

Analysis

Novel biomarkers and drug correlations of non-canonical WNT signaling in prostate and breast cancer

Yongming Huang¹ · Meiyin Fan² · Yushuai Liu³ · Xiaoying Jiang² · Kevin Du⁴ · Alice Wu⁴ · Qingyi Li⁵ · Yingying Wu⁶ · Jiaqian Liang⁷ · Keshan Wang⁸

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Abstract

Prostate cancer (PCa) and breast cancer (BC) present formidable challenges in global cancer-related mortality, necessitating effective management strategies. The present study explores non-canonical Wnt signaling in PCa and BC, aiming to identify biomarkers and assess their clinical and therapeutic implications. Co-expression analyses reveal distinct gene patterns, with five overlapping genes (SULF1, ALG3, IL16, PLXNA2 and RASGRF2) exhibiting divergent expression in both cancers. Clinical relevance investigations demonstrate correlations with TNM stages and biochemical recurrence. Drug correlation analyses unveil potential therapeutic avenues, indicating that Wnt5a and ROR2 expressions are related to MEK inhibitor sensitivity in cancers. Meanwhile, further correlation analyses were conducted between drugs and the other novel non-canonical WNT genes (ALG3, IL16, SULF1, PLXNA2, and RASGRF2). Our findings contribute to understanding non-canonical Wnt signaling, offering insights into cancer progression and potential personalized treatment approaches.

1 Introduction

Prostate cancer (PCa) and breast cancer (BC) represent significant challenges in the realm of cancer-related mortality, impacting both men and women globally [1, 2]. These two malignancies have garnered substantial attention due to their prevalence and the critical need for effective strategies in managing and treating these diseases [3]. As estimated, PCa will account for 29% of diagnosed male tumor cases and BC will represent 32% of diagnosed female cases, both two have the highest incidence rates among males and females respectively [4]. As two of the most

Yongming Huang, Meiyin Fan and Yushuai Liu contributed equally.

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✉ Yingying Wu, ywu68@uh.edu; ✉ Jiaqian Liang, 5959170@qq.com; ✉ Keshan Wang, wks4869@hust.edu.cn; Yongming Huang, huangym90@outlook.com; Meiyin Fan, 895462105@qq.com; Yushuai Liu, 395421396@qq.com; Xiaoying Jiang, 842130048@qq.com; Kevin Du, kevindu@college.harvard.edu; Alice Wu, awu1@college.harvard.edu; Qingyi Li, 973248926@qq.com | ¹Gastrointestinal Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China. ²Health Management Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China. ³Ophthalmology Department, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China. ⁴Harvard University, Boston, USA. ⁵Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China. ⁶Department of Mathematics, University of Houston, Houston, USA. ⁷Department of Urology, Wuhan No.1 Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. ⁸Department of Urology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China.



representative hormone-related tumors, PCa and BC have certain correlations. It is reported that the increased risk of developing BC was associated with a positive family history of PCa, and women with a family history of both BC and PCa among first-degree relatives had a whopping two fold increase in the risk of developing BC [5]. Moreover, a recent study reveals that individuals with familial BC experience a 21% higher risk of developing PCa and a 34% greater risk of lethal disease [6]. From the perspective of tumor molecular subtyping, Breast and Ovarian Cancer Susceptibility Protein 2 (BRCA2) mutations could lead to poor prognosis in PCa patients, also suggesting the correlation between BC and PCa [7]. However, suitable and integrated biomarkers for PCa and BC together still lack.

Emerging research has shed light on the intricate role of aberrant Wnt signaling in the pathogenesis of various cancers, with a particular focus on PCa and BC [8, 9]. For instance, Menck et al. demonstrated that ROR2, a WNT receptor highly expressed in aggressive breast tumors, promoted an auto-stimulatory loop by inducing the expression of its ligand, WNT11, leading to increased tumor invasiveness and metastasis in BC, revealing potential therapeutic targets for intervention [10]. The Wnt/ β -Catenin pathway is pivotal in embryonic development and tissue repair, particularly in generating growth stimulatory factors (GSF) from Wnt-related genes, contributing to tissue morphogenesis [11]. This pathway orchestrates downstream signaling cascades and assumes a non-canonical state under abnormal Wnt signaling conditions. Anomalies in extracellular signals can activate this pathway, leading to the overexpression of GSF, a phenomenon associated with the development of numerous cancers [12, 13]. While canonical Wnt signaling has been extensively studied, non-canonical Wnt signaling pathways have received comparatively less attention. Though these diverse pathways exhibit significant overlap among them, a dearth of research focuses on indicators of high non-canonical Wnt signaling [14, 15]. Consequently, identifying biomarkers indicative of high non-canonical Wnt activity in cancers is crucial for enhancing diagnostic capabilities and advancing the development of targeted therapeutics against Wnt signaling in cancer [16, 17].

The present study is driven by two primary objectives: (i) identifying biomarkers associated with high non-canonical Wnt activity in PCa and BC cancer cells. (ii) Exploring the clinical relevance of these biomarkers, along with the identification of drugs that demonstrate hypersensitivity or resistance in cancer cells exhibiting high non-canonical Wnt activity. These results will deepen our understanding of the intricate role of non-canonical Wnt signaling in the progression of PCa and BC, providing novel therapeutic strategies for patients affected by these malignancies.

2 Method and materials

2.1 Multi-objective function optimization

Using RNA sequencing data from PCa cell lines (LAPC4 and DU145) provided by Dr. Wang from Balk Lab and BC cell lines (MCF7) from GSE74383 [18] for multi-objective function optimization. Based on the data from RNA sequencing (Prostate cancer, LAPC4 and DU145) and GEO dataset (Breast cancer, GSE74383) and performed analysis, significant genes shared between PCa and BC were obtained and selected for subsequent analysis. Quality control of the RNA-seq data and GEO datasets was performed, including Remove the low-quality samples, inspecting metrics such as sequencing depth and quality scores to exclude low-quality samples;

Filter low-quality genes, exclude genes with extremely low expression levels or many missing values; Batch effect correction, adjusting for systematic differences between batches using methods like ComBat. Meanwhile, RNA-Seq raw data quality was checked by examining sequencing depth, quality scores (e.g., Phred quality scores), and other indicators to ensure data quality meets analysis requirements.

RNA-seq data was cleaned and Filtered by removing low-quality sequencing reads, adapter sequences, contaminants, etc., to reduce noise impacting subsequent analyses, and then followed by aligning cleaned sequencing reads to a reference genome and calculating gene or transcript expression levels. Also, data normalization of expression matrices was performed, and standardized quantification results were used to eliminate the effects of sequencing depth, gene length, and other factors across samples by FPKM and TPM. A histogram of the Pearson correlation for every pair of genes was drawn to understand the distribution described by the dataset.

2.2 Co-expression analysis

Download public sequencing data of BC (TCGA, Firehose Legacy), metastatic prostate cancer (SU2C/PCF Dream Team, Cell 2015), and prostate cancer (TCGA, Firehose Legacy) from the cBioPortal website [19]. Use Pearson correlation analysis to obtain the co-expression analysis results for each cancer type with two corresponding high non-canonical Wnt activity biomarkers (WNT5a or ROR2).

2.3 Clinical relevance analysis

Based on the studies and methods we described before [20, 21], the clinical information contained pathologic stages and survival time of Prostate adenocarcinoma (PRAD) and Breast invasive carcinoma (BRCA) were obtained from Xena Functional Genomics Explorer [22]. Significant results from mRNA comparison between BCa and PCa and non-cancer patients using Mann–Whitney test. Significant results from mRNA expression between overall 5-year survival for BC patients using Mann–Whitney Test, showing the survival difference between breast cancer patients with “genes high expression vs genes low expression”.

2.4 Drug correlation analysis

Download primary PCa cell lines, metastatic PCa cell lines, and BC cell lines, along with cell line drug sensitivity data from DepMap website [23] for analyzing the correlation between targeted genes and drugs.

2.5 Statistical analysis

Small sample size from the DepMap datasets proved to be a limitation in narrowing down, a smaller amount of reliable correlated biomarkers and compounds. The amount of samples per Pearson correlation test was very small ($n = 3$) in the cases utilizing Primary and Metastatic PCa cell lines respectively, similarly small ($n = 10$) in the case utilizing BC cell lines. As a result, the p-value and its FDR adjusted p-value were not utilized as they would not be reliable due to such a small sample size. Instead, the Pearson coefficient was used as a cutoff Tests with $|\rho| > 0.15$ were kept.

3 Results

3.1 Multi-objective function optimization

The problem of selecting a set of genes representing the Wnt signaling signature can be formulated as a combinatorial optimization problem: given a set of genes V , select a subset of size s that has the minimum pairwise average distance, where $\text{distance} = 1 - \text{correlation}$ is a measure of “closeness” of two genes. This problem is in general NP-hard, since the problem of finding a clique of size s on a graph G can be reduced to finding a minimum-weight set of size s on the corresponding binary weighted graph with a 0 if an edge is present in G and a 1 otherwise. In this analysis, we apply the Metropolis–Hastings (MH) algorithm to search for a Wnt signature.

MH is a general-purpose Markov chain Monte Carlo algorithm commonly applied to graph problems. To use MH in our case, we want to sample from X , the collection of sets of genes with size s , according to a distribution π that favors low-distance elements. The distribution we chose was

$$\pi \sim \left(\frac{1}{\text{distance}} \right)^c$$

where c is a distribution parameter that controls how much the distribution is skewed towards lower distance. Other distributions are possible, but we found experimentally that this distribution performs sufficiently well.

In this project, we use a neighbor function determined by an undirected graph G whose vertices are genes in V and edges are highly correlated pairs of genes in V . Then, we can call two elements $x, y \in X$ neighbors if they differ by only one gene and the two genes that they differ by are connected by an edge on G .

Thus, to define the probability density function to select the new candidate, we define

$$g(x'|x) = \frac{1}{s|\text{adj}_G(x-x') - (x \cap x')|}$$

for any two neighbors x and x' , where $\text{adj}_G(v)$ is the set of vertices adjacent to v in G .

A single trial of MH for 106 samples provided a set of 100 genes; these 100 significant genes will act as possible biomarkers for the co-expression analysis (Fig. 1 and S1).

3.2 Co-expression analysis with Wnt5a and ROR2 in PCa and BC

As we and other researchers demonstrated in previous studies, Wnt5a and ROR2 are two typical biomarkers of non-canonical WNT signals in cancers [24–26]. Utilizing cBioPortal, a comprehensive co-expression analysis was conducted to evaluate the correlation between a set of 100 potential biomarkers and two well-established biomarkers associated with high non-canonical Wnt activity, specifically WNT5a and ROR2. This analysis involved gene expression datasets from primary PCa, metastatic PCa, and BC samples. The results of this study led to the identification of 17 genes in PCa (Fig. 2A) and 18 genes in BC (Fig. 2B) that were deemed particularly relevant. A comparison between primary and metastatic PCa revealed the selection of 11 and 8 distinct genes, respectively, with an overlap of two genes (RPL24 and MTX1) (Figure S2A & S2B).

Upon conducting a more detailed analysis, it was revealed that, in PCa, 4 out of the 17 genes associated with non-canonical Wnt signaling exhibited increased expression, while 11 genes showed decreased expression, and 2 genes displayed no significant change (Fig. 3A). Similarly, in BC, 9 out of the 18 genes associated with non-canonical Wnt signaling exhibited increased expression, whereas 9 genes demonstrated decreased expression (Fig. 3B).

Interestingly, there were 5 overlapping genes associated with non-canonical Wnt signaling between PCa and BC (Fig. 2C). Notably, three genes (IL16, PLXNA2, RASGRF2) were downregulated in tumor samples, while ALG3 demonstrated increased expression in both PCa and BC. Intriguingly, the gene SULF1 displayed decreased expression in PCa but increased expression in BC. SULF1, which was down-regulated in several types of cancer, has been demonstrated to influence the formation of Wnt signaling complexes to modulate the activation of both canonical and non-canonical WNT pathways [27, 28]. Besides, SULF1 suppressed the Wnt3A-driven growth of bone metastatic prostate cancer cells in 3D models [29]. However, the correlations between the other four genes (ALG3, IL16, PLXNA2, RASGRF2) and WNT signaling still remain unclear. ALG3, mainly located in the endoplasmic reticulum and the Golgi apparatus, is related to Nglycans synthesis signaling. In radioresistant breast cancer, ALG3 expression is significantly elevated, and ALG3 serves as an effective target to decrease radioresistance by regulating glycosylation of TGFBR2 in breast cancer [30]. IL16 and PLXNA2 have been reported to regulate prostate cancer development through inflammation or TMPRSS2:ERG gene fusion, while the correlations between RASGRF2 and breast cancer/prostate cancer have not been clearly demonstrated yet [31–33].

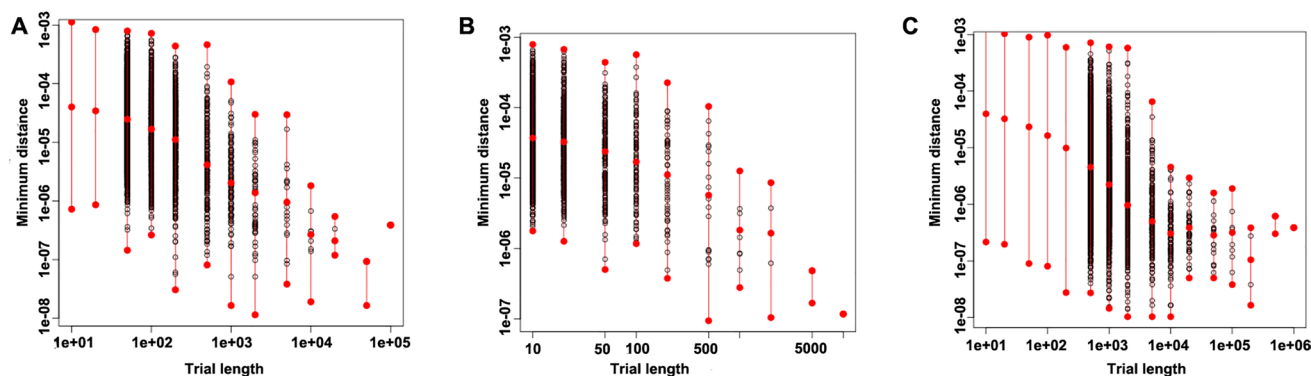


Fig. 1 Range and median of distances from trials of MH with different trial lengths

Fig. 2 Co-expression analysis with Wnt5a and ROR2 in prostate cancer and breast cancer

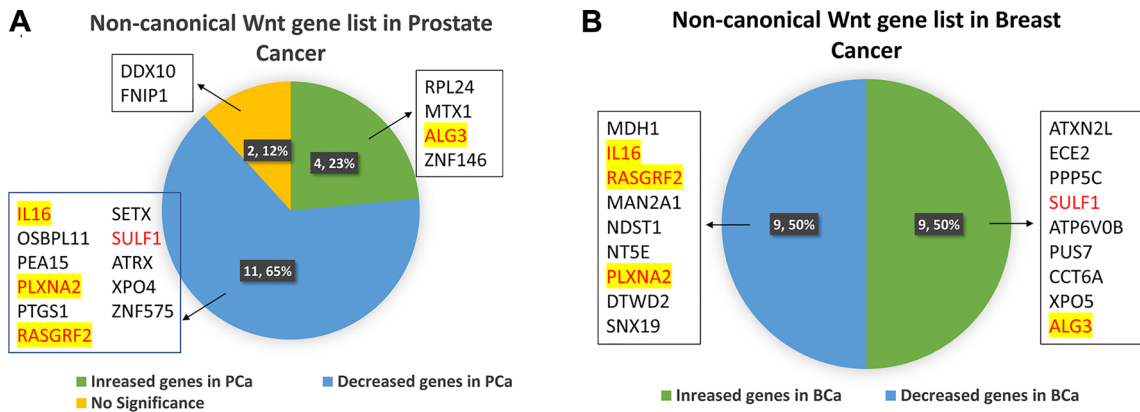
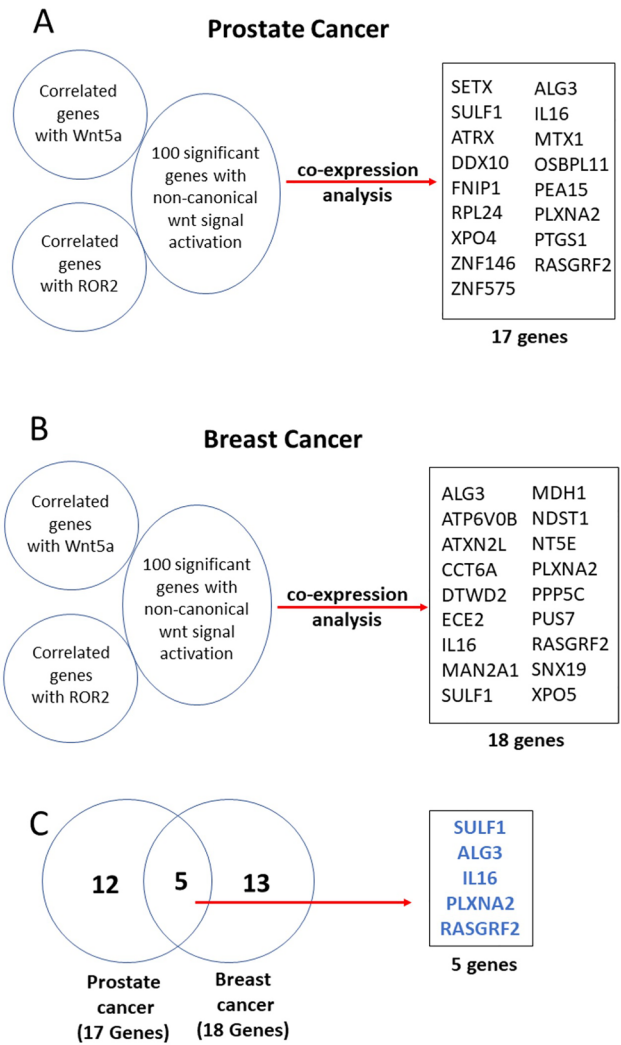


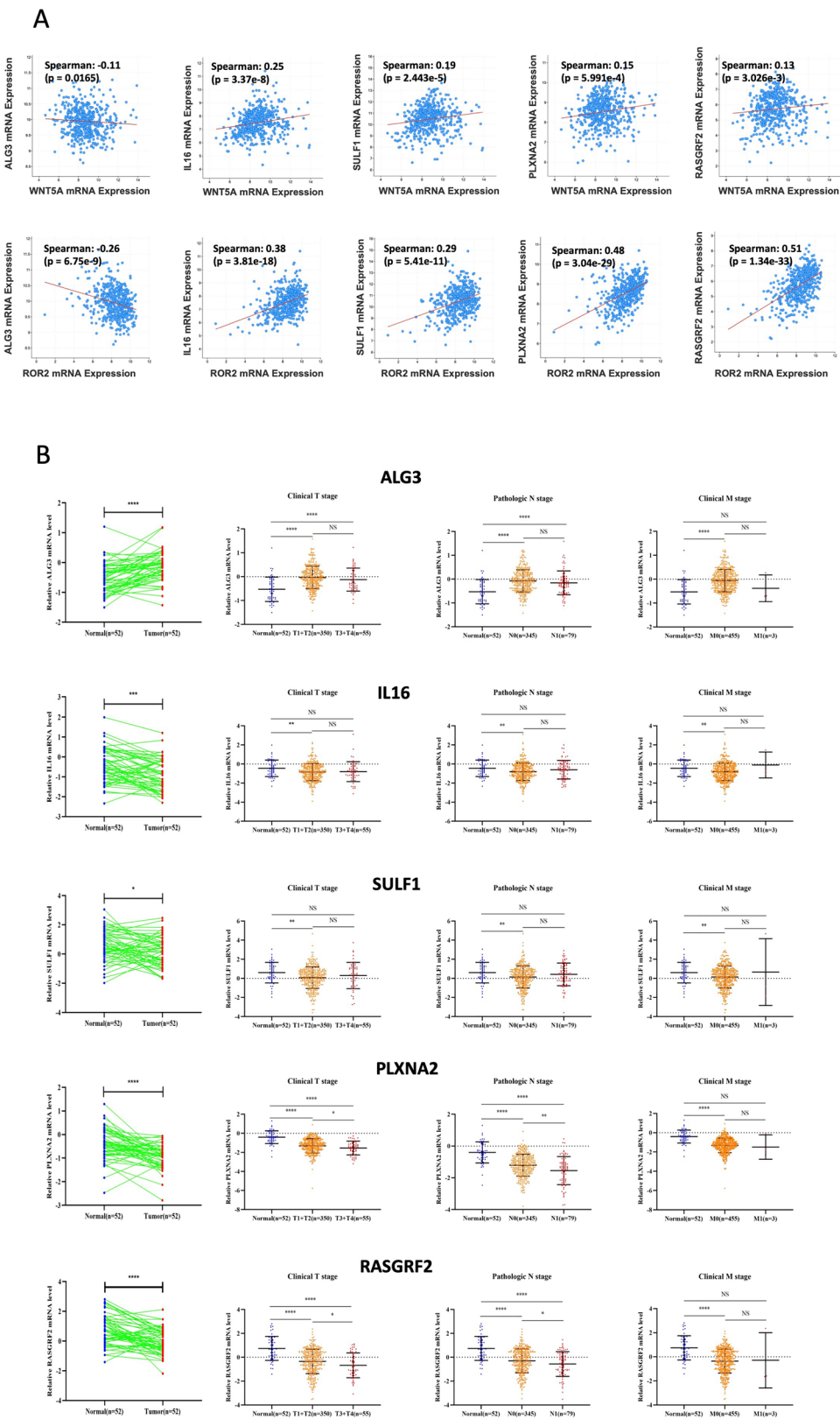
Fig. 3 The non-canonical Wnt gene list in prostate cancer and breast cancer, respectively

3.3 The clinical relevance of the novel non-canonical Wnt gene in PCa and BC

As previously highlighted, our subsequent focus centered on the five newly identified overlapping genes. We sought to investigate the correlation between WNT5a and these novel non-canonical Wnt genes in clinical samples. Notably,

Fig. 4 The clinical relevance of the novel non-canonical Wnt gene in prostate cancer

Prostate Cancer



in the TCGA dataset of PCa, WNT5a exhibited a significant positive correlation with the expression of IL16 (Spearman R value = 0.25), SULF1 (Spearman R value = 0.19), PLXNA2 (Spearman R value = 0.15), and RASGRF2 (Spearman R value = 0.13), but displayed a negative correlation with the expression of ALG3 (Spearman R value = -0.11) (Fig. 4A). Similar positive or negative correlations were observed when examining the association of ROR2 with these genes in prostate cancer (Fig. 4A).

Comparing expression levels to normal prostate tissues, ALG3 showed a significant increase in PCa, while IL16, SULF1, PLXNA2, and RASGRF2 exhibited significantly lower expression in PCa (Fig. 4B and S3). Subsequently, we explored the correlation of these genes across different TNM stages, biochemical recurrence, and specific metrics related to PCa (Gleason Score and PSA values). Significant differences were observed in tumor TNM stages (Fig. 4B). However, only SULF1 displayed a significant difference in biochemical recurrence, and ALG3, IL16, and SULF1 showed significance in Gleason Score (Figure S3). Among the five novel non-canonical Wnt signaling markers we demonstrated, IL16 was reported to correlate with diagnosis and survival in prostate cancer [33, 34]. More interestingly, SULF1 suppresses Canonical Wnt signaling-driven growth of bone metastatic prostate cancer while PLXNA2 expression contributed to TMPRSS2:ERG-mediated enhancements of prostate cancer cell migration and invasion according to the previous studies [29, 31]. However, the roles of ALG3 and RASGRF2 in prostate cancer still remain unclear.

In the context of breast cancer, the correlations between WNT5a (or ROR2) and the novel non-canonical Wnt genes were identified: WNT5a exhibited a significant positive correlation with the expression of IL16 (Spearman R value = 0.24), SULF1 (Spearman R value = 0.37), PLXNA2 (Spearman R value = 0.18), and RASGRF2 (Spearman R value = 0.48), but displayed a negative correlation with the expression of ALG3 (Spearman R value = -0.25) (Fig. 5A). Meanwhile, similar positive or negative correlations were observed when examining the association of ROR2 with these genes in breast cancer (Fig. 5A). Notably, the correlations between Wnt5a/ROR2 with the five genes (ALG3, IL16, SULF1, PLXNA2, and RASGRF2) we demonstrated were poorly investigated in cancer up to now. Differing from PCa, the expression levels of ALG3 and SULF1 were significantly higher in BC than in normal breast tissues, while the expression levels of IL16, PLXNA2, and RASGRF2 were significantly lower in BC (Fig. 5B and S4). Further comparisons of these genes across different TNM stages revealed significant differences (Fig. 5B). However, these five genes did not exhibit significance in overall survival (OS), and only BC patients with low levels of ALG3 expression had a significantly longer disease-free survival (DFS) than those with high levels of ALG3 expression (Figure S4). According to the reports, high expression of SULF1 is correlated with poor prognosis in advanced breast cancer brain metastasis [35], while ALG3 expression predicts poor prognosis and increases resistance to anti-PD-1 therapy in breast cancer [36]. Although the roles of IL16, PLXNA2 and RASGRF2 have been demonstrated in some other cancers (colorectal cancer, neuroblastoma, hepatocellular carcinoma, etc.) [37–39], their biological functions in breast cancer are still lack of direct and detailed reporting.

3.4 Drug correlation of selected biomarkers

To assess the responsiveness of a variety of drugs in high non-canonical Wnt activity cancers, we employed a drug-versus-biomarker correlation analysis. Utilizing the Pearson product-moment correlation coefficient, we gauged the strength and direction of the relationship between biomarker expression and drug sensitivity data.

Our analysis revealed that Wnt5a exhibited a positive correlation with ten drugs, while one drug displayed a negative correlation (Fig. 6A and S5). Conversely, ROR2 demonstrated a positive correlation with twelve drugs (Fig. 6B and S6). Notably, eight compounds (AZD8330, PD-0325901, AS-703026, SELUMETINIB, MEK162, TRAMETINIB, COBIMETINIB, RO-4987655) were identified as red-marked, indicating a shared positive correlation between Wnt5a and ROR2 (Fig. 8A and S7). Mitogen-activated protein kinase kinases 1 and 2 (MEK1/2) are crucial to the ERK pathway and regulate various cancer cell cellular processes [40]. Targeting the MEK signal has become an important strategy for cancer therapy. For example, among the eight MEK inhibitors we found, PD-0325901, also named Mirdametinib, has been included in several Phase 1 studies in cancer therapy [41]. Besides, Cobimetinib and Trametinib were developed to treat melanomas, which had been approved by the FDA [42].

Overall, MEK inhibitors have demonstrated promising application prospects in the clinical treatment of both breast cancer and prostate cancer. However, several challenges persist, with drug resistance being a significant issue among them [43–45]. MEK1/2 inhibitors performed as activators of Wnt/ β -catenin signaling in colorectal cancer by down-regulating AXIN1, which induces stem cell plasticity and an unknown side effect [46, 47]. Here, our results showed that the Wnt5a and ROR2 expressions in cancer cells are potential biomarkers of the anti-tumor efficiency of MEK1/2 inhibitors, which might be useful of the clinical application of MEK1/2 inhibitors.

Fig. 5 The clinical relevance of the novel non-canonical Wnt gene in breast cancer

Breast Cancer

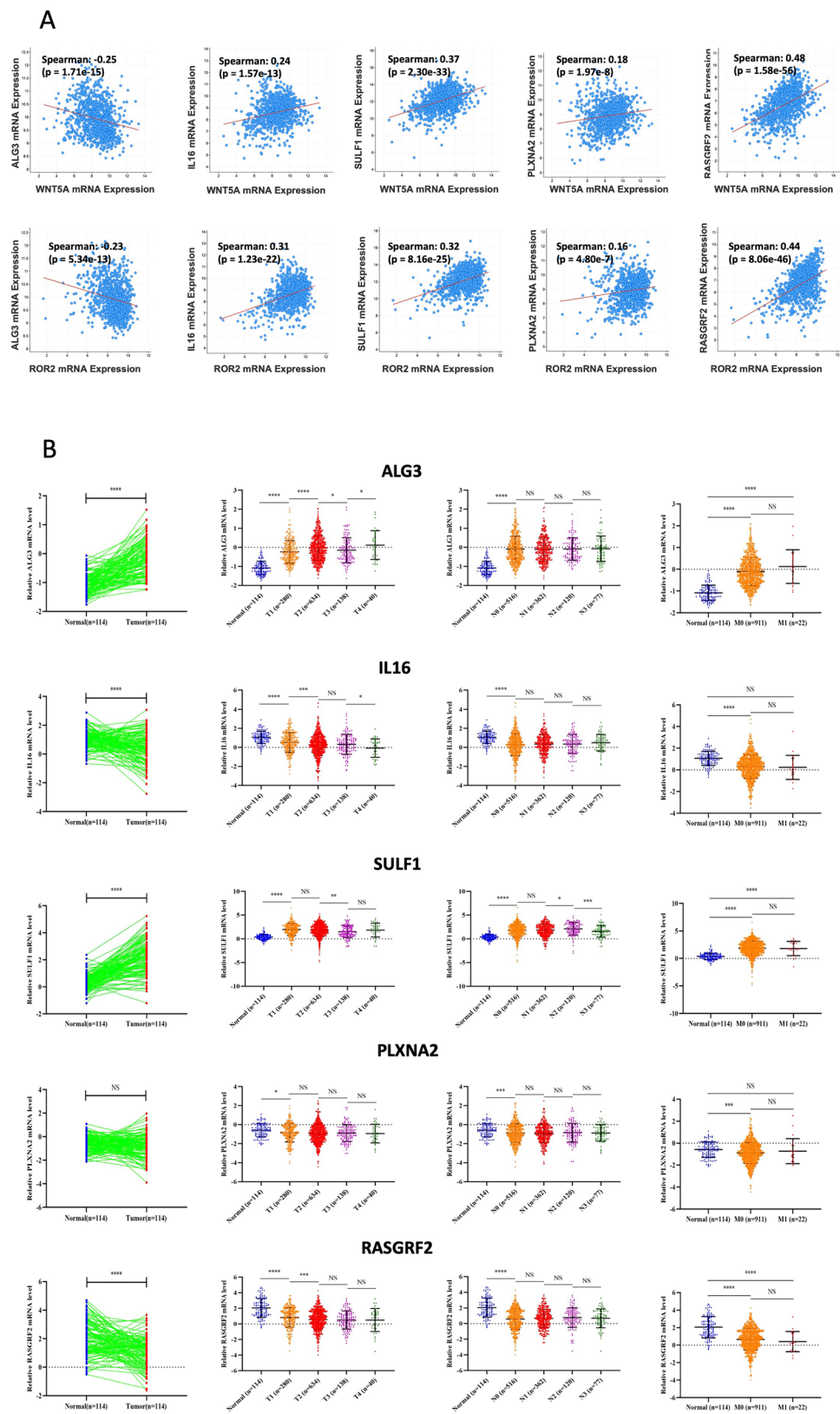


Fig. 6 The related compounds with Wnt5a (A) or ROR2 (B) gene expression

A

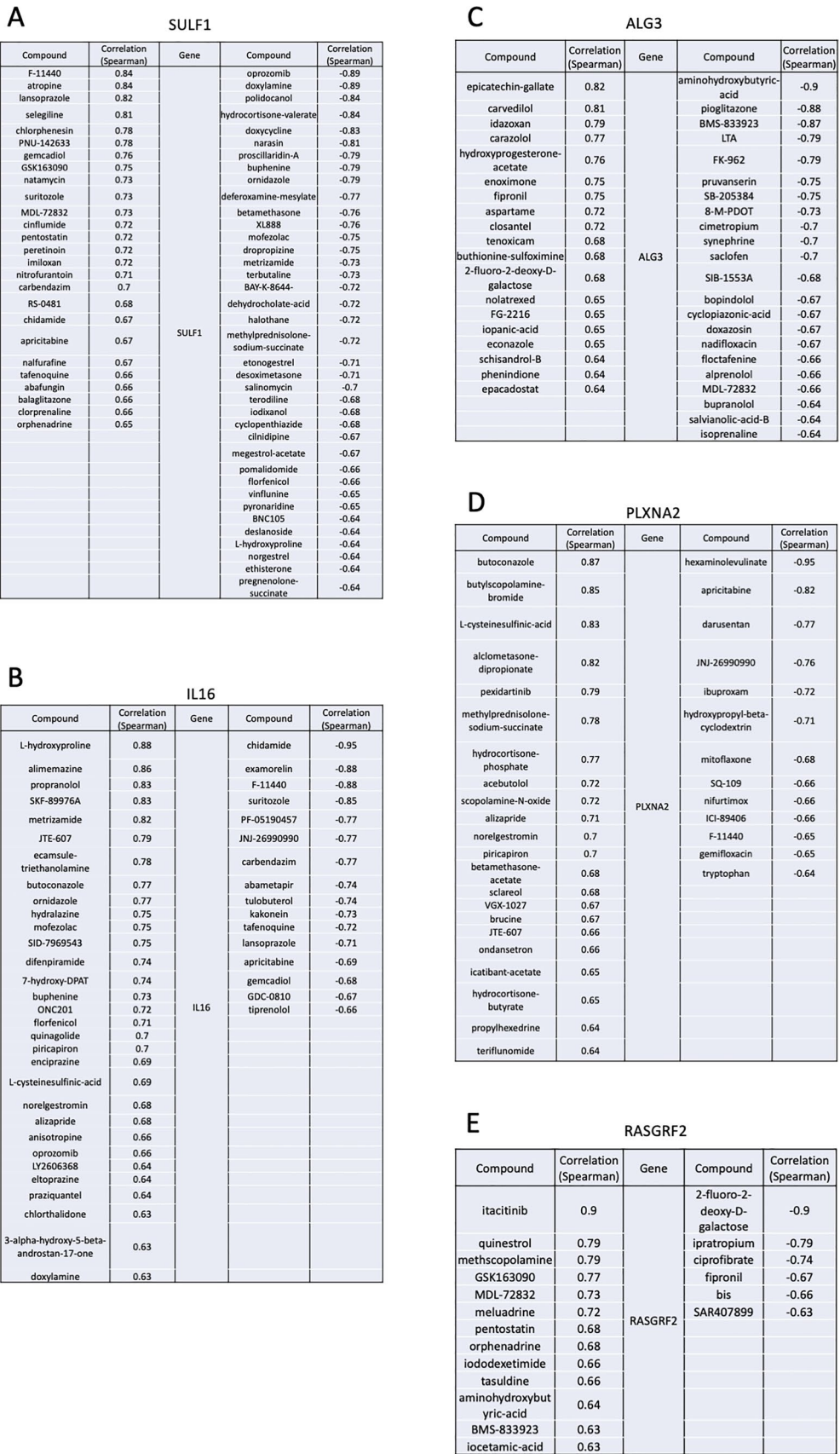
Related Compounds with Wnt5a gene expression	Target/Signal	Spearman Correlation
TRAMETINIB	MEK1/2	0.243
AZD8330	MEK1/2	0.241
AS-703026	MEK1/2	0.239
RO-4987655	MEK1/2	0.239
MEK162	MEK/Autophagy	0.227
BVD-523	ERK2/1	0.222
PD-0325901	MEK/1/2	0.221
COBIMETINIB	MEK1	0.22
SELUMETINIB	MEK/1/2	0.207
MIDOSTAURIN	MEK/1/2	-0.205
AST-1306	EGFR/ErbB2	0.203

B

Related Compounds with ROR2 gene expression	Target/Signal	Spearman Correlation
AZD8330	MEK1/2	0.236
REFAMETINIB	MEK	0.23
RO-4987655	MEK1/2	0.211
PD-0325901	MEK/1/2	0.211
AS-703026	MEK1/2	0.209
TAK-733	MEK	0.208
MEK162	MEK/Autophagy	0.207
COBIMETINIB	MEK1	0.205
TRAMETINIB	MEK1/2	0.203
SELUMETINIB	MEK/1/2	0.202
PD-318088	MEK1/2	0.202
PD-198306	MEK	0.201

Besides Wnt5a and ROR2, further correlation analyses were conducted between drugs and the other novel non-canonical WNT genes (ALG3, IL16, SULF1, PLXNA2, and RASGRF2) we demonstrated before (Fig. 7). Since we involved five genes in drug correlation analysis here, different from Wnt5a/ROR2 related drugs analysis, there is no drug correlated with those five genes simultaneously. The intersection of related compounds across these genes is illustrated in Fig. 8B, highlighting MDL-72832 associated with ALG3, RASGRF2, and SULF1, as well as apricitabine and F-11440 associated with IL16, PLXNA2, and SULF1 (Fig. 8B). MDL 72832, a selective 5-HT1A receptor ligand, is mainly applied in neural signal transduction studies and remains unclear in cancer biology functions [48]. F-11440 (Eptapirone) is another selective and efficacious 5-HT1A receptor agonist with notable potential for anxiolytic and antidepressant treatment [49, 50]. Besides, Apricitabine has shown good application prospects in AIDS and has entered some clinical trials. However, its role in tumor treatment and WNT signal regulation is still unclear and needs further research [51, 52]. Given the many hypothesis tests conducted, we applied the Benjamini–Hochberg procedure to adjust the returned p-values, minimizing false positive results (Supplementary Table 1). This refinement narrowed down the number of drugs within each drug compound dataset that exhibited correlations with specific biomarkers in primary prostate, metastatic prostate, and breast cancer. In summary, based on the further drug-related analysis of these five potential biomarkers of non-canonical WNT signaling, we propose several compounds that may have further research value and clinical application potential in cancer patients with abnormal non-canonical WNT signaling. Of course, these findings require further extensive biological experiments for detailed validation and exploration. The research flowchart is presented in Fig. 8C.

Fig. 7 The related compounds with the novel non-canonical Wnt genes



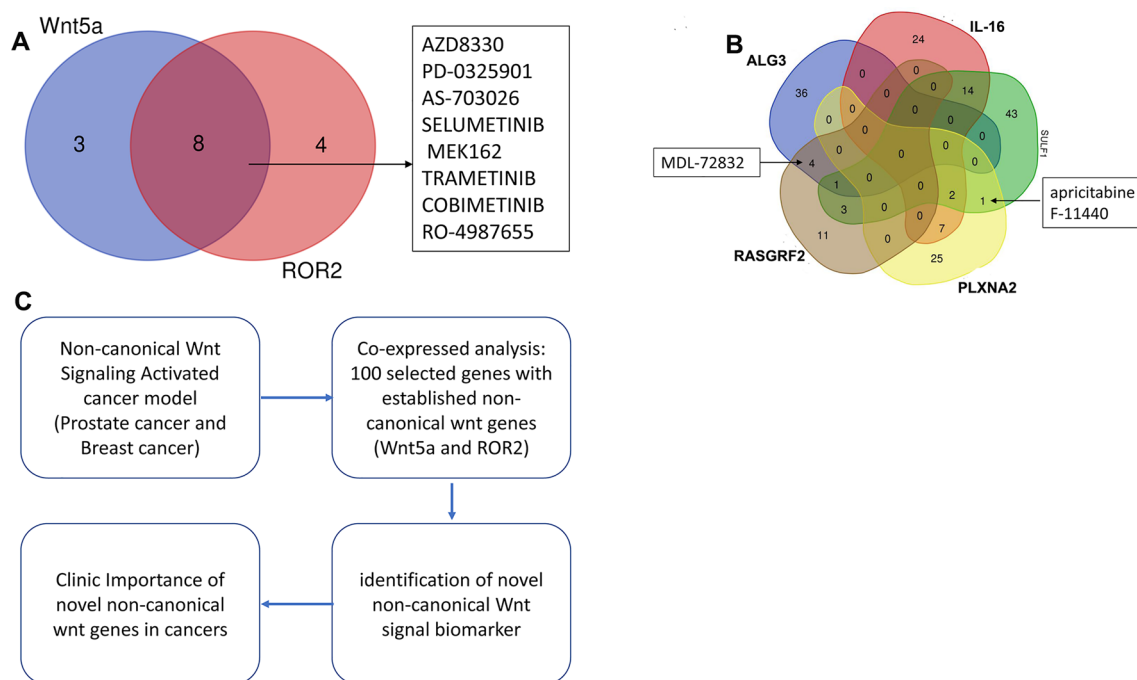


Fig. 8 The intersection of related compounds with the non-canonical Wnt gene expression

4 Discussion

The landscape of PCa and BC remains challenging, necessitating a nuanced understanding of the molecular mechanisms governing their progression [53, 54]. Our study delves into the intricate content of Wnt signaling, with a specific focus on the less-explored non-canonical pathway. The prevalence of these cancers and the critical need for effective strategies underscore the importance of unraveling the role of aberrant Wnt signaling in their pathogenesis.

The identified biomarkers associated with high non-canonical Wnt activity present promising avenues for both diagnostic and therapeutic advancements [55]. For instance, in PCa, ETS status influences the transcriptional repertoire of the androgen receptor, revealing distinct AR-dependent transcriptional programs driving tumorigenesis in ETS- tumors, with dysregulation of ETS-dependent AR-target genes linked to clinical outcomes [56]. The differential expression patterns observed in PCa and BC shed light on the complexity of non-canonical Wnt signaling, emphasizing the need for tailored approaches in each malignancy. The shared genes between these cancers, notably with opposing expression patterns for SULF1, raise intriguing questions about the context-dependent nature of non-canonical Wnt signaling. In the context of breast cancer, the investigation of a single nucleotide polymorphism in SULF1 reveals a significant association with breast cancer susceptibility, suggesting that the rs2623047 SNP in SULF1, specifically the T allele, may increase the risk of developing breast cancer, highlighting the potential for markers like SULF1 to enhance early detection and improve patient treatment and prognosis in this prevalent malignancy [57]. Furthermore, SULF1 acts as the main glycosaminoglycanase in the desmoplastic stroma of PCa bone metastases, suppressing Wnt3A-driven growth signals and influencing the progression of metastatic PCa, even in the presence of pro-tumorigenic tumor-associated macrophages [29]. ALG3 has been demonstrated to be correlated with several cancer developments, such as non-small cell lung cancer (NSCLC), ovarian cancer and bladder cancer, etc. [58–60]. In Breast cancer, ALG3 promotes cancer cell growth and increases anti-PD-1 therapy resistance through different mechanisms [30, 36]. However, the role and mechanism of ALG3 regulating prostate cancer still remain unclear. Exploring the clinical relevance of the identified genes adds depth to their potential as prognostic indicators [61, 62]. The correlations with TNM stages and biochemical recurrence in prostate cancer, along with their distinct expression profiles in breast cancer, highlight the multifaceted roles these genes may play in cancer progression [63]. It is essential to recognize the dynamic nature of these biomarkers and their implications in diverse clinical scenarios. The present study revealed distinct correlations with WNT5a and ROR2 in prostate cancer. ALG3, displaying increased

expression, and IL16, SULF1, PLXNA2, and RASGRF2, with decreased expression, exhibited noteworthy associations. Notably, these genes demonstrated differential expression across TNM stages and biochemical recurrence in prostate cancer. In breast cancer, the correlation patterns were consistent, albeit with different expression profiles. ALG3 and SULF1 exhibited increased expression, while IL16, PLXNA2, and RASGRF2 showed decreased expression. These genes also displayed significant differences across TNM stages, emphasizing their potential as prognostic indicators.

The role of IL-16 in prostate cancer and breast cancer is multifaceted and significant, though its exact mechanisms may vary depending on the cancer type. IL-16 has been identified as a biomarker for prostate cancer. Studies have shown a correlation between pre-diagnostic serum IL-16 levels and prostate cancer risk. Further research indicates that higher pre-diagnostic IL-16 levels are significantly associated with an increased risk of high-grade prostate cancer, supporting the notion that sexually transmitted inflammation may elevate prostate cancer risk [34, 64]. In breast cancer, IL-16 may exert antitumor effects. By enhancing the antitumor effector function of CD4⁺ T cells, IL-16 may inhibit breast tumor growth. Given IL-16's role in modulating the tumor immune microenvironment, it holds potential as a new strategy for breast cancer immunotherapy [65–67]. Although there are some studies indicating that PLXNA2 regulates prostate cancer cell development by SEMA3C/PlexinA2/NRP1-cMET signaling or TMPRSS2:ERG gene fusion [31, 68], research on the specific roles of PLXNA2 in prostate cancer and breast cancer is relatively limited and mostly exploratory. PLXNA2, as a receptor in the Semaphorin (Sema) signaling pathway, is involved in regulating cell migration and invasion. In both breast cancer and prostate cancer, these processes are crucial steps in tumor progression and metastasis. Therefore, PLXNA2 may play a role in prostate cancer or breast cancer development by influencing these processes. The RASGRF2 gene is located on human chromosome 5q13, a region frequently undergoing allelic loss in various cancers, suggesting a potential tumor suppressor function. The roles of RASGRF2 in cancer are different and cancer-type dependent. The precise role of RASGRF2 (RAS Guanine Nucleotide Releasing Factor 2) in breast cancer and prostate cancer remains relatively unexplored, with most research focusing on its functions in other types of cancer, such as colorectal cancer (CRC), lung cancer [69, 70]. As a component of the RAS signaling pathway, RASGRF2 may regulate RAS activity, thereby influencing cell growth, proliferation, and differentiation [71]. The precise roles of RASGRF2 in breast cancer and prostate cancer remain to be fully elucidated. Future research should aim to validate the expression levels and functional status of RASGRF2 in these cancers and explore its interactions with other breast and prostate cancer-related genes and signaling pathways. Assess the feasibility and effectiveness of RASGRF2 as a potential therapeutic target.

The drug correlation analysis bridges molecular insights and therapeutic applications [72, 73]. For instance, Qiang et al. establish a robust prognostic signature of 13 immune-related genes for Head and Neck Squamous Cell Carcinoma (HNSC), offering high predictive accuracy, a prognostic nomogram, and identifying potential drugs (doxorubicin and daunorubicin) for combination therapy, providing a foundation for individualized cancer [74]. The positive correlations of Wnt5a and ROR2 with specific drugs suggest targeted interventions for cancers with high non-canonical Wnt activity. As we demonstrated before, the expression of Wnt5a and ROR2 are valuable biomarkers for YAP/Hippo pathway inhibitors in prostate cancer [24]. What we found in this study identifying compounds showing consistent positive correlations with both Wnt5a and ROR2, such as AZD8330 and PD-0325901, offers potential candidates for further exploration. Based on this, the expression level of Wnt5a/ROR2 might be a novel indicator for the clinical application of MEK inhibitors in both breast cancer and prostate cancer. Meanwhile, the analysis of the further related compounds indicated that 5-HT1A receptor agonist (MDL 72832, F-11440) and Apricitabine might be correlated with the other five non-canonical WNT signaling makers' expression in cancers. The compound-specific correlations with the overlapping genes underscore the potential for tailored therapeutic strategies based on the molecular profile of individual patients.

While our study contributes valuable insights, it is crucial to acknowledge its limitations. The findings are based on computational analyses, and further experimental validation is imperative to confirm the observed correlations and establish the functional significance of the identified biomarkers. Additionally, the dynamic nature of cancer progression suggests the necessity of longitudinal studies to capture temporal changes in non-canonical Wnt signaling.

In conclusion, our research endeavors to unravel the intricate web of non-canonical Wnt signaling in prostate and breast cancer, providing potential biomarkers and therapeutic leads. As we navigate the complexities of these malignancies, personalized and targeted approaches based on the identified molecular signatures hold promise for improving diagnostics and treatment outcomes.

Author contributions Yongming Huang, Meiyin Fan and Yushuai Liu carried out the analysis and experiments. Xiaoying Jiang participated in material preparation and data collection. Kevin Du, Alice Wu, and Qingyi Li conducted the statistical analysis. Kevin Du and Alice Wu also work on manuscript writing and language checking. Jiaqian Liang assisted in collecting tissue samples or conducting animal experiments. Yingying

Wu and Keshan Wang conceived experiments and analyzed data. All authors were involved in writing the paper and had final approval of the submitted and published versions.

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Data availability This study did not generate new data. The data that support the findings of this study are openly available in DepMap (<https://depmap.org/portal/>), GEO (<https://www.ncbi.nlm.nih.gov/geo/>), cBioPortal (<https://www.cbioportal.org/>) and Xena (<https://xena.ucsc.edu/>) websites.

Declarations

Competing interests The authors declare no competing interests.

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