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Sustainable healthcare: A future for all

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Establishment and characterisation of a novel tumour cell line model of the bladder – the QB1s

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Background and clinical case study

Bladder cancer (BC) is the 10th most common malignancy worldwide[1]. Given the large disparity in clinical responses, it is presently challenging to predict whether individual patients will respond to specific therapies. Currently, there is a need to develop personalised models of BC to provide a platform for investigating patient-specific drug responses. Here, we present **the Queensland Bladder-1 (QB1) cell line**; a novel patient-derived cell line encompassing the genomic and phenotypic characteristics of bladder cancer (BC), a lethal urological malignancy which is hampered by a lack of relevant *in vitro* models.

Donor patient clinical history:

- 71-year-old male
- Presented with macrohaematuria

- High grade bladder cancer with squamous differentiation
- pT3bN3

Molecular profiling classifies the QB1s of basal urothelial origin in 2D and 3D culture

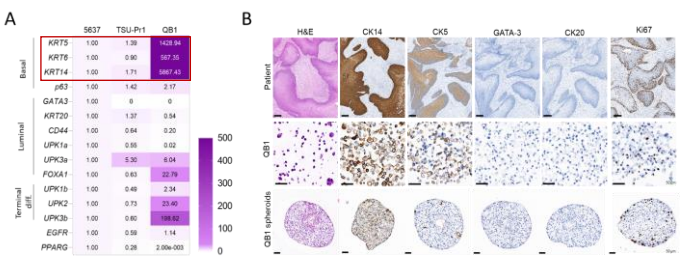

Figure 2. Gene expression and immunohistochemical analyses demonstrate a urothelial origin, basal cell phenotype of the QB1s. **(A)** When compared to the basal cell line, 5637 and luminal TSU-Pr1 cells, the QB1s demonstrated increased expression of basal high molecular weight keratin gene markers, *KRT5*, *KRT6*, *KRT14* and *p63*. In comparison, a luminal gene set consisting of *GATA3*, *KRT20*, *CD44*, *UPK1a* were reduced when compared to the 5637 and TSU-Pr1 BC cell lines. **(B)** The QB1s maintain a consistent phenotype when comparing the parental patient tumour, single cells and QB1 3D spheroids.

Genetic characterization of the QB1s using whole exome sequencing (WES)

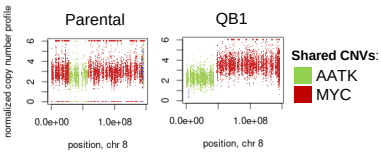

Figure 3. CNVs exclusive to Chr8 in patient tissue and QB1 cell line. green is normal copy number, red is duplication, blue is deletion. Red box indicates amplified 41,850 bp region of chromosome 8q in the QB1 cell line.

The QB1s are highly dependent on glucose as their energy source

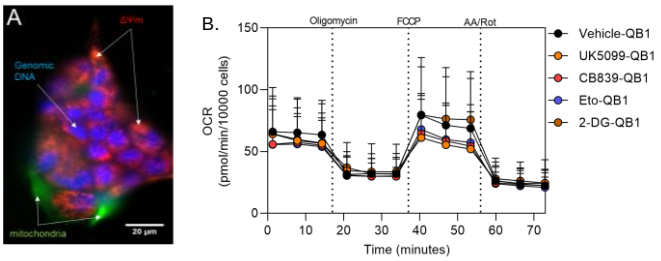

Figure 4. The QB1s have a distinct metabolic phenotype. **(A)** Immunofluorescent staining for mitochondria (green) and the mitochondrial membrane potential (ΔΨm; red) in basal QB1 cells. Nuclei indicated with hoescht staining (blue). **(B)** Profiling the bioenergetic profile of the QB1s in the Seahorse XFe96 showed that the QB1s are highly dependent on glucose, demonstrated by a loss of OXPHOS following blockade of UK5099 (72% loss in spare respiratory capacity versus 60% and 35% in the 5637 and TSU-Pr1, respectively).

Derivation of the novel cell line – the QB1s - following surgical excision and selective culture

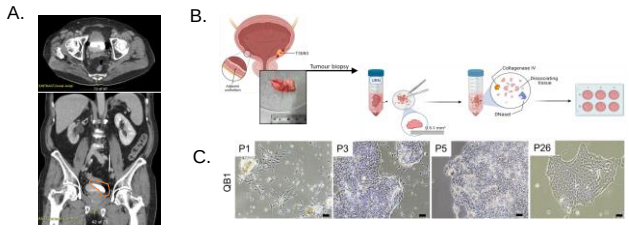

Figure 1. The QB1 cell line favors a distinct morphology. **(A)** Computed tomography image showing patient tumour. **(B)** Radical cystectomy tissue from a T3 urothelial cancer with features of squamous differentiation. schematic showing the manual dissection of whole tissue and enzymatic digestion before plating in a 6-well cell culture dish. **(C)** Representative phase contrast morphology of QB1 cell line at initial passage, passage 1, 3, 5 and 24. Below, short tandem repeat (STR) profiling showed 15 tetranucleotide repeat loci and Amelogen sex-determining markers distributed across passage 10 and 26 of the QB1s. The QB1s did not match for existing deposited cell lines, indicating they represent a distinct and unique entity. Red arrow indicates passage used for WES studies.

Table 1. STR profiling of passage 7, 10 and 26 of the QB1 cell line.

Designation	Passage	AMEL	D5S818	D13S317	D7S820	D16S539	vWA	TH01	TPOX	CSF1PO
QB1	7	X, Y	7, 11	10	6, 7	8, 9	12, 13	6, 7	7, 8	11
QB1	10	X, Y	7, 11, 12	7, 11, 12	8, 11	9, 11	18	7, 9.3	8	11
QB1	26	X, Y	7, 11, 12	12, 15	8, 11	9, 11	18	7, 9.3	8	11

QB1, Queensland Bladder Cancer 1; AMEL, Amelogenin

The QB1s demonstrates a biphasic growth pattern in culture and readily form spheroids

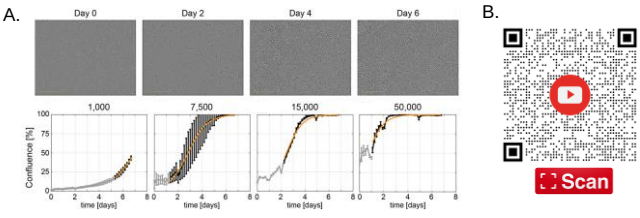

Figure 3. Biphasic population growth in two-dimensional tissue growth conditions with the QB1 bladder cancer cell line. **(A).** Experimental phase contrast images of the QB1s (P22, 5x10[3] cells/ well) at t = 0 hr to 6 days of growth in complete culture medium. Biphasic changepoint indicated in various densities of initial cell seeding by black experimental data point. **(B)** 3D spheroid formation of QB1s in round-bottom ultra low attachment conditions over 24 hr. Scan for timelapse video over time period.

The QB1s are sensitive to standard of care chemotherapy agents

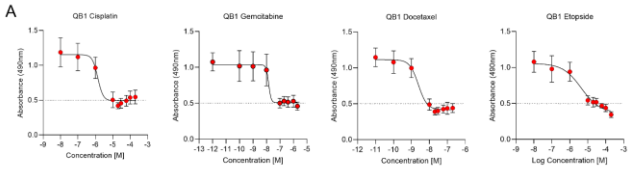

Table 2. Comparison of drug IC_{50} values for QB1 cells in 2D

Cell line	Cisplatin (µM)	Gemcitabine (nM)	Docetaxel (nM)	Etoposide (µM)
QB1	2.722	33.65	5.27	18.11

Figure 5. IC_{50} dose response curves of QB1s over 72 h treatment **(A)** with cisplatin, gemcitabine, docetaxel and etoposide. Estimated IC_{50} values calculated by non-linear regression following normalized to untreated controls (n=3). Error bars represent standard deviation. All experiments were performed with at least three different passages of the QB1s.

Conclusions

- The QB1s are a novel – and unique - basal BC cell line that was derived from a high grade, MIBC.
- Harbour amplifications in Chr8 (including MYC) and deletions in tumour suppressive cyclin-dependent kinase inhibitors and AATK
- The QB1s grow in serum-free conditions, are highly dependent on glucose to fuel mitochondrial activity and readily form viable spheroids
- The QB1s are sensitive to bladder cancer standard of care cytotoxic therapies

Thus, this will be a useful *in vitro* model system for tumour biology and the development of future treatments for BC.

References/ Acknowledgements

1. Australian Institute of Health and Welfare, 2019; Available at: https://canceraustralia.gov.au/affected-cancer/cancer-types/bladder-cancer/bladder-cancer-statistics.

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The QB1s have been profiled by PARF – scan QR code for release: Answers for bladder cancer