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Data Article

Dataset of cell viability and analytes released by cancer cell lines exposed to low pH and conditioned medium

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Dataset link: Dataset of cell viability and secreted analytes tumour cell lines treated with low pH and conditioned medium (Original data)

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ABSTRACT

Cancer cells influence their microenvironment by secreting factors that promote tumour growth and survival while evading immune-mediated destruction. We previously determined the expression of secreted factors in breast and oesophageal squamous cell carcinoma cell lines (MCF-7 and WHCO6, respectively) using Luminex assays. These cells were subsequently treated with low pH medium to mimic *in vivo* acid exposure, and the effects on cell viability, proliferation, and secretion of cytokines, chemokines and growth factors were described [1]. Here, we present the datasets from these experiments in addition to data obtained from treating cell lines with conditioned medium from apoptotic cell cultures.

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Specifications Table

Subject	Biology
Specific subject area	Oncology
Data format	Raw data and analysed data.
Type of data	Tables and graphs
Data collection	Breast and oesophageal squamous cell carcinoma cell lines were treated with low pH medium and conditioned medium from apoptotic cells. Cell culture medium was removed from treated and untreated cell cultures after 24 h and was stored at −80 °C. Apoptosis was detected, and cytokines, chemokines, and growth factors released into the culture medium were tested. Fresh cell cultures were exposed to conditioned medium from apoptotic cells and secreted factors were measured by Luminex ™.
Data source location	Data are currently stored at the University of Pretoria (25.7545° S, 28.2314° E).
Data accessibility	The analysed data is available in this article and the raw data set is in the linked public repository [2]. Repository name: Mendeley Data Data identification reference: Worsley CM, Veale RB, Mayne ES. Dataset of cell viability and secreted analytes tumour cell lines treated with low pH and conditioned medium. 2024. 10.17632/6zhwcznnft.1. URL: https://data.mendeley.com/datasets/6zhwcznnft/1
Related research article	C.M. Worsley, R.B. Veale, E.S. Mayne. The effect of acute acid exposure on immunological protein secretion, cell survival, and cell cycle progression in tumour cell lines. Cytokine (2023) 162: 156,118. https://doi.org/10.1016/j.cyto.2022.156118 [1]

1. Value of the Data

- Low pH caused by hypoxia and glycolysis can select for tumour cells with enhanced survival strategies, while causing apoptosis in other cancer cells.
- Apoptotic cancer cells release factors that impact on survival and growth of related cancer cells that is likely tumour-type specific.
- Identifying factors released by tumour cells under these conditions may provide insight into bi-directional communication in the tumour microenvironment that can be useful for development of suitable treatment.

2. Background

Cancer develops in the context of a conducive microenvironment where multiple systems fail to suppress uncontrolled cell proliferation. Solid tumours typically occur in acidic microenvironments which impact on cell proliferation, apoptosis, and the interactions with surrounding cells to support tumour growth and immune evasion. This study aimed to evaluate the impact of the acidic microenvironment on the production of pro-tumorigenic and immunomodulatory factors in breast cancer and oesophageal squamous cell carcinoma cell lines. Cell lines were treated with low pH medium to mimic *in vivo* conditions, and the effects on apoptosis and cell cycle progression were measured by flow cytometry. The secretion of immunomodulatory proteins and growth factors was measured by Luminex multiplex assay. As cells undergoing apoptosis release factors that affect bystander cells, conditioned medium obtained from apoptotic cells was incubated with previously untreated cells. The effects on cell survival, cell cycle progression, and secreted factors were measured.

3. Data Description

This article describes the dataset obtained from both flow cytometric and Luminex assays. The associated repository contains both raw data and statistical analyses of experiments conducted on breast (MCF-7) and oesophageal squamous cell carcinoma (OSCC) (WHCO6) cell lines.

Table 1
Contents of associated database. The linked database contains a spreadsheet with 7 sheets. Here the contents contained within each sheet are described.

Sheet	Title	Contents
1	Cell viability pH treatment	Percentage of viable cells after treatment with low pH medium, DMSO, and STS. ANOVA and post-hoc Dunnett's comparisons of treatments compared to the serum free (untreated) control.
2	Luminex protein concentrations	Concentration (pg/ml) of analytes detected by Luminex in culture media of all samples at baseline (untreated), cells treated with low pH (analytes present in CM), and cells treated with CM (analytes present in PCM).
3	ANOVA protein concentrations	ANOVA and Dunnett's comparisons per analyte after low pH treatment compared to the untreated control
4	Cell viability CM	Percentage of viable cells after treatment with conditioned medium
5	CM-PCM MCF-7	Comparison of analyte concentrations between conditioned medium (CM) and post-conditioned medium (PCM) (ANOVA and Dunnett's comparison) in MCF-7 cell line
6	CM-PCM WHCO6	Comparison of analyte concentrations between CM and PCM (ANOVA and Dunnett's comparison) in WHCO6 cell line
7	BrdU WHCO6	The percentage of cells throughout the cell cycle after treatment with low pH medium (6.5 and 3.2) and after exposure to conditioned medium from cells previously treated (pH 6.5)

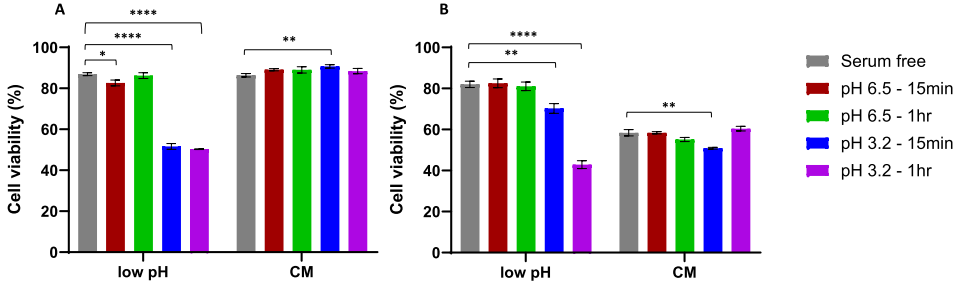

Fig. 1. Comparison of cell viability after low pH treatment and exposure to CM. (A) WHCO6 and (B) MCF-7 cells were treated with low pH medium (pH 6.5 and 3.2) for 15 min and 1 h, and with conditioned medium (CM) overnight. Apoptosis and cell viability were assessed by flow cytometric staining with Annexin V antibody and propidium iodide. The percentage of viable cells was compared to the serum-free untreated control for each treatment type. Statistical comparisons were performed using one-way ANOVA. (****$p < 0.0001$, **$p < 0.01$, *$p < 0.05$).

Cell lines were acutely exposed to low pH culture medium (6.5 and 3.2) to mimic conditions seen within solid tumours *in vivo* [3–5]. The effects on cell viability, cell cycle progression, and the release of soluble factors were measured after 24 h. Conditioned medium (CM) was then removed and was added to additional untreated cell cultures for 24 h. The effect of CM on cell viability, cell cycle progression, and factor secretion was assessed. The dataset in the repository contains 7 separate sheets in one spreadsheet, with an overview of the contents shown in Table 1.

Fig. 1 is a summary of the percentage of viable cells after treatment with low pH or conditioned medium (CM). Cells that were viable or in early or late stage apoptosis were identified using Annexin V and propidium iodide by flow cytometry. Both MCF-7 and WHCO6 cells lines were acutely exposed to low pH medium (pH 3.2 and 6.5) which caused a decrease in cell viability compared to the untreated controls. This was statistically significant at pH 3.2 ($p < 0.0001$) in both cell lines. Dimethyl sulfoxide (DMSO) and staurosporine (STS) were included as positive controls as agents known to induce apoptosis [6,7] (see sheet 1 in linked database - [2]). CM was then used to treat additional cell cultures and flow cytometric analysis of Annexin V and propidium iodide was performed. Treatment with CM did not adversely affect viability in WHCO6, but did cause increased cell death in MCF-7 (Fig. 1).

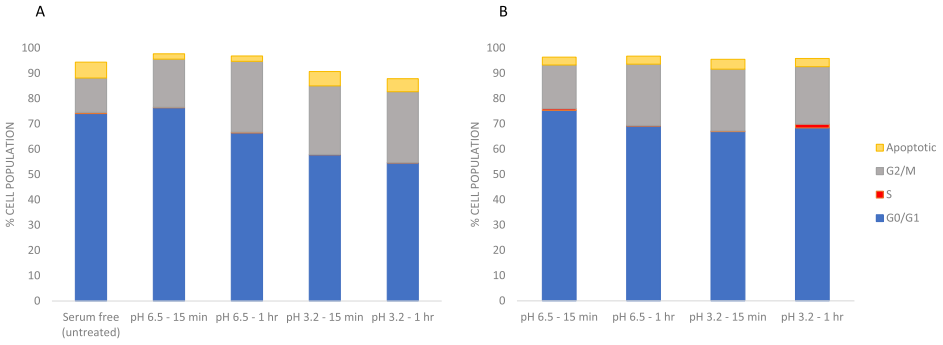

Fig. 2. Cell cycle analysis via BrdU incorporation in the WHCO6 cell line treated with low pH and CM. (A) WHCO6 cells were treated with low pH medium (pH 6.5 and 3.2) for 15 min and 1 h. (B) WHCO6 cells were treated with CM from cells previously treated with low pH medium. BrdU incorporation was measured by flow cytometry. The percentage of cells based on anti-BrdU and 7-AAD staining of cells in various stages of the cell cycle (G_0/G_1, S, G_2/M), and apoptotic cells is shown.

Fig. 2 shows the percentage of WHCO6 cells in the different phases of the cell cycle (G_0/G_1, S, G_2/M) after treatment with low pH medium (Fig. 2A), and treatment with CM (Fig. 2B). This was assessed by BrdU incorporation over 24 h post-treatment. Treatment with low pH medium caused a decrease in cells the percentage of cells in S phase compared to the untreated control, whereas treatment with CM (pH 3.2) caused more cells to progress through the cell cycle to G_2/M phase (see database sheet 7).

Table 2 shows the mean concentrations (pg/ml) of cytokines, chemokines, and growth factors released by WHCO6 and MCF-7 cell lines in untreated cultures (baseline), and after treatment with low pH medium and CM (present in CM and PCM respectively). IP-10, VEGF, G-CSF, EGF, FGF, GDF-15, Lipocalin-2, myoglobin, and sICAM-1 were detected in both cell lines, while IL-6 and IL-8 were only found in WHCO6. Several analytes tested were not found in either cell line including IL-1β, IL-2, IL-4, IL-5, IL-10, GM-CSF, IFN-γ, TNF-α, Eotaxin, Rantes, MCP-1, MCP-2, MCP-3, GRO-α, MIG, MIP-1α, MIP-1β, SDF-1α, ADAMTS13, MPO, sVCAM-1, and P-selectin. Concentrations of all detected analytes were higher in untreated baseline cultures than in serum-free controls or in cultures treated with low pH or CM. Statistical differences observed between treated samples and untreated controls are reported (see database - sheet 3) [2]. All analytes that were present in CM were also detected in the PCM in both cell lines (Fig. 3). G-CSF was significantly increased in WHCO6, while other growth factors including VEGF, epidermal growth factor (EGF), and fibroblast growth factor (FGF) increased to a lesser extent.

4. Experimental Design, Materials and Methods

4.1. Cell culture

Breast carcinoma (MCF-7) and oesophageal squamous cell carcinoma (WHCO6) cell lines were obtained from the Cell Biology Laboratory in the School of Molecular and Cell Biology at the University of the Witwatersrand. They were *Mycoplasma* spp. free and were maintained in Dulbecco's Modified Eagle's Medium (DMEM)/Hams F12 (Sigma Aldrich, USA) (3:1) with 10 % foetal calf serum (FCS) (Sigma Aldrich, USA) and 2 % penicillin/streptomycin (Sigma Aldrich, USA). Cultures were grown at 37 °C with 5 % CO_2 to 80 % confluency, before being harvested with trypsin and ethylenediaminetetra-acetic acid (EDTA) (Merck Millipore, USA), counted, and seeded into 6-cm culture dishes. Cells were maintained in DMEM/Hams F12 with 10 % FCS for 24 h before further treatment.

Table 2
Dataset of mean concentrations of cytokines, chemokines, and growth factors detected in culture media from breast carcinoma (MCF-7) and OSCC (WHCO6) cell lines. Multiplex Luminex assays were performed in duplicate to assess secretion of these analytes from untreated cells at baseline, serum-free controls, after cells were treated with low pH medium (pH 6.5 and 3.2 for 15 min and 1 h) (conditioned medium assessment), and after cells were treated with conditioned medium (post-conditioned medium assessment – PCM). Mean concentrations (pg/ml) and standard deviations are reported.

Sample ID	IL-8	IL-6	VEGF	G-CSF	EGF	FGF	IP-10	GDF-15	Lipocalin-2	sICAM-1	Myoglobin
WHCO6 cell line											
Baseline	31 057.50 ± 10 497.00	442.50 ± 6.36	1631.50 ± 304.76	2534.50 ± 201.53	220.50 ± 23.33	1044.50 ± 283.55	13118.00 ± 2 333.45	8356.50 ± 5 651.90	4320.00 ± 1627.76	1265.00 ± 346.48	309.50 ± 45.96
Serum free	687.50 ± 70.00	59.00 ± 4.24	94.50 ± 4.95	298.00 ± 18.38	107.00 ± 2.83	96.00 ± 7.07	393.00 ± 1.41	2 365.50 ± 48.79	488.00 ± 31.11	128.50 ± 2.12	185.50 ± 4.95
pH 6.5 – 15 min	33.50 ± 4.95	12.50 ± 2.12	62.50 ± 3.54	199.50 ± 38.89	97.50 ± 0.61	73.00 ± 12.73	23.00 ± 7.07	1 75.00 ± 258.80	514.00 ± 66.47	136.50 ± 27.58	144.50 ± 21.92
pH 6.5 – 1 h	568.00 ± 79.20	51.00 ± 1.41	70.50 ± 2.12	239.00 ± 2.83	92.00 ± 7.07	86.00 ± 5.66	227.50 ± 37.48	2 104.00 ± 65.05	435.50 ± 95.46	130.00 ± 16.97	146.50 ± 20.51
pH 3.2 – 15 min	123.00 ± 8.49	21.00 ± 0.00	68.00 ± 2.83	205.00 ± 25.46	96.50 ± 3.54	72.00 ± 11.31	36.50 ± 9.19	551.00 ± 48.08	18.50 ± 2.12	16.00 ± 1.41	61.00 ± 2.83
pH 3.2 – 1 h	1 064.50 ± 0.71	50.50 ± 0.71	103.50 ± 7.78	317.00 ± 26.87	111.00 ± 22.63	109.00 ± 1.41	151.50 ± 17.68	666.50 ± 193.04	84.50 ± 21.92	603.50 ± 133.64	585.50 ± 144.96
Serum free PCM	136.50 ± 16.26	19.50 ± 3.54	91.50 ± 0.71	263.00 ± 7.07	67.50 ± 3.54	100.00 ± 2.83	52.00 ± 0.00	1 550.00 ± 229.10	72.50 ± 2.12	27.50 ± 2.12	54.50 ± 3.54
pH 6.5 – 15 min PCM	147.00 ± 2.83	19.50 ± 0.71	103.00 ± 19.80	331.00 ± 19.80	94.50 ± 13.44	114.50 ± 13.44	53.00 ± 5.66	1 655.00 ± 39.60	49.50 ± 6.36	15.50 ± 2.12	62.50 ± 2.12
pH 6.5 – 1 h PCM	247.00 ± 12.73	50.00 ± 1.41	96.50 ± 14.85	342.00 ± 8.49	108.00 ± 41.01	102.50 ± 9.19	35.50 ± 14.85	1 710.00 ± 132.94	51.00 ± 5.66	13.50 ± 0.71	66.50 ± 2.12
pH 3.2 – 15 min PCM	95.50 ± 6.36	30.50 ± 0.71	134.00 ± 2.83	434.00 ± 9.90	90.00 ± 16.67	122.50 ± 10.61	33.00 ± 7.07	719.50 ± 150.61	35.00 ± 0.00	12.00 ± 4.24	23.50 ± 2.12
pH 3.2 – 1 h PCM	710.50 ± 14.85	76.50 ± 3.54	113.00 ± 18.38	419.00 ± 48.08	104.00 ± 16.97	141.00 ± 1.41	82.00 ± 5.66	938.00 ± 74.95	46.50 ± 0.71	13.00 ± 1.41	92.50 ± 3.54
MCF-7 cell line											
Baseline	–	18.50 ± 0.71	294.00 ± 29.70	905.50 ± 136.47	167.50 ± 7.78	235.00 ± 11.31	30.50 ± 0.71	9 681.50 ± 1 150.46	36.50 ± 0.36	1 308.50 ± 424.97	82.00 ± 18.38
Serum free	–	–	62.00 ± 1.41	266.50 ± 12.02	75.00 ± 11.31	70.00 ± 2.83	32.00 ± 0.00	1 378.50 ± 178.90	8.00 ± 1.41	171.50 ± 0.71	33.50 ± 2.12
pH 6.5 – 15 min	–	–	43.50 ± 2.12	263.00 ± 7.07	61.00 ± 5.66	59.50 ± 7.78	14.50 ± 14.85	217.00 ± 43.84	7.00 ± 0.00	21.00 ± 1.41	33.50 ± 6.36
pH 6.5 – 1 h	–	–	46.50 ± 4.95	255.50 ± 60.10	46.50 ± 4.95	59.50 ± 2.12	30.50 ± 2.12	3 823.50 ± 267.99	10.00 ± 1.41	462.50 ± 2.12	30.50 ± 2.12
pH 3.2 – 15 min	–	–	50.50 ± 3.54	285.50 ± 48.79	63.00 ± 7.07	57.00 ± 4.24	17.50 ± 12.02	14.50 ± 2.12	7.00 ± 0.00	10.00 ± 1.41	34.00 ± 0.00
pH 3.2 – 1 h	–	–	40.50 ± 0.71	158.00 ± 31.11	82.50 ± 3.54	49.50 ± 3.54	38.00 ± 1.41	46.50 ± 6.36	6.00 ± 0.00	11.50 ± 0.71	34.50 ± 3.54
Serum free PCM	–	–	34.50 ± 40.31	266.50 ± 12.02	75.00 ± 11.31	70.00 ± 2.83	29.00 ± 0.00	2 073.00 ± 89.10	9.50 ± 0.71	319.50 ± 9.19	38.00 ± 0.00
pH 6.5 – 15 min PCM	–	–	58.00 ± 7.07	237.00 ± 9.90	117.50 ± 41.72	71.00 ± 5.66	21.50 ± 9.19	1 232.50 ± 37.48	8.00 ± 0.00	243.00 ± 18.38	37.50 ± 6.36
pH 6.5 – 1 h PCM	–	–	65.00 ± 2.83	240.50 ± 28.99	102.00 ± 22.63	79.00 ± 2.83	23.00 ± 8.49	924.50 ± 17.68	8.00 ± 1.41	165.00 ± 19.80	36.50 ± 6.36
pH 3.2 – 15 min PCM	–	–	67.50 ± 2.12	262.50 ± 26.16	76.00 ± 7.07	75.00 ± 4.24	27.00 ± 11.31	947.00 ± 42.43	8.00 ± 1.41	182.50 ± 51.62	31.00 ± 2.83
pH 3.2 – 1 h PCM	–	–	64.00 ± 1.41	246.00 ± 2.83	125.00 ± 19.80	68.50 ± 10.61	28.50 ± 0.71	2 163.50 ± 27.58	9.50 ± 0.71	330.00 ± 21.21	33.50 ± 3.54

"-" represents analytes not detected in the assays. Units – pg/ml. Abbreviations used in table: EGF – epidermal growth factor, FGF – fibroblast growth factor, G-CSF – granulocyte colony-stimulating factor, GDF-15 – growth/differentiation factor 15, IL-6 – interleukin-6, IL-8 – interleukin-8, IP-10 – interferon-γ inducible protein-10, PCM – post conditioned media, sICAM-1 – soluble intracellular cell adhesion molecule-1, VEGF – vascular endothelial growth factor.

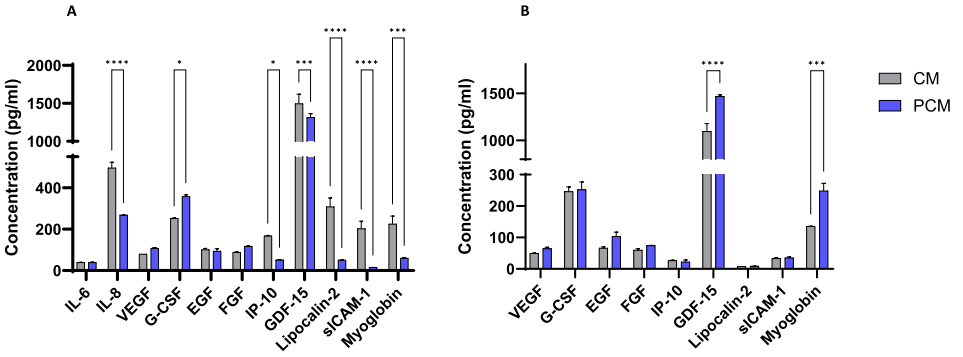

Fig. 3. Comparison of secreted factors in WHCO6 and MCF-7 cell lines assessed in conditioned media (CM) and post-conditioned media (PCM). (A) WHCO6 and (B) MCF-7 cell lines were treated with low pH medium and CM. Luminex assays were used to detect analytes present in medium of both cell lines after low pH treatment (in CM), and after CM treatment (in PCM). Two-way ANOVA tests were used to compare factor concentrations between CM and PCM. (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

4.2. Cell treatment with low *pH*, DMSO, and STS

Low pH media, which did not contain FCS, was adjusted to pH 3.2 or 6.5 using 1 N hydrochloric acid (HCl) (Sigma Aldrich, USA). Cultured cells were washed with phosphate buffered saline (PBS) (Merck Millipore, USA), and were then incubated for either 15 min or 1 h in low pH medium which was then removed, and pH 7.4 media was used to recondition cells overnight. Staurosporine (STS) and dimethyl sulfoxide (DMSO) were used as positive controls of apoptosis and 10 µl per ml of each were added to cell cultures incubated overnight as described [8]. An untreated culture of cells served as an untreated control.

4.3. Cell treatment with conditioned media (CM) and post conditioned media (PCM)

After overnight incubation after low pH treatment, conditioned media (CM) was removed from cell cultures. Some was stored at −80 °C for later Luminex assays (500 µl), while the rest (5.5 ml) was added to additional cell cultures for 24 h (cells that had not previously been treated with low pH medium). The medium was removed from these cells after 24 h – post conditioned medium (PCM). This PCM was added to additional cell cultures for 24 h (cells that had not previously been treated). Effects on cell viability, cell cycle progression, and protein concentration were then determined as described previously [1].

4.4. Annexin V assay

CM or PCM were removed from both cell lines, and cells were washed with PBS. Non-adherent cells present in media were collected by centrifugation (1 000 rpm for 5 min). Trypsin EDTA (1 ml) was used to detach adherent cells. These were combined with the non-adherent cells to which 5 ml PBS was added, and the suspension was centrifuged as before to pellet the cells. Annexin V binding buffer (BD Biosciences, USA) (1–2 ml) was added to the cell pellet, and 100 µl of cell suspension was stained with Annexin V fluorescein isothiocyanate (FITC) antibody and propidium iodide (PI) (BD Biosciences, USA) in the dark for 15 min. Binding buffer was added (200 µl), the sample was briefly vortexed, and cells were acquired on the BD LSR II flow cytometer (BD Biosciences, USA). Testing was conducted in duplicate. The percentage of viable cells was determined by how many cells excluded PI staining.

4.5. BrdU assay

Cell cycle analysis was performed using the allophycocyanin (APC) BrdU Flow kit (BD Pharmingen, BD Biosciences, USA). BrdU was added to WHCO6 cultures overnight (1 µl of BrdU per 1 ml of culture media). Non-adherent cells were collected from culture media by centrifugation, and adherent cells were harvested using trypsin-EDTA. Cells were incubated with cytofix/cytoperm buffer at 4 °C for 30 min followed by washing with PBS. The cytofix/cytoperm and was repeated twice for 10 min and once for 5 min followed by PBS washes and centrifugation. The cell pellet was resuspended in DNAse for 1 h at 37 °C followed by addition of 50 µl of perm/wash buffer and anti-BrdU APC antibody. Samples were incubated in the dark at room temperature for 20 min, and then centrifuged in perm/wash buffer at 300 g for 5 min. 7-Amino Actinomycin D (7-AAD) viability dye was added and the suspension was acquired on the BD LSR II flow cytometer (BD Biosciences, USA). Replicate testing was not performed.

4.6. xMAP luminex assays

The Luminex™ Bio-Plex 200 analyzer (Bio-Rad, USA) was used to detect concentrations of proteins found in culture media of untreated cells that were incubated with FCS serum (baseline), serum-free controls, and those present in CM and PCM respectively. Culture media was incubated with beads labelled with antibodies of interest including those against IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, GM-CSF, IFN-γ, TNF-α, IP-10, Eotaxin, Rantes, MCP-1, MCP-2, MCP-3, GRO-α, MIG, MIP-1α, MIP-1β, VEGF, G-CSF, EGF, FGF, SDF-1α, ADAMTS13, GDF-15, lipocalin-2, MPO, myoglobin, sICAM-1, sVCAM-1, and P-selectin in a 96-well plate. Standard controls and samples were assayed in duplicate for all analytes. Unbound proteins were removed with wash buffer, and streptavidin-phycoerythrin (PE) was added to each well to detect antigen-antibody complexes. After incubation, wash buffer was added and samples were acquired on the Luminex ™ Bio-Plex 200 analyzer (Bio-Rad, USA) [1].

4.7. Data analysis

Flow cytometric data was analyzed by importing .fcs files into FlowJo™ v 10.8 software (BD Biosciences, USA). Gating was applied to untreated controls, with debris excluded as described [8]. For cell viability assessments, discrete cell populations were gated namely live (Annexin V- PI-), early apoptotic (Annexin *V*+ PI-), late apoptotic (Annexin *V*+ PI+), and dead cells (Annexin V- PI+). The same gates were applied to all samples. For cell cycle analysis, discrete populations were gated including G_0/G_1, S phase, G_2/M, and apoptotic cells. Luminex data were analyzed using Bio-Plex Manager v. 5.0 software (Bio-Rad, USA) from a 5-prime logistic standard curve.

4.8. Statistical analysis

Descriptive statistics including mean percentages or concentration, standard deviations, and confidence intervals (CI) were determined where relevant, and one- and two-way analysis of variance (ANOVA) tests and post-hoc Dunnett's tests were used for multiple comparisons. All analyses were performed using GraphPad Prism for Windows v 9.4 or 9.5 (California, USA) and are included in the linked database [2].

Limitations

For cell cycle analysis of the WHCO6 line, the significance of these results were not statistically assessed due to limited test replicates.

Ethics Statement

This study was conducted on well-established cancer cell lines and was approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (approval M120205).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Dataset of cell viability and secreted analytes tumour cell lines treated with low pH and conditioned medium (Original data) (Mendeley Data).

CRediT Author Statement

Catherine M. Worsley: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Writing – original draft, Writing – review & editing; **Rob B. Veale:** Resources, Supervision, Writing – review & editing; **Elizabeth S. Mayne:** Resources, Supervision, Writing – review & editing.

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