cancers

MDPI

Supplementary Materials: Chemosensitivity of 3D Pancreatic Cancer Organoids Is not Affected by Transformation to 2D Culture or Switch to Physiological Culture Medium

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Table S1. Comparison of human plasma composition with Plasmax and DMEM/F-12.

Proteinogenic Amino Acids	Human Plasma	Plasmax™	DMEM/ F-12
L-Alanine	230 - 510	510	50
L-Arginine	13 - 64	64	699
L-Asparagine	45 -130	41	50
L-Aspartic acid	0 - 6	6	50
L-Cysteine	23.2 - 43.8	33	100
L-Glutamate	32 -140	98	50
L-Glutamine	420 -720	650	2500
Glycine	170 - 330	330	250
L-Histidine	26 - 120	120	150
L-Isoleucine	42 - 100	140	416
L-Leucine	66 - 170	170	451
L-Lysine	150 - 220	220	499
L-Methionine	16 - 30	30	116
L-Phenylalanine	41 - 68	68	215
L-Proline	110 -360	360	150
L-Serine	56 - 140	140	250
L-Threonine	92 - 240	240	449
L-Tryptophan	44.8 - 64.2	78	44
L-Tyrosine	45 - 74	74	214
L-Valine	150 - 310	230	452
Non-proteinogenic Amino Acids			
α-Aminobutyrate	15 - 41	41	
L-Citrulline	16 - 55	55	
L-Cystine	30 - 65	65	100
L-Homocysteine	6.1 - 12.1	9	
4-Hydroxy-L-proline	3 - 23	13	
L-Ornitine	27 - 80	80	
L-Pyroglutamate	12.2 - 15.3	20	
Amino Acids Derivatives			
L-Acetyl glycine	69.7	70	
L-Carnosine	5.5 - 7.5	6	
Glutathione (reduced)	32.2 - 41.8	37	
Putrescine	0.1 - 0.3		0.5
Taurine	45 - 130	130	
N-Trimethylglycine (betaine)	49.6 - 94.4	72	

Other Components			
Acetate	26.8 - 57	42	
Acetone	24.8 - 84	55	
Acetyl carnitine	2.5 - 8.6	5	
Citrate	87.2 - 141.2	114	
Carnitine	34.1 - 57.3	46	
Creatine	8.4 - 65	37	
Creatinine	60.5 - 87.7	74	
Formate	19.5 - 46.1	33	
Fructose	28.0 - 34.0		
Galactose	53.6 - 123		
D-Glucose	4598.5 - 5344.1	5560	17506
	Human Plasma	**Plasmax™**	**DMEM/ F-12**
Glycerol	331.2 - 532	82	
2-Hydroxybutyrate	23.5 - 39.1	31	
3-Hydroxybutyrate	10.6 - 143.2	77	
3-Hydroxyisobutyrate	19.0 - 23.0	20	
Hypoxanthine	4.5 - 5.3	5	15
Lactate	1118.2 - 1860.6	500	
Linoleic Acid	45.8 - 121.8		0.15
Lipoic Acid	0.060 - 0.094		0.5
Malonate	12.3 - 14.7		
Methyl acetoacetate	for acetoacetate 4.1 - 77.1	41	
Phenol Red		25.0	21.5
Pyruvate	9.3 - 59.7	100	500
Succinate	23.5	23	
Thymidine	0.1 - 0.3		1.5
Uracil	1.1 - 3.1	2	
Urate	228.9 - 315.1	270	
Urea	3920.4 - 8228.8	3000	
Uridine	1.8 - 4.4	3	
Inorganic Salts			
Ammonium Chloride	for NH_4^+ for Cl^-	50	
Calcium Chloride	for Ca^{2+} and Cl^-	1800	1050
Calcium Nitrate	for Ca^{2+} for NO_3^-		
Magnesium Chloride	for Mg^{2+} and Cl^-		302
Magnesium Sulfate	for Mg^{2+} and SO_4^{2-}	813	407
Potassium Chloride	for K^+ and Cl^-	5330	4157
Potassium Nitrate	for K^+ for NO_3^-		
Sodium Bicarbonate	for Na^+ and HCO_3^-	26191	29024
Sodium Chloride	for Na^+ and Cl^-	118706	120612
Sodium Phosphate monobasic	for Na^+ and PO_4^{3-}	1010	453
Sodium Phosphate dibasic	for Na^+ and PO_4^{3-}		500
Trace Elements			
Ammonium Metavanadate	for NH_4^+ for V	0.0026	
Cupric Sulfate	for Cu for SO_4^{2-}	0.0052	0.0052
Ferric Chloride	for Fe for Cl^-		
Ferric Nitrate	for Fe for NO_3^-	0.1238	0.1238

Ferric Sulfate	for Fe for SO_4^{2-}	1.0428	1.5000
Manganous Chloride	for Mn for Cl^-	0.0002	
Sodium Selenite	for Na^+ for Se	0.0289	
Zinc Sulfate	for Zn for SO_4^{2-}	1.50	1.50
Vitamins			
p-Aminobenzoate	5.0 - 32.0		
Ascorbate	57.9 - 67.3	62	
D-Biotin	0.0006 - 0.0019	4.100	0.014
Choline	9.2 - 19.8	7.1	64.1
Folate	0.017 - 0.025	2.30	6.00
myo-Inositol	17.1	11.1	70.0
Niacinamide	0.435 - 0.445	8.2	16.6
D-Calcium pantothenate	4.5 - 5.3	2.10	4.70
Pyridoxine	0.007 - 0.060	4.90	9.80
Riboflavin	0.0054 - 0.028	0.30	0.58
Thiamine	0.078 - 0.114	3.0	6.4
Vitamin B12	0.00017 - 0.00033	0.0050	0.5000

Adjusted from Tobias Ackermann and Saverio Tardito [16].

Table S2. Media composition of Tumor 2 medium.

Growth factor	**Final conc. T2**
Adv. DMEM/F12+++ /	base
Wnt3a	50 % (v/v)
Noggin	10% (v/v)
Rspondin1	10% (v/v)
B27 supplement (B27)	1:50
N-acetyl-cysteine (nAc)	1.25 mM
Primocin	1:500
Nicotinamide (N)	10 mM
A83.01 (T)	500 nM
FGF10 (F)	100 ng/mL
Gastrin (G)	10 nM
Rho kinase Inhibitor Y-27632 (Y)	10 µM

Table S3. Medium composition of Tumor 2 and Plasmax Tumor 2 media for 2D organoids .

Growth factor	**Final conc. 2DT2/2DPlxT2 (adv.DMEM/F-12 / Plasmax)**
Adv. DMEM/F12+++ /Plasmax™+++	base
Wnt3a	25 % (v/v)
Noggin	5% (v/v)
Rspondin1	5% (v/v)
B27 supplement (B27)	1:100
N-acetyl-cysteine (nAc)	0.625 mM
Primocin	1:1000
Nicotinamide (N)	5 mM
A83.01 (T)	250 nM
FGF10 (F)	50 ng/mL
Gastrin (G)	5 nM

Rho kinase Inhibitor Y-27632 (Y)	5 μM

Table S4. Proliferation metrics of PANCO09b and PANCO11b cultured in 3D versus 2D.

Cell culture	Parameter	3D	2D	*p*-value
PANCO09b	doubling time (h)	75.85 ± 22.76	74.73 ± 1.810	0.9364
	growth rate/ day	0.236 ± 0.085	0.222 ± 0.005	0.7863
PANCO11b	doubling time (h)	80.26 ± 40.36	52.35 ± 6.09	0.3051
	growth rate/ day	0.237 ± 0.119	0.320 ± 0.035	0.2984

Data are presented as mean ± SD of three independent experiments performed in triplicate. Independent sample t-test was used to assess differences. Significance was reached when $p < 0.05$.

Table S5. Differentially expressed genes between 3D organoids and corresponding 2D transformed cell cultures.

Name	Ensembl ID	Function [19–21]
None	ENSG00000263244	• RNA gene
RSC1A1	ENSG00000215695	• involved in Glucose cotransporter 1 inhibition
HERC2P4	ENSG00000230267	• Pseudogene
LY75-CD302	ENSG00000248672	• Unknown function
None	ENSG00000260772	• RNA gene
ATP2A3	ENSG00000074370	• Magnesium-dependent ATPase • Calcium transporter
None	ENSG00000273489	• RNA gene
CDKL3	ENSG00000006837	• Cyclin dependent protein kinase • Regulator of cell cycle progression
PSAPL1	ENSG00000178597	• Lysosomal degradation of sphingolipids
GCA	ENSG00000115271	• Calcium-binding protein • Abundant in neutrophils and macrophages
AQP12B	ENSG00000185176	• Water and small neutrals carrier
SLC13A2	ENSG00000007216	• Cotransport of sodium ions and dicarboxylates such as succinate and citrate
MIR17HG	ENSG00000215417	• RNA gene
RBP4	ENSG00000138207	• Retinol-binding protein • Transports retinol from liver storage through plasma
IHH	ENSG00000163501	• Intercellular signaling • Involved in events during development
R3HDML-AS1	ENSG00000226812	• RNA gene
JAML	ENSG00000160593	• transmembrane protein of leukocytes • leukocyte migration
ADGRV1	ENSG00000164199	• G-protein coupled receptor • involved in development of hearing and vision
HOXC10	ENSG00000180818	• Sequence-specific transcription factor • Developmental regulatory system
MUC5AC	ENSG00000215182	• Gel forming glycoprotein • Gastric and respiratory tract • Protects mucosa from infection
LINC01301	ENSG00000251396	• RNA gene
GPI	ENSG00000105220	• Glycolytic enzyme • Catalyzes G-6-P → F-6-P
PLA2G4F	ENSG00000168907	• Lipase • Potential role in membrane lipid remodeling and biosynthesis of lipid mediators

Name	EnsemblID	• **Function** [19–21]
OTC	ENSG00000036473	• Enzyme involved in urea cycle
None	ENSG00000224739	• RNA gene
GORASP2	ENSG00000115806	• Involved in membrane stacking of Golgi cisternae • Involved in process by which Golgi stacks reform after breakdown during mitosis and meiosis
MON1B	ENSG00000103111	• Required for acrosome formation during spermiogenesis • Involved in early viral transcription
NDRG1	ENSG00000104419	• Stress responsive protein involved in hormone response, cell growth, and differentiation • Tumor suppressor • Necessary but not sufficient for p53/TP53-mediated caspase activation and apoptosis
IL1R2	ENSG00000115590	• Role in cell trafficking • Interleukin receptor
MYCN	ENSG00000134323	• Member of MYC family • Located in nucleus • Amplification associated with variety of tumors
FFAR4	ENSG00000186188	• G-protein coupled receptor involved in adipogenesis, energy metabolism, inflammation

Table S6. IC_{50} values of PANCO09b and PANCO11b cultured in 2D versus 3D exposed to gemcitabine, paclitaxel, SN-38, 5-FU, and oxaliplatin.

Cell culture	Drugs	IC_{50} (μM) ± SD Adv. DMEM/F-12		*p*-value
		2D	3D	
PANCO09b	**Gemcitabine**	$6.24 * 10^{-3} \pm 0.50 * 10^{-3}$	$8.12 * 10^{-3} \pm 4.24 * 10^{-3}$	0.323
	Paclitaxel	$1.66 * 10^{-3} \pm 0.51 * 10^{-3}$	$1.07 * 10^{-3} \pm 0.79 * 10^{-3}$	0.342
	SN-38	$5.3 * 10^{-3} \pm 0.76 * 10^{-3}$	$4.25 * 10^{-3} \pm 0.88 * 10^{-3}$	0.128
	5-FU	13.20 ± 1.44	11.87 ± 7.84	0.836
	Oxaliplatin	10.38 ± 0.69	8.61 ± 0.91	0.170
PANCO11b	Gemcitabine	$6.02 * 10^{-3} \pm 2.15 * 10^{-3}$	$6.87 * 10^{-3} \pm 1.24 * 10^{-3}$	0.656
	Paclitaxel	$2.20 * 10^{-3} \pm 0.67 * 10^{-3}$	$4.48 * 10^{-3} \pm 2.46 * 10^{-3}$	0.200
	SN-38	$8.20 * 10^{-3} \pm 2.31 * 10^{-3}$	$1.13 * 10^{-3} \pm 0.59 * 10^{-3}$	0.027
	5-FU	14.21 ± 3.41	13.25 ± 6.31	0.867
	Oxaliplatin	5.36 ± 0.86	32.30 ± 32.34	0.212

Data are presented as mean ± SD of three independent experiments performed in triplicate. Independent sample t-test was used to assess differences. Significance was reached when $p < 0.05$.

Table S7. GR_{50} values of PANCO09b and PANCO11b 2D versus 3D exposed to gemcitabine, paclitaxel, SN-38, 5-FU, and oxaliplatin.

Cell culture	Drugs	GR_{50} (μM) ± SD Adv. DMEM/F-12		*p*-value
		2D	3D	
PANCO09b	**Gemcitabine**	$6.24 * 10^{-3} \pm 0.50 * 10^{-3}$	$7.44 * 10^{-3} \pm 6.09 * 10^{-3}$	0.595
	Paclitaxel	$1.66 * 10^{-3} \pm 0.51 * 10^{-3}$	$0.87 * 10^{-3} \pm 0.60 * 10^{-3}$	0.143
	SN-38	$5.3 * 10^{-3} \pm 0.76 * 10^{-3}$	$1.96 * 10^{-3} \pm 1.43 * 10^{-3}$	0.076
	5-FU	13.20 ± 1.44	5.24 ± 3.61	0.061
	Oxaliplatin	11.67 ± 2.47	6.53 ± 3.10	0.128
PANCO11b	Gemcitabine	$7.42 * 10^{-3} \pm 1.75 * 10^{-3}$	$2.55 * 10^{-3} \pm 2.20 * 10^{-3}$	0.069
	Paclitaxel	$3.48 * 10^{-3} \pm 0.99 * 10^{-3}$	$1.41 * 10^{-3} \pm 1.23 * 10^{-3}$	0.126
	SN-38	$9.82 * 10^{-3} \pm 0.71 * 10^{-3}$	$3.80 * 10^{-3} \pm 0.26 * 10^{-3}$	0.001
	5-FU	44.85 ± 12.23	11.90 ± 0.99	0.063
	Oxaliplatin	28.65 ± 15.49	6.11 ± 3.08	0.181

Data are presented as mean ± SD of three independent experiments performed in triplicate. Independent sample t-test was used to measure differences. Significance was reached when $p < 0.05$.

Table S8. Doubling time and growth rate of PANCO09b and PANCO11b growing in conventional versus physiological medium.

Cell culture	Parameter	Adv.DMEM/F-12	Plasmax	*p*-value
PANCO09b	doubling time (h)	74.73 ± 1.81	63.36 ± 3.67	< 0.001
	growth rate/ day	0.222 ± 0.005	0.263 ± 0.014	< 0.001
PANCO11b	doubling time (h)	52.35 ± 6.09	62.68 ± 2.89	0.057
	growth rate/ day	0.320 ± 0.035	0.266 ± 0.012	0.063

Data are presented as mean ± SD of three independent experiments performed in triplicates. Independent sample t-test was used to measure differences. Significance was reached when $p < 0.05$.

Table S9. IC_{50} values of PANCO09b and PANCO11b growing in conventional versus physiological media exposed to gemcitabine, paclitaxel, SN-38, 5-FU, and oxaliplatin.

Cell culture	Drugs	IC_{50} (μM) ± SD Adv.DMEM/F-12	Plasmax	*p*-value
PANCO09b	**Gemcitabine**	$6.24 * 10^{-3} \pm 0.50 * 10^{-3}$	$8.12 * 10^{-3} \pm 4.24 * 10^{-3}$	0.488
	Paclitaxel	$1.66 * 10^{-3} \pm 0.51 * 10^{-3}$	$1.45 * 10^{-3} \pm 0.51 * 10^{-3}$	0.647
	SN-38	$5.3 * 10^{-3} \pm 0.76 * 10^{-3}$	$4.25 * 10^{-3} \pm 0.88 * 10^{-3}$	0.177
	5-FU	13.20 ± 1.44	11.55 ± 1.32	0.355
	Oxaliplatin	10.38 ± 0.69	7.68 ± 3.39	0.247
PANCO11b	Gemcitabine	$6.02 * 10^{-3} \pm 2.15 * 10^{-3}$	$5.28 * 10^{-3} \pm 0.69 * 10^{-3}$	0.602
	Paclitaxel	$2.20 * 10^{-3} \pm 0.67 * 10^{-3}$	$1.55 * 10^{-3} \pm 0.35 * 10^{-3}$	0.211
	SN-38	$8.20 * 10^{-3} \pm 0.23 * 10^{-3}$	$5.46 * 10^{-3} \pm 0.78 * 10^{-3}$	0.123
	5-FU	14.21 ± 3.41	11.42 ± 6.82	0.656
	Oxaliplatin	5.36 ± 0.86	5.89 ± 1.10	0.545

Data are presented as mean ± SD of three independent experiments performed in triplicate. Independent sample t-test was used to assess differences. Significance was reached when $p < 0.05$.

Table S10. GR_{50} values of PANCO09b and PANCO11b growing in conventional versus physiological media exposed to gemcitabine, paclitaxel, SN-38, 5-FU, and oxaliplatin.

Cell culture	Drugs	IC_{50} (μM) ± SD		*p*-value
		Adv.DMEM/F-12	Plasmax	
PANCO09b	**Gemcitabine**	$5.33 * 10^{-3} \pm 1.04 * 10^{-3}$	$9.11 * 10^{-3} \pm 4.55 * 10^{-3}$	0.239
	Paclitaxel	$1.81 * 10^{-3} \pm 0.66 * 10^{-3}$	$2.11 * 10^{-3} \pm 0.66 * 10^{-3}$	0.605
	SN-38	$5.56 * 10^{-3} \pm 2.19 * 10^{-3}$	$6.34 * 10^{-3} \pm 2.72 * 10^{-3}$	0.392
	5-FU	17.45 ± 2.62	35.75 ± 19.02	0.31
	Oxaliplatin	11.67 ± 2.47	15.03 ± 8.22	0.535
PANCO11b	Gemcitabine	$7.42 * 10^{-3} \pm 1.75 * 10^{-3}$	$6.30 * 10^{-3} \pm 0.651 * 10^{-3}$	0.357
	Paclitaxel	$3.48 * 10^{-3} \pm 0.99 * 10^{-3}$	$2.26 * 10^{-3} \pm 0.74 * 10^{-3}$	0.164
	SN-38	$9.82 * 10^{-3} \pm 0.71 * 10^{-3}$	$5.18 * 10^{-3} \pm 1.83 * 10^{-3}$	0.015
	5-FU	44.85 ± 12.23	30.20 ± 14.28	0.563
	Oxaliplatin	28.65 ± 15.49	13.30 ± 3.85	0.174

Data are presented as mean ± SD of three independent experiments performed in triplicate. Independent sample t-test was used to assess differences. Significance was reached when $p < 0.05$.