

Title: Metformin Increases Sensitivity of Pancreatic Cancer Cells to Gemcitabine by Reducing CD133⁺ Cell Populations and Suppressing ERK/P70S6K Signaling

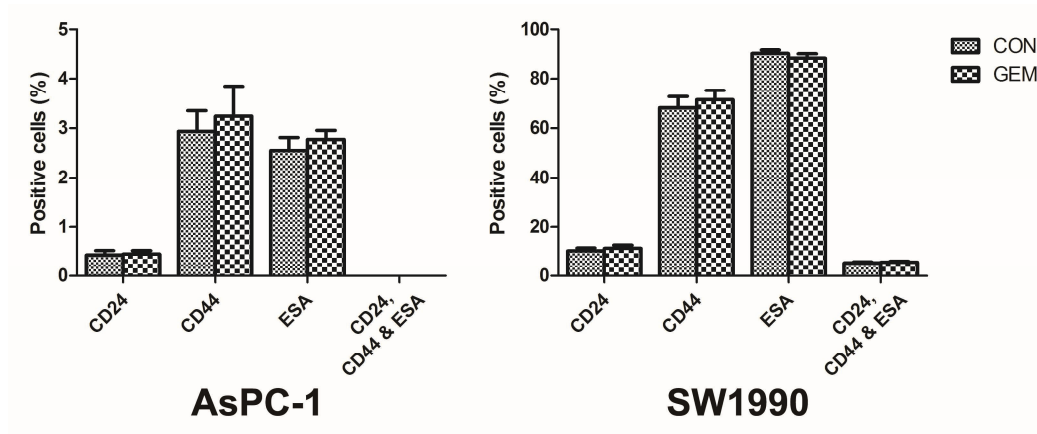
Authors:

Xinqun Chai¹, Hongpeng Chu¹, Xuan Yang¹, Yuanpu Meng¹, Pengfei Shi², Shanmiao Gou³.

¹ Department of Hepatobiliary Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology.

² Department of Breast and Thyroid Surgery, Central Hospital of Wuhan.

³ Pancreatic Disease Institute, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology.



Supplementary Figure S1. Gemcitabine didn't change the proportion of CD24⁺CD44⁺ESA⁺ cells in pancreatic cancer cells. AsPC-1 and SW1990 pancreatic cancer cells were treated with 300 nM gemcitabine for 48 h, and the proportion of CD24⁺, CD44⁺, ESA⁺ and CD24⁺CD44⁺ESA⁺ cells was determined by flow cytometry. Gemcitabine did not affect CD24⁺, CD44⁺, ESA⁺ or CD24⁺CD44⁺ESA⁺ cells (CD24⁺CD44⁺ESA⁺ cells are not detectable among AsPC-1 cells). CON, control; GEM, gemcitabine.

Supplementary Table S1. Effect of gemcitabine on CD133⁺ and CD133⁻ pancreatic cancer cells

	Control			Gemcitabine (300 nM, 48 hours)				
	A: Cell numbers (relative to control, mean \pm standard deviation, %)	B: CD133 ⁺ cells (mean \pm standard deviation, %)	C: CD133 ⁻ cells (mean \pm standard deviation, %)	D: Cell numbers (relative to control, mean \pm standard deviation, %)	E: CD133 ⁺ cells (mean \pm standard deviation, %)	F: CD133 ⁻ cells (mean \pm standard deviation, %)	G: Proportion of survived CD133 ⁺ cells ((D*E)/(A*B), %)	H: Proportion of survived CD133 ⁻ cells ((D*F)/(A*C), %)
AsPC-1	100 \pm 2.83	5.18 \pm 0.94	94.82 \pm 0.94	40.73 \pm 2.51	11.00 \pm 2.34	89.00 \pm 2.34	86.41	38.23
SW1990	100 \pm 2.51	4.38 \pm 1.08	95.62 \pm 1.08	31.33 \pm 1.93	8.40 \pm 1.70	91.60 \pm 1.70	60.14	30.02

Supplementary Table S2. Correlation between 193 proteins and histological grade of pancreatic cancer using reverse phase protein array (RPPA)

Protein	CC	95% CI (Lower)	95% CI (Upper)	P	t	df
beta	0.0995	-0.132	0.3207	0.3991	0.8482	72
epsilon	-0.026	-0.253	0.2037	0.826	-0.2207	72
zeta	-0.0996	-0.3208	0.1319	0.3986	-0.8492	72
4E-BP1	-0.1277	-0.3461	0.1039	0.2783	-1.0923	72
4E-BP1_pS65	-0.0936	-0.3153	0.1379	0.4278	-0.7975	72
4E-BP1_pT37_T46	-0.0963	-0.3178	0.1352	0.4143	-0.8211	72
4E-BP1_pT70	0.0694	-0.1616	0.2933	0.5567	0.5906	72
53BP1	0.0697	-0.1614	0.2935	0.5553	0.5926	72
A-Raf_pS299	-0.1267	-0.3452	0.1048	0.2819	-1.0842	72
ACC_pS79	0.1251	-0.1064	0.3438	0.2881	1.0701	72
ACC1	-0.0121	-0.24	0.217	0.9184	-0.1028	72
Acetyl-a-Tubulin-Lys40	0.0495	-0.181	0.2749	0.6752	0.4207	72
ACVRL1	-0.0371	-0.2634	0.193	0.7534	-0.3153	72
ADAR1	0.0071	-0.2217	0.2352	0.952	0.0604	72
Akt	0.1417	-0.0897	0.3586	0.2284	1.2149	72
Akt_pS473	0.0888	-0.1426	0.311	0.4518	0.7564	72
Akt_pT308	0.0161	-0.2132	0.2437	0.8918	0.1365	72
alpha-Catenin	-0.1482	-0.3643	0.0832	0.2077	-1.2712	72
AMPK_alpha	-0.0453	-0.271	0.1851	0.7015	-0.3848	72
AMPK_pT172	0.2365	0.0085	0.4412	0.0425	2.0653	72
Annexin-1	0.0695	-0.1616	0.2933	0.5564	0.5909	72
Annexin_VII	-0.2238	-0.4303	0.0049	0.0552	-1.9489	72
AR	0.0851	-0.1462	0.3076	0.471	0.7247	72
ARHI	-0.1072	-0.3277	0.1243	0.3631	-0.9152	72
ASNS	-0.0378	-0.264	0.1923	0.749	-0.3212	72
ATM	0.1152	-0.1164	0.3348	0.3286	0.9837	72
B-Raf	0.1457	-0.0856	0.3621	0.2155	1.2497	72
Bad_pS112	-0.0224	-0.2496	0.2071	0.8496	-0.1903	72
Bak	0.1118	-0.1197	0.3318	0.3428	0.9549	72
Bap1c-4	0.0735	-0.1576	0.297	0.5337	0.6254	72
Bax	0.04	-0.1903	0.266	0.7354	0.3393	72
Bcl-2	-0.0054	-0.2336	0.2234	0.9635	-0.0459	72
Bcl-xL	-0.0473	-0.2729	0.1831	0.6888	-0.4021	72
Beclin	0.0184	-0.211	0.2459	0.8763	0.1562	72
beta-Catenin	0.0541	-0.1766	0.2791	0.6473	0.4594	72
Bid	0.1336	-0.0979	0.3514	0.2563	1.1442	72
Bim	0.125	-0.1066	0.3437	0.2887	1.0689	72
BRCA2	0.0867	-0.1446	0.3091	0.4625	0.7388	72

c-Jun_pS73	-0.1051	-0.3258	0.1264	0.3729	-0.8967	72
c-Kit	-0.0837	-0.3064	0.1476	0.4782	-0.7129	72
c-Met_pY1235	-0.0802	-0.3032	0.1511	0.4969	-0.6828	72
c-Myc	-0.2441	-0.4477	-0.0166	0.0361	-2.1362	72
C-Raf	0.1563	-0.0749	0.3715	0.1836	1.3428	72
C-Raf_pS338	-0.0187	-0.2462	0.2107	0.8742	-0.1589	72
Caspase-7_cleavedD198	0.1411	-0.0903	0.358	0.2305	1.2092	72
Caveolin-1	0.0583	-0.1725	0.283	0.6219	0.4953	72
CD20	0.1229	-0.1086	0.3418	0.2968	1.051	72
CD31	-0.0708	-0.2946	0.1602	0.5486	-0.6027	72
CD49b	-0.0736	-0.2971	0.1575	0.533	-0.6265	72
CDK1	-0.0896	-0.3117	0.1418	0.4476	-0.7635	72
Chk1	0.186	-0.0444	0.3976	0.1126	1.6062	72
Chk1_pS345	-0.0871	-0.3095	0.1442	0.4603	-0.7423	72
Chk2	-0.137	-0.3544	0.0945	0.2445	-1.1734	72
Chk2_pT68	-0.0824	-0.3052	0.1489	0.485	-0.7019	72
cIAP	0.0614	-0.1695	0.2859	0.6034	0.5219	72
Claudin-7	-0.1579	-0.373	0.0732	0.1789	-1.3573	72
Collagen_VI	0.0278	-0.2019	0.2547	0.8139	0.2363	72
Cyclin_B1	0.1054	-0.1262	0.326	0.3716	0.8991	72
Cyclin_D1	0.124	-0.1075	0.3428	0.2923	1.0608	72
Cyclin_E1	0.0576	-0.1731	0.2824	0.6257	0.4899	72
Cyclin_E2	0.0843	-0.147	0.3069	0.4749	0.7182	72
DJ-1	-0.2208	-0.4277	0.0081	0.0587	-1.9208	72
Dvl3	0.0329	-0.1971	0.2594	0.7808	0.2793	72
E-Cadherin	-0.0309	-0.2576	0.199	0.7935	-0.2627	72
eEF2	-0.0332	-0.2597	0.1968	0.7791	-0.2815	72
eEF2K	-0.0382	-0.2643	0.192	0.7469	-0.324	72
EGFR	0.1643	-0.0667	0.3786	0.1619	1.4134	72
EGFR_pY1068	-0.0328	-0.2593	0.1972	0.7815	-0.2784	72
EGFR_pY1173	0.0618	-0.1691	0.2863	0.6008	0.5256	72
eIF4E	-0.2292	-0.4349	-8.00E-04	0.0495	-1.9982	72
eIF4G	0.0714	-0.1597	0.2951	0.5454	0.6076	72
ER-alpha	0.1886	-0.0417	0.3999	0.1076	1.6294	72
ER-alpha_pS118	0.0934	-0.138	0.3152	0.4285	0.7963	72
ERCC1	0.1383	-0.0931	0.3556	0.24	1.1849	72
ERK2	0.0327	-0.1973	0.2592	0.7823	0.2774	72
ETS-1	0.1862	-0.0442	0.3977	0.1123	1.6077	72
FASN	0.0642	-0.1667	0.2885	0.5867	0.546	72
Fibronectin	0.1393	-0.0921	0.3565	0.2365	1.1936	72
FoxM1	0.0224	-0.2071	0.2497	0.8495	0.1904	72
FOXO3a	-0.1608	-0.3755	0.0703	0.1711	-1.3825	72
FOXO3a_pS318_S321	-0.1412	-0.3582	0.0902	0.23	-1.2106	72
G6PD	-0.0234	-0.2505	0.2062	0.8434	-0.1982	72

Gab2	0.0842	-0.1471	0.3068	0.4758	0.7168	72
GAPDH	0.0393	-0.1909	0.2654	0.7394	0.334	72
GATA3	0.1152	-0.1164	0.3348	0.3286	0.9837	72
GSK3-alpha-beta	-0.0489	-0.2743	0.1816	0.6791	-0.4153	72
GSK3-alpha-beta_pS21_S9	-0.0018	-0.2302	0.2268	0.9876	-0.0156	72
GSK3_pS9	0.0912	-0.1402	0.3132	0.4397	0.7771	72
HER2	0.0602	-0.1706	0.2848	0.6102	0.5121	72
HER2_pY1248	0.0261	-0.2037	0.253	0.8256	0.2212	72
HER3	0.0759	-0.1553	0.2992	0.5204	0.6459	72
HER3_pY1289	0.0299	-0.1999	0.2567	0.8002	0.254	72
Heregulin	0.1615	-0.0695	0.3761	0.1692	1.3888	72
HSP70	0.1496	-0.0817	0.3656	0.2033	1.284	72
IGFBP2	-0.1047	-0.3254	0.1268	0.3746	-0.8935	72
INPP4B	0.0343	-0.1957	0.2607	0.7718	0.2911	72
IRS1	0.1536	-0.0776	0.3691	0.1914	1.3188	72
JNK_pT183_pY185	-0.0196	-0.247	0.2098	0.868	-0.1668	72
JNK2	8.00E-04	-0.2277	0.2293	0.9944	0.007	72
Ku80	0.0887	-0.1427	0.3109	0.4522	0.7559	72
Lck	0.0926	-0.1388	0.3145	0.4326	0.7892	72
LKB1	-0.019	-0.2464	0.2105	0.8726	-0.1609	72
MAPK_pT202_Y204	0.047	-0.1835	0.2726	0.6908	0.3994	72
MEK1	-0.0286	-0.2554	0.2012	0.8088	-0.2429	72
MEK1_pS217_S221	0.0043	-0.2244	0.2326	0.9711	0.0364	72
MIG-6	-2.00E-04	-0.2287	0.2283	0.9984	-0.002	72
Mre11	0.048	-0.1825	0.2735	0.6844	0.4081	72
MSH2	0.1119	-0.1197	0.3319	0.3426	0.9553	72
MSH6	0.1509	-0.0803	0.3668	0.1992	1.2956	72
mTOR	0.0309	-0.199	0.2576	0.794	0.2621	72
mTOR_pS2448	0.0625	-0.1684	0.2869	0.5968	0.5313	72
MYH11	0.0183	-0.2111	0.2457	0.8772	0.155	72
Myosin-IIa-pS1943	-0.0107	-0.2387	0.2183	0.9276	-0.0911	72
N-Cadherin	0.1432	-0.0882	0.3599	0.2235	1.2278	72
N-Ras	0.1115	-0.1201	0.3315	0.3443	0.952	72
NDRG1_pT346	0.0382	-0.192	0.2644	0.7468	0.3241	72
NF-kB-p65_pS536	0.1331	-0.0983	0.351	0.2581	1.1399	72
NF2	0.0088	-0.2201	0.2368	0.9407	0.0747	72
Notch1	0.0599	-0.1709	0.2845	0.612	0.5094	72
P-Cadherin	0.12	-0.1116	0.3392	0.3085	1.0256	72
p21	0.088	-0.1434	0.3102	0.4561	0.7493	72
p27	-0.0203	-0.2477	0.2092	0.8637	-0.1723	72
p27_pT157	-0.0291	-0.2559	0.2007	0.8055	-0.2472	72
p27_pT198	-0.0561	-0.281	0.1747	0.6352	-0.4765	72
p38	0.0706	-0.1605	0.2943	0.5502	0.6003	72
p38_pT180_Y182	-0.0437	-0.2696	0.1866	0.7113	-0.3716	72

p53	-0.0641	-0.2884	0.1669	0.5876	-0.5448	72
p62-LCK-ligand	0.1677	-0.0632	0.3816	0.1532	1.4434	72
p70S6K	0.0966	-0.1348	0.3181	0.4128	0.8238	72
p70S6K_pT389	0.2292	7.00E-04	0.4349	0.0495	1.998	72
p90RSK	0.0554	-0.1753	0.2803	0.6394	0.4706	72
p90RSK_pT359_S363	0.0765	-0.1547	0.2998	0.517	0.6513	72
PAI-1	0.1024	-0.1291	0.3233	0.3853	0.8734	72
Paxillin	0.1432	-0.0882	0.3599	0.2235	1.2278	72
PCNA	-0.0594	-0.2841	0.1714	0.6151	-0.505	72
PDCD4	-0.1865	-0.398	0.0439	0.1116	-1.6106	72
PDK1	0.0211	-0.2084	0.2484	0.8586	0.1788	72
PDK1_pS241	-0.0512	-0.2765	0.1794	0.6646	-0.4353	72
PEA15	-0.133	-0.3508	0.0985	0.2587	-1.1385	72
PEA15_pS116	0.1255	-0.106	0.3441	0.2867	1.0734	72
PI3K-p110-alpha	-0.0105	-0.2384	0.2185	0.9292	-0.0891	72
PI3K-p85	0.0506	-0.18	0.2759	0.6685	0.43	72
PKC-alpha	-0.1195	-0.3388	0.1121	0.3105	-1.0213	72
PKC-alpha_pS657	-0.0436	-0.2694	0.1868	0.7123	-0.3702	72
PKC-delta_pS664	0.0077	-0.2212	0.2358	0.948	0.0654	72
PKC-pan_BetaII_pS660	0.0062	-0.2226	0.2343	0.9583	0.0524	72
PR	-0.0534	-0.2785	0.1772	0.6512	-0.454	72
PRAS40_pT246	0.0379	-0.1923	0.2641	0.7486	0.3217	72
PRDX1	-0.1545	-0.37	0.0767	0.1886	-1.3273	72
PREX1	-0.0277	-0.2546	0.2021	0.815	-0.2349	72
PTEN	0.0412	-0.189	0.2672	0.7272	0.3502	72
Rab11	0.1243	-0.1073	0.343	0.2915	1.0627	72
Rab25	-0.1367	-0.3541	0.0947	0.2455	-1.171	72
Rad50	0.0385	-0.1917	0.2646	0.7448	0.3267	72
Rad51	0.0999	-0.1316	0.3211	0.3971	0.8519	72
Raptor	-0.0169	-0.2444	0.2124	0.8865	-0.1433	72
Rb-M-QC	-0.1813	-0.3935	0.0492	0.1221	-1.5643	72
Rb_pS807_S811	-0.0207	-0.248	0.2088	0.861	-0.1758	72
RBM15	0.102	-0.1295	0.323	0.3872	0.87	72
Rictor	0.1098	-0.1218	0.33	0.3518	0.9372	72
Rictor_pT1135	0.0851	-0.1462	0.3076	0.4709	0.7248	72
S6	-0.064	-0.2883	0.167	0.5882	-0.5439	72
S6_pS235_S236	7.00E-04	-0.2279	0.2291	0.9956	0.0056	72
S6_pS240_S244	0.0354	-0.1947	0.2617	0.7649	0.3002	72
SCD1	-0.209	-0.4176	0.0204	0.0739	-1.8137	72
SF2	-0.0265	-0.2534	0.2032	0.8228	-0.2248	72
Shc_pY317	-0.0874	-0.3097	0.1439	0.4589	-0.7447	72
Smad1	0.0645	-0.1664	0.2888	0.5848	0.5488	72
Smad3	0.1242	-0.1074	0.3429	0.2919	1.0619	72
Smad4	0.042	-0.1883	0.2679	0.7226	0.3564	72

Src	-0.1168	-0.3363	0.1148	0.3219	-0.9975	72
Src_pY416	-0.0894	-0.3115	0.142	0.4487	-0.7617	72
Src_pY527	-0.0362	-0.2625	0.1939	0.7595	-0.3073	72
STAT3_pY705	0.0584	-0.1724	0.2832	0.6209	0.4967	72
STAT5-alpha	0.1285	-0.103	0.3468	0.2752	1.0996	72
Stathmin	0.037	-0.1931	0.2633	0.7544	0.314	72
Syk	-0.0153	-0.2429	0.214	0.8973	-0.1295	72
TAZ	0.1077	-0.1239	0.3281	0.3612	0.9189	72
TIGAR	-9.00E-04	-0.2293	0.2277	0.9942	-0.0073	72
Transglutaminase	0.126	-0.1056	0.3446	0.2849	1.0775	72
TFRC	-0.0169	-0.2445	0.2124	0.886	-0.1438	72
TSC1	0.0496	-0.181	0.275	0.6748	0.4213	72
TTF1	0.1042	-0.1274	0.3249	0.3772	0.8886	72
Tuberin	0.1071	-0.1244	0.3276	0.3635	0.9145	72
Tuberin_pT1462	-0.0174	-0.2449	0.212	0.8833	-0.1473	72
VEGFR2	0.0618	-0.1691	0.2863	0.6008	0.5256	72
VHL	0.0606	-0.1703	0.2851	0.6082	0.5149	72
XBP1	0.0905	-0.1409	0.3125	0.4433	0.7709	72
XRCC1	-0.1005	-0.3216	0.131	0.3943	-0.8571	72
YAP	1.00E-04	-0.2284	0.2286	0.9994	8.00E-04	72
YAP_pS127	-0.0602	-0.2848	0.1707	0.6106	-0.5115	72
YB-1	-0.1665	-0.3806	0.0644	0.1561	-1.4331	72
YB-1_pS102	-0.0415	-0.2675	0.1888	0.7256	-0.3524	72

CC, correlation coefficient; CI, confidence interval; df, degree of freedom.