

A NEW ISOFORM OF THE ZNF217 ONCOGENE: DECIPHERING THE FUNCTIONAL IMPACT AND THE PROGNOSTIC VALUE IN BREAST CANCER

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INTRODUCTION

ZNF217 is an oncogene which has a deleterious role in various types of cancers. Our group focuses on ZNF217 role in breast cancer and has reported that it promotes cell proliferation, drug resistance, migration and epithelio-mesenchymal transition [1]. We have also shown that ZNF217 is a new biomarker for poor prognosis associated with shorter relapse free survival (RFS) in breast cancer. In particular, ZNF217 prognostic is more powerful in Estrogen Receptor alpha positive (ER+) breast cancer [2]. Here we find and demonstrate for the first time the existence of a new *ZNF217* isoform (named *ZNF217-A*) in breast tumor samples. We aim at elucidating the impact and the possible role of this new isoform in breast cancer cells regarding on cellular proliferation and on chemotherapy resistance in comparison with the impact of *ZNF217* wild type (*ZNF217-WT*). The prognostic value of *ZNF217-WT* and *ZNF217-A* in a set of 113 primary breast tumor samples is also investigated by qRT-PCR, focusing on ER+ and ER- subclasses.

METHODS

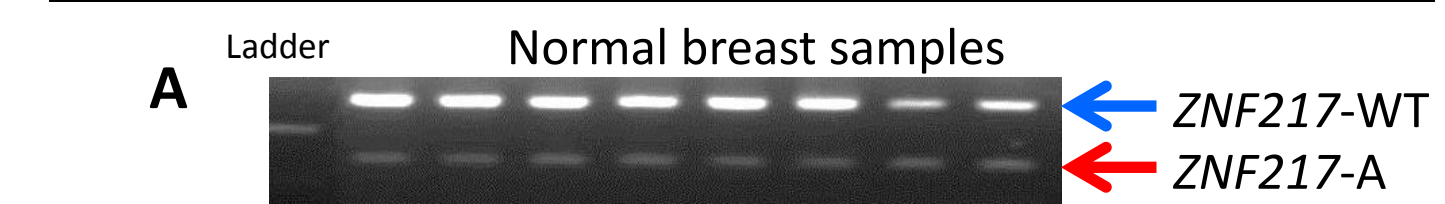
- Design of highly specific qRT-PCR primer sets targeting each isoform of ZNF217
- Establishment of cell clones overexpressing either ZNF217-WT or ZNF217-A, by transfection and cellular cloning
- Comparison of phenotypic characteristics developed by MDA-MB-231 cells following ZNF217-WT or ZNF217-A overexpression regarding proliferation and chemoresistance
- Exploration of ZNF217-WT and ZNF217-A prognostic value in breast cancer by qRT-PCR and univariate statistical analysis

RESULTS

1. Heterogeneous pattern of ZNF217-A isoform expression in breast tumor samples

Classical PCR

Homogeneous pattern of expression in normal breast



Heterogeneous pattern of expression in breast tumors

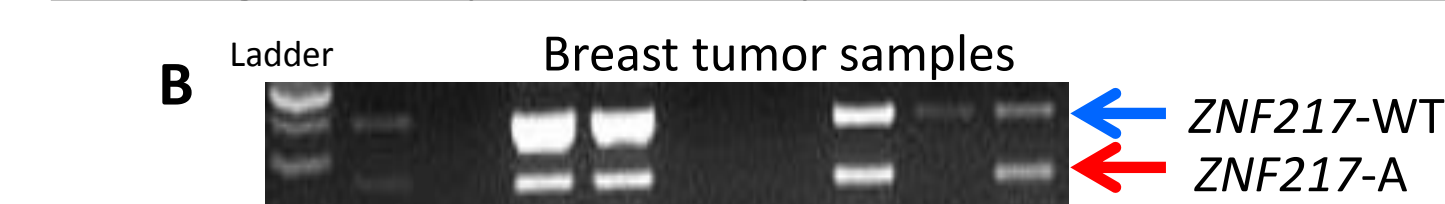


Figure 1 : Primers were designed to amplify both *ZNF217-WT* and *ZNF217-A* isoforms. **A** : Homogenous pattern of *ZNF217-WT* and *ZNF217-A* expression in normal breast samples. **B** : Heterogeneous pattern of *ZNF217-WT* and *ZNF217-A* in breast tumor samples

2. Validation of specific qRT-PCR primer sets for ZNF217 isoforms discrimination

Quantitative measurement of ZNF217 isoforms by qRT-PCR in an isoforms mix context

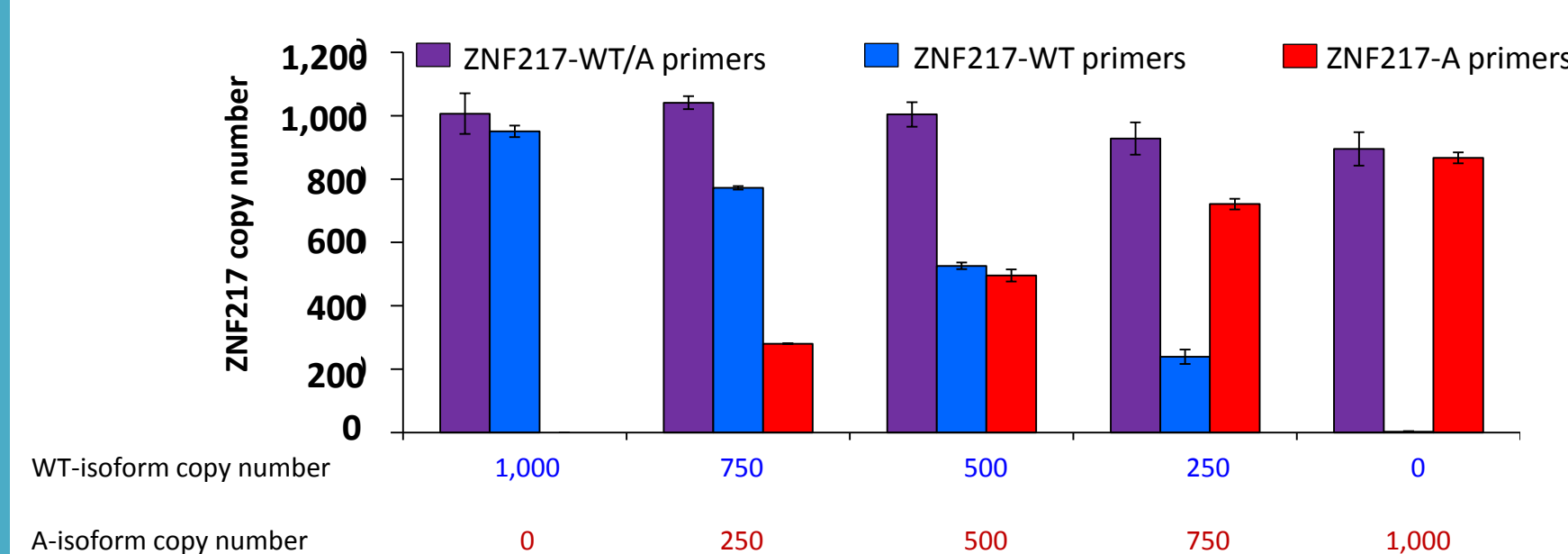
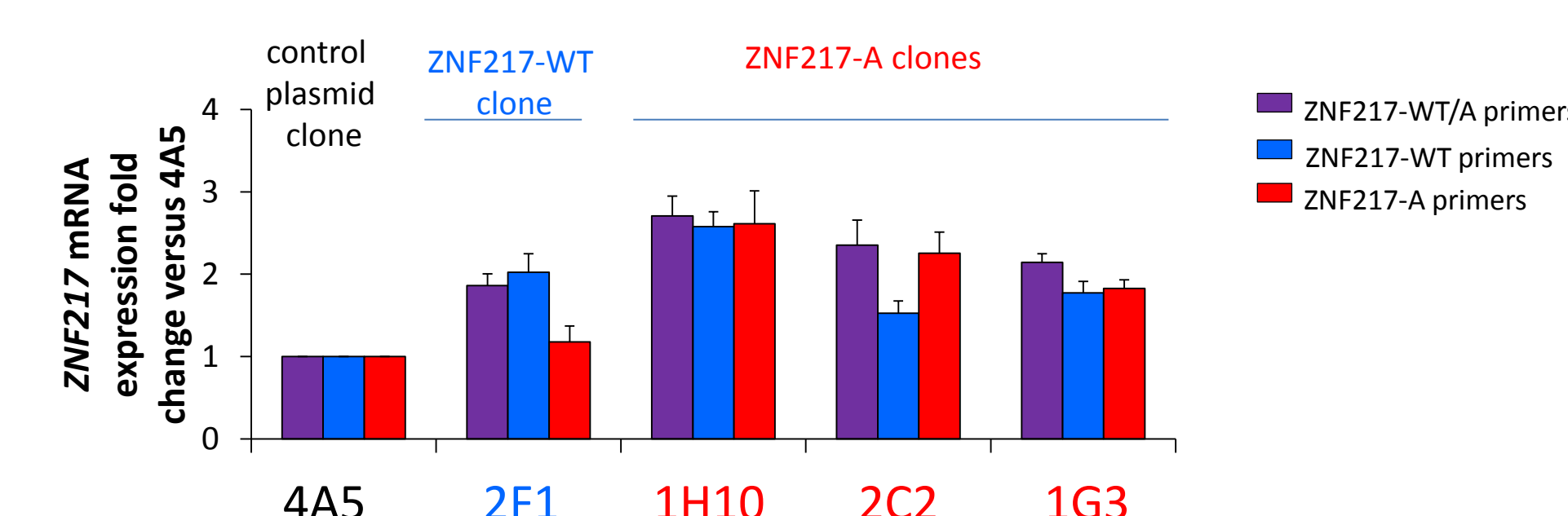


Figure 2 : DNA mixtures containing 1,000 copies of pcDNA6-ZNF217-WT or pcDNA6-ZNF217-A or different ratios of pcDNA6-ZNF217-WT /A plasmids were used as template for ZNF217-isoforms primers.

3. Establishment and validation of ZNF217-WT and ZNF217-A stable transfectants (MDA-MB-231 cells)

A qRT-PCR measurement of ZNF217-isoforms expression



B Western blot

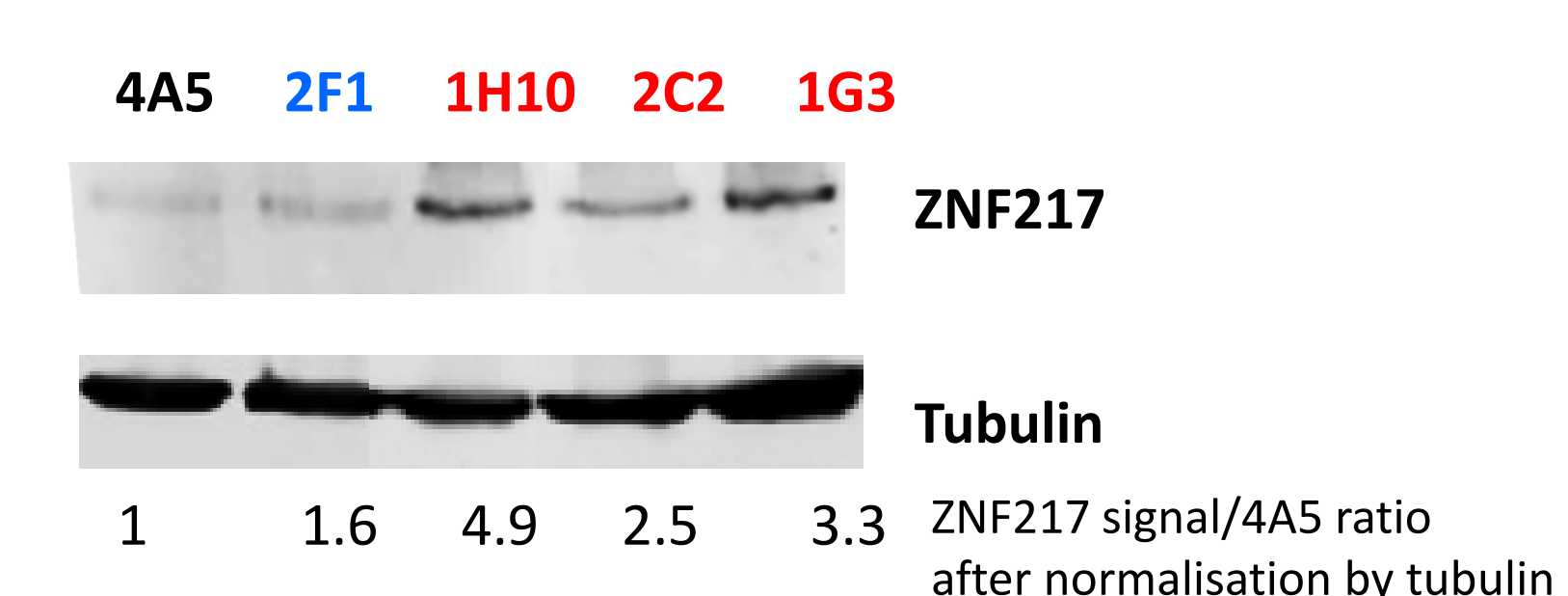
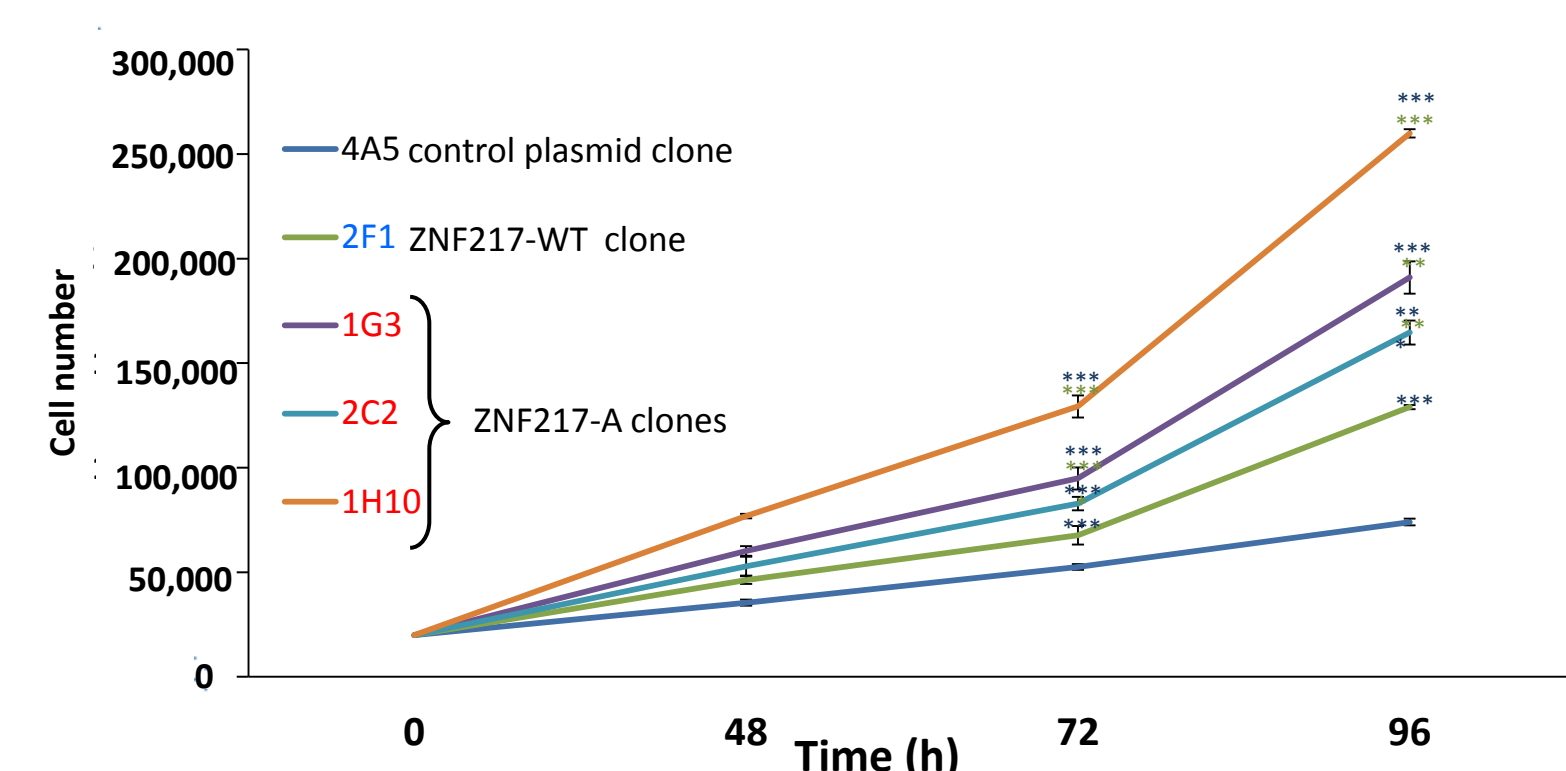


Figure 3 : MDA-MB-231 cells were transfected with pcDNA6 plasmid as a negative control, with ZNF217-WT or ZNF217-A pcDNA6 expressing plasmid. Cell cloning was performed using the limit dilution method. 4A5 is a cell clone obtained after transfection with pcDNA6 plasmid. 2F1 is a cell clone obtained after transfection with pcDNA6-ZNF217-WT plasmid. 1H10, 2C2 and 1G3 are cell clones obtained after transfection with pcDNA6-ZNF217-A plasmid. **A**. qRT-PCR analysis of ZNF217-WT and ZNF217-A expression in each cell clone. Results are mean \pm Standard deviation (SD). **B**. Western blot analysis of ZNF217 protein expression.

4. ZNF217-A isoform overexpression confers a worse phenotype than ZNF217-WT regarding cell proliferation and paclitaxel resistance in MDA-MB-231 cell line

A ZNF217-A leads to a higher increase in cell proliferation than ZNF217-WT



B ZNF217-A leads to a higher increase in paclitaxel resistance than ZNF217-WT

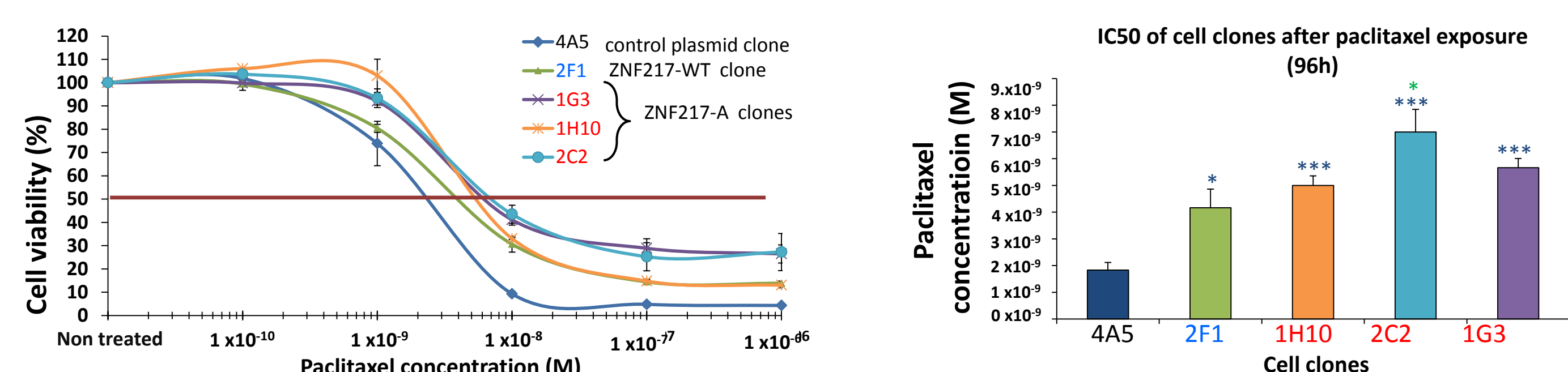


Figure 4 : **A**. Cells were seeded at 20,000 cells/ well in a 24-well culture plate. Cell proliferation was measured at 48h, 72h and 96h after seeding. Results represent one experiment performed in triplicate and representative of three independent experiments. **B**. Cells were treated for 96h with paclitaxel and cell viability was assessed by cytotoxicity assays (MTS). Results are mean \pm SD. * : $p < 0,05$; ** : $p < 0,01$; *** : $p < 0,001$ (Student t test). Blue stars represent the Student t test value of clones versus 4A5, green stars represent the Student t test value of clones versus 2F1.

5. High ZNF217-A isoform mRNA levels are associated with poor prognosis in ER+ but not in ER- breast cancers

A As expected, supporting Nguyen *et al.*, 2014, the prognostic value of ZNF217 is present in ER+ breast tumors but not in ER- breast tumors

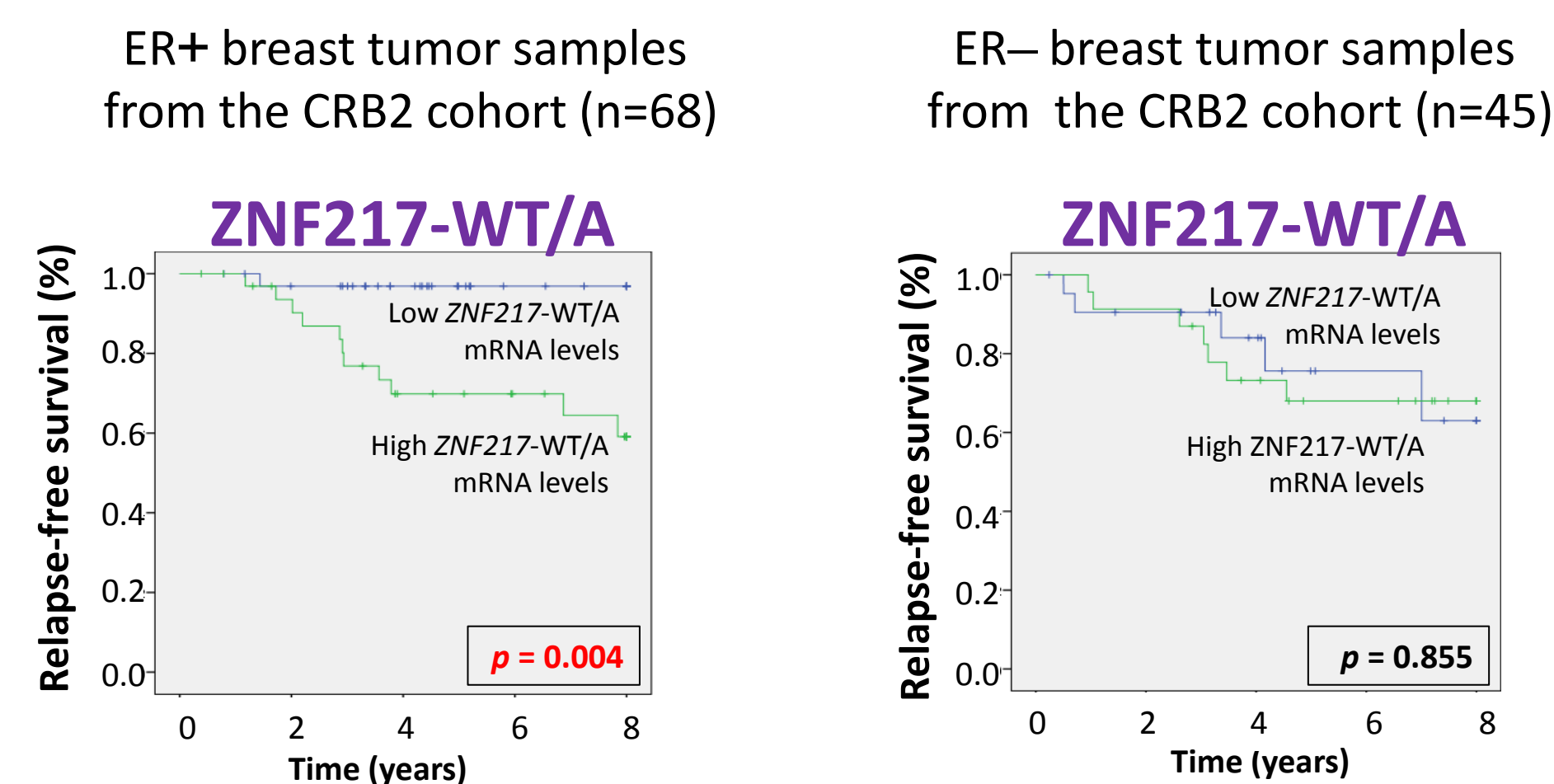
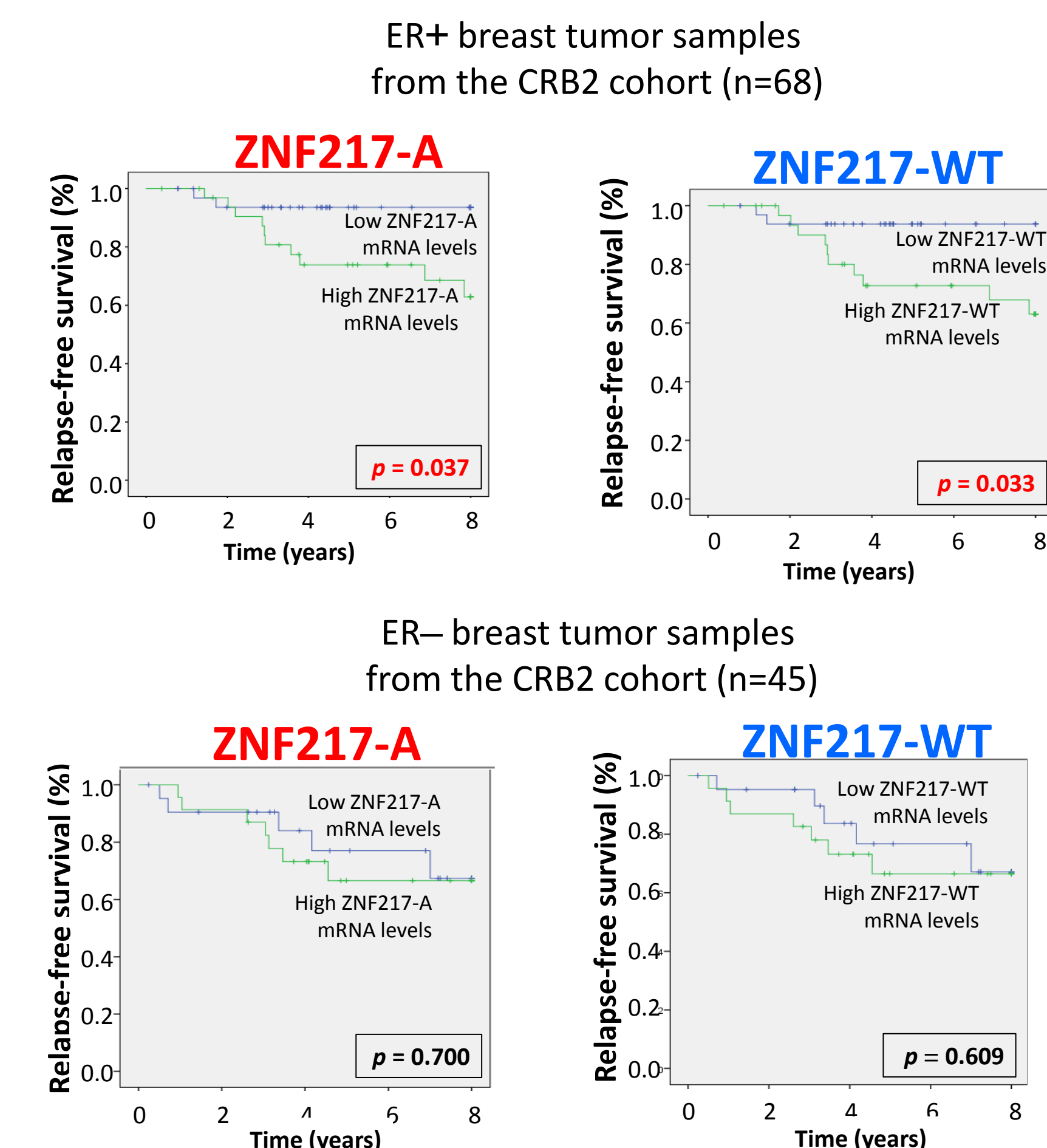


Figure 5 : **A**. Prognostic value analysis of *ZNF217-WT/A* mRNA expression in primary ER+ and ER- breast tumor samples from the CRB2 cohort determined using univariate analysis. **B**. Prognostic value analysis of *ZNF217-A* and *ZNF217-WT* mRNA expression in ER+ and in ER- breast tumor samples from the CRB2 cohort [2] determined using univariate analysis. IBM SPSS software (IBM, Armonk, NY, USA) was used for all statistical analyses in which the prognostic value of each *ZNF217* mRNA was analyzed. The data are divided, at the value representing the median level of expression of a particular *ZNF217* mRNA, into two groups with either high or low expression.

B Both ZNF217-A and ZNF217-WT isoforms are significantly associated with shorter RFS in ER+ breast tumors but not in ER- breast tumors



CONCLUSIONS

- *ZNF217-A* is expressed with a heterogeneous pattern in breast tumors
- *ZNF217-A* demonstrates a phenotypic impact by increasing cell proliferation and seems to lead to increased paclitaxel resistance with a greater magnitude compared to *ZNF217-WT* in breast cancer cells
- *ZNF217-WT/A* mRNA demonstrates the best prognostic significance in ER+ breast tumors
- Both *ZNF217-A* and *ZNF217-WT* high mRNA expression levels are associated with a shorter RFS in ER+ breast tumors

Conclusion : Altogether, this work highlights the discovery of a new functional *ZNF217* isoform having a deleterious impact on breast cancer