

Divergent Tissue and Circulatory Expression of miR-10a in Canine Hepatocellular Carcinoma: Comparative Insights from Human HCC

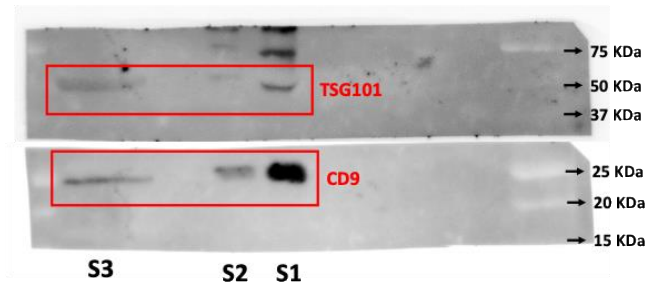
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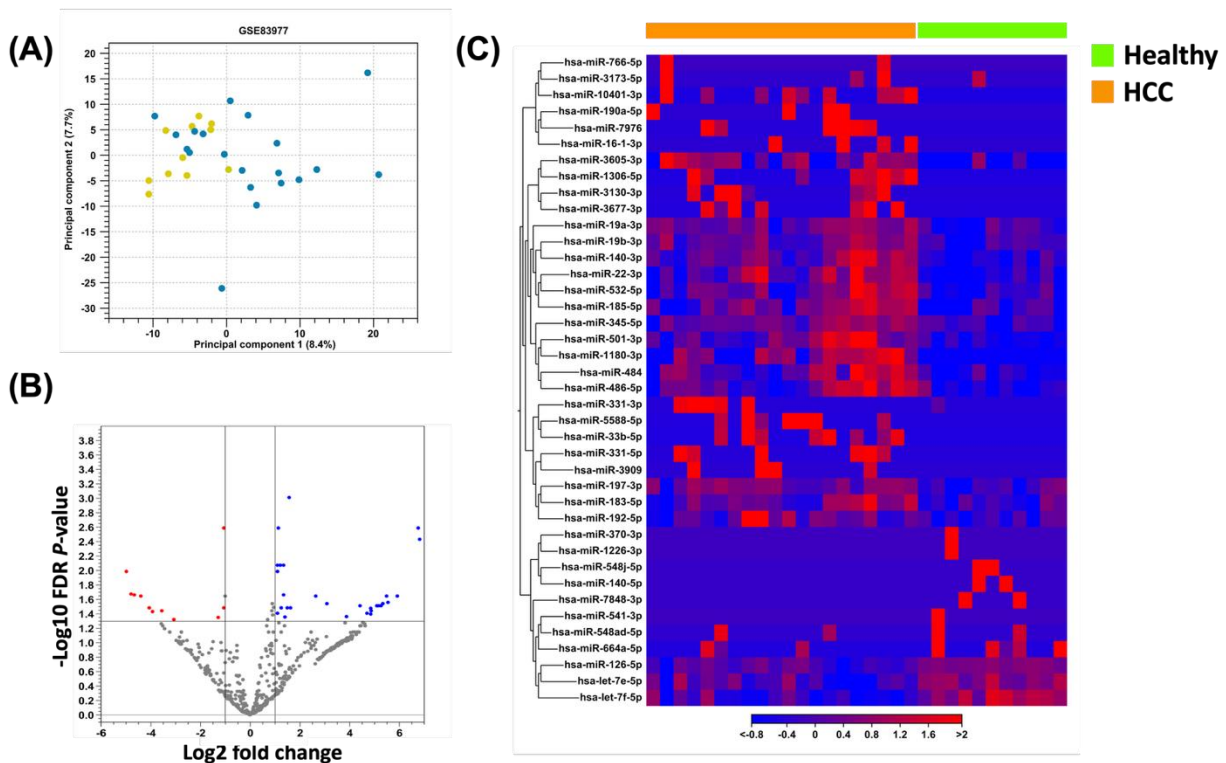
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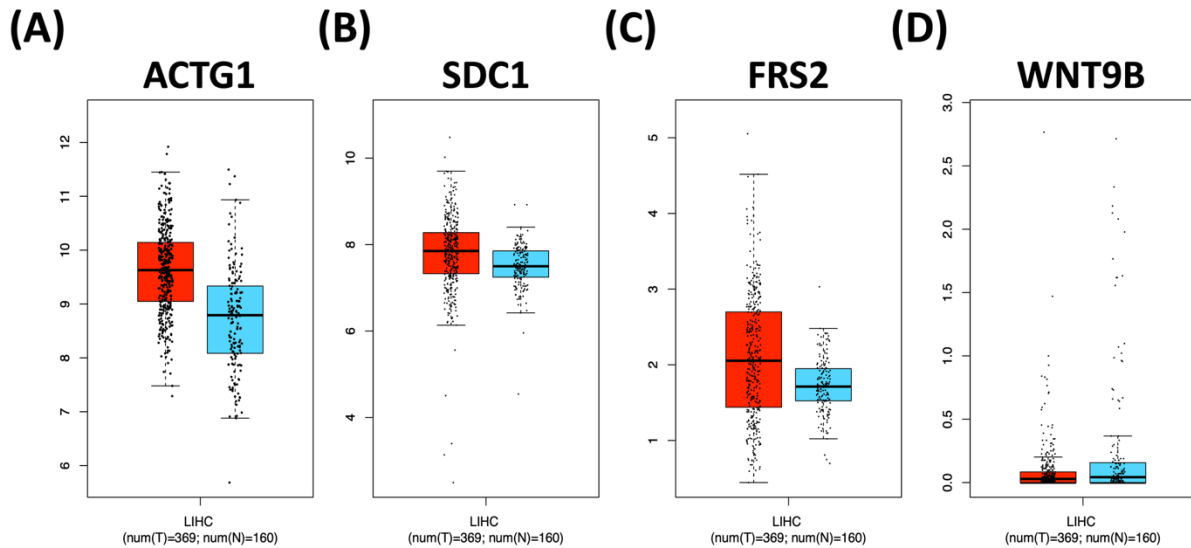
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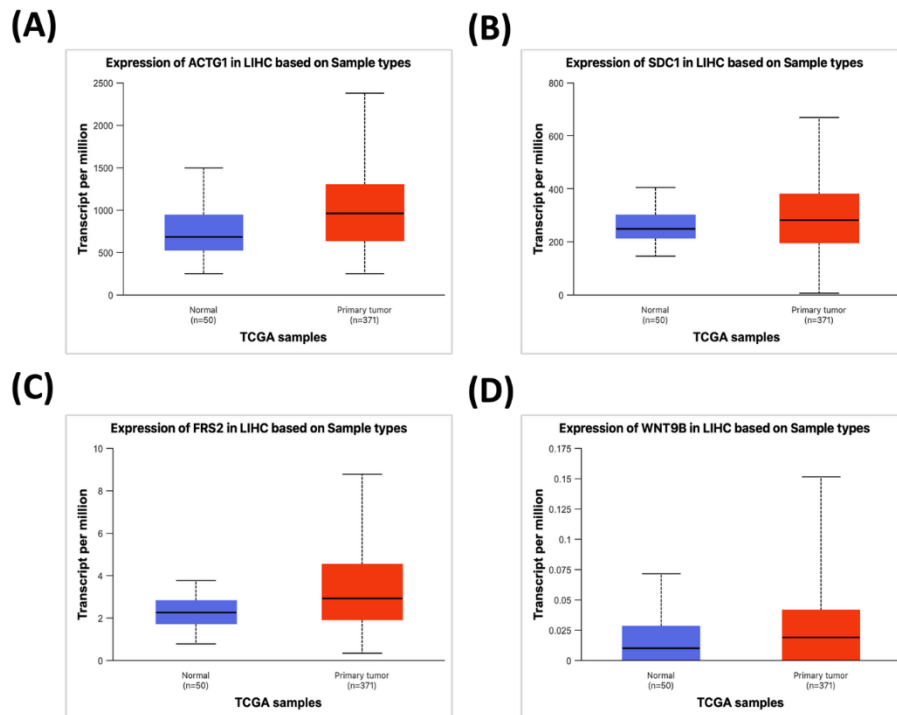
Supplementary Figure S1: Full western blot image showing specific bands for the exosomal markers CD9 and TSG101. S1, S2 and S3 correspond to three independent exosome preparations. Molecular weight markers (kDa) are indicated on the right side.



Supplementary Figure S2: Transcriptomic overview of differentially expressed serum exosomal miRNAs in human HCC from the GEO dataset (GSE83977). (A) PCA plot showing the clustering of HCC serum exosomes and healthy serum exosomes. Yellow dots represent healthy exosomes, and blue dots represent HCC exosomes. (B) Volcano plot displaying differentially expressed miRNAs. Blue dots represent upregulated miRNAs, red dots represent downregulated miRNAs, and gray dots represent miRNAs that were not differentially expressed. Differentially expressed miRNAs were identified using the criteria $|\text{fold change}| > 2$ and FDR-adjusted $p\text{-value} < 0.05$. (C) Hierarchical clustering of differentially expressed exosome-derived miRNAs in human HCC based on their expression profiles.



Supplementary Figure S3: Expression analysis of target genes contributing to the enrichment of the Proteoglycans in cancer pathway in canine and human HCC using the GEPIA2 online database. Expression levels of ACTG1 (A), SDC1 (B), FRS2 (C), and WNT9B (D) in HCC tissues. Red box represents tumor tissues and blue one represents normal liver tissues.



Supplementary Figure S4: Expression analysis of target genes contributing to the enrichment of the Proteoglycans in Cancer pathway in canine and human HCC using the UALCAN online database. Expression levels of ACTG1 (A), SDC1 (B), FRS2 (C), and WNT9B (D) in HCC tissues. Red box represents tumor tissues and blue one represents normal liver tissues.

Supplementary Table S1: Details of samples included in this study

Samples	Age	Diagnosis	Sex	Spayed/ Neutered	Breed	Tissue	Blood
NL-1	-	Healthy	-	-	Beagle	6	-
NL-2	-	Healthy	-	-	Beagle		
NL-3	-	Healthy	-	-	Beagle		
NL-4	-	Healthy	-	-	Beagle		
NL-5	-	Healthy	-	-	Beagle		
NL-6	-	Healthy	-	-	Beagle		
HB-1	4 Y 8 M	Healthy	M	No	Toy Poodle	-	7
HB-2	5 Y 3 M	Healthy	M	Yes	Mong		
HB-3	5 Y 4 M	Healthy	M	No	Pomeranian		
HB-4	7 Y 5 M	Healthy	F	Yes	Toy Poodle		
HB-5	11 Y 2 M	Healthy	F	No	Chihuahua		
HB-6	6 Y 4 M	Healthy	F	No	Shiba		
HB-7	7 Y	Healthy	F	Yes	Mong		
P1	11 Y 7 M	HCC	F	Yes	Shiba Inu	14	14
P2	11 Y 10 M	HCC	F	Yes	Shiba Inu		
P3	14 Y	HCC	F	Yes	Crossbreed		
P4	10 Y 9 M	HCC	F	No	Crossbreed		
P5	10 Y 3 M	HCC	F	No	Beagle		
P6	10 Y 9 M	HCC	F	No	Yorkshire Terrier		
P7	12 Y	HCC	F	No	Miniature Schnauzer		
P8	13 Y 10 M	HCC	F	No	Shetland Sheepdog		
P9	12 Y 3 M	HCC	F	No	Chihuahua		
P10	10 Y 8 M	HCC	M	Yes	Shiba		
P11	11 Y 7 M	HCC	M	Yes	Welsh Corgi		
P12	11 Y 6 M	HCC	M	No	Shiba		
P13	11 Y 10 M	HCC	M	No	Yorkshire Terrier		
P14	10 Y 4 M	HCC	M	Yes	Crossbreed		

NL - Normal liver tissue from experimental animal at Shin Nippon Biomedical Laboratories (SNBL, Kagoshima, Japan); HB- Blood samples from apparently healthy dogs collected from Chuou Aiken Animal Hospital, Kagoshima, Japan; P – Patient; Y – Year; M – Month; HCC - Hepatocellular carcinoma

Supplementary Table S2: A list of differentially expressed miRNAs in canine HCC. Following criteria were applied to filter the differentially expressed miRNAs: |fold change| > 2, FDR *p*-value < 0.05, and maximum group mean > 10.

Differential expression (No.)	Name	Max group mean	Log ₂ fold change	Fold change	<i>p</i> -value	FDR <i>p</i> -value	Bonferroni
Upregulated (46)	hsa-let-7i-5p	273,077.33	2	4.01	2.46E-03	0.03	1
	cfa-miR-222	47,745.33	2.36	5.13	1.39E-03	0.02	1
	cfa-miR-182	32,497.67	3.32	9.96	1.05E-06	6.39E-05	7.97E-04
	hsa-miR-193a-3p	30,260.33	2.55	5.88	4.46E-04	8.77E-03	0.34
	cfa-miR-301a	18,187.67	1.8	3.47	2.63E-03	0.03	1
	cfa-miR-8908a-3p	15,266.33	11.76	3,465.02	2.39E-15	1.45E-12	1.81E-12
	cfa-miR-34a	8,445.33	3.15	8.88	3.58E-09	5.45E-07	2.72E-06
	cfa-miR-483	5,589.67	4.26	19.11	9.52E-04	0.02	0.72
	cfa-miR-183	2,248.33	2.89	7.44	3.13E-05	9.53E-04	0.02
	hsa-let-7i-3p	1,867.00	2.08	4.22	2.22E-03	0.03	1
	hsa-miR-210-3p	1,595.00	3.03	8.16	3.36E-05	9.73E-04	0.03
	cfa-miR-551a	1,204.67	2.78	6.85	2.59E-04	5.43E-03	0.2
	cfa-miR-184	1,076.67	7.95	248.03	1.94E-09	3.94E-07	1.47E-06
	hsa-miR-675-3p	783.33	6.29	78	8.78E-08	1.07E-05	6.67E-05
	cfa-miR-8908b	760.67	12.06	4,263.99	6.34E-06	2.52E-04	4.82E-03
	hsa-miR-5701	609	2.94	7.7	6.52E-04	0.01	0.5
	cfa-miR-135b	602.67	5.4	42.18	2.62E-05	8.87E-04	0.02
	hsa-miR-105-5p	596	5.54	46.38	1.39E-06	7.72E-05	1.06E-03
	cfa-miR-8865	353.33	5.35	40.72	1.25E-09	3.80E-07	9.50E-07
	cfa-miR-8908c	344	10.91	1,928.36	5.89E-05	1.50E-03	0.04
	cfa-miR-8908d	341.33	10.9	1,913.42	5.92E-05	1.50E-03	0.04
	cfa-miR-210	322.67	3.49	11.25	9.44E-04	0.02	0.72
	cfa-miR-6529	222.67	3.03	8.19	2.95E-03	0.03	1
	hsa-miR-105-3p	208.33	5.1	34.31	1.42E-05	5.08E-04	0.01
	hsa-miR-1271-3p	177.33	5.12	34.87	1.00E-06	6.39E-05	7.60E-04
	cfa-miR-96	172.33	2.56	5.89	5.85E-04	0.01	0.44
	hsa-miR-1224-5p	171	2.91	7.51	9.98E-04	0.02	0.76
	cfa-miR-454	166.67	2.08	4.23	1.77E-03	0.02	1
	hsa-miR-10395-3p	166	2.8	6.99	1.17E-03	0.02	0.89
	hsa-miR-877-5p	163	3.42	10.67	2.49E-04	5.41E-03	0.19
	hsa-miR-34a-3p	146.67	3.85	14.45	6.63E-06	2.52E-04	5.04E-03
	cfa-miR-105a	142	4.81	27.96	1.35E-04	3.16E-03	0.1
	cfa-miR-8908a-5p	127.33	9.48	714.49	3.15E-04	6.39E-03	0.24
	hsa-miR-4508	85	2.94	7.65	2.36E-04	5.33E-03	0.18

	hsa-miR-7975	65.33	3.15	8.9	1.39E-03	0.02	1
	cfa-miR-2387	65	2.48	5.6	3.01E-03	0.03	1
	hsa-miR-190a-3p	60.67	2.46	5.5	1.84E-03	0.02	1
	hsa-miR-4443	59.33	3.49	11.21	4.62E-05	1.28E-03	0.04
	hsa-miR-4492	45	3.74	13.33	4.05E-06	1.89E-04	3.07E-03
	cfa-miR-551b	38	2.7	6.51	2.07E-03	0.03	1
	cfa-miR-105b	30.33	5.61	48.96	1.18E-03	0.02	0.9
	hsa-miR-302b-3p	27	4.51	22.78	1.10E-03	0.02	0.83
	cfa-miR-196a	20.33	4.53	23.07	1.85E-03	0.02	1
	cfa-miR-205	19	3.43	10.8	3.89E-03	0.04	1
	hsa-miR-4485-3p	18.67	7.07	134.53	4.02E-03	0.04	1
	hsa-miR-183-3p	14.33	4.81	28.06	1.21E-03	0.02	0.92
Downregulated (13)	cfa-miR-10a	1,629,083.33	-2.43	-5.37	5.20E-04	9.91E-03	0.4
	hsa-miR-30a-5p	961,197.00	-1.93	-3.81	1.32E-03	0.02	1
	cfa-miR-375	108,425.33	-3.54	-11.61	2.30E-06	1.17E-04	1.75E-03
	cfa-miR-146a	36,973.67	-3.16	-8.95	8.19E-07	6.24E-05	6.23E-04
	cfa-miR-215	3,816.67	-2.65	-6.27	4.87E-06	2.12E-04	3.70E-03
	cfa-miR-214	3,796.33	-1.88	-3.67	2.77E-03	0.03	1
	cfa-miR-338	1,401.00	-2.63	-6.2	2.88E-05	9.23E-04	0.02
	hsa-miR-338-3p	1,058.67	-2.37	-5.15	1.32E-03	0.02	1
	cfa-miR-490	437.33	-4.24	-18.96	4.62E-07	4.02E-05	3.51E-04
	hsa-miR-590-5p	69.67	-7.42	-170.8	2.00E-07	2.03E-05	1.52E-04
	hsa-miR-1-3p	58.67	-3.45	-10.92	1.35E-04	3.16E-03	0.1
	cfa-miR-1	16	-3.28	-9.73	4.23E-03	0.04	1
	hsa-miR-323b-3p	12.33	-4.9	-29.91	1.34E-03	0.02	1

Supplementary Table S3: A list of differentially expressed exosomal miRNAs in human HCC using GSE83977 dataset. Following criteria were applied to filter the differentially expressed miRNAs: $|\text{fold change}| > 2$, and FDR p -value < 0.05 .

Name	Max group mean	Log ₂ fold change	Fold change	p -value	FDR p -value	Bonferroni
hsa-miR-486-5p	1.15E+07	1.11	2.16	1.76E-04	0.01	0.13
hsa-let-7f-5p	19,246.82	-1.05	-2.07	1.32E-05	2.59E-03	9.81E-03
hsa-miR-22-3p	9,907.45	1.14	2.2	1.77E-05	2.60E-03	0.01
hsa-miR-192-5p	7,255.65	1.11	2.16	2.55E-03	0.04	1
hsa-miR-126-5p	1,970.09	-1.06	-2.09	1.77E-03	0.03	1
hsa-miR-501-3p	1,148.70	1.57	2.96	1.66E-06	9.76E-04	1.23E-03
hsa-miR-484	523.95	1.1	2.14	1.02E-04	8.50E-03	0.08
hsa-miR-19b-3p	444.15	1.35	2.55	4.81E-04	0.02	0.36
hsa-miR-532-5p	410.85	1.35	2.55	1.09E-04	8.50E-03	0.08
hsa-miR-140-3p	348.1	1.21	2.31	1.16E-04	8.50E-03	0.09
hsa-miR-197-3p	267.9	1.62	3.07	1.60E-03	0.03	1
hsa-miR-183-5p	208.3	1.49	2.81	1.79E-03	0.03	1
hsa-miR-19a-3p	197.75	1.25	2.38	1.71E-03	0.03	1
hsa-let-7e-5p	167.64	-1.28	-2.42	3.38E-03	0.05	1
hsa-miR-345-5p	58.7	1.39	2.63	3.25E-03	0.04	1
hsa-miR-185-5p	19.95	2.65	6.26	5.50E-04	0.02	0.41
hsa-miR-664a-5p	12.55	-3.07	-8.37	3.67E-03	0.05	1
hsa-miR-3605-3p	11.1	3.08	8.48	1.04E-03	0.03	0.77
hsa-miR-548ad-5p	8.55	-3.55	-11.74	2.18E-03	0.04	1
hsa-miR-10401-3p	7.05	6.82	113.01	3.18E-05	3.74E-03	0.02
hsa-miR-1180-3p	6.5	6.78	109.69	1.22E-05	2.59E-03	9.03E-03
hsa-miR-541-3p	6.18	-4.8	-27.8	4.00E-04	0.02	0.3
hsa-miR-548j-5p	5.09	-4.06	-16.64	1.68E-03	0.03	1
hsa-miR-7848-3p	4.91	-4.98	-31.57	1.73E-04	0.01	0.13
hsa-miR-190a-5p	4.4	5.56	47.07	9.00E-04	0.03	0.67
hsa-miR-3173-5p	4.25	3.88	14.75	3.14E-03	0.04	1
hsa-miR-1226-3p	4	-4.66	-25.32	4.82E-04	0.02	0.36
hsa-miR-5588-5p	3.7	5.49	44.98	6.35E-04	0.02	0.47
hsa-miR-766-5p	3.65	5.92	60.7	6.32E-04	0.02	0.47
hsa-miR-140-5p	3.45	-4.39	-21.02	6.97E-04	0.02	0.52
hsa-miR-3130-3p	3.4	5.34	40.5	1.02E-03	0.03	0.75
hsa-miR-331-3p	3.25	4.43	21.56	1.24E-03	0.03	0.92
hsa-miR-370-3p	2.36	-3.93	-15.23	2.34E-03	0.04	1
hsa-miR-3677-3p	2.35	5.1	34.22	1.37E-03	0.03	1
hsa-miR-3909	2.25	4.86	29.03	2.73E-03	0.04	1
hsa-miR-16-1-3p	2.15	5.26	38.45	1.37E-03	0.03	1

hsa-miR-7976	2.05	5.18	36.23	1.32E-03	0.03	0.98
hsa-miR-33b-5p	1.9	4.85	28.76	1.88E-03	0.03	1
hsa-miR-331-5p	1.65	4.86	29.14	2.23E-03	0.04	1
hsa-miR-1306-5p	1.5	4.7	25.93	2.61E-03	0.04	1