Investigation of drug resistance in breast cancer using genomic analysis

1.ABSTRACT

Breast cancer is the second most common cancer in the world. The main purpose of this study is to investigate the genetic biomarkers of drug resistance in breast cancer. Patient derived explants (PDEs) were used as drug-testing platform. Multi-immunofluorescence (mIF) staining was for measuring the drug response. Sequencing data were generated successfully in 36 samples by whole exome sequencing. *PIK3CA* mutation especially *PIK3CA* H1047R was found to promote the drug resistance in breast cancer. The relationship between breast cancer biomarkers such as tumor mutation burden/mutant-allele tumour heterogeneity and several tumour clinical features were revealed in this study.

2.Aims and objectives

In this study, our overall aim is to use next generation sequencing (NGS) of breast cancer PDEs to identify genetic biomarkers predictive of sensitivity and resistance to standard of care (SoC) chemotherapy.

Objectives are to:

- Analyse WES data derived from breast cancer PDEs,
- Identify genetic lesions that segregate in PDEs that are sensitive and resistance to standard of care SoC
- Assess resistance markers using mIF.

3. MATERIALS AND METHODS

3.1 Ex vivo patient derived explants culture and drug treatment

3.2 Multi-immunofluorescence (mIF) staining and Quantitation of staining with digital slide scanning

Tumour and stroma were distinguished by pan-Cytokeratin. Necrosis and background were distinguished by built-in machine learning algorithms of the sofeware Inform which was trained manually. Each cell was labelled with DAPI for segmentation. Cells with cPARP+ were classified as apoptotic cells. Total cell death response = Fold change value of apoptosis + Fold change value of necrosis = Drug response.

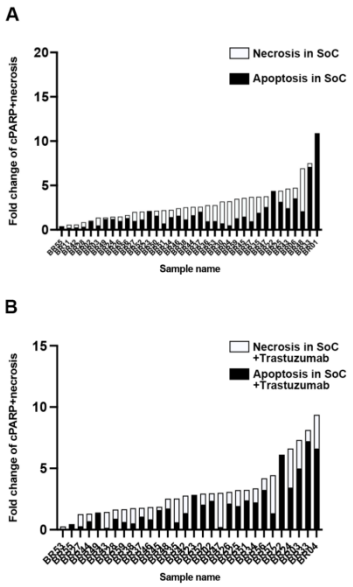
3.3 Whole exome sequencing and genome analysis using R

Novogene standard analysis pipeline was used for data pre-processing. After that, annotated data of somatic SNP, somatic INDEL and somatic CNV were converted into MAF files. R 4.1.0 was used for genome analysis based on the pre-processed sequencing data. A summary of genetic mutations, affected signaling pathways, lollipop plots for amino acid changes of *PIK3CA* mutations, tumour mutation burden (TMB) values and values for mutant-allele tumor heterogeneity (MATH) were generated by Maftools (Mayakonda et al., 2018).

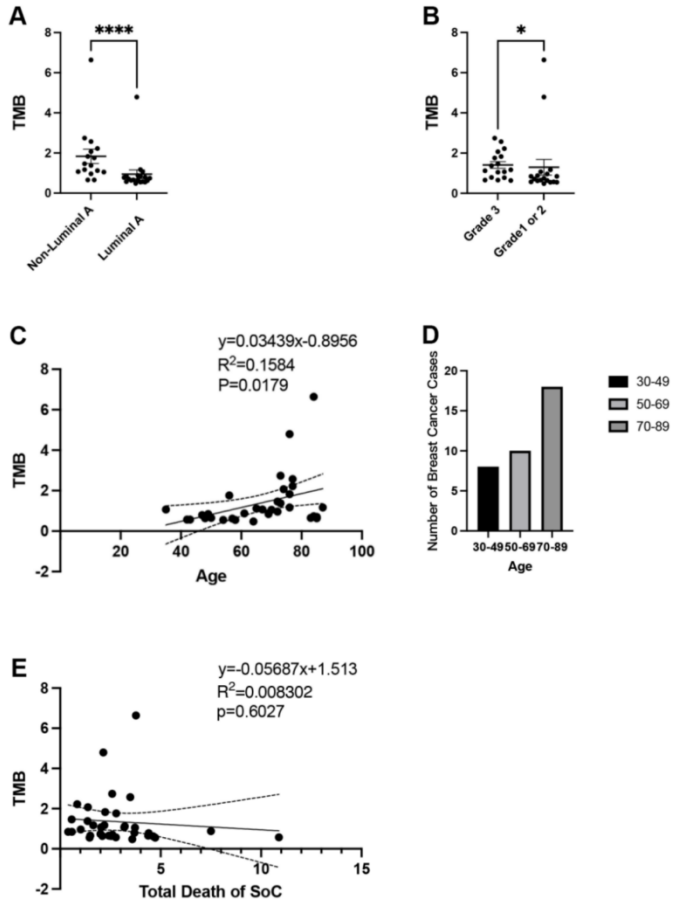
3.4 Analysis of breast cancer datasets of TCGA and METABRIC using R as supporting evidence

4 RESULTS

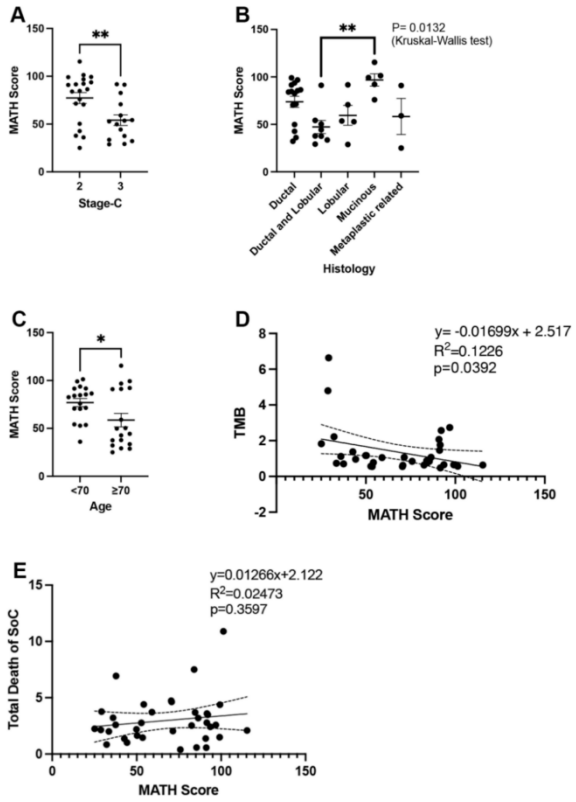
4.1 Tumour drug response in all samples



4.2 Relationship between tumor mutation burden (TMB) and tumour clinical features

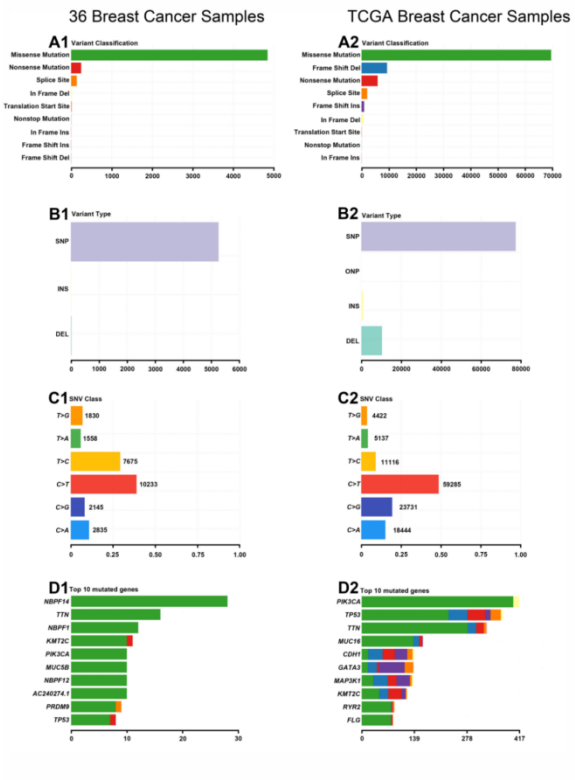


4.3 Relationship between mutant-allele tumour heterogeneity (MATH) and tumour clinical features



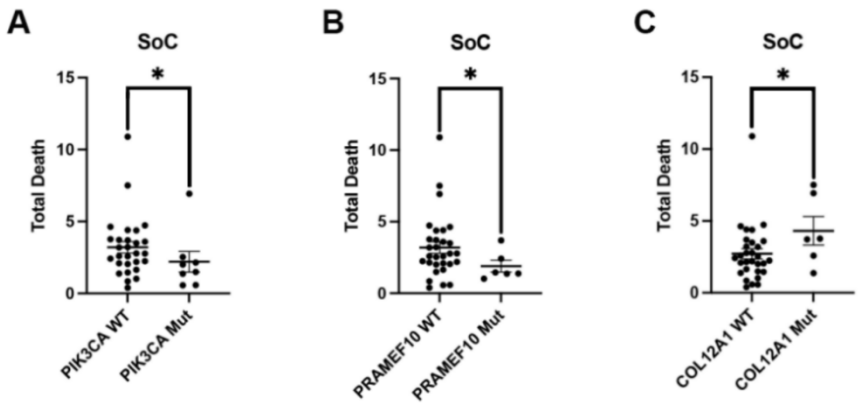
4.4 Summary of genetic mutations in our samples and TCGA datasets of breast cancer samples

PIK3CA is among the top 10 mutated genes

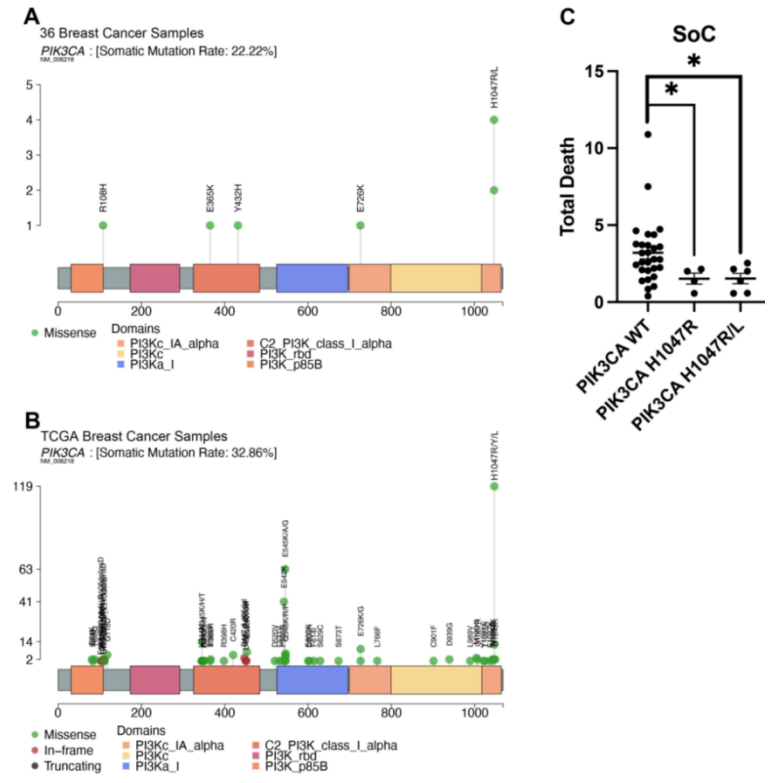


4.5 Genes that are significantly different between wild type and mutation type linked to SoC drug response

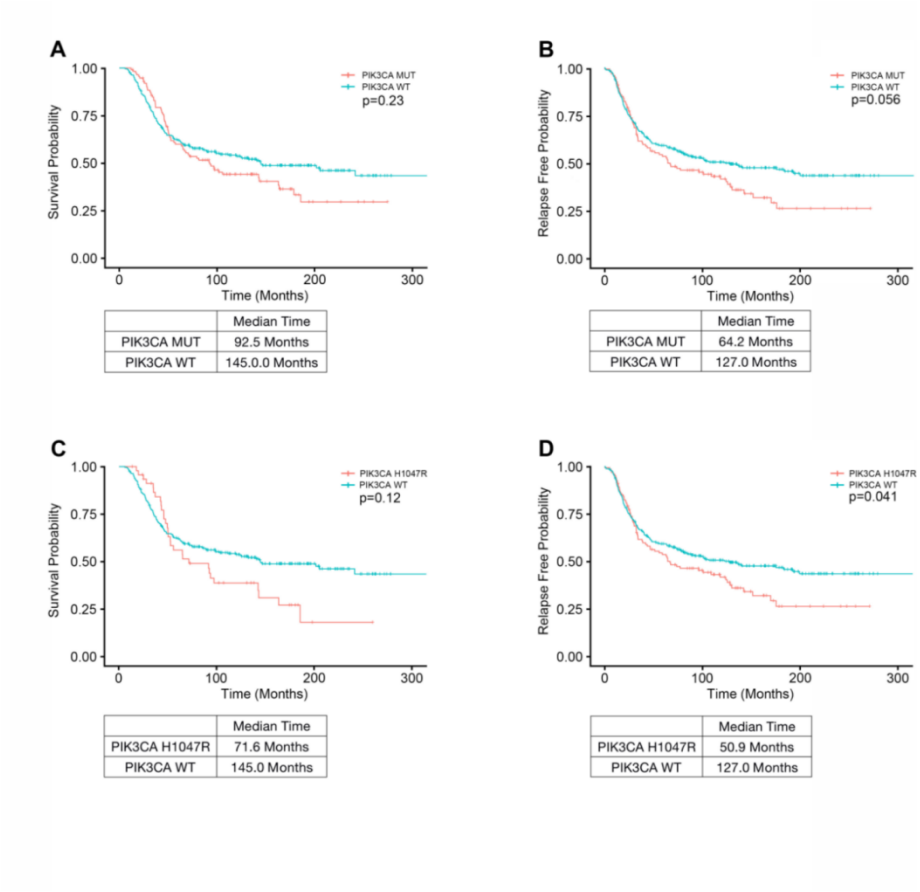
Samples with *PIK3CA* mutation are more resistant to SoC drug treatment.



4.6 H1047R is the most frequent *PIK3CA* mutation in breast cancer and *PIK3CA* H1047R/L mutations promote SoC drug resistance in breast cancer



4.7 *PIK3CA* H1047R is associated with shorter relapse free time in patients receiving chemotherapy based on METABRIC dataset analysis



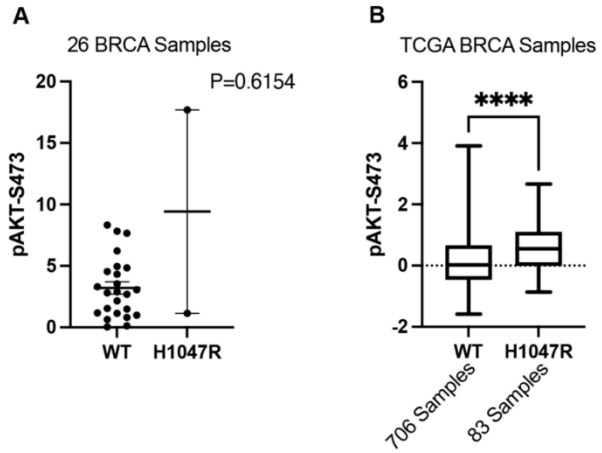
	Median Time
PIK3CA MUT	92.5 Months
PIK3CA WT	145.0.0 Months

	Median Time
PIK3CA MUT	64.2 Months
PIK3CA WT	127.0 Months

	Median Time
PIK3CA H1047R	71.6 Months
PIK3CA WT	145.0 Months

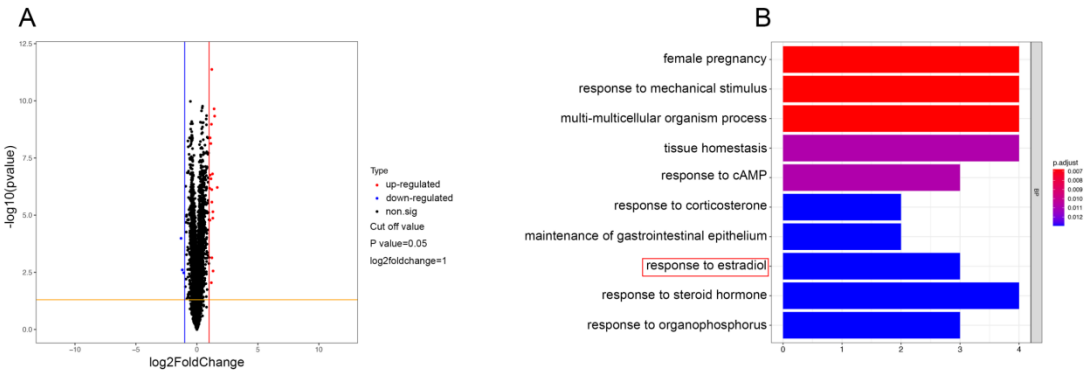
	Median Time
PIK3CA H1047R	50.9 Months
PIK3CA WT	127.0 Months

4.8 PIK3CA H1047R activates the PI3K/AKT signaling pathway



4.9 The drug resistance induced by *PIK3CA* H1047R is associated with breast cancer's response to estradiol.

mRNA differential expression between patients with PIK3CA wild type and PIK3CA H1047R mutation in METABRIC.



4.10 Significantly altered regions linked to drug response