Supporting Information

Therapeutic targeting of pancreatic cancer stem cells by dexamethasone modulation of the MKP-1 - JNK axis

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Running title: GR-MKP-1 -JNK axis in pancreatic cancer stem cells

Contents: Supporting Figures S1-S12



Supporting Figure S1. Dexamethasone effects on non-cancer cell viability. IMR90 human fetal lung fibroblasts (*A*) and rat cortical neural stem cells (Rat NSC) (*B*) either treated with DEX at the indicated concentrations or left untreated (Control) for six days were stained with trypan blue to determine cell viability. Data are shown as means \pm standard deviation from triplicate samples of a representative experiment repeated with similar results. **p* < 0.05 (compared with control).



Supporting Figure S2. Dexamethasone-induced changes in differentiation and stem cell marker expression in pancreatic CSCs persist even after dexamethasone withdrawal. Cells maintained in either the presence or absence of 1 μ M DEX for six days were subsequently cultured in DEX-free medium for the indicated time periods. Indicated proteins were detected by immunoblotting.



Supporting Figure S3. Dexamethasone promotes differentiation and loss of stemness in lung and ovarian CSCs. *A*, A549 CSLC (non-small cell lung cancer) and A2780 CSLC (ovarian cancer) human cancer stem cells cultured either with 1 μ M DEX or without drug (Control) for six days were subjected to flow cytometry to detect cell-surface expression of CD133; percentage of CD133-positive cells was determined. Data are shown as mean ±

standard deviation (SD) from three independent experiments (*p < 0.05). **B** and **C**, Cells cultured as described in (**A**) were subjected to either immunoblotting for indicated proteins (**B**) or to a sphere formation assay in the absence of DEX (**C**). **C**, Percentage of wells in which a sphere formed from a single cell is shown (*Top*). Data are shown as mean \pm SD from three independent experiments. *p < 0.05. *Bottom*, photomicrographs of representative wells. Scale bars, 200 µm.



Supporting Figure S4. Prednisolone activates glucocorticoid receptor and promotes loss of stemness in pancreatic CSCs. *A*, Cells cultured either with 1 μ M prednisolone (PSL) or without PSL (Control) for six days were subjected to flow cytometry to detect cell-surface expression of CD133; percentage of CD133-positive cells was determined. Data are shown as mean \pm standard deviation from three independent experiments. **p* < 0.05. *B*, Cells cultured as described in (*A*) were subjected to immunoblotting for the indicated proteins. GR: glucocorticoid receptor.



Supporting Figure S5. Multi-phasic change in JNK pathway activity after dexamethasone treatment. PANC-1 CSLC cells were cultured either with 1 µM DEXfor indicated times, or without DEX (Control) for 14 days. Indicated proteins were detected by immunoblotting. GR: glucocorticoid receptor.



Supporting Figure S6. The effect of systemic dexamethasone administration on mouse body weight. Two groups of mice (n = 5 per group) were injected intraperitoneally with either vehicle only or 1 mg/kg DEX three times a week. Mouse body weight was measured at indicated time points. Data are shown as mean \pm standard deviation for each treatment group.



Supporting Figure S7. Role of survivin expression in chemoresistance of pancreatic CSCs.

A, Survivin levels were determined by immunoblotting in cells transiently transfected with either an siRNA against survivin (siSurvivin) or a control RNA (siControl). PANC-1 CSLC (*B*, *C*) and PSN-1 CSLC (*D*, *E*) cells transiently transfected with either siSurvivin or siControl were either treated on the day with the indicated concentrations of GEM or 5-FU or left untreated for three days. *Left*, Viable and dead cell numbers and *Right*, percentage of dead cells were determined by trypan blue staining. Data are shown as mean \pm standard deviation from triplicate samples of a representative experiment. **p* < 0.05.



Supporting Figure S8. The effect of dexamethasone pretreatment on gemcitabine-induced survivin expression in pancreatic CSCs. PANC-1 CSLC cells pretreated with or without DEX (1 μ M) for 6 days were treated with or without GEM (1 μ M) for 3 days in the absence of DEX. Cells were then subjected to immunoblotting for survivin expression. The longer exposure panel is presented to show that the basal level of survivin expression is inhibited by DEX pretreatment.



Supporting Figure S9. Forced JNK1 activation fails to prevent dexamethasone inhibition of survivin expression. PANC-1 CSLC cells transiently transfected with activated JNK1 protein expression plasmid (JNK1 CA) or with empty control vector (Vector) for 24 h were either treated with 1 μM DEX or left untreated for six days. Indicated proteins were detected by immunoblotting.



Supporting Figure S10. Differential requirement for JNK1 and JNK2 in the maintenance

of stemness and survivin expression in pancreatic CSCs. PANC-1 CSLC cells were

transiently transfected either with siRNA(s) against JNK1, JNK2, or both, or with a control

RNA (siControl) for four days. Indicated proteins were detected by immunoblotting.

																														(Days)
CEM (mpkg) DEX (mpkg)		0 1	2 3 4	5 6 3	40	10 11 1 40 105 05 0	12 13 14	13 14 40 9.25	17 18 19 40 0.125 8.125	29 21 22 2 40 8.1 0.1	3 24 25	26 27 28	29 3	31 32 2	3 M M 1	6 37 38 5 9,25 9,125	29 40 4 43	41 42 43 44 40 1 1 0.5 8.25	45 45 40 9.125	47 48 49 50 40 1 1 0.5	51 52 53 40 (25 0)25	54 55 56	57 58 55	60 61	62 63 64 40	65 66 67 48 0.25 8.125	68 69 79 71 49 1 1 0.5	72 73 74 40 9.25 0.125	75 76 77 78 48	79 80 81 40 10.25 0.125
Necks								2		3			4					6		2			8				19			
Centrel		0			46	32		199	62.5	165	126		185	256	2	1	258	288	365	446	365			847	1916	1352	1352	NA	NA	NA
	2	0			40	22.5		87.5	87.5	\$2.5	144		228.5	220.5		*	352	486	384	567	354			847	968	1296	1286	NA	NA	NA
	3	0			8	27		48	75	87.5	188		126		- P	4	3.20	435	680	864	787			1372	1568	1568	1800	1764	NO.	NA.
	4	9			32	64		190	199	155	190		155	245		*	320	600	680	1085	1005			1115	1568	1764	1665	1962	NA	NA
	5	0			13.5	- 4		32	32	-40	40		185	75			185	196	221	352	609			847	847	847	1103	1268	NA.	NA
	- 6	0			22.5	22.5		40	43	- 41	45		185	105	P	2	126	162	162	352	563			1112	11.52	1437	1655	1264	NA	NA.
OEM	1	0			27	27		75	87.5	55	87.5		144	144	1	4	196	320	485	527	653			847	1152	1192	1437	1521	NA	NA.
	2	9			13.5	48		75	\$7.5	87.5	190		144	144		8	144	221	255	495	609			689	847	1083	1268	1568	NA.	NA NA
	3	0			- 6	56		64	56	109	126		162	144		8	245	446	3.20	446	726			3008	1470	1568	1913	N/A	NA	NA
	4	0			15	45		75	\$7.5	-40	75		100	126		×.	221	320	485	446	726			3004	1268	1568	1913	NA	NA	NA
	5	0			22.5	75		40	35	25	75		126	144		4	221	320	3.20	486	660			1004	1268	NA	NA	NA	NA	NA
	- 6	0			22.5	18		40	40	28	75		126	126		×	196	320	485	600	935			1479	1968	NA	204	N/A	N.X.	NA NA
	7	0			27	13.5		32	49 7	13.5	- 11		48	-43		3	188	126	172	256	465			485	660	5.50	936	1908	1668	NA NA
	- 8	9			22.5	18		75	43	-43	75	_	140	144	- 2		221	245	255	352	485			659	847	595	1650	233	1103	NA NA
DEX+GEM	1	0			-0	0.5		-4	8	6			18	13.5			18	18	3.2	15	168			195	199	288	213	445	144	196
	- 2	0			- 0				18		- C.4		4	13.5			18	18	. 12	63	- 63			165	105	172	108	159	196	500
	3	9			13.5			5	1.4	6			13.5	13.5			32	48	40	14	32			14	14	14	14	14	40	14
	4	0			- 4			5	1.6	18	- 4			6			32	32	32	15				14	4	14		6		14
	5	0						. 6	13.5	13.5	13.5		6	18			63	198	188	126	166				- 41	126	196	196	172	365
	6	0			- 6		_	18	13.5	13.5			18	40			88	144	126	196	195			168	144	256	365	165	188	6
	7	0			13.5	13.5		6	6	4	- 6		18	13.5			18	14	14	11	6			4	1	1	1	4	3.2	
	- X	9			- 0		_		4	0		_	13.5	18		2	18	23	32	18	32	_	_		6	8	15	18	32	16

Supporting Figure S11. Combination treatment with systemic dexamethasone and

gemcitabine. Treatment schedule and effect on growth of tumors initiated by pancreatic CSCs are shown. Details of treatment schedule and tumor volume data from the mouse study shown in Fig. 8. Note that gray-shaded boxes indicate presence of subcutaneous tumor.













Supporting Figure S12. Unprocessed immunoblots. Unprocessed immunoblot images for Figs. 1B, 2A, 2B, 3A, 3D, 3E, 4B, 7B, 7C, 7D, 7E, 7F and for Supporting Figs. S2, S3, S4, S5, S7, S8, S9 and S10 are shown.