Differential expression of the protein phosphatase 2 (PP2A) inhibitors following PP2A activation by FTY720 in specific breast cancer cell lines

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Background

Triple negative breast cancer (TNBC) patients derive little benefit from target-specific therapies due to lack of the favourable prognostic targets oestrogen/progesterone receptor (ER/PR) and human epidermal growth factor receptor type 2 (HER2). Data from the cBioPortal and The Cancer Genome Atlas (TCGA) demonstrate that PP2A function is likely to be reduced in up to 60% of basal breast tumours. PP2A is vital tumour suppressor that regulates cell proliferation and cell survival. Tumours exhibit either homozygous deletion or underexpression of PP2A, but also overexpression of PP2A inhibitors namely CIP2A, IGBP1, ANP32A, SET and/or SETBP1.

In this study we assessed the effect of FTY720, an activator of PP2A, on the viability of breast cancer cell lines. The viability is then correlated with the cellular localisation of the PP2A inhibitory subunits and the mTOR targets, phosphorylated S6K (pS6K) and phosphorylated AKT (pAKT).

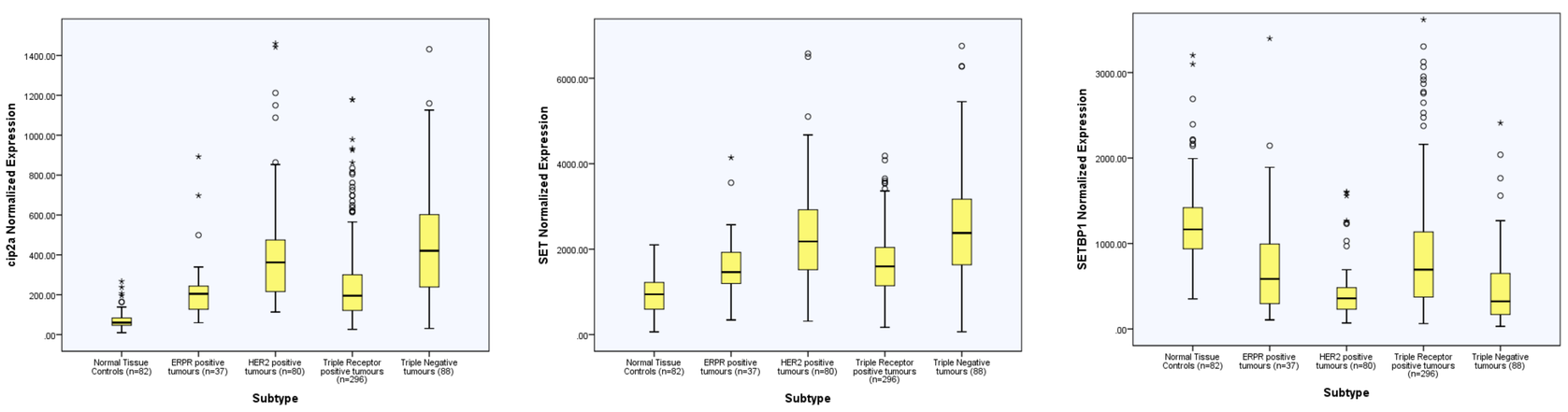

Figure 1: Box plots representing RNASeq V2 expression data from TCGA database across different breast subtypes. Comparison of normalised expression of CIP2A (**A**), SET (**B**) and SETBP1 (**C**) was performed against a set of normal tissue controls available in the dataset, using One-Way Analysis of Variance (ANOVA). CIP2A was significantly upregulated in the HER2 positive patients and the TNBC patients ($p<0.001$). Of interest, although SET (**B**) was significantly upregulated in all subtypes, SETBP1 expression was downregulated, hence compromising stability of SET.

Methodology & Results

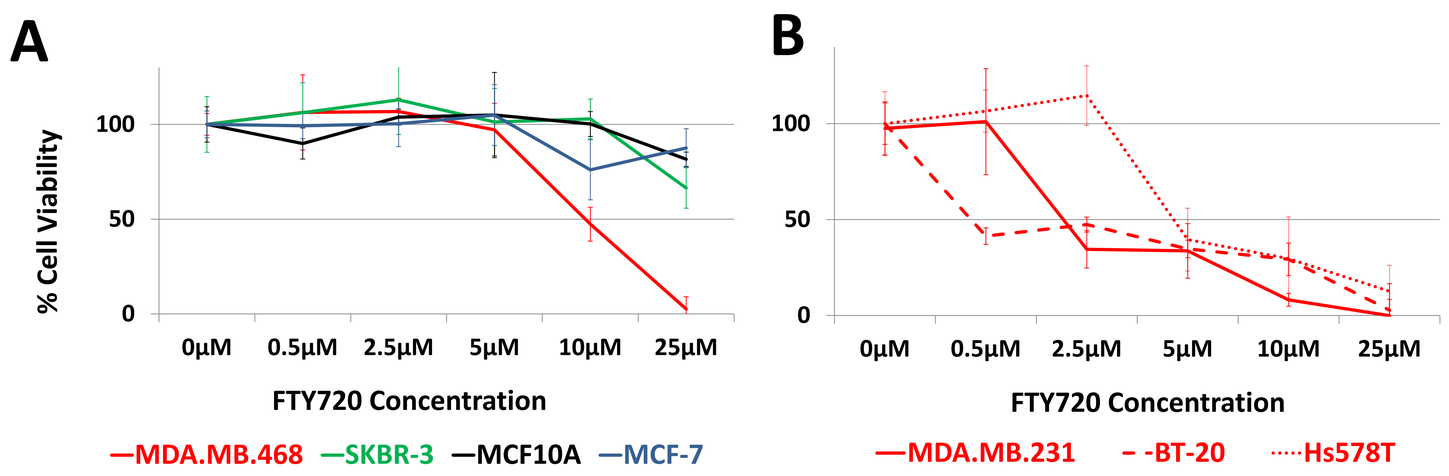

Figure 2: A Sensitivity of representative breast cancer cell lines to FTY720. MDA.MB.468 (**Red**) represents a TNBC subtype cell line with suppressed viability at high dose FTY720, SKBR-3 (**Green**) represents the HER2 positive subtype, MCF-7 (**Blue**) is the ERPR positive cell line and MCF10A (**Black**) as a normal breast epithelium cell line.
B Viability of sensitive cell lines across the FTY720 concentration gradient. 3 out of 6 TNBCs cell lines were sensitive at varying concentrations of FTY720. The highest non-cytotoxic effective dose of 5μM was selected for assessing the localisation of PP2A inhibitors and targets.

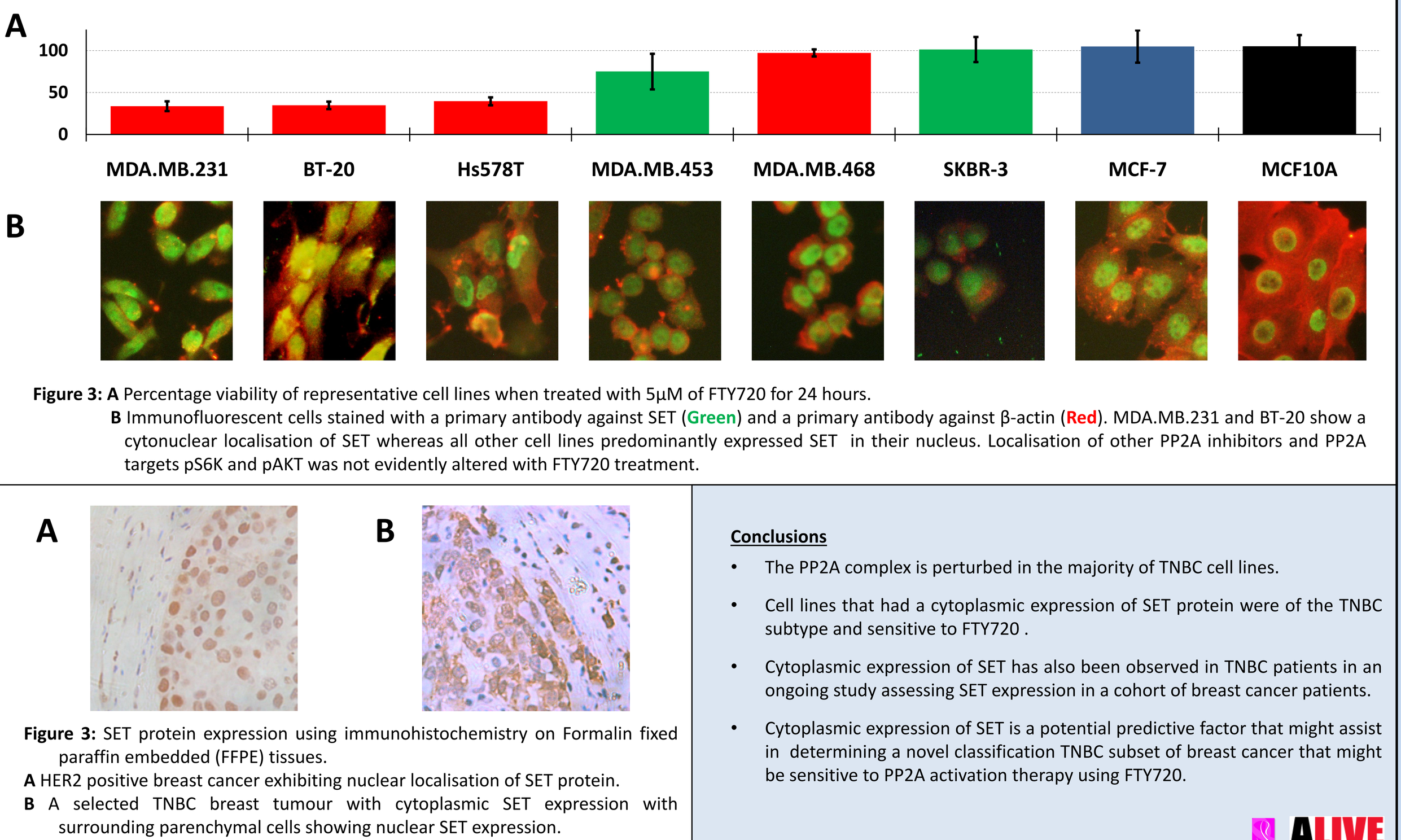

Figure 3: A Percentage viability of representative cell lines when treated with 5μM of FTY720 for 24 hours.
B Immunofluorescent cells stained with a primary antibody against SET (**Green**) and a primary antibody against β-actin (**Red**). MDA.MB.231 and BT-20 show a cytonuclear localisation of SET whereas all other cell lines predominantly expressed SET in their nucleus. Localisation of other PP2A inhibitors and PP2A targets pS6K and pAKT was not evidently altered with FTY720 treatment.

Figure 3: SET protein expression using immunohistochemistry on Formalin fixed paraffin embedded (FFPE) tissues.
A HER2 positive breast cancer exhibiting nuclear localisation of SET protein.
B A selected TNBC breast tumour with cytoplasmic SET expression with surrounding parenchymal cells showing nuclear SET expression.

Conclusions

- The PP2A complex is perturbed in the majority of TNBC cell lines.
- Cell lines that had a cytoplasmic expression of SET protein were of the TNBC subtype and sensitive to FTY720 .
- Cytoplasmic expression of SET has also been observed in TNBC patients in an ongoing study assessing SET expression in a cohort of breast cancer patients.
- Cytoplasmic expression of SET is a potential predictive factor that might assist in determining a novel classification TNBC subset of breast cancer that might be sensitive to PP2A activation therapy using FTY720.

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