Methods

Acquisition and analysis of miRNA expression

The data of microRNA expression in ccRCC and normal tissues from BC Cancer Canada's Michael Smith Genome Sciences Centre (BSGSC, https://www.bcgsc.ca/) was acquired and analyzed with Sangerbox, a free online platform for data analysis (http://www.sangerbox.com/tool). According to Oncomir, an open access database (26). The relationship between the expression of microRNAs and the survival outcomes of ccRCC patients were analyzed. Diana TarBase V8 (27) and Encyclopedia of RNA Interactomes (ENCORI) (28) were used to predict the target genes. GEPIA (29) was used to preliminarily explore the possible relationship between the genes and the outcome of ccRCC patients.

Cell culture, sEVs isolation and identification

Human RCC cell lines A498, 786-O, OS-RC-2, and human renal tubular epithelial cell line HK2 were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Human ccRCC cell line SW839, ACHN was purchased from Suran Biotechnology (Shanghai, China). The culture conditions are as follows, ACHN and A498: MEM medium (GIBCO, USA) +10% FBS (GIBCO, USA) +1% penicillin streptomycin(GIBCO, USA), 5% CO2, 37 ℃. OS-RS-2, SW839, 786-O and HK2: RPMI-1640 medium (GIBCO, USA) +10% FBS+1% penicillin, 5% CO2, 37 ℃. Spheres formation culture medium: serum-free DMEM/F12 (Invitrogen, USA), supplemented with B27 (1:50, Invitrogen), 20 ng ml EGF (Peprotech), 10 ng ml bFGF (Invitrogen), and 4 mgml insulin (Sigma).

When cells are cultured for sEVs isolation, we use ultrafiltration EV-depleted FBS (UF-dFBS). According to Kornilov *et al.* (30), the FBS aforementioned is centrifuged at 3,000 g for 55 min using Amicon® Ultra-15 ultrafiltration centrifuge tubes (100kDa, Millipore, USA) to remove sEVs from bovine.

sEVs were isolated as existing reports and our previous study (32-34). Cell culture medium was replaced with serum-free conditioned medium (CM, Gibco, USA) when reaches the density of 90%. 48 hours later, CM was collected and centrifuged at 300×g for 10 minutes at 4 ℃ (Xiangyi, China). The supernatant was collected and centrifuge at 2000×g, 4 ℃ for 10 minutes (Beckman, CA, USA). Then filter the supernatant with a 0.22 μm filter (Millipore, USA) in the vertical clean benches to remove cell debris. The filtered supernatant was centrifuged at 100,000×g at 4 ℃ for 2 hours using an ultracentrifuge (Beckman, CA, USA) to precipitate sEVs. Then the pellet was washed and resuspended in sterile 50 uL PBS,centrifuged at 4,000×g for 10 minutes at 4 ℃ (Beckman, CA, USA) to make sure there was no cell debris. Finally, in total 200 mL sterile PBS was used to resuspend the sEVs pellet. The extracted sEVs suspension was frozen and stored in a refrigerator at -80 ℃.

The obtained sEVs suspension was placed under a transmission electron microscope (TEM, Hitachi H-7650) to observe and evaluate the morphology. A Nanoparticle Tracking Analyzer (Particle Metrix, Germany) was used to detect the size and distribution of the sEVs. Western blot was applied to detect the sEVs marker proteins.

Operation of mouse kidney local injection

Mice were nesthetized using 1% sodium pentobarbital, 0.08 mg/g and subcutaneously injected with butorphanol (0.001 mg/g) before injection. The mice were then depilated on the back, placed prone on the operating table, and the operation area was disinfected. The adipose tissue could be seen after cutting the skin at 1 cm from the left side of the spine and 2 cm from the lower edge of the ribs. Then, cut the fascia along the middle of the adipose tissue and removed the adipose to expose the kidney. 150 μL liquid was injected into kidney tissue around the renal artery.

Information of antibodies and primers

Anti-CD105, abcam, UK. Anti-CD133, abcam. Anti-CD9, abcam. Anti-CD63, abcam. Anti-CD81: abcam. Anti-TSG101, santa cruz, USA. Anti-Caspase-3, abcam. Anti-Caspase12, abcam. Anti-GRP78, abcam. Anti-PERK, abcam. Anti-IRE1α, abcam. Anti-ATF6, abcam. Anti-ATF4, abcam. Anti-CHOP, abcam. Anti-ERP44, abcam. Anti-β-Actin, abcam. Goat anti-

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rabbit IgG-HRP secondary antibody, santa cruz.

The microRNA primers were designed and synthesized by RiboBio (RiboBio Biotechnology Ltd., Guangzhou, China). The primers for CSCs identification were follows: CD105, Forward (5'-3') CACTAGCCAGGTCTCGAAGG, Reverse (5'-3') CTGAGGACCAGAAGCACCTC; CD133, Forward (5'-3')GCAGCAGTCTGACCAGCGTGAA, Reverse (5'-3') ACGGGTGGAAGCTGCCTCAGTT; c-Myc, Forward (5'-3')CATCATCATCCAGGACTGTATGTG, Reverse (5'-3') GGCTGCCGCTGTCTTTGC; Klf4, Forward (5'-3') GCCCCTCGGGCGGCTTCGTGGCCGAGCTC, Reverse (5'-3') CGTACTCGCTGCCAGGGGCG; Nanog, Forward (5'-3') AATACCTCAGCCTCCAGCAGATG, Reverse (5'-3') TGCGTCACACCATTGCTATTCTTC; Sox2, Forward (5'-3') AAATGGGAGGGGTGCAAAAGAGGAG, Reverse (5'-3') CAGCTGTCATTTGCTGTGGGTGATG; Oct4, Forward (5'-3') CTTGCTGCAGAAGTGGGTGGAGGAA, Reverse (5'-3') CTGCAGTGTGGGTTTCGGGCA.

Statical analysis

Box plots were drawn for the data in each group, and the outlier standard was 3 times more than SD. After testing, there was no obvious outlier in each group. The Shapiro-Wilk normality test was used to detect the distribution of dependent variables (including 24-h urinary protein and Scr) within each group. Most of the results showed $P>0.05$, indicating that the data obeyed the normal distribution. For dependent variables that do not follow a normal distribution ($P<0.05$), non-parametric tests are used according to the study design. The comparison of the two and multiple groups means were respectively analyzed using the Mann-Whitney U test and Kruskal-Wallis H test. Considering the non-parametric test did not change the conclusion, the effect size and significance level were uniformly presented using the results of student's t test and ANOVA to maintain the consistency of the results report.

https://dx.doi.org/10.21037/tau-21-1007

Table S1 Up-regulated or down-regulated microRNAs in ccRCC

row.names(et$table)	logFC	logCPM	PValue	FDR	regulated
hsa-mir-106b	1.345475	8.278517	6.87E-96	1.29E-92	Up-Regulated
hsa-mir-122	6.156104	4.734137	5.39E-74	1.01E-70	Up-Regulated
hsa-mir-1228	1.510224	1.456964	6.92E-10	1.18E-06	Up-Regulated
hsa-mir-1269b	2.999629	4.567055	3.07E-05	0.04956	Up-Regulated
hsa-mir-1270	1.880128	4.305779	2.99E-24	5.39E-21	Up-Regulated
hsa-mir-1271	1.501855	3.6106	3.40E-22	6.11E-19	Up-Regulated
hsa-mir-1277	1.067413	1.958075	6.76E-11	1.16E-07	Up-Regulated
hsa-mir-1293	2.984457	1.511091	2.05E-13	3.56E-10	Up-Regulated
hsa-mir-1295a	1.266404	1.462018	1.65E-07	0.000277	Up-Regulated
hsa-mir-130b	1.119691	3.838239	6.72E-27	1.22E-23	Up-Regulated
hsa-mir-142	2.133956	11.52664	1.41E-43	2.61E-40	Up-Regulated
hsa-mir-144	1.994261	8.624419	2.31E-20	4.12E-17	Up-Regulated
hsa-mir-146a	1.284047	6.543904	1.71E-21	3.07E-18	Up-Regulated
hsa-mir-146b	1.607339	9.475595	1.48E-17	2.61E-14	Up-Regulated
hsa-mir-153-2	1.315191	3.553436	1.62E-13	2.83E-10	Up-Regulated
hsa-mir-155	3.695546	9.262616	1.58E-67	2.93E-64	Up-Regulated
hsa-mir-15a	1.331327	7.186753	3.37E-73	6.29E-70	Up-Regulated
hsa-mir-16-1	1.209316	8.444548	1.29E-64	2.40E-61	Up-Regulated
hsa-mir-16-2	1.215486	8.45293	4.73E-64	8.77E-61	Up-Regulated
hsa-mir-181a-1	1.055438	9.775196	3.17E-26	5.75E-23	Up-Regulated
hsa-mir-181b-1	1.420349	7.448525	1.83E-41	3.36E-38	Up-Regulated
hsa-mir-181b-2	1.399268	7.31536	8.38E-37	1.54E-33	Up-Regulated
hsa-mir-18a	1.104123	3.117546	1.06E-18	1.89E-15	Up-Regulated
hsa-mir-193a	1.032486	7.800495	1.09E-27	1.98E-24	Up-Regulated
hsa-mir-21	2.339519	17.41738	5.75E-78	1.07E-74	Up-Regulated
hsa-mir-210	3.250836	11.62392	4.19E-82	7.82E-79	Up-Regulated
hsa-mir-215	1.749446	6.301706	3.77E-16	6.65E-13	Up-Regulated
hsa-mir-221	1.157826	7.040486	1.18E-09	2.00E-06	Up-Regulated
hsa-mir-223	1.142185	7.002875	5.81E-16	1.02E-12	Up-Regulated
hsa-mir-224	2.570346	5.15238	3.99E-41	7.35E-38	Up-Regulated
hsa-mir-2277	1.463943	1.835638	7.11E-15	1.24E-11	Up-Regulated
hsa-mir-2355	1.687088	6.120418	7.83E-71	1.46E-67	Up-Regulated
hsa-mir-25	1.088127	12.21046	2.22E-50	4.11E-47	Up-Regulated
hsa-mir-301b	1.263244	1.415144	7.88E-08	0.000132	Up-Regulated
hsa-mir-3130-1	1.427266	2.023684	1.66E-15	2.92E-12	Up-Regulated
hsa-mir-3130-2	1.505952	2.046906	6.73E-18	1.19E-14	Up-Regulated
hsa-mir-3170	1.096728	2.952769	8.37E-14	1.46E-10	Up-Regulated
hsa-mir-3191	1.289114	1.300534	1.63E-06	0.002694	Up-Regulated
hsa-mir-320c-1	1.204009	1.293245	3.31E-06	0.005422	Up-Regulated
hsa-mir-320d-2	1.211028	1.257172	7.61E-06	0.012385	Up-Regulated
hsa-mir-330	1.124373	4.136204	2.03E-29	3.70E-26	Up-Regulated
hsa-mir-33b	1.442921	2.20491	4.24E-13	7.36E-10	Up-Regulated

Table S1 (*continued*)

https://dx.doi.org/10.21037/tau-21-1007

Table S1 (*continued*)

row.names(et$table)	logFC	logCPM	PValue	FDR	regulated
hsa-mir-342	1.263284	7.178999	3.56E-34	6.51E-31	Up-Regulated
hsa-mir-3591	1.669085	1.267662	2.74E-09	4.63E-06	Up-Regulated
hsa-mir-3609	2.133627	1.566439	4.63E-07	0.00077	Up-Regulated
hsa-mir-3613	1.79747	4.171263	1.46E-66	2.72E-63	Up-Regulated
hsa-mir-3614	1.249641	1.476819	2.38E-08	4.01E-05	Up-Regulated
hsa-mir-3615	1.599853	1.930363	6.01E-17	1.06E-13	Up-Regulated
hsa-mir-3653	1.02637	4.662008	3.23E-07	0.000538	Up-Regulated
hsa-mir-365a	1.15475	5.993383	3.38E-24	6.11E-21	Up-Regulated
hsa-mir-365b	1.160107	5.996155	1.40E-24	2.53E-21	Up-Regulated
hsa-mir-3678	1.666652	1.407218	1.10E-10	1.88E-07	Up-Regulated
hsa-mir-3690-1	1.287488	1.413983	2.33E-07	0.000388	Up-Regulated
hsa-mir-374a	1.087263	9.719855	7.04E-43	1.30E-39	Up-Regulated
hsa-mir-374c	2.690249	2.265415	2.85E-13	4.96E-10	Up-Regulated
hsa-mir-3940	1.310685	1.395912	1.16E-06	0.001911	Up-Regulated
hsa-mir-3941	2.176489	1.658295	8.90E-21	1.59E-17	Up-Regulated
hsa-mir-451a	1.606532	10.16607	1.46E-13	2.54E-10	Up-Regulated
hsa-mir-452	2.139804	6.102088	1.54E-44	2.84E-41	Up-Regulated
hsa-mir-4652	2.788864	1.788183	3.74E-18	6.63E-15	Up-Regulated
hsa-mir-4677	1.249206	3.113801	1.19E-33	2.17E-30	Up-Regulated
hsa-mir-4746	1.365468	1.562548	5.49E-10	9.36E-07	Up-Regulated
hsa-mir-4772	2.082579	2.648554	5.78E-38	1.06E-34	Up-Regulated
hsa-mir-4773-1	2.400136	1.273233	1.82E-15	3.19E-12	Up-Regulated
hsa-mir-4773-2	2.45798	1.286346	4.19E-16	7.38E-13	Up-Regulated
hsa-mir-4784	1.71081	1.208849	3.29E-06	0.005393	Up-Regulated
hsa-mir-486-1	1.31577	7.012406	2.63E-10	4.50E-07	Up-Regulated
hsa-mir-486-2	1.348452	7.011024	9.80E-11	1.68E-07	Up-Regulated
hsa-mir-4999	1.128808	1.486251	5.27E-07	0.000876	Up-Regulated
hsa-mir-5000	1.073655	2.226023	4.31E-14	7.53E-11	Up-Regulated
hsa-mir-550a-1	1.101411	2.09581	1.07E-11	1.85E-08	Up-Regulated
hsa-mir-550a-3	1.216265	1.673367	8.72E-10	1.48E-06	Up-Regulated
hsa-mir-5586	1.465211	2.255373	1.71E-16	3.02E-13	Up-Regulated
hsa-mir-5588	1.175318	1.579854	8.88E-09	1.50E-05	Up-Regulated
hsa-mir-5683	1.088071	2.001164	1.70E-06	0.00281	Up-Regulated
hsa-mir-576	1.170472	3.886686	1.60E-36	2.93E-33	Up-Regulated
hsa-mir-584	2.297806	5.655345	5.30E-69	9.86E-66	Up-Regulated
hsa-mir-590	1.113889	4.491627	5.79E-37	1.06E-33	Up-Regulated
hsa-mir-592	3.194073	4.750831	8.99E-40	1.65E-36	Up-Regulated
hsa-mir-599	3.479489	3.029658	9.40E-12	1.62E-08	Up-Regulated
hsa-mir-616	1.366744	2.887106	2.64E-21	4.73E-18	Up-Regulated
hsa-mir-618	1.388311	1.75801	3.66E-10	6.24E-07	Up-Regulated
hsa-mir-625	1.219445	6.621009	3.82E-24	6.89E-21	Up-Regulated

Table S1 (*continued*)

https://dx.doi.org/10.21037/tau-21-1007

Table S1 (*continued*)

row.names(et$table)	logFC	logCPM	PValue	FDR	regulated
hsa-mir-629	1.67041	6.254662	6.05E-69	1.13E-65	Up-Regulated
hsa-mir-643	1.186165	1.37535	1.98E-06	0.003264	Up-Regulated
hsa-mir-6509	1.987878	1.830431	1.10E-21	1.98E-18	Up-Regulated
hsa-mir-653	1.534865	6.996749	3.78E-11	6.51E-08	Up-Regulated
hsa-mir-6718	1.556049	2.017569	1.11E-06	0.00183	Up-Regulated
hsa-mir-708	1.009815	6.499353	1.31E-10	2.23E-07	Up-Regulated
hsa-mir-760	1.76683	1.745474	1.19E-15	2.09E-12	Up-Regulated
hsa-mir-7702	1.570163	1.694958	8.03E-08	0.000135	Up-Regulated
hsa-mir-7978	1.309768	1.300901	6.23E-07	0.001036	Up-Regulated
hsa-mir-875	3.338812	1.669042	5.11E-12	8.83E-09	Up-Regulated
hsa-mir-885	3.780009	4.243653	1.64E-38	3.01E-35	Up-Regulated
hsa-mir-891a	3.732082	9.497127	9.05E-07	0.0015	Up-Regulated
hsa-mir-93	1.217163	11.37587	3.15E-40	5.79E-37	Up-Regulated
hsa-mir-935	1.255269	1.74411	9.21E-07	0.001524	Up-Regulated
hsa-mir-937	1.143806	1.382899	1.34E-06	0.002219	Up-Regulated
hsa-let-7e	-1.03931	10.27405	1.18E-29	2.16E-26	Down-Regulated
hsa-mir-1-1	-1.5815	3.051193	9.88E-25	1.79E-21	Down-Regulated
hsa-mir-1-2	-1.52958	3.113564	3.43E-23	6.18E-20	Down-Regulated
hsa-mir-1251	-2.07951	3.492983	3.05E-21	5.45E-18	Down-Regulated
hsa-mir-129-1	-3.72706	2.326702	3.71E-89	6.93E-86	Down-Regulated
hsa-mir-129-2	-3.49805	2.384484	3.86E-73	7.20E-70	Down-Regulated
hsa-mir-135a-1	-1.38004	3.397755	1.30E-11	2.25E-08	Down-Regulated
hsa-mir-135a-2	-1.44984	3.503521	1.42E-12	2.46E-09	Down-Regulated
hsa-mir-136	-1.14728	3.925742	7.46E-19	1.33E-15	Down-Regulated
hsa-mir-138-1	-2.25323	1.79101	1.75E-25	3.18E-22	Down-Regulated
hsa-mir-138-2	-1.97901	1.617763	1.21E-22	2.17E-19	Down-Regulated
hsa-mir-141	-2.28464	6.441843	1.93E-16	3.41E-13	Down-Regulated
hsa-mir-149	-1.45669	3.715346	7.88E-30	1.44E-26	Down-Regulated
hsa-mir-184	-3.16844	2.238006	6.56E-23	1.18E-19	Down-Regulated
hsa-mir-187	-1.15289	4.38913	1.36E-05	0.022056	Down-Regulated
hsa-mir-188	-1.77682	2.682317	6.16E-65	1.14E-61	Down-Regulated
hsa-mir-199a-1	-1.20231	8.833864	5.95E-27	1.08E-23	Down-Regulated
hsa-mir-199a-2	-1.15613	9.555863	5.04E-25	9.11E-22	Down-Regulated
hsa-mir-199b	-1.08988	9.814238	1.42E-21	2.55E-18	Down-Regulated
hsa-mir-200a	-1.22558	8.210674	4.43E-35	8.12E-32	Down-Regulated
hsa-mir-200b	-1.38119	7.860998	7.23E-37	1.33E-33	Down-Regulated
hsa-mir-200c	-2.98381	8.603762	2.68E-38	4.92E-35	Down-Regulated
hsa-mir-203a	-1.75469	9.159506	1.92E-33	3.51E-30	Down-Regulated
hsa-mir-203b	-2.42411	2.958851	1.13E-25	2.05E-22	Down-Regulated
hsa-mir-204	-1.39098	8.744475	8.23E-16	1.45E-12	Down-Regulated
hsa-mir-20b	-1.08785	4.921541	1.22E-18	2.16E-15	Down-Regulated

Table S1 (*continued*)

https://dx.doi.org/10.21037/tau-21-1007

Table S1 (*continued*)

row.names(et$table)	logFC	logCPM	PValue	FDR	regulated
hsa-mir-214	-1.18799	3.305088	1.12E-20	2.00E-17	Down-Regulated
hsa-mir-216b	-2.9701	1.314272	2.08E-30	3.79E-27	Down-Regulated
hsa-mir-217	-1.37703	5.84582	4.69E-11	8.06E-08	Down-Regulated
hsa-mir-3065	-1.22912	4.965322	4.48E-12	7.74E-09	Down-Regulated
hsa-mir-323a	-1.06108	1.808893	1.88E-06	0.003105	Down-Regulated
hsa-mir-323b	-1.30545	2.189406	1.91E-13	3.32E-10	Down-Regulated
hsa-mir-362	-2.45774	4.605934	3.03E-106	5.68E-103	Down-Regulated
hsa-mir-363	-1.73483	3.960189	4.66E-53	8.62E-50	Down-Regulated
hsa-mir-372	-1.88256	1.125039	1.41E-18	2.50E-15	Down-Regulated
hsa-mir-376a-1	-1.08515	1.369097	9.54E-08	0.00016	Down-Regulated
hsa-mir-411	-1.01942	1.980453	1.81E-09	3.07E-06	Down-Regulated
hsa-mir-429	-1.69119	5.285152	9.21E-54	1.71E-50	Down-Regulated
hsa-mir-433	-1.3458	1.301413	5.39E-14	9.41E-11	Down-Regulated
hsa-mir-4484	-1.08616	1.380582	8.82E-06	0.014357	Down-Regulated
hsa-mir-500a	-1.4449	8.238412	1.25E-63	2.31E-60	Down-Regulated
hsa-mir-500b	-1.08849	3.172918	9.64E-27	1.75E-23	Down-Regulated
hsa-mir-501	-1.22784	6.239633	2.12E-39	3.90E-36	Down-Regulated
hsa-mir-506	-5.09899	1.597163	2.42E-165	4.54E-162	Down-Regulated
hsa-mir-507	-3.41432	1.100654	6.19E-58	1.15E-54	Down-Regulated
hsa-mir-508	-4.33007	5.42692	1.51E-187	2.85E-184	Down-Regulated
hsa-mir-509-1	-2.97281	2.429234	8.37E-94	1.57E-90	Down-Regulated
hsa-mir-509-2	-2.98874	2.455304	7.17E-102	1.34E-98	Down-Regulated
hsa-mir-509-3	-3.2217	2.590325	3.42E-112	6.40E-109	Down-Regulated
hsa-mir-510	-1.83937	0.994094	6.15E-18	1.09E-14	Down-Regulated
hsa-mir-513a-1	-2.11675	1.020481	1.55E-21	2.77E-18	Down-Regulated
hsa-mir-513a-2	-2.00606	1.007569	2.04E-19	3.64E-16	Down-Regulated
hsa-mir-513b	-1.80319	1.017562	1.33E-14	2.33E-11	Down-Regulated
hsa-mir-513c	-2.98688	1.129884	5.02E-47	9.29E-44	Down-Regulated
hsa-mir-514a-1	-4.30775	3.652777	4.46E-164	8.38E-161	Down-Regulated
hsa-mir-514a-2	-4.27812	3.652255	1.13E-155	2.13E-152	Down-Regulated
hsa-mir-514a-3	-4.33247	3.650037	3.98E-163	7.47E-160	Down-Regulated
hsa-mir-514b	-5.06829	1.379762	1.13E-127	2.12E-124	Down-Regulated
hsa-mir-532	-1.13405	9.87942	1.28E-34	2.34E-31	Down-Regulated
hsa-mir-5708	-1.14922	1.022273	7.66E-07	0.001271	Down-Regulated
hsa-mir-6507	-1.38923	1.036311	1.08E-09	1.84E-06	Down-Regulated
hsa-mir-660	-1.47239	5.538949	1.46E-45	2.71E-42	Down-Regulated
hsa-mir-6723	-1.14686	1.038441	3.91E-06	0.006411	Down-Regulated
hsa-mir-675	-1.5572	5.90678	1.28E-11	2.21E-08	Down-Regulated
hsa-mir-6863	-1.54622	1.014219	1.54E-08	2.60E-05	Down-Regulated
hsa-mir-934	-4.92701	1.354789	4.99E-129	9.36E-126	Down-Regulated

https://dx.doi.org/10.21037/tau-21-1007

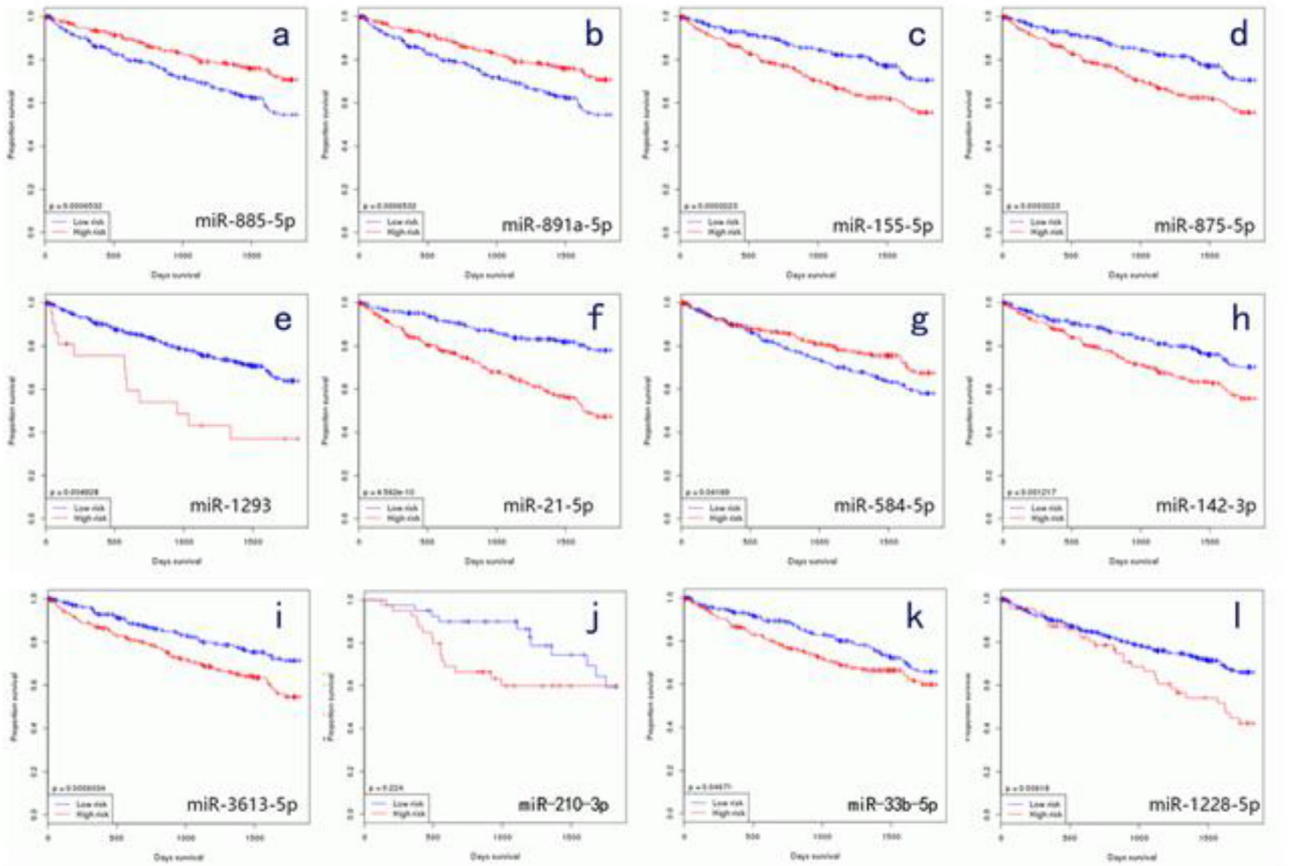

Figure S1 Survival outcomes of patients with specific microRNAs highly expressed, 12 microRNAs were able to affect the survival outcomes of ccRCC patients.

https://dx.doi.org/10.21037/tau-21-1007