

Supplementary data to:

Original article:

**SIMULTANEOUS MICRORNA-612 RESTORATION AND 5-FU
TREATMENT INHIBIT THE GROWTH AND MIGRATION OF
HUMAN PANC-1 PANCREATIC CANCER CELLS**

Darya Javadrashid^{1,2}, Reza Mohammadzadeh^{2*}, Amir Baghbanzadeh¹, Sahar Safae¹,
Mohammad Amini¹, Ziba Lotfi¹, Elham Baghbani¹, Vahid Khaze Shahgoli¹,
Behzad Baradaran^{1*}

¹ Immunology Research Center, Tabriz University of Medical Sciences, Iran

² Faculty of Basic Sciences, Department of Biology, University of Maragheh, Iran

* **Corresponding authors:** Behzad Baradaran, Immunology Research Center, Tabriz
University of Medical Sciences, Iran. Tel: +98 413 337 1440;

E-mail: behzad_im@yahoo.com

Reza Mohammadzadeh, Faculty of Basic Sciences, Department of Biology, University
of Maragheh, Iran. Tel: +989381983364; E-mail: rmohamadzadeh82@gmail.com

<https://orcid.org/0000-0002-8642-6795> (Behzad Baradaran)

<https://orcid.org/0000-0002-7893-5151> (Reza Mohammadzadeh)

<http://dx.doi.org/10.17179/excli2020-2900>

This is an Open Access article distributed under the terms of the Creative Commons Attribution License
(<http://creativecommons.org/licenses/by/4.0/>).

Supplementary Table 1: Raw data showing investigated genes' expression after transfection of PANC-1 cells with the miR-612 or their corresponding control group. The relative expression of each gene was analyzed by comparative threshold cycle (Ct). Ct value was normalized using the formula $\Delta Ct = Ct$ (investigated genes) - Ct (SNORD and GAPDH). Then formula $\Delta\Delta Ct = \Delta Ct$ (treated) - ΔCt (control) was used. Finally, the formula $2^{-\Delta\Delta Ct}$ was used for estimating relative expression of each gene.

Group	CT values SNORD				CT values miR-612				Fold induction				
PANC1	R1	R2	R3	mean	R1	R2	R3	mean	R1	R2	R3	mean	SD
Dose 60	25.87	25.75	26.2	25.94	29.83	29.42	29.42	29.42	156.498	164.2785	147.0334	155.9366	7.051464
Dose 80	25.53	25.52	25.81	25.62	28.09	27.81	27.81	27.81	413.0006	427.565	415.8732	418.8129	6.298781
Dose 100	26.29	26.28	25.83	26.13333	28.91	28.7	28.7	28.7	396.1766	390.7224	385.3432	390.7474	4.422752
Control	25.7	25.89	25.71	25.76667	36.95	36.92	36.92	36.92	1	1	1	1	0
PANC1	CT values SNORD				CT values miR-612				Fold induction				
PANC1	R1	R2	R3	mean	R1	R2	R3	mean	R1	R2	R3	mean	SD
24h	23.31	23.78	23.01	23.36667	26.04	26.26	26.03	26.11	367.0925	380.038	352.1387	366.4231	11.39969
48h	26.22	26.38	26.43	26.34333	28.74	28.68	29.16	28.86	424.6116	430.539	430.539	428.5632	2.794183
72h	27.18	27.31	27.04	27.17667	31.44	31.43	31.45	31.44	127.1158	121.9377	134.3637	127.8057	5.096325
Control	25.7	25.87	25.71	25.76	36.95	36.92	37.19	37.02	1	1	1	1	0
PANC1	CT values GAPDH				CT values BAX				Fold induction				
PANC1	R1	R2	R3	mean	R1	R2	R3	mean	R1	R2	R3	mean	SD
5FU	19.95	19.82	20.13	19.96667	26.3	26.1	26.32	26.24	2.42839	2.297397	2.114036	2.279941	0.128927
miR	20.51	20.89	21.07	20.82333	27.82	28.2	27.98	28	1.248331	1.125058	1.283426	1.218938	0.067912
miR+5FU	20.23	20.46	20.88	20.52333	26.24	26.24	26.42	26.3	3.07375	3.24901	3.317278	3.213346	0.102568
Control	21.67	22.03	21.88	21.86	29.3	29.51	29.15	29.32	1	1	1	1	0
PANC1	CT values GAPDH				CT values CAS3				Fold induction				
PANC1	R1	R2	R3	mean	R1	R2	R3	mean	R1	R2	R3	mean	SD
5FU	19.95	19.82	20.13	19.96667	32.27	31.45	31.62	31.78	30.06473	35.0174	33.59093	32.89102	2.081609
miR	20.51	20.89	21.07	20.82333	35.91	35.48	35.7	35.69667	3.555371	4.500234	3.810552	3.955386	0.399102
miR+5FU	20.23	20.46	20.88	20.52333	30.9	30.54	30.82	30.75333	94.35323	102.5369	98.36001	98.41673	3.341228
Control	21.67	22.03	21.88	21.86	38.9	38.79	38.44	38.71	1	1	1	1	0

Supplementary Table 1 (cont.): Raw data showing investigated genes' expression after transfection of PANC-1 cells with the miR-612 or their corresponding control group. The relative expression of each gene was analyzed by comparative threshold cycle (Ct). Ct value was normalized using the formula $\Delta Ct = Ct$ (investigated genes) - Ct (SNORD and GAPDH). Then formula $\Delta\Delta Ct = \Delta Ct$ (treated) - ΔCt (control) was used. Finally, the formula $2^{-\Delta\Delta Ct}$ was used for estimating relative expression of each gene.

Group	CT values GAPDH				CT values BCL2				Fold induction				
PANC1	R1	R2	R3	mean	R1	R2	R3	mean	R1	R2	R3	mean	SD
5FU	19.95	19.82	20.13	19.96667	34.45	32.31	32.96	33.24	0.051119	0.086569	0.115824	0.084504	0.026456
miR	20.51	20.89	21.07	20.82333	30.9	30.32	31.14	30.78667	0.882703	0.721965	0.784584	0.796417	0.066152
miR+5FU	20.23	20.46	20.88	20.52333	36.22	38.54	40.3	38.35333	0.018199	0.001797	0.001202	0.007066	0.007876
Control	21.67	22.03	21.88	21.86	31.88	30.99	31.6	31.49	1	1	1	1	0
	CT values GAPDH				CT values MMP9				Fold induction				
PANC1	R1	R2	R3	mean	R1	R2	R3	mean	R1	R2	R3	mean	SD
5FU	19.95	19.82	20.13	19.96667	39.36	39.46	39.05	39.29	0.285191	0.351111	0.438303	0.358202	0.062708
miR	20.51	20.89	21.07	20.82333	39.21	40.33	39.68	39.74	0.466516	0.403321	0.543367	0.471068	0.057264
miR+5FU	20.23	20.46	20.88	20.52333	41.77	42.03	43.34	42.38	0.065154	0.092142	0.037681	0.064992	0.022234
Control	21.67	22.03	21.88	21.86	39.27	40.16	39.61	39.68	1	1	1	1	0
	CT values GAPDH				CT values PD-L1				Fold induction				
PANC1	R1	R2	R3	mean	R1	R2	R3	mean	R1	R2	R3	mean	SD
5FU	19.95	19.82	20.13	19.96667	33.34	33.12	33.77	33.41	0.895025	0.742262	0.707107	0.781465	0.081572
miR	20.51	20.89	21.07	20.82333	34.73	34.54	35.34	34.87	0.503478	0.582367	0.456916	0.514253	0.051779
miR+5FU	20.23	20.46	20.88	20.52333	35.75	34.84	35.84	35.47667	0.204476	0.351111	0.283221	0.279603	0.059918
Control	21.67	22.03	21.88	21.86	34.9	34.9	35.02	34.94	1	1	1	1	0
	CT values SNORD				CT values miR612				Ratio				
PANC1	R1	R2	R3	mean	R1	R2	R3	mean	R1	R2	R3	mean	SD
Mia paca2	26.4	26.89	26.21	26.5	33.36	33.71	32.91	33.3267	0.00803	0.008851	0.009618	0.00881	0.000649
	24.56	24.81	25.02	24.79667	29.06	29.52	29.98	29.52	0.044	0.038	0.032	0.038	0.004899

Supplementary Table 2: Raw data of MTT assay analysis showing the capacity of cell proliferation of the miR-612-transfected cells and 5-FU treated groups in comparison with the control group

8000 cell/well	OD1	OD2	OD3
control	100.000	92.730	94.880
Just shocked	88.005	83.350	94.850
5-FU IC25	83.76402	70.7947	68.2225
5-FU IC50	60.2231	51.0365	56.0702
miR-612	81.1896	86.8844	80.5948
miR-612+IC25	43.83715	47.78157	44.96831
miR-612+IC50	15.92394	11.53584	25.56330