Investigation of *in vitro* prostate-specific membrane antigen expression in MCF-7 and MDA-MB-231 breast tumour cells

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BACKGROUND

Breast and prostate cancer mutually represent the most commonly occurring malignancies worldwide in women and men, respectively.[1] The mutative state, recurrence capacity, resistance to conventional chemotherapy, low success rate of surgery and risks associated with radiotherapy confound the management of both these malignancies. There are several similarities between breast and prostate cancer, like growth hormone dependence and similar chemotherapeutic interventions.[2] Radiopharmaceutical based therapy targeting the prostate-specific membrane antigen (PSMA) is proving to be an effective theranostics intervention for prostate cancer. Clinical positron emission tomography (PET) scans have located anti-PMSA binding sites in breast cancer *in vivo*.[3] This indicates possible non-prostatic expression of PSMA, initiating research focussed on understanding the cellular kinetics, protein expression profiles and genomic variation of breast cancer. This may lead to discovery of underlying biomarkers such as PSMA or similar surface molecules that can aid in development of more selective, effective and safe diagnostic and anti-cancer therapeutic alternatives, which are financially considerate within the African demographic.

AIM

The aim of this study was to evaluate PSMA receptor expression in the two breast cancer cell lines MCF-7 and MDA-MB-231 compared to a positive control prostate tissue cell line (LNCaP).

METHODS

Cells

MCF-7	MDA-MB-231		LNCaP
Experimental cell lines			Positive control

PSMA

Identification	**Localisation**	**Quantification**
- Flow cytometry	- Confocal microscopy	- ELISA
LNCaP	LNCaP	LNCaP
MCF-7	MCF-7	MCF-7
MDA-MB-231	MDA-MB-231	MDA-MB-231

- Confocal microscopy - Tissue

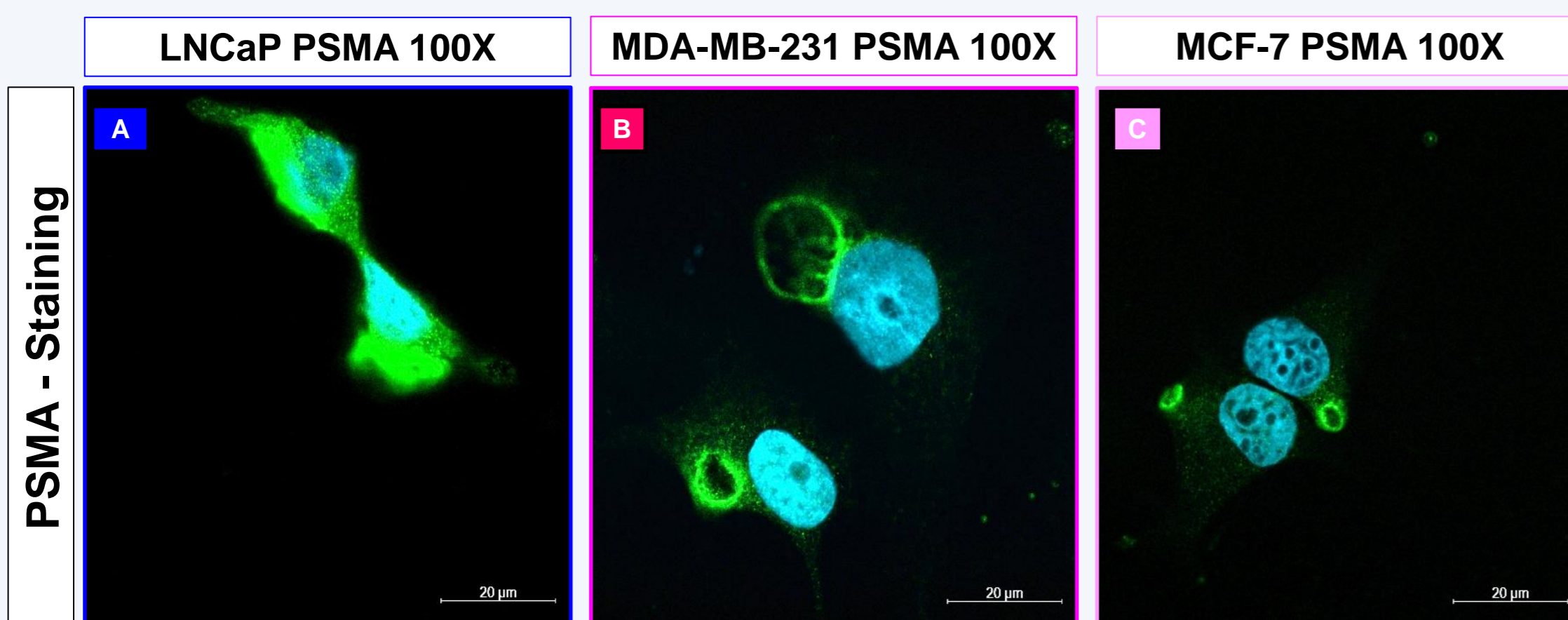| Luminal - A	HER-2

RESULTS

Figure 1. PSMA Stained confocal microscopy images of LNCaP (A), MDA-MB-231 (B), and MCF-7 (C) monolayer cells. (Green = FITC; Turquoise = DAPI)
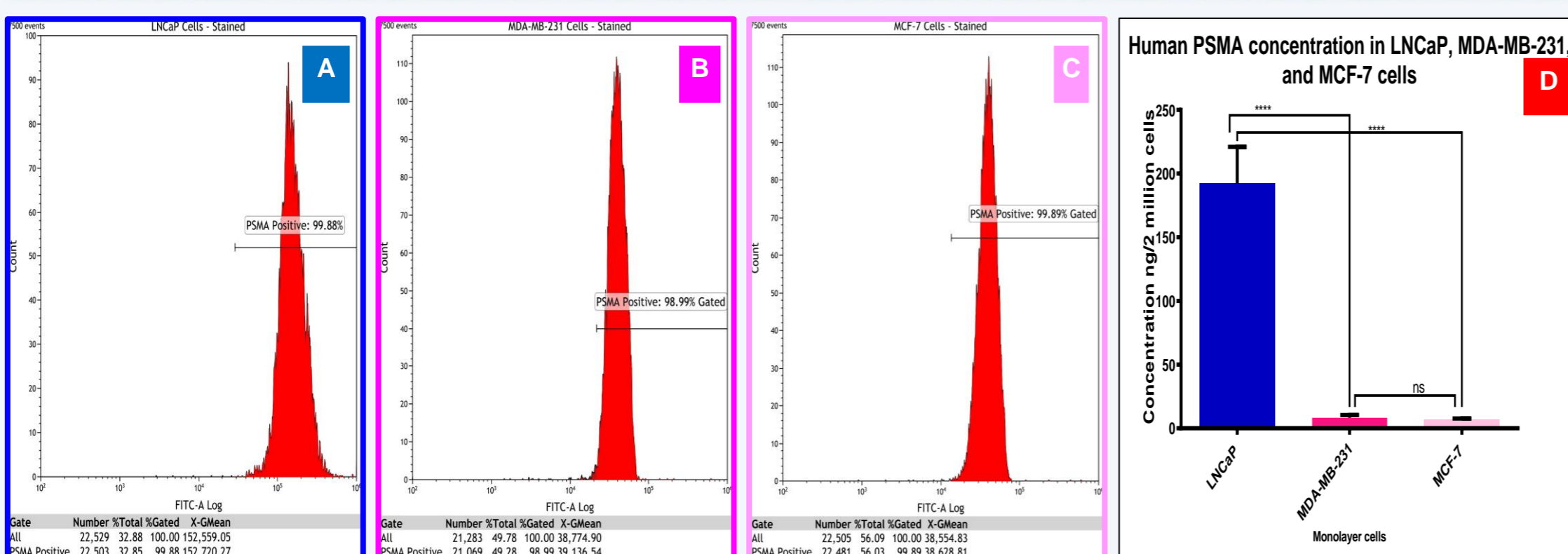

Figure 2. Univariate flow cytometry histograms of PSMA stained LNCaP (A), MDA-MB-231 (B), and MCF-7 (C) cells. Protein quantification of all cells utilising ELISA (D).
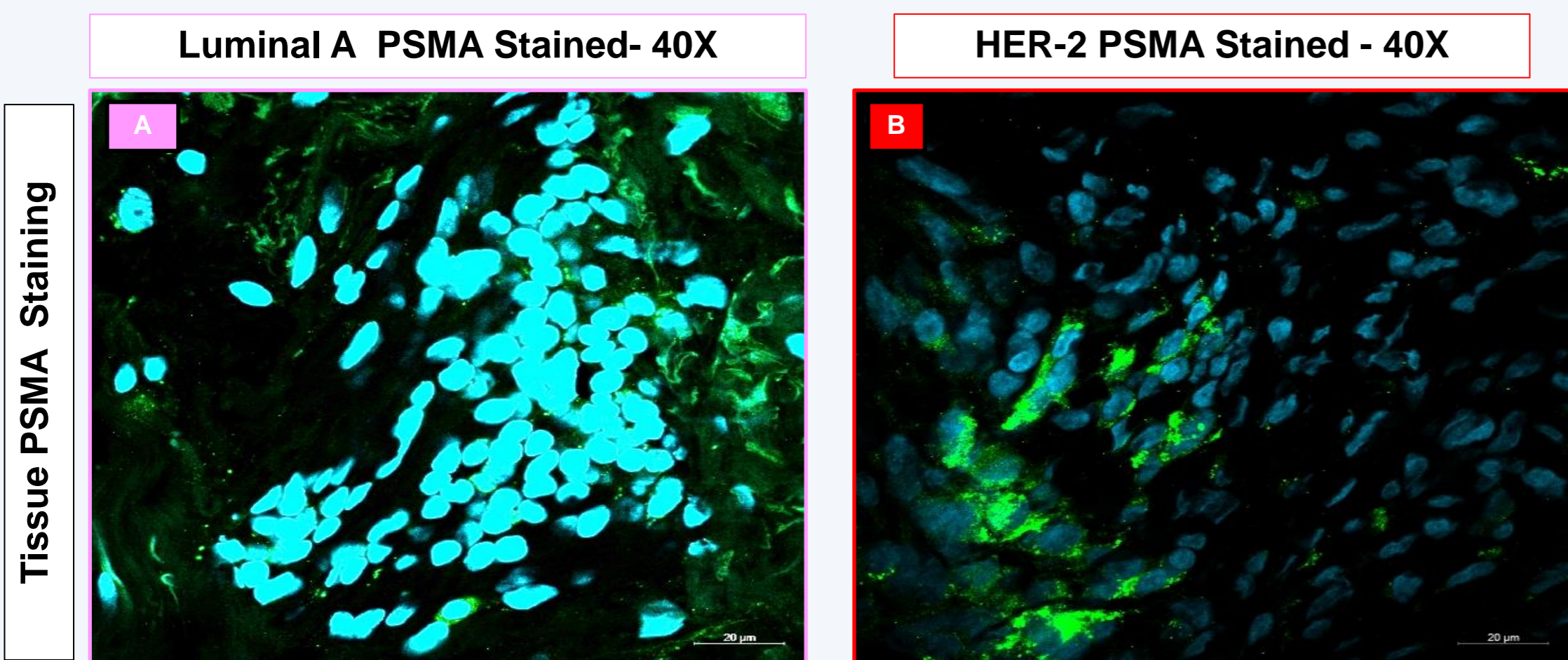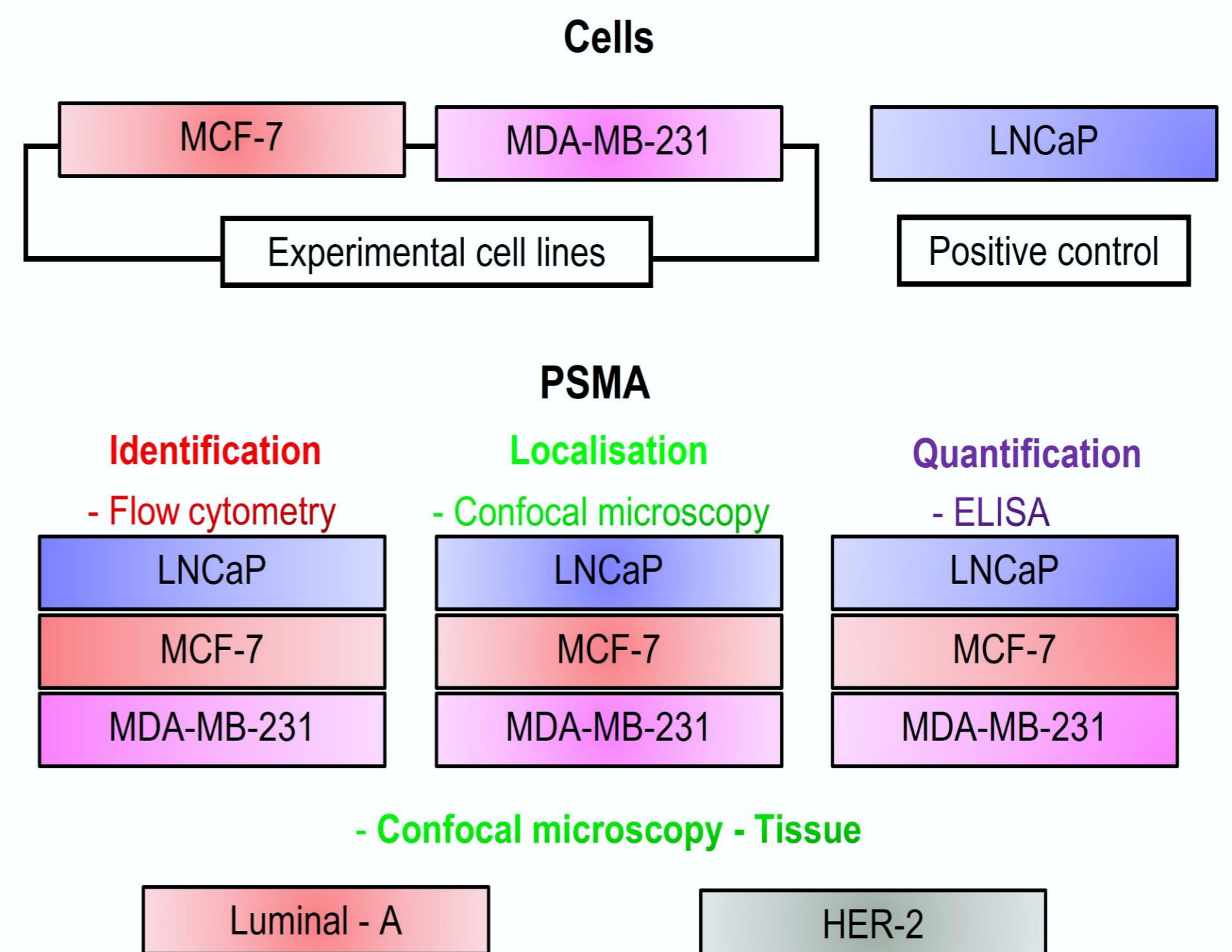

Figure 3. Delipidated PSMA stained Luminal A (A) and HER-2 (B) breast cancer tissue slices visualised utilizing confocal microscopy. (Green = FITC; Turquoise = DAPI)

DISCUSSION

Despite not having a prostate origin, findings from three complementary techniques demonstrate positive PSMA identification, localisation, and quantification in both breast cancer cell lines (Figure 1 and Figure 2). In the LNCaP cells, PSMA was localised on the cell membrane and dispersed within the cytosol (Figure 1A). The MDA-MB-231 and MCF-7 cells exhibited a differential expression pattern of PSMA. These cells showed diffuse cytosolic accumulation with intense circular regions of accumulation apparently bordering the cell membrane and the cell nucleus (Figure 1B and 1C). Quantitatively, PSMA was higher in the metastatic MDA-MB-231 cell line than in the MCF-7 cell line but still had significantly lower concentrations (~100 fold) to those found in LNCaP's (Figure 2D). Luminal A, which are analogous to MCF-7 cells and HER-2 cryotome sections of tumour biopsies from breast cancer patients equally exhibited detectable PSMA expression showing continuity between simple cell cultures and heterogenous breast tissue samples (Figure 3). Receptor internalisation, vesicle transportation to membrane, receptor recycling or mitochondrial expression are all plausible reasons that can be tabled to explain the intracellular accumulation of PSMA in the breast cancer cells. However, further studies need to be undertaken to pinpoint PSMA's exact location and possibly, function in breast cancer. The identification of PSMA in triple-negative MDA-MB-231 cells may represent a previously unrealised paradigm in triple-negative breast cancer treatment.

CONCLUSION

Through this study, PSMA expression in non-prostate cancer types, particularly in breast cancer was positively identified. Continuity was demonstrated between literature and data collected from this study when considering PSMA membrane expression and receptor abundance in LNCaP cells. In contrast the expression pattern localised in MCF-7 and MDA-MB-231 cells was in some cases observed for the first time. It may be plausible that the shown evidence of PSMA can be used to target breast cancer and as a result the success being realised in prostate cancer theranostics through PSMA targeting, may conceivably be realised in other carcinomas, particularly breast carcinoma.

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