structures | Expression | Analysis 39 | Downloads

Results for: UNIPROT (39) ▾ | Type: Overrepresentation | [Data: Gene coding for differentially expressed transcripts in Female Liver Tumors] | Results | Identifiers not found: 1

Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
Plasmalogen biosynthesis	1	2	0	1.63E-3	7.17E-2	2	2	0	Homo sapiens
Phosphate bond hydrolysis by NTPDase proteins	1	8	0.001	6.51E-3	8.4E-2	1	12	0.001	Homo sapiens
Glycogen breakdown (glycogenolysis)	1	16	0.001	1.3E-2	8.4E-2	1	16	0.001	Homo sapiens
Metabolism	5	2,110	0.191	1.62E-2	8.4E-2	6	1,868	0.17	Homo sapiens
Miscellaneous transport and binding events	1	26	0.002	2.1E-2	8.4E-2	1	13	0.001	Homo sapiens
Glycogen metabolism	1	27	0.002	2.18E-2	8.4E-2	1	38	0.003	Homo sapiens
Activation of the pre-replicative complex	1	32	0.003	2.58E-2	8.4E-2	6	8	0.001	Homo sapiens
Activation of ATR in response to replication stress	1	37	0.003	2.98E-2	8.4E-2	2	9	0.001	Homo sapiens
Nucleobase catabolism	1	39	0.004	3.14E-2	8.4E-2	1	56	0.005	Homo sapiens
Retinoid metabolism and transport	1	44	0.004	3.53E-2	8.4E-2	1	26	0.002	Homo sapiens
Metabolism of fat-soluble vitamins	1	48	0.004	3.85E-2	8.4E-2	1	31	0.003	Homo sapiens
Neutrophil degranulation	2	480	0.043	5.56E-2	8.4E-2	2	10	0.001	Homo sapiens
DNA Replication Pre-Initiation	1	85	0.008	6.72E-2	8.4E-2	6	20	0.002	Homo sapiens
M/G1 Transition	1	85	0.008	6.72E-2	8.4E-2	6	20	0.002	Homo sapiens
Visual phototransduction	1	100	0.009	7.87E-2	8.4E-2	1	80	0.007	Homo sapiens
Metabolism of nucleotides	1	105	0.01	8.24E-2	8.4E-2	1	133	0.012	Homo sapiens
Phase I - Functionalization of compounds	1	106	0.01	8.32E-2	8.4E-2	1	98	0.009	Homo sapiens
Post-translational protein phosphorylation	1	107	0.01	8.4E-2	8.4E-2	1	1	0	Homo sapiens
DNA Replication	1	108	0.01	8.47E-2	8.47E-2	6	47	0.004	Homo sapiens
Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)	1	124	0.011	9.67E-2	9.67E-2	1	14	0.001	Homo sapiens

Figure 16. Reactome pathway analysis representing top affected pathways in Female liver tumor individuals.

5. Cox Regression of Survival in different stages of liver tumor individuals

Liver tumor individuals were classified into three groups depending on the stage of the liver tumor; Stage I, Stage II and Stage III.

5a. Cox Regression of Survival in Stage I liver tumor individuals

HEXB, C2orf83, SEC61A1, ABCB5, RRP12, GLRX2, CDKL3, RIOK2 and ***RPL26L1*** are the top differentially expressed transcripts in individuals with Stage I liver tumor [Table 13]. The main affected pathways in Stage I liver tumor are SRP-dependent cotranslational protein targeting to membrane, Major pathway of rRNA processing in the nucleolus and cytosol, rRNA processing in the nucleus and cytosol, Translation, Peptide chain elongation, Eukaryotic Translation Termination and Selenoamino acid metabolism [Figure 17].

Table 13. Top differentially expressed transcripts in Stage I liver tumor individuals

Transcript_ID	Gene Coding	mean_expression	sc_stage_1_P_value	sc_stage_1_FDR
ENSG00000049860	***HEXB***	6.12892506817414	3.97031174270701e-07	0.000321198219984997
ENSG00000042304	***C2orf83***	0.00186425440639726	1.00417962634047e-06	0.00040619065885472
ENSG00000058262	***SEC61A1***	8.84325138209975	1.21212540146187e-05	0.00326869816594218
ENSG00000004846	***ABCB5***	8.57671820360321	0.00010416834993876	0.0210680487751142
ENSG00000052749	***RRP12***	0.27474225530191	0.000133497973872743	0.0215999721726098
ENSG00000023572	***GLRX2***	11.7608726421299	0.000347372794884726	0.0468374318436239
ENSG00000006837	***CDKL3***	6.83378649860745	0.000517123400052721	0.0597646900918073
ENSG00000058729	***RIOK2***	7.10900554084715	0.000664516862260989	0.0671992676961425
ENSG00000037241	***RPL26L1***	0.0988300630572247	0.000771398026133241	0.0693401114601991

Description | Molecules | Structures | Expression | Analysis 55 | Downloads

Results for: UNIPROT (55) ▾ | Type: Overrepresentation | [Data: Gene Coding for deferentially expressed transcripts in Stage I_liver tumors] | Results | Identifiers not found:

Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
SRP-dependent cotranslational protein targeting to membrane	3	113	0.01	1.22E-4	6.72E-3	5	5	0	Homo sapiens
Major pathway of rRNA processing in the nucleolus and cytosol	3	183	0.017	5.01E-4	8.81E-3	2	7	0.001	Homo sapiens
rRNA processing in the nucleus and cytosol	3	193	0.017	5.85E-4	8.81E-3	2	15	0.001	Homo sapiens
rRNA processing	3	203	0.018	6.78E-4	8.81E-3	2	21	0.002	Homo sapiens
Translation	3	293	0.027	1.95E-3	1E-2	16	99	0.009	Homo sapiens
Peptide chain elongation	2	90	0.008	2.87E-3	1E-2	4	5	0	Homo sapiens
Eukaryotic Translation Termination	2	94	0.009	3.12E-3	1E-2	3	5	0	Homo sapiens
Selenocysteine synthesis	2	94	0.009	3.12E-3	1E-2	2	7	0.001	Homo sapiens
Eukaryotic Translation Elongation	2	95	0.009	3.19E-3	1E-2	4	9	0.001	Homo sapiens
Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC)	2	96	0.009	3.25E-3	1E-2	1	1	0	Homo sapiens
Viral mRNA Translation	2	101	0.009	3.59E-3	1E-2	2	2	0	Homo sapiens
Formation of a pool of free 40S subunits	2	102	0.009	3.66E-3	1E-2	1	2	0	Homo sapiens
L13a-mediated translational silencing of Ceruloplasmin expression	2	112	0.01	4.39E-3	1E-2	1	3	0	Homo sapiens
GTP hydrolysis and joining of the 60S ribosomal subunit	2	113	0.01	4.47E-3	1E-2	2	3	0	Homo sapiens
Nonsense-Mediated Decay (NMD)	2	117	0.011	4.78E-3	1E-2	5	6	0.001	Homo sapiens
Nonsense Mediated Decay (NMD) enhanced by the Exon Junction Complex (EJC)	2	117	0.011	4.78E-3	1E-2	4	5	0	Homo sapiens
Selenoamino acid metabolism	2	118	0.011	4.86E-3	1E-2	2	23	0.002	Homo sapiens
Eukaryotic Translation Initiation	2	120	0.011	5.02E-3	1E-2	4	21	0.002	Homo sapiens
Cap-dependent Translation Initiation	2	120	0.011	5.02E-3	1E-2	3	18	0.002	Homo sapiens
Influenza Viral RNA Transcription and Replication	2	149	0.014	7.63E-3	1.53E-2	2	14	0.001	Homo sapiens

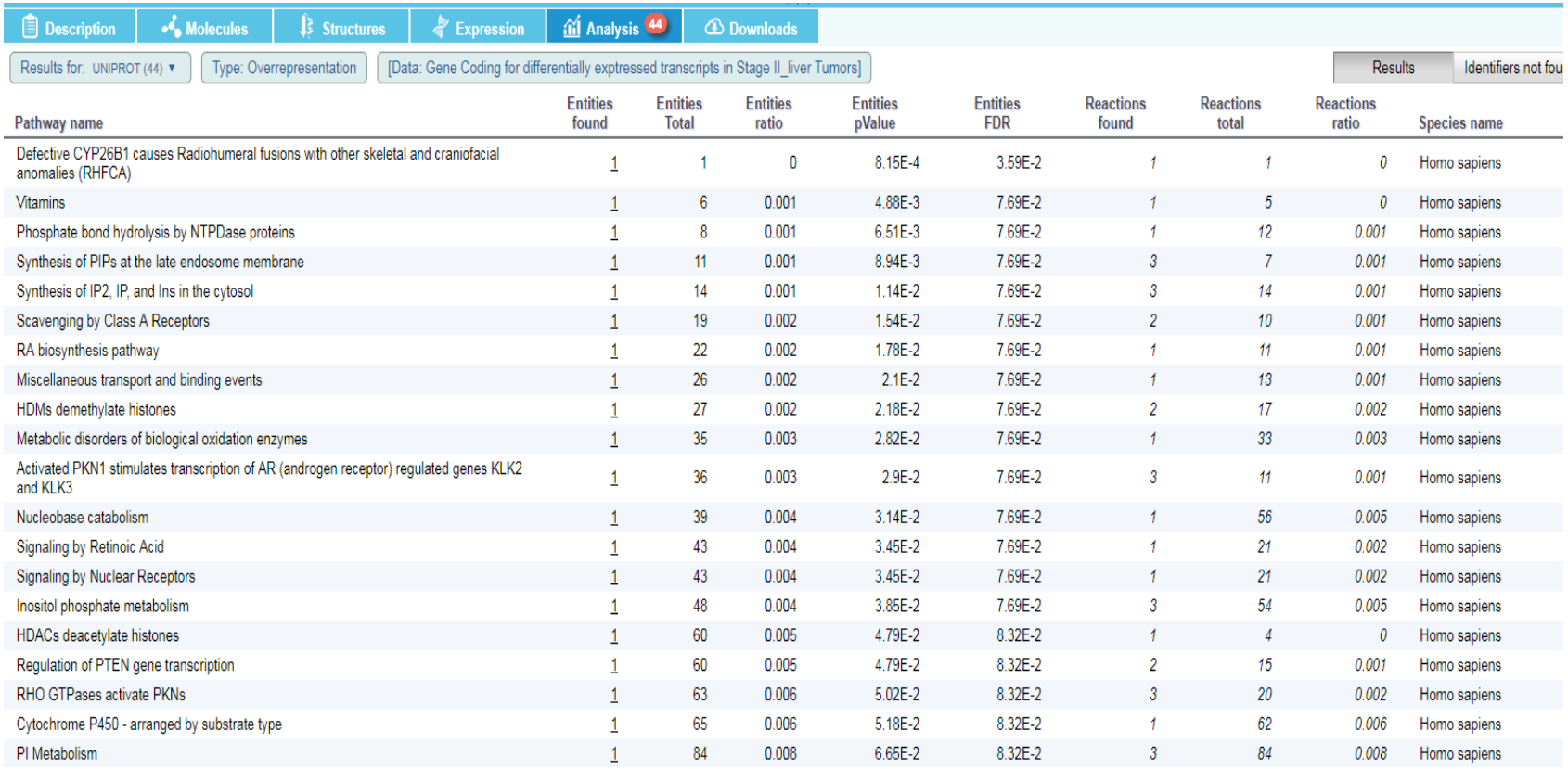
Figure 17. Reactome pathway analysis representing top affected pathways in Stage I liver tumor individuals.

5b. Cox Regression of Survival in Stage II liver tumor individuals

CYP26B1, MTMR7, TKTL1, LYPLA2 and ***ZIC2*** are the top differentially expressed transcripts in individuals with Stage II liver tumor. These differentially expressed transcripts are not GWAS significant [Table 14]. The main affected pathways in the Stage II liver tumors are Defective CYP26B1 causes Radiohumeral fusions with other skeletal and craniofacial anomalies (RHFCA), Vitamins, Phosphate bond hydrolysis by NTPDase proteins, Synthesis of PIPs at the late endosome membrane, Synthesis of IP2, IP, and Ins in the cytosol, Scavenging by Class A Receptors, HDMs demethylate histones and Activated PKN1 stimulates transcription of AR (androgen receptor) regulated genes KLK2 and KLK3 [Figure 18].

Table 14. Top differentially expressed transcripts Stage II liver tumor individuals

Transcript_ID	Gene Coding	mean_expression	sc_stage_II_P_value	sc_stage_II_FDR
ENSG00000003137	***CYP26B1***	2.97120530774537	0.0010632571166187	0.214247390707714
ENSG00000003987	***MTMR7***	0.782705827398134	0.00158897941192371	0.214247390707714
ENSG00000007350	***TKTL1***	35.9357215848277	0.00045639681630627	0.214247390707714
ENSG00000011009	***LYPLA2***	2.93368419874735	0.00139689124784237	0.214247390707714
ENSG00000043355	***ZIC2***	7.40768008946923	0.00144132779911532	0.214247390707714
ENSG00000054179	***ENTPD2***	2.41803151159627	0.00082721141490627	0.214247390707714
ENSG00000004487	***KDM1A***	34.7931889120134	0.00433606977811407	0.389764494499365
ENSG00000019169	***MARCO***	2.54992075215313	0.00413466283448349	0.389764494499365
ENSG00000040487	***PQLC2***	14.526932697851	0.00346292990478991	0.389764494499365



Description | Molecules | Structures | Expression | Analysis 44 | Downloads

Results for: UNIPROT (44) ▾ | Type: Overrepresentation | [Data: Gene Coding for differentially exptressed transcripts in Stage II_liver Tumors] | Results | Identifiers not fou

Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
Defective CYP26B1 causes Radiohumeral fusions with other skeletal and craniofacial anomalies (RHFCA)	1	1	0	8.15E-4	3.59E-2	1	1	0	Homo sapiens
Vitamins	1	6	0.001	4.88E-3	7.69E-2	1	5	0	Homo sapiens
Phosphate bond hydrolysis by NTPDase proteins	1	8	0.001	6.51E-3	7.69E-2	1	12	0.001	Homo sapiens
Synthesis of PIPs at the late endosome membrane	1	11	0.001	8.94E-3	7.69E-2	3	7	0.001	Homo sapiens
Synthesis of IP2, IP, and Ins in the cytosol	1	14	0.001	1.14E-2	7.69E-2	3	14	0.001	Homo sapiens
Scavenging by Class A Receptors	1	19	0.002	1.54E-2	7.69E-2	2	10	0.001	Homo sapiens
RA biosynthesis pathway	1	22	0.002	1.78E-2	7.69E-2	1	11	0.001	Homo sapiens
Miscellaneous transport and binding events	1	26	0.002	2.1E-2	7.69E-2	1	13	0.001	Homo sapiens
HDMs demethylate histones	1	27	0.002	2.18E-2	7.69E-2	2	17	0.002	Homo sapiens
Metabolic disorders of biological oxidation enzymes	1	35	0.003	2.82E-2	7.69E-2	1	33	0.003	Homo sapiens
Activated PKN1 stimulates transcription of AR (androgen receptor) regulated genes KLK2 and KLK3	1	36	0.003	2.9E-2	7.69E-2	3	11	0.001	Homo sapiens
Nucleobase catabolism	1	39	0.004	3.14E-2	7.69E-2	1	56	0.005	Homo sapiens
Signaling by Retinoic Acid	1	43	0.004	3.45E-2	7.69E-2	1	21	0.002	Homo sapiens
Signaling by Nuclear Receptors	1	43	0.004	3.45E-2	7.69E-2	1	21	0.002	Homo sapiens
Inositol phosphate metabolism	1	48	0.004	3.85E-2	7.69E-2	3	54	0.005	Homo sapiens
HDACs deacetylate histones	1	60	0.005	4.79E-2	8.32E-2	1	4	0	Homo sapiens
Regulation of PTEN gene transcription	1	60	0.005	4.79E-2	8.32E-2	2	15	0.001	Homo sapiens
RHO GTPases activate PKNs	1	63	0.006	5.02E-2	8.32E-2	3	20	0.002	Homo sapiens
Cytochrome P450 - arranged by substrate type	1	65	0.006	5.18E-2	8.32E-2	1	62	0.006	Homo sapiens
PI Metabolism	1	84	0.008	6.65E-2	8.32E-2	3	84	0.008	Homo sapiens

Figure 18. Reactome pathway analysis representing top affected pathways in Stage II liver tumor individuals.

5c. Cox Regression of Survival in Stage III liver tumor individuals

ANLN, NCAPD2, DBF4, NDC1, VRK2, NOP58 are the top differentially expressed transcripts Stage III liver tumors; GWAS significant (p< 5e-08), while ***ABCF2, GDI2, GCLM, FUCA2, FAM136A, KDM1A and PQLC2*** were not GWAS significant (p < 5e-05) [Table 15]. The main affected pathways in the Stage III liver tumors are Cell Cycle, Mitosis, SUMOylation of RNA binding proteins, Nuclear Envelope Breakdown, Glutathione synthesis and recycling, Signaling by Rho GTPases, NOTCH3 Activation and Transmission of Signal to the Nucleus and HDMs demethylate histones [Figure 19].

Table 15. Top differentially expressed transcripts in Stage III liver tumor individuals

Transcript_ID	Gene Coding	mean_expression	sc_stage_iii_P_value	sc_stage_iii_FDR
ENSG00000011426	***ANLN***	3.98177489501828	5.25815280205677e-10	4.25384561686393e-07
ENSG00000010292	***NCAPD2***	2.4760328105841	1.15920018028959e-09	4.68896472927139e-07
ENSG00000006634	***DBF4***	4.53422080012747	4.87653684100309e-09	1.3150394347905e-06
ENSG00000058804	***NDC1***	4.24041585547021	6.8631726968249e-09	1.38807667793284e-06
ENSG00000028116	***VRK2***	1.59638074915355	2.58738304115269e-08	4.18638576058505e-06
ENSG00000055044	***NOP58***	13.1395426473703	4.09022065106512e-08	5.51498084451947e-06
ENSG00000033050	***ABCF2***	1.81746063109551	1.12507946181672e-07	1.21131181032713e-05
ENSG00000057608	***GDI2***	2.9933462787894	1.19783615359914e-07	1.21131181032713e-05
ENSG00000023909	***GCLM***	2.76541465545887	2.33535984106581e-07	2.09922901269138e-05
ENSG00000001036	***FUCA2***	4.3691496147043	2.83983955795541e-07	2.29743020238593e-05
ENSG00000035141	***FAM136A***	18.9408532553226	3.51879791526954e-07	2.58791592132096e-05
ENSG00000004487	***KDM1A***	34.7931889120134	4.25102467671579e-07	2.86589913621923e-05
ENSG00000040487	***PQLC2***	14.526932697851	5.55667995727482e-07	3.4579646811041e-05
ENSG00000040275	***SPDL1***	0.931267456282186	7.46265198636742e-07	4.31234675497946e-05
ENSG00000018699	***TTC27***	1.91420168041969	9.05816038287988e-07	4.88536783316655e-05
ENSG00000013810	***TACC3***	5.27965029048592	1.10777573381071e-06	5.6011910540804e-05
ENSG00000053372	***MRTO4***	2.20101293347763	1.35376356691363e-06	6.44232191548898e-05
ENSG00000009844	***VTA1***	1.25381663644389	1.98289010333585e-06	8.91198940888168e-05
ENSG00000020256	***ZFP64***	1.43324891461347	2.09727257960424e-06	8.92996587842016e-05

Description | Molecules | Structures | Expression | Analysis 124 | Downloads

Results for: UNIPROT (124) ▾ | Type: Overrepresentation | [Data: Gene Coding for differentially expressed transcripts in Stage III liver tumors] | Results | Identifiers not found: 6

Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
Cell Cycle, Mitotic	5	513	0.046	1.46E-3	1.04E-1	21	334	0.03	Homo sapiens
SUMOylation of RNA binding proteins	2	49	0.004	3.21E-3	1.04E-1	3	4	0	Homo sapiens
M Phase	4	371	0.034	3.3E-3	1.04E-1	15	66	0.006	Homo sapiens
Cell Cycle	5	620	0.056	3.35E-3	1.04E-1	27	422	0.038	Homo sapiens
Nuclear Envelope Breakdown	2	56	0.005	4.16E-3	1.04E-1	3	13	0.001	Homo sapiens
SUMO E3 ligases SUMOylate target proteins	2	116	0.011	1.68E-2	1.26E-1	11	62	0.006	Homo sapiens
Mitotic Prophase	2	117	0.011	1.71E-2	1.26E-1	3	34	0.003	Homo sapiens
Clearance of Nuclear Envelope Membranes from Chromatin	1	10	0.001	1.71E-2	1.26E-1	1	1	0	Homo sapiens
SUMOylation	2	122	0.011	1.84E-2	1.26E-1	11	71	0.006	Homo sapiens
Condensation of Prometaphase Chromosomes	1	11	0.001	1.88E-2	1.26E-1	4	4	0	Homo sapiens
Glutathione synthesis and recycling	1	13	0.001	2.21E-2	1.26E-1	1	7	0.001	Homo sapiens
Initiation of Nuclear Envelope Reformation	1	14	0.001	2.38E-2	1.26E-1	1	3	0	Homo sapiens
Nuclear Envelope Reassembly	1	14	0.001	2.38E-2	1.26E-1	1	3	0	Homo sapiens
Late Phase of HIV Life Cycle	2	150	0.014	2.71E-2	1.26E-1	4	74	0.007	Homo sapiens
HIV Life Cycle	2	163	0.015	3.16E-2	1.26E-1	4	112	0.01	Homo sapiens
Signaling by Rho GTPases	3	416	0.038	3.3E-2	1.26E-1	8	112	0.01	Homo sapiens
NOTCH3 Activation and Transmission of Signal to the Nucleus	1	25	0.002	4.22E-2	1.26E-1	1	15	0.001	Homo sapiens
Miscellaneous transport and binding events	1	26	0.002	4.38E-2	1.26E-1	1	13	0.001	Homo sapiens
Mitotic Prometaphase	2	199	0.018	4.54E-2	1.26E-1	9	15	0.001	Homo sapiens
HDMs demethylate histones	1	27	0.002	4.55E-2	1.26E-1	2	17	0.002	Homo sapiens

Figure 19. Reactome pathway analysis representing top affected pathways in Stage III liver tumor individuals.

Discussion

Liver cancers are the third life threating cancer in the world, especially in developing countries. Gender, age, race and stage are the main parameters that has direct effect on the disease prognosis and hence the survival of the affected patients (14).

In this study, 336 individuals RNAseq data for 816 transcripts were analyzed to determine the differentially expressed transcripts and the gene that code for these transcripts. RNAseq data survival analysis was done in R using survival package "Survival". Liver tumor individuals were categorized into many subgroups based on their age, gender, race and stage of disease. Kaplan Meier fit for all liver tumor individuals' calculated median survival of 1685 days (4.6 years). The categorization of individuals based on age is difficult as there is not a proper definition for the young age, adult age and old age. In this study, liver tumor individuals were categorized based on their age to three groups; "Youth-group" that includes all individuals below the age of thirty years, "Adult-group" that includes all the individuals of age between thirty years and below sixty years old and "Old-group" that includes all individuals that are sixty years old or more. Median survival was about 1685 days, 1622 days and 827 days respectively. There is a significant difference in the genes coding for the transcript that are differentially expressed in the three age groups as well as the affected pathway. ***ANLN***, ***NCAPD2, DBF4, NDC1, VRK2*** and ***NOP58*** are the genes coding for the significantly differentially expressed transcripts ($p<5e-08$) in the adult group. On the other hand these adult-group genes are not significant in the old-group; ***DBF4*** and ***ANLN*** are only slightly associative in the old-age group. ***HEXB*** and ***STARD3NL*** were differentially expressed in the old-group but not in adult-group. Adult group and old-group differ in the affected metabolic pathways. Cell cycle and nuclear membrane defects are the major defective pathways in the adult group while hyaluronan uptake and degradation in the old group. Hyaluronan catabolism plays an important role in tumor suppression. Defective hyaluronan catabolism pathway may lead to liver tumors especially hyaluronan uptake is only by liver cells and hyaluronidases enzymes that breakdown hyaluronan are in the liver (15, 16). Young liver tumor individuals had totally different set of genes that are coding for the differentially expressed

transcripts; ***ABCC8, STMN4, EPN3 and LGALS14***. These genes were not differentially expressed in either Adult-group or Old-group. Glycemic pathways and ATP- dependent potassium channels ere the defective pathways in the young liver tumor individuals.

Race of liver tumor individual had a strong impact on the liver tumor survival and disease prognosis. High risk races represented by Asian; China (55% of liver tumors new cases), African, and low risk races White (17). In this study, the median survival of Asian and Afro-American individuals was not calculated for data inavailability. However the difference between Asian and Afro-American individuals was explained by the differentially expressed transcripts in both groups. ***NDC1, DSG2, DEPDC1, LAMC2, ZNF207, VRK2, NCAPD2, DEPDC1B, ANLN, UBA6, SCMH1, CDC27, E2F2 and ZZZ3*** are the genes coding for the top GWAS significant differentially expressed transcripts in the Asian liver tumor individuals. ***SPDL1, MAP3K9, ARNTL2, GCLM and DBF4*** were differentially expressed in Asian liver tumors but were not significant. In Afro-Americans, Asian differentially expressed genes were not significant instead another group of genes ***MTMR11, FUCA2, GRAMD1B, SEMA3F, HECW1, TMEM176A, USH1C***, ***BID and DNAH5*** were differentially expressed (not GWAS significant p > 5e-08). Cell cycle, SUMOylation of proteins and nuclear envelop were the defective pathways in Asian liver tumor individuals. TP53 regulated pathways, apoptosis and programmed cell death were the affected pathways in Afro-American liver tumor individuals accounting for the shorter median survival. In the White liver tumor individuals, median survival was about 1687 days. ***TKTL1, CDH10, ISL1, FUCA2 and PHF21B*** were the genes coding for the differentially expressed transcripts in the White liver tumor individulas, with FUCA2 in common with the Afro-American. Glucose and Insulin pathways were the main affected pathways in the White liver tumor samples and this is different from the underlying pathways in Asian and Afro-american liver tumor individuals.

Liver tumor staging for the primary hepatocellular liver tumors and intrahepatic bile duct; TMN classification (8) [Figure 20 Appendix II] is a very important parameter that affects disease prognosis and disease management in liver tumor individuals. In this study, the median survivals were 2500,

1600 and 665 days in Stage I, Stage II and Stage III respectively. Stage III had the shortest median survival of the three liver tumor stages. ***ANLN, NCAPD2, DBF4, NDC1, VRK2, NOP58*** are the top differentially expressed transcripts Stage III liver tumors; GWAS significant (p< 5e-08), while ***ABCF2, GDI2, GCLM, FUCA2, FAM136A, KDM1A and PQLC2*** were not GWAS significant (p < 5e-05). Cell cycle, NOTCH3 signaling, Glutathione synthesis and recycling, Signaling by Rho GTPase and DNA histone demethylation were the major affected pathways in Stage III liver tumors. ***CYP26B1, MTMR7, TKTL1, LYPLA2*** and ***ZIC2*** were the genes coding for the differentially expressed transcripts in Stage II. Defective Cytochrome CYP26B1, Phosphate bond hydrolysis by NTPDase proteins, Synthesis of PIPs at the late endosome membrane, Scavenging by Class A Receptors, HDMs demethylate histones and Activated PKN1 stimulates transcription of AR (androgen receptor) regulated genes KLK2 and KLK3 were the defective pathways in Stage II liver tumors. ***HEXB, C2orf83, SEC61A1, ABCB5, RRP12, GLRX2, CDKL3, RIOK2*** and ***RPL26L1*** (p > 5e-08); are genes coding for the differentially expressed transcripts in Stage I liver tumor individuals. Defective translation pathways were the majority of the affected pathways in Stage I. The three stage of liver tumors had different gene sets and defective metabolic pathways that their management strategies will also be different.

Liver tumors affect Males more than females (8). In this study, genetic and pathway analysis illustrated the difference between Male and Female liver tumor individuals. Median survivals were 1791 days and 1490 days in Males and Females respectively. Males lived longer than Females. This can be explained by the differentially expressed transcripts in the two groups and their affected pathways. Males had GWAS significant differentially expressed transcripts. The genes coding for the differentially expressed transcripts in Male liver tumor individuals were ***NDC1, ANLN, VRK2, DEPDC1, NCDN, MAP3K9, NCAPD2 and NOP58*** (p < 5e-08). Cell cycle, SUMOylation of target proteins and nuclear membrane were the defective pathways. On the other hand, ***SLC7A14*** was the gene coding for the GWAS significant differentially expressed transcript and ***ENTPD2, PHKA2, AOC1, PQLC2, DBF4, FUCA2, RETSAT and AGPS*** were the genes coding for not GWAS

significant differentially expressed transcripts in Female liver tumor individuals. Plasmalogen biosynthesis, NTPDase protein hydrolyze the phosphate bond, glycogen metabolism, activation of pre-replicative complex and activation of ATR in response to replication stress were the candidate affected pathways in Female liver tumors.

In conclusion, Liver tumor diseases have heterogeneous genetic makeup depending on the age, gender, race and stage of the liver tumor individual. Genetic and pathway analysis highlight the genetic architecture as well as the behavior of the liver tumor making therapeutic approaches more diverse in liver tumor individuals and help development of new drug targets for the aim of a better disease prognosis and survival. ***NCAPD2, ANLN, NOP58, FUCA2, DBF4, VRK2 and FAM136A*** were differentially expressed in different age, sex, race and stage groups. Cell cycle pathways, nuclear membrane, SUMOylation of target proteins were the main affected pathways in liver tumors.

References

1. Menche N. (ed.) Biologie Anatomie Physiologie. Munich: Urban & Fischer/ Elsevier; 2012.
2. Pschyrembel W. Klinisches Wörterbuch. Berlin: De Gruyter; 2014.
3. Rui L. Energy Metabolism in the Liver. *Comprehensive Physiology*. 2014;4(1):177-197. doi:10.1002/cphy.c130024.
4. Van den Berghe, G. J Inherit Metab Dis (1991) 14: 407.https://doi.org/10.1007/BF01797914
5. Roberts KB. Essentials of Human Physiology. Canadian Medical Association Journal. 1979;121(3):335-336.
6. Ishibashi, H., Nakamura, M., Komori, A. et al. Semin Immunopathol (2009) 31: 399. https://doi.org/10.1007/s00281-009-0155-6.
7. Ghouri, Y.A.; Misn, I.; Rowe, J.H. Review of hepatocellular carcinoma: Epidemiology, etiology, and carcinogenesis. J. Carcinog. 2017, 16, 1.
8. International Agency for Research on Cancer.WHO histological classification of tumours of the liver and intrahepatic bile ducts; Chapter 8. Available from https://www.iarc.fr/en/publications/pdfs-online/pat-gen/bb2/bb2-chap8.pdf.
9. Ananthakrishnan A, Gogineni V, Saeian K. Epidemiology of Primary and Secondary Liver Cancers. Seminars in Interventional Radiology. 2006;23(1):47-63. doi:10.1055/s-2006-939841.
10. R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.
11. Therneau T (2015). A Package for Survival Analysis in S. version 2.38, https://CRAN.R-project.org/package=survival.
12. National Center for Biotechnology Information (NCBI) [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2017 Apr 06]. Available from: https://www.ncbi.nlm.nih.gov/
13. Fabregat A, Jupe S, Matthews L et al. The Reactome Pathway Knowledgebase.Nucleic Acids Res. 2018 Jan 4;46 (D1):D649-D655. doi: 10.1093/nar/gkx1132
14. Zhang W, Sun B. Impact of age on the survival of patients with liver cancer: an analysis of 27,255 patients in the SEER database. Oncotarget. 2015;6(2):633-641.
15. Robert Stern; Devising a pathway for hyaluronan catabolism: are we there yet?, Glycobiology, Volume 13, Issue 12, 1 December 2003, Pages 105R–115R, https://doi.org/10.1093/glycob/cwg112
16. Bård Smedsrød Cellular events in the uptake and degradation of hyaluronan Advanced Drug Delivery Reviews1991, (7): 265-278. https://doi.org/10.1016/0169-409X(91)90006-X.
17. Ananthakrishnan A et al. Epidemiology of Primary and Secondary Liver Cancers. Seminars in interventional Radiology, 2006. 23(1).

Appendix I

List of Gene ID and Gene Names

ABCB5 ATP binding cassette subfamily B member 5 (Chromosome 7)
ABCC8 ATP binding cassette subfamily C member 8 (Chromosome 11)
ABCF2 ATP binding cassette subfamily F member 2 (Chromosome 7)
AGPS alkylglycerone phosphate synthase (Chromosome 2)
ANLN anillin actin binding protein (Chromosome 7)
AOC1 amine oxidase, copper containing 1 (Chromosome 7)
ARNTL2 aryl hydrocarbon receptor nuclear translocator like 2 (Chromosome 12)
BID BH3 interacting domain death agonist (Chromosome 22)
C2orf83 chromosome 2 open reading frame 83 (Chromosome 2)
CDC27 cell division cycle 27 (Chromosome 17)
CDH10 cadherin 10 (Chromosome 5)
CDKL3 cyclin dependent kinase like 3 (Chromosome 5)
CYP26B1 cytochrome P450 family 26 subfamily B member 1 (Chromosome 2)
DBF4 DBF4 zinc finger (Chromosome 7)
DEPDC1 DEP domain containing 1 (Chromosome 1)
DNAH5 dynein axonemal heavy chain 5 dynein axonemal heavy chain 5 (Chromosome 5)
DSG2 desmoglein 2 (Chromosome 18)
DVL2 dishevelled segment polarity protein 2 (Chromosome 17)
E2F2 E2F transcription factor 2 (Chromosome 1)
ENTPD2 ectonucleoside triphosphate diphosphohydrolase 2 (Chromosome 9)
EPN3 epsin 3 (Chromosome 17)
FAM136a family with sequence similarity 136 member A (Chromosome 2)
FUCA2 alpha-L-fucosidase 2 (Chromosome 6)
GCLM glutamate-cysteine ligase modifier subunit (Chromosome 1)
GDI2 GDP dissociation inhibitor 2 (Chromosome 10)
GLRX2 glutaredoxin 2 (Chromosome 1)
GRAMD1B GRAM domain containing 1B (Chromosome 11)
GUCA1A guanylate cyclase activator 1A (Chromosome 6)
HECW1 HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1 (Chromosome 7)
HEXB hexosaminidase subunit beta (Chromosome 5)
HSF2 heat shock transcription factor 2 (Chromosome 6)
ISL1 ISL LIM homeobox 1 (Chromosome 5)
KDM1A lysine demethylase 1A (Chromosome 1)
LAMC2 laminin subunit gamma 2 (Chromosome 1)
LGALS14 galectin 14 (Chromosome 19)
LYPLA2 lysophospholipase II (Chromosome 1)
MAP3K9 mitogen-activated protein kinase kinase kinase 9 (Chromosome 14)
MARCO macrophage receptor with collagenous structure (Chromosome 2)
MRTO4 MRT4 homolog, ribosome maturation factor (Chromosome 1)
MTA3 metastasis associated 1 family member 3 (Chromosome 2)
MTMR11 myotubularin related protein 11 (Chromosome 1)
MTMR7 myotubularin related protein 7 (Chromosome 8)
NCAPD2 non-SMC condensin I complex subunit D2 (Chromosome 12)
NDC1 NDC1 transmembrane nucleoporin (Chromosome 1)
NLRP2 NLR family pyrin domain containing 2 (Chromosome 19)
NOP58 NOP58 ribonucleoprotein (Chromosome 2)
PHF21B PHD finger protein 21B (Chromosome 22)

PHKA2 phosphorylase kinase regulatory subunit alpha 2 (Chromosome X)
PQLC2 PQ loop repeat containing 2 (Chromosome 1)
RETSAT retinol saturase (Chromosome 2)
RIOK2 RIO kinase 2 (Chromosome 5)
RPL26L1 ribosomal protein L26 like 1(Chromosome 5)
SCIN scinderin (Chromosome 7)
SCMH1 Scm polycomb group protein homolog 1 (Chromosome 1)
SEC61A1 Sec61 translocon alpha 1 subunit (Chromosome 3)
SEMA3F semaphorin 3F (Chromosome 3)
SLC7A14 solute carrier family 7 member 14 (Chromosome 3)
SPDL1 spindle apparatus coiled-coil protein 1 (Chromosome 5)
ST7L suppression of tumorigenicity 7 like (Chromosome 1)
STARD3NL STARD3 N-terminal like (Chromosome 7)
STMN4 stathmin 4 (Chromosome 8)
TACC3 transforming acidic coiled-coil containing protein 3 (Chromosome 4)
TKTL1 transketolase like 1(Chromosome X)
TMEM176A transmembrane protein 176A (Chromosome 7)
TTC27 tetratricopeptide repeat domain 27 (Chromosome 2)
UBA6 ubiquitin like modifier activating enzyme 6 (Chromosome 4)
USH1C USH1 protein network component harmonin (Chromosome 11)
VRK2 vaccinia related kinase 2 (Chromosome 2)
VTA1 vesicle trafficking 1 (Chromosome 6)
ZIC2 Zic family member 2 (Chromosome 13)
ZNF207 zinc finger protein 207 (Chromosome 17)
ZZZ3 zinc finger ZZ-type containing 3 (Chromosome 1)

Appendix II

WHO histological classification of tumours of the liver and intrahepatic bile ducts

Epithelial tumours	
Benign	
Hepatocellular adenoma (liver cell adenoma)	8170/0[1]
Focal nodular hyperplasia	
Intrahepatic bile duct adenoma	8160/0
Intrahepatic bile duct cystadenoma	8161/0
Biliary papillomatosis	8264/0
Malignant	
Hepatocellular carcinoma (liver cell carcinoma)	8170/3
Intrahepatic cholangiocarcinoma (peripheral bile duct carcinoma)	8160/3
Bile duct cystadenocarcinoma	8161/3
Combined hepatocellular and cholangiocarcinoma	8180/3
Hepatoblastoma	8970/3
Undifferentiated carcinoma	8020/3
Non-epithelial tumours	
Benign	
Angiomyolipoma	8860/0
Lymphangioma and lymphangiomatosis	9170/0
Haemangioma	9120/0
Infantile haemangioendothelioma	9130/0
Malignant	
Epithelioid haemangioendothelioma	9133/1
Angiosarcoma	9120/3
Embryonal sarcoma (undifferentiated sarcoma)	8991/3
Rhabdomyosarcoma	8900/3
Others	
Miscellaneous Tumours	
Solitary fibrous tumour	8815/0
Teratoma	9080/1
Yolk sac tumour (endodermal sinus tumour)	9071/3
Carcinosarcoma	8980/3
Kaposi sarcoma	9140/3
Rhabdoid tumour	8963/3
Others	
Haemopoietic and lymphoid tumours	
Secondary tumours	
Epithelial abnormalities	
Liver cell dysplasia (liver cell change)	
Large cell type (large cell change)	
Small cell type (small cell change)	
Dysplastic nodules (adenomatous hyperplasia)	
Low-grade	
High-grade (atypical adenomatous hyperplasia)	
Bile duct abnormalities	
Hyperplasia (bile duct epithelium and peribiliary glands)	
Dysplasia (bile duct epithelium and peribiliary glands)	
Intraepithelial carcinoma (carcinoma in situ)	8500/211
Miscellaneous lesions	
Mesenchymal hamartoma	
Nodular transformation (nodular regenerative hyperplasia)	
Inflammatory pseudotumour	

[1] Morphology code of the International Classification of Diseases for Oncology (ICD-O) {542} and the Systematized Nomenclature of Medicine (http://snomed.org). Behaviour is coded /0 for benign tumours, /1 for unspecified, borderline or uncertain behaviour, /2 for in situ carcinomas and grade III intraepithelial neoplasia and /3 for malignant tumours.

TNM classification of tumours of the liver and intrahepatic bile ducts

TNM classification[1, 2, 3]

T	Primary Tumour
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
T1	Solitary tumour 2 cm or less in greatest dimension without vascular invasion
T2	Solitary tumour 2 cm or less in greatest dimension with vascular invasion; or multiple tumours limited to one lobe, none more than 2 cm in greatest dimension without vascular invasion; or solitary tumour more than 2 cm in greatest dimension without vascular invasion.
T3	Solitary tumour more than 2 cm in greatest dimension with vascular invasion; or multiple tumours limited to one lobe, none more than 2 cm in greatest dimension with vascular invasion; or multiple tumours limited to one lobe, any more than 2 cm in greatest dimension with or without vascular invasion.
T4	Multiple tumours in more than one lobe; or tumour(s) involve(s) a major branch of the portal or hepatic vein(s); or tumour(s) with direct invasion of adjacent organs other than gallbladder; or tumour(s) with perforation of visceral peritoneum.

N – Regional Lymph Nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M – Distant Metastasis	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Stage Grouping

Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage IIIA	T3	N0	M0
Stage IIIB	T1	N1	M0
	T2	N1	M0
	T3	N1	M0
Stage IVA	T4	Any N	M0
Stage IVB	Any T	Any N	M1

[1] {1, 66}. This classification applies only to primary hepatocellular and cholangio-(intrahepatic bile duct) carcinomas of the liver.
[2] A help desk for specific questions about the TNM classification is available at http://tnm.uicc.org.
[3] For classification, the plane projecting between the bed of the gallbladder and the inferior vena cava divides the liver in two lobes.

Figure 20: WHO and TMN classification of the tumors of the liver and intrahepatic bile ducts