

Anandamide-modulated changes in metabolism, glycosylation profile and migration of metastatic melanoma cells

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Supplementary materials

Table S1. The full results of the QCM-D kinetic analysis of the studied lectin-glycan interaction performed on melanoma cells. Legend: k_{on} – the association rate constant, k_{off} – the dissociation rate constants, K_D – the dissociation constant / lectin affinity towards cell surface glycans. The K_D ratio was calculated as the AEA/DMSO relation.

Cell Type/Cell Line	Sample	k_{on} [1/M*s]	k_{off} [1/s]	K_D [nM]	K_D Ratio
RGP site – WM35	CTR	5.010	0.0009	1.80	1.8
	AEA	2.860	0.0009	3.15	
VGP site – WM115	CTR	8.456	0.0008	0.95	1.5
	AEA	6.303	0.0008	1.43	
Metastasis to the lymph node – WM266-4	CTR	30.843	0.0007	0.23	3.1
	AEA	12.586	0.0009	0.72	
Solid tumor metastasis – A375-P	CTR	19.627	0.0007	0.36	4.6
	AEA	4.810	0.0008	1.66	

Table S2. The full results of the lectin-ELISA analysis of the studied lectin-glycan interaction performed on AEA-treated melanoma cells. The b/a relation was calculated by using the parameters of the linear regression, where a is the slope and b the intercept. The b/a ratio was calculated as the AEA/DMSO relation.

Cell Type/Cell Line	Sample	a Values	b Values	b/a Relation	b/a Ratio
RGP site – WM35	CTR	433.2	8987.2	20.7	1.0
	AEA	407.7	8588.7	21.1	
VGP site – WM115	CTR	454.6	2531.9	5.6	1.3
	AEA	338	2363.7	7.0	
Metastasis to the lymph node – WM266-4	CTR	733.2	3877.8	5.3	3.0
	AEA	279.5	4482.4	16.0	
Solid tumor metastasis – A375-P	CTR	1113.4	5011.9	4.5	4.1
	AEA	317.6	5897.4	18.6	

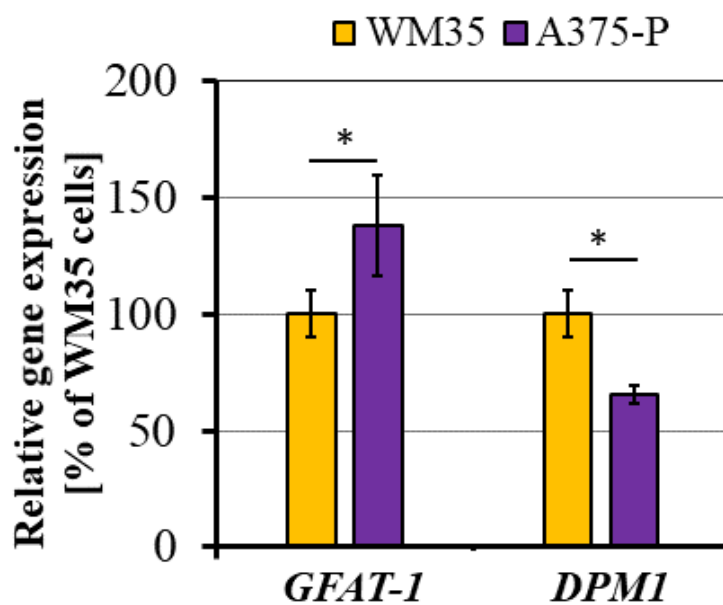


Figure S1. RT-qPCR analysis of *GFAT-1* and *DPM1* expression in melanoma cells from the RGP site (WM35 - yellow) and from the solid tumor site (A375-P - violet). The statistical significance of the p-value below 0.05 (*) was marked on the graph.

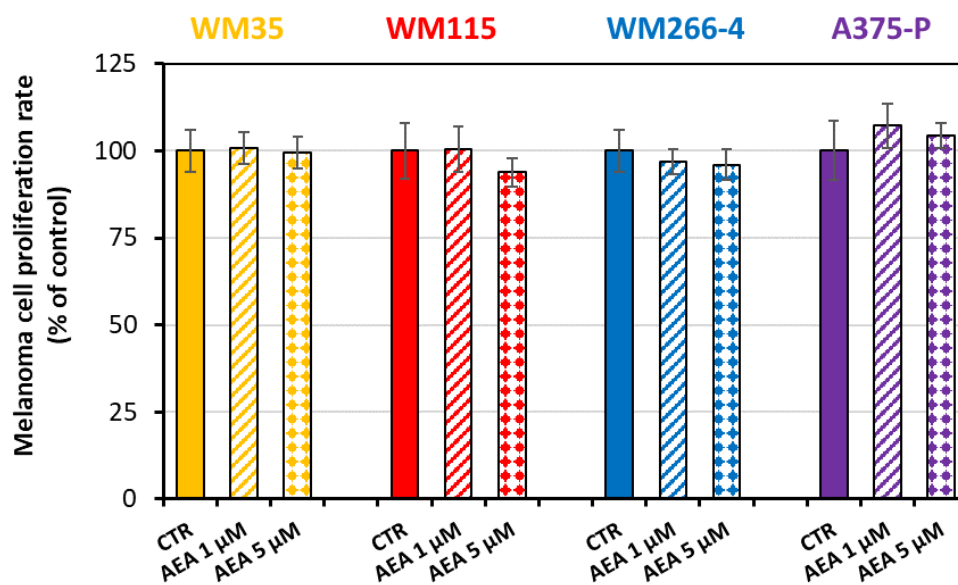


Figure S2. Proliferation rate analysis of melanoma cells after 24 hour treatment with 1 μM and 5 μM anandamide, followed by a 2 hour incubation with 10 μM BrdU. Then, melanoma cell proliferation rate was evaluated with the use of the BrdU Cell Proliferation Assay Kit (Merck) according to the producers protocol. Cell lines: WM35 (RGP site), WM115 (VGP site), WM266-4 (lymph node metastasis), A375-P (solid tumor metastasis).