

Additional File 1

Acidic ascites inhibits ovarian cancer cell proliferation and correlates with the metabolomic, lipidomic and inflammatory phenotype of human patients

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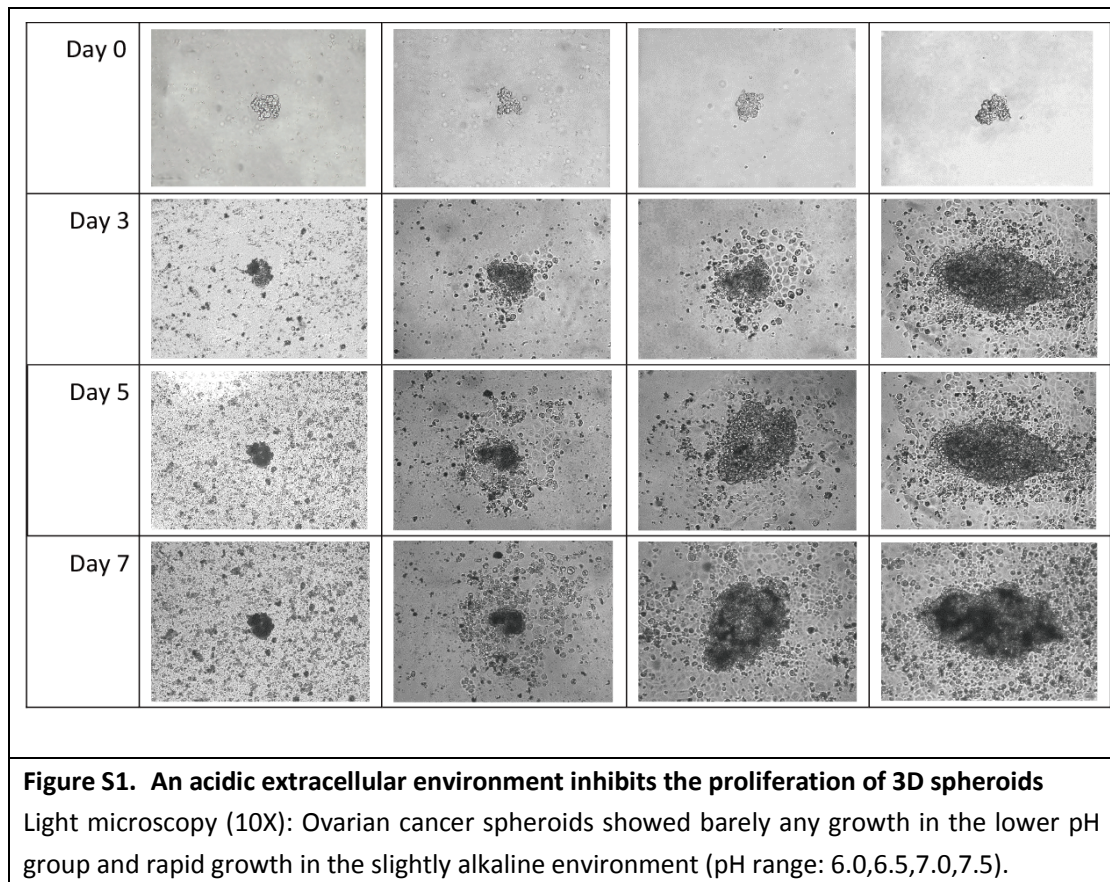
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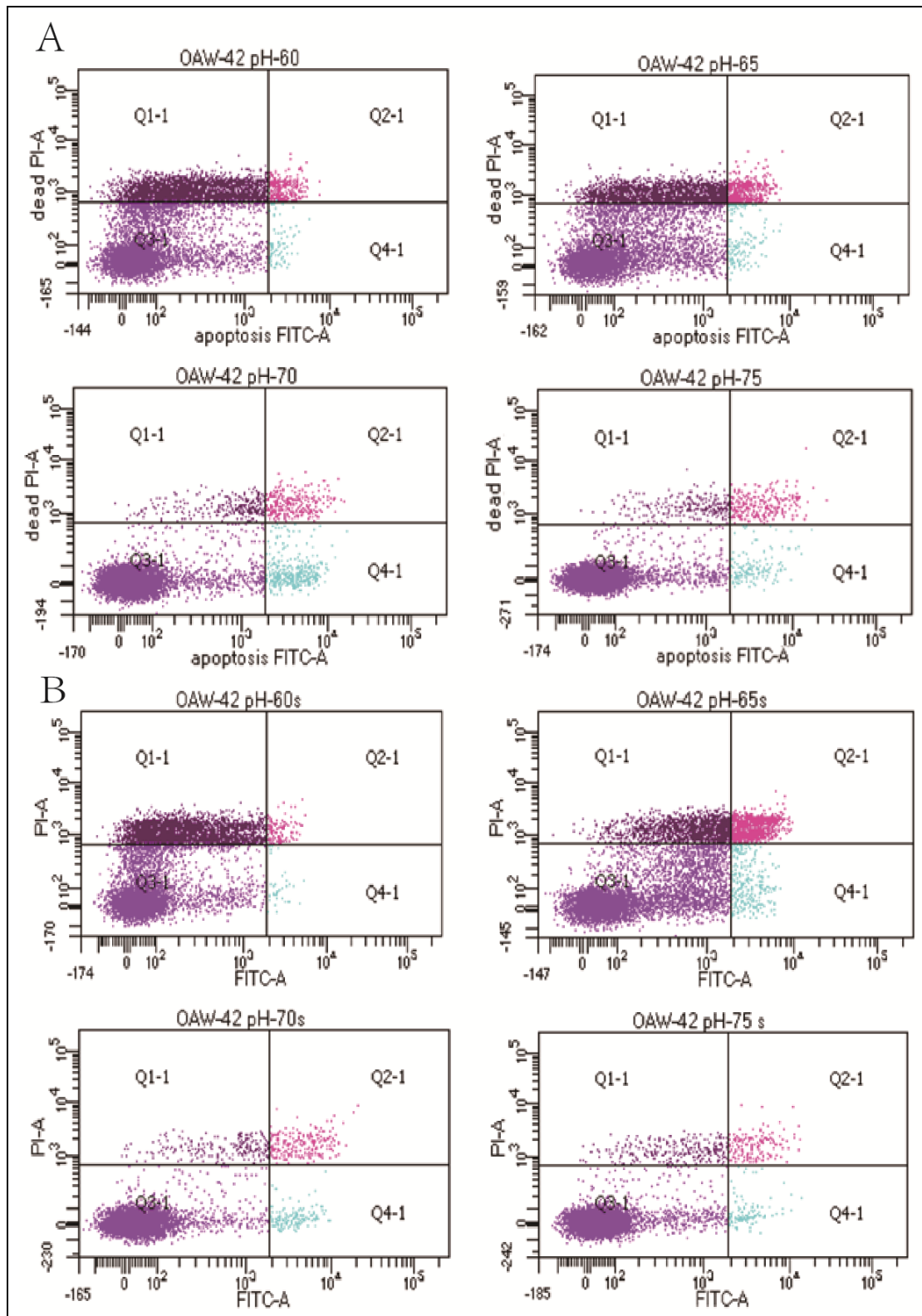


Figure S2. Flow cytometric analysis of the effect of pH on apoptosis of OAW42 cells in 10% malignant ovarian ascites at different pHs

A) Apoptosis rate of OAW45 cells at different pHs (6.0, 6.5, 7.0, 7.5). B) Apoptosis rate of OAW45 cells at different pHs with 10% ascites.

Table S1 – Fold changes of polar metabolites on ovarian cancers with II-III vs IV.

| | FC | log2(FC) | raw.pval | -log10(p) |
|-------------------|---------|----------|-----------|-----------|
| Alanine | 0.44628 | -1.164 | 0.002351 | 2.6288 |
| Isoleucine | 0.79325 | -0.33415 | 0.0040014 | 2.3978 |
| Phenylalanine | 0.39805 | -1.329 | 0.014645 | 1.8343 |
| 3-Hydroxybutyrate | 2.3475 | 1.2311 | 0.02715 | 1.5662 |
| Glutamine | 0.66784 | -0.58243 | 0.049762 | 1.3031 |

Table S2 – Full matrix of all obtained molecular and physical-chemical ascites parameters of stages II-III and IV.

| Parameter | Stage II- III | Stage IV |
|--------------------------|---------------|--------------|
| pH | 7.51±0.16 | 7.78±0.16 |
| pCO2 (mmHg) | 22.05±14.56 | 17.33±2.08 |
| Na+ (mmol/L) | 142.00±8.04 | 136.22±1.35 |
| K+(mmol/L) | 3.24±1.61 | 4.34±0.63 |
| Ca2+(mmol/L) | 0.89±0.38 | 1.12±0.06 |
| Cl-(mmol/L) | 121.24±17.67 | 108.77±5.59 |
| Glu (mg/dL) | 65.81±33.54 | 102.78±13.42 |
| Lac (mmol/L) | 1.73±1.36 | 2.10±0.85 |
| 2-Hydroxybutyrate | 2.13±1.42 | 2.22±0.96 |
| 3-Hydroxybutyrate | 25.64±15.04 | 11.31±17.39 |
| 3-Hydroxyisobutyrate | 0.32±0.17 | 0.68±0.45 |
| 3-Hydroxyisovaleric acid | 1.91±1.01 | 1.81±0.66 |
| Acetate | 3.29±0.15 | 3.29±0.21 |
| Acetone | 2.69±4.96 | 0.57±0.72 |
| Alanine | 3.87±1.92 | 9.73±3.35 |
| Asparagine | 1.45±0.62 | 1.37±0.81 |
| Carnitine | 0.74±0.41 | 1.15±0.87 |
| Choline | 0.22±0.19 | 0.32±0.11 |
| Citric acid | 0.74±0.34 | 0.98±0.19 |
| Creatine | 1.34±0.83 | 1.21±1.21 |
| Creatine phosphate | 1.06±0.56 | 1.44±0.26 |
| Formate | 0.86±0.57 | 0.96±0.22 |
| Glucose | 75.72±33.67 | 138.16±47.17 |
| Glutamate | 4.60±3.54 | 3.75±3.09 |
| Glutamine | 7.95±3.73 | 14.15±1.68 |
| Glutathione | 0.45±0.24 | 0.41±0.07 |
| Glycerol | 6.23±2.95 | 7.27±1.72 |
| Glycine | 5.69±3.02 | 8.58±1.96 |
| Histidine | 1.41±0.72 | 1.79±0.28 |

| | | |
|------------------------------------|-----------------|-------------------|
| Hypotaurine | 1.13±0.61 | 1.92±1.86 |
| Isoleucine | 1.49±0.55 | 2.21±0.31 |
| Lactate | 38.13±30.99 | 69.74±35.05 |
| Leucine | 3.25±1.59 | 3.66±0.60 |
| Lysine | 3.36±1.43 | 4.86±0.82 |
| O-Phosphocholine | 0.43±0.19 | 0.482±0.08 |
| Ornithine | 1.47±0.44 | 1.921±0.68 |
| Phenylalanine | 1.08±0.43 | 3.378±2.75 |
| Proline | 2.83±1.73 | 3.358±1.11 |
| Threonine | 4.09±3.09 | 5.09±0.89 |
| Tyrosine | 1.10±0.51 | 1.69±0.18 |
| Valine | 5.09±2.11 | 7.97±0.93 |
| sn-Glycerol-3-phosphocholine | 1.34±0.54 | 1.26±0.65 |
| CE (Cholesterol Ester) | 0.39±0.32 | 0.32±0.07 |
| FA (Fatty Acids) | 0.01±0.01 | 0.006±0.00 |
| FC (Free Cholesterol) | 0.29±0.19 | 0.26±0.13 |
| LA (Linoleic Acid) | 0.03±0.02 | 0.02±0.01 |
| MUFA (Monounsaturated Fatty Acids) | 0.01±0.01 | 0.008±0.00 |
| PL (Phospholipids) | 0.32±0.06 | 0.20±0.02 |
| PUFA (Polyunsaturated Fatty Acids) | 0.03±0.02 | 0.02±0.00 |
| SFA (Saturated Fatty Acids) | 0.10±0.07 | 0.07±0.01 |
| TC (Total Cholesterol) | 0.362±0.29 | 0.29±0.06 |
| TG (Triglycerides) | 0.008±0.01 | 0.004±0.00 |
| UFA (Unsaturated Fatty Acids) | 0.04±0.03 | 0.03±0.01 |
| w-3 FA (Omega-3 Fatty Acids) | 0.08±0.06 | 0.06±0.01 |
| MCP-1 (A8) | 840.45±1359.80 | 1998.66±3120.40 |
| IL-6 (A10) | 4510.35±3502.17 | 15267.12±14619.95 |
| IL-8 (B2) | 650.87±1138.10 | 281.04±297.60 |
| IL-10 (B3) | 115.67±76.64 | 166.62±228.32 |
| IL-18 (B6) | 179.14±162.49 | 322.41±283.25 |

Table S3 – Patient Survival Time Information.

| ID | Survival (Months) | State | ID | Survival (Months) | State |
|-------------|-------------------|-------|-------------|-------------------|-------|
| ASC-GC-B-2 | 0.3 | dead | ASC-OC-B-11 | 2 | dead |
| ASC-OC-T-4 | 15 | alive | ASC-OC-T-12 | 11 | dead |
| ASC-OC-T-6 | 2 | dead | ASC-OC-T-13 | 12 | alive |
| ASC-OC-T-7 | 15 | alive | ASC-OC-T-14 | 12 | alive |
| ASC-OC-T-10 | 6 | dead | ASC-OC-T-15 | 1 | dead |

Table S4 – Patient peritoneal metastasis information.

| ID | peritoneal metastasis |
|-------------|--|
| ASC-GC-B-2 | Extensive peritoneal metastasis, M1b: Gastric metastasis |
| ASC-OC-T-4 | omentum majus: 0.9cm, colonic mesentery:0.2cm, splenic hilum:0.5cm, hepatoduodenal ligament:0.3cm |
| ASC-OC-T-6 | Extensive peritoneal metastasis |
| ASC-OC-T-7 | omentum majus: 0.7cm, diaphragm: 3.5cm, hepatoduodenal ligament: 1.5cm, gallbladder peritoneum: 0.3cm, pancreas: 2.5cm, omentum minus:0.2cm, M1b: pleura |
| ASC-OC-T-10 | omentum majus: multiple (3.5cm max), small intestinal plasma membrane: 0.3cm, hepatoduodenal ligament: 0.5cm, hepatic hilar: 1.2cm, small intestinal plasma membrane: 2.5cm |
| ASC-OC-B-11 | Extensive peritoneal metastasis, M1b: pleura |
| ASC-OC-T-12 | omentum majus: 5.5cm, omentum minus: 1.0cm, gallbladder peritoneum: 0.5cm, ileocecal: 3.5cm, bladder peritoneum: 3.5cm, left septum adipose tissue: 9cm, spleen peritoneum: 9.5cm |
| ASC-OC-T-13 | No |
| ASC-OC-T-14 | omental capsule: 0.5cm, colonic mesentery: 0.3cm, splenic peritoneum: 6.5cm, pancreas: 0.3cm |
| ASC-OC-T-15 | omentum majus: 3.2cm, multiple rectal wall: 1.9cm, colonic plasma membrane: 0.5cm, small intestinal plasma membrane: 1cm, colonic plasma membrane: 1.5cm, peripancreatic tissue: 2.8cm, splenic peritoneum: 2.5cm, renal peritoneum: 0.9cm |