Expression pattern, prognostic value and potential microRNA silencing of FZD8 in breast cancer

MARYAM H. AL-ZAHRANI[1], MOURAD ASSIDI[2], PETER NATESAN PUSHPARAJ[2,3], JAUDAH AL-MAGHRABI[4,5], ALI ZARI[6], ATLAL ABUSANAD[7], ABDELBASET BUHMEIDA[2] and MUHAMMAD ABU-ELMAGD[2]

[1]Department of Biochemistry, Faculty of Science, King Abdulaziz University; [2]Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah 21589, Saudi Arabia; [3]Center for Transdisciplinary Research, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai 600077, India; [4]Department of Pathology, Faculty of Medicine, King Abdulaziz University Hospital, King Abdulaziz University; [5]Department of Pathology, King Faisal Specialist Hospital and Research Center; [6]Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University; [7]Department of Medicine, Faculty of Medicine, King Abdulaziz University Hospital, King Abdulaziz University, Jeddah 21589, Saudi Arabia

Received January 31, 2023; Accepted September 7, 2023

DOI: 10.3892/ol.2023.14065

Abstract. Breast cancer (BC) is one of the most widespread types of cancer affecting females, and therefore, early diagnosis is critical. BC is a complex heterogeneous disease affected by several key pathways. Among these, WNT proteins and their frizzled receptors (FZD) have been demonstrated to be crucial in regulating a number of cellular and molecular events in BC tumorigenesis. The role of the WNT receptor, FZD8, in BC has received minimal attention; for that reason, the present study examined the prognostic value of its protein expression pattern in a BC cohort. FZD8 cytoplasmic expression pattern analysis revealed that ~38% of the primary samples presented with a high expression profile, whereas ~63% of the samples had a low expression profile. Overall, ~46% of the malignant tissues in the lymph node-positive samples exhibited an increased FZD8 cytoplasmic expression, whereas 54% exhibited low expression levels. An increased expression of FZD8 was associated with several clinicopathological characteristics of the patients, including a low survival rate, tumor vascular invasion, tumor size and grade, and molecular subtypes. Affymetrix microarray triple-negative BC datasets were analyzed and compared with healthy breast tissues in order to predict the potential interfering microRNAs (miRNAs) in the WNT/FZD8 signaling pathway. A total of 29 miRNAs with the potential to interact with the WNT/FZD8 signaling pathway were identified, eight of which exhibited a significant prediction score. The target genes for each predicted miRNA were identified. On the whole, the findings of the present study suggest that FZD8 is a potential prognostic marker for BC, shedding some light onto the silencing mechanisms involved in the complex BC signaling.

Correspondence to: Professor Muhammad Abu-Elmagd, Center of Excellence in Genomic Medicine Research, King Abdulaziz University, 106 Ali Al-Murtada Street, Al-Sulaymaniyah, Jeddah 21589, Saudi Arabia
E-mail: mabuelmagd@kau.edu.sa

Key words: WNT signaling, FZD8, breast cancer, prognosis, miRNA, biomarkers discovery

Introduction

Recent advances in cellular, molecular, and genomic technologies and approaches have markedly improved disease diagnosis and treatment (1). However, the early prediction and diagnosis of certain diseases, including cancer, remains to be successfully achieved. In 2018, the WHO estimated the occurrence of ≥9.6 million deaths worldwide, which is expected to be <21.4 million by 2030 (2,3). Female breast cancer (BC) is a highly prevalent type of cancer among young and aged women in the Kingdom of Saudi Arabia, with an incidence rate of 29.7% (4). Regardless of the effective and globally available BC therapies for the treatment of BC, diagnosis at the earliest stage of the disease remains a challenge (5,6). Consequently, early detection followed by prompt referral to the patient BC clinics are crucial for overcoming many of the challenges in disease management (7); thus there is an urgent need for the discovery of powerful BC biomarkers.

It has been well documented that multiple signaling pathways are involved in BC pathophysiology, including development, proliferation, differentiation, motility and metastasis (8,9). These mainly include the WNT (10), NOTCH (11), bone morphogenetic protein-2 (12), STAT3 (13), estrogen receptor (ER) (14), human epidermal growth factor receptor 2 (HER2) (15), MAPK (9,16), PI3K/Akt/NF-κB (17), TGF-β (18), Hedgehog (19) and HIPPO (20) pathways. In addition, inflammation has been strongly linked to BC progression through TLR activities (21-23). Furthermore, microRNAs (miRNAs/miRs) have been demonstrated to be critical in orchestrating and fine-tuning the signaling during BC stem cell self-renewal, proliferation, tumor metastasis and drug resistance (8,24).

Frizzled receptors (FZDs) and their WNT ligands have been revealed to regulate several cellular processes during embryonic development and tumorigenesis (25). Mutations in the WNT components pathway can trigger diseases, specifically cancer (25-27). Several FZDs have been reported to play a crucial role in cancer progression (28-31). FZD8 receptor is included in this category, cloned and characterized in humans on chromosome 10pll.2 (32). FZD8 has been demonstrated in several studies to play differential roles in various types of cancer (33-37). In BC, FZD8 has been demonstrated to mediate resistance to chemotherapy in patients with triple-negative (TN)BC, thus rendering it an important candidate as a therapeutic target (33). In gastric cancer (34), FZD8 has been demonstrated to promote metastasis through WNT/β-catenin. It also exhibits the same function in prostate cancer, in addition to the activation of TGF-β signaling (36). In lung cancer, FZD8 has been reported as a novel powerful prognostic marker (37).

Regardless of the important role of FZD8 in different types of cancer, a limited number of studies have previously analyzed its role in BC (33,38). Therefore, the aim of the present study was to assess the prognostic value of FZD8 expression in a BC cohort using immunohistochemistry (IHC), multivariate Cox analysis and Kaplan-Meier univariate survival analysis. The association between the FZD8 expression pattern and patient clinicopathological parameters was determined. In addition, using BC microarray datasets in the Gene Expression Omnibus (GEO) and miRNA target prediction bioinformatics tools, the silencing machinery involving FZD8 in BC was investigated, and potential miRNAs targeting FZD8 expression were identified.

Materials and methods

Patients. The present study was reviewed and approved by the Ethics Committee of the Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University (Jeddah, Saudi Arabia; approval no. 012-CEGMR-ETH). All patients who participated in the study provided written informed consent in accordance with The Declaration of Helsinki (39). The procedures for collecting patient specimens adhered to the King Abdulaziz University Hospital (KAUH) guidelines. The BC specimens used in the present study were obtained from the Pathology Department, KAUH, and covered the period between January, 1995 and December, 2017. A total of 562 patients aged between 25-70 years with an age median of 50 years were enrolled in the present study. The surgery was performed on almost all patients, usually in the form of a lumpectomy, or a radical or modified radical mastectomy with axillary clearance. Per patient, one sample/biopsy was included in the study and in all further analyses. The clinicopathological data of the patients were obtained from the KAUH medical records. The inclusion criteria that were applied included all female breast cancer patients diagnosed, monitored and followed-up at KAUH. Only patients with available clinical records and retrospective samples were included in the study. Patients with other concomitant diseases or with no medical records or available samples were excluded from the study. The present study did not include patients who had received neoadjuvant therapy. Following surgery, the BC and lymph node specimens of the patients were immediately fixed in 10% formalin buffer overnight at 4˚C with shaking and processed for the typical formalin-fixed paraffin-embedded (FFPE) blocks, followed by paraffin sectioning at a thickness of 4 μm. For the assessment of the histopathological characteristics, histological grading, and tumor, node and metastasis-based staging of the biopsies, the paraffin sections were stained at room temperature according to the manufacturer's protocol of the hematoxylin and eosin kit (ab245880, Abcam), and then examined and analyzed by pathologists. For the BC grading system, the method published by Schauer *et al* (40) was followed. Briefly, upon histological examination, tumor cells were classified based on their similarity or dissimilarity to the normal cells, as well as their mitotic activity: i) When tumor cells were closely similar to normal cells or well-differentiated with lower mitotic count, they were classified as grade 1; ii) when tumor cells were moderately-differentiated with moderate mitotic activity, they were considered as grade 2; iii) when tumor cells were poorly-differentiated with high mitotic activity, they were classified as grade 3; and iv) undifferentiated tumor cells with very high mitotic activity were classified as grade 4.

Treatment and follow-up. For post-operative early adjuvant systemic therapy, 75, 59 and 34% of the patients received chemotherapy, radiation therapy and hormone therapy, respectively. Patients who completed the therapy were followed-up every 6-12 months until they either succumbed or reached the end of follow-up by December, 2017. During the follow-up, some patients did not survive. The mean follow-up time for the entire series was 99 months (range, 2-630 months). In addition to routine clinical check-ups throughout the follow-up period, the patients were also subjected to any necessary chest, abdominal-pelvic and bone isotope scans. In total, ~19% of the patients experienced recurrence and 23% succumbed to the disease. Disease-specific survival (DSS) was determined as the interval between the time of diagnosis and the disease-related mortality or the last time a patient was seen alive. Patients who succumbed due to unspecified or unrelated causes were not included in the DSS calculation. Based on the clinical evidence alone, the causes of mortality were typically clear. In those cases, no autopsy was carried out. The date on which patients were last seen disease-free was used to calculate disease-free survival (DFS), which was also determined as the interval between the diagnosis and the occurrence of disease recurrence.

Tissue microarray and IHC. Tissue microarray (TMA) slides (41) were prepared using a total number of 562 BC FFPE blocks. In brief, BC tissue cores were extracted from the donor block and placed into a recipient paraffin block using a computerized TMA platform (TMA Master 1.14 SP3; 3DHISTECH, Ltd.). FZD8 protein expression patterns in BC and lymph node tissues were detected by staining the TMA slides with anti-FZD8 antibody using a fully automated Ventana IHC-staining system (BenchMark XT automated slide preparation system; Roche Tissue Diagnostics). The Ventana protocol included paraffin removal from the 4-μm-thick sections with Ventana EZ-Prep at 75˚C for 15 min and treatment with human anti-FZD8 monoclonal primary antibody (cat. no. ab155093, Abcam; dilution, 1:100)

for 16 min at 37˚C. Chromogen color staining was developed at room temperature using the iView DAB Detection Kit (cat. no. 760-091, Ventana Medical Systems), which was carried out as previously reported (42). The TMA-stained sections were counterstained with Hematoxylin II (Roche Tissue Diagnostics) for 3-5 min at room temperature and treated with running tap water for bluing for 4 min, followed by washing with PBS for 1 min. This was followed by immersion in ascending grades of ethanol buffer (50, 70, 80, 90, 95 and 100%) for 3 min for each change. The stained sections were mounted with Tissue-Tek xylene-based mounting media (Sakura Finetek USA, Inc.) and covered with a glass coverslip (Corning, Inc.).

FZD8 protein expression scoring. The evaluation and scoring of the IHC-stained FZD8 expression in BC and lymph node TMA sections was performed by two experienced pathologists blinded to the clinical data. The IHC Index Score System, which has already been previously validated (43,44), was used for scoring. The BC tumor cells with FZD8 cytoplasmic staining were classified into four groups as follows: i) 0, negative or no detectable staining; ii) 1+, weak but still detectable staining; iii) 2+, moderate or clearly positive; and iv) 3+, strong or highly strong (heavy staining). The cytoplasmic index was determined using the following formula, considering the intensity of the staining, as well as the fraction of the positively stained cells (45): I=0xf0 + 1xf1 + 2xf2 + 3xf3, where (I) is the staining index and (f0-f3) are the proportions of cells exhibiting a given staining intensity (0-3+). The index value may vary between 0 and 300. Different cut-off points were used to test their potential value as prognostic indicators. The FZD8 expression pattern was examined using a Nikon light microscope (model no. 6132; Nikon Corporation) at a magnification of x40 and imaged using a Coolsnap Pro Color camera equipped with Image Pro Plus software, v6 (Media Cybernetics, Inc.).

miRNA target-prediction analysis. Microarray datasets from the GEO (accession no. GSE65194) (46) were used for *in silico* analysis. GEO2R was used to identify the differentially expressed genes (DEGs) in TNBC patient samples compared with healthy breast tissue. Herein, the raw P-value was calculated using unpaired Student's t-test (47). The DEGs were subsequently investigated using the iPathwayGuide high-throughput knowledge discovery tool (Advaita Corporation) using a cut-off fold change of 1.5 and a cut-off of P-value at <0.05 based on unpaired Student's t-test, as previously described (48). TargetScanHuman (version 7.2, http://www.targetscan.org/vert_72/) was used to validate the miRNAs acquired from the iPathwayGuide study for FZD8 and WNT ligand-target prediction (49) and then validated further using the new interface of miRabel miRNAs target-prediction platform (http://bioinfo.univ-rouen.fr/mirabel/) (50). In the miRabel platform, four miRNA target-prediction bioinformatics tools (miRanda, PITA, SVmicrO and TargetScan) are merged into one database.

Statistical analysis. SPSS, Inc. (v19, IBM SPSS Statistics for Windows) software packages were used to conduct the statistical analysis. To determine the significance of the association between the various categorical variables, Fisher's exact test was used. For the univariate survival analysis of the survival outcome measures (DSS and DFS), the Kaplan-Meier method with a log-rank (Mantel-Cox) comparison test was used. P<0.05 was considered to indicate a statistically significant difference.

Results

Frequency of FZD8 protein expression pattern profiling in BC. FZD8 cytoplasmic expression pattern analysis revealed that 37.5% of the primary samples exhibited a high expression profile (2+ and 3+), whereas almost 63% of the samples exhibited a low expression profile (0 and 1+) (Table I). Furthermore, ~46% of the malignant tissues from the lymph node-positive samples exhibited a high FZD8 cytoplasmic expression (2+ and 3+), with 54% exhibiting a decreased expression in the cytoplasm (0 and 1+) (Table I). The cytoplasmic protein expression patterns in the primary BC samples (Fig. 1), as well as those in the lymph nodes (Fig. 2), varied in intensity, ranging from no expression (0), to weak (1+), moderate (2+) and strong expression (3+).

Association between the FZD8 expression pattern and the patient clinicopathological characteristics. In this analysis, a cut-off point of a low cytoplasmic expression (0 and 1+ scores) compared to a high expression in the cytoplasm (2+ and 3+ scores) was used (Table II). There was a significant association between the FZD8 expression pattern and various clinicopathological features. Tumors with vascular invasion exhibited a stronger FZD8 expression (P<0.03) than the tumors without invasion, while low/intermediate grade tumors exhibited a stronger FZD8 expression (P<0.003). Of note, and consistent with the molecular dichotomy of BC [triple-positive (TP) vs. TN], TP tumors [ER-, progesterone receptor (PR)- and HER2-positive] exhibited a higher FZD8 expression compared with TN tumors (P<0.001). However, the HER2-enriched BC molecular subtype was very rare in the present study cohort and hence, the analysis of its association with FZD8 expression was not possible. A marginally significant association was identified between tumor size and FZD8 cytoplasmic expression (P=0.06), whereas small tumors exhibited a high expression of FZD8. However, there was no significant association between FZD8 cytoplasmic expression, and patient age (P=0.41), lymph node status (P=0.23) and histopathological type (P=0.26).

Association between FZD8 expression status and survival outcomes. The Kaplan-Meier survival analysis revealed a significant (log-rank test, P<0.007) association between the FZD8 cytoplasmic expression and DSS. In this case, patients exhibiting a low expression of FZD8 survived for a significantly longer period of time (longer DSS) than those with a higher FZD8 expression (Fig. 3). Of note, the present study demonstrated that at the median follow-up time (200 months), ~50% of all patients exhibiting FZD8 overexpression were deceased, as compared with only 18% of those exhibiting a low expression of FZD8, suggesting that patients with a low FZD8 expression had a better prognosis.

Table I. FZD8 cytoplasmic expression pattern intensities and corresponding percentages in the primary BC tissue and secondary lymph node samples.

	BC tissues		Lymph nodes	
FZD8 cytoplasmic expression intensity	No. of samples	Percentage	No. of samples	Percentage
Negative (0)	133	23	23	6.5
Weak (1+)	229	39.5	166	47.3
Moderate (2+)	119	20.5	73	20.8
Strong (3+)	98	17	89	25.4

FZD8, frizzled receptor 8; BC, breast cancer.

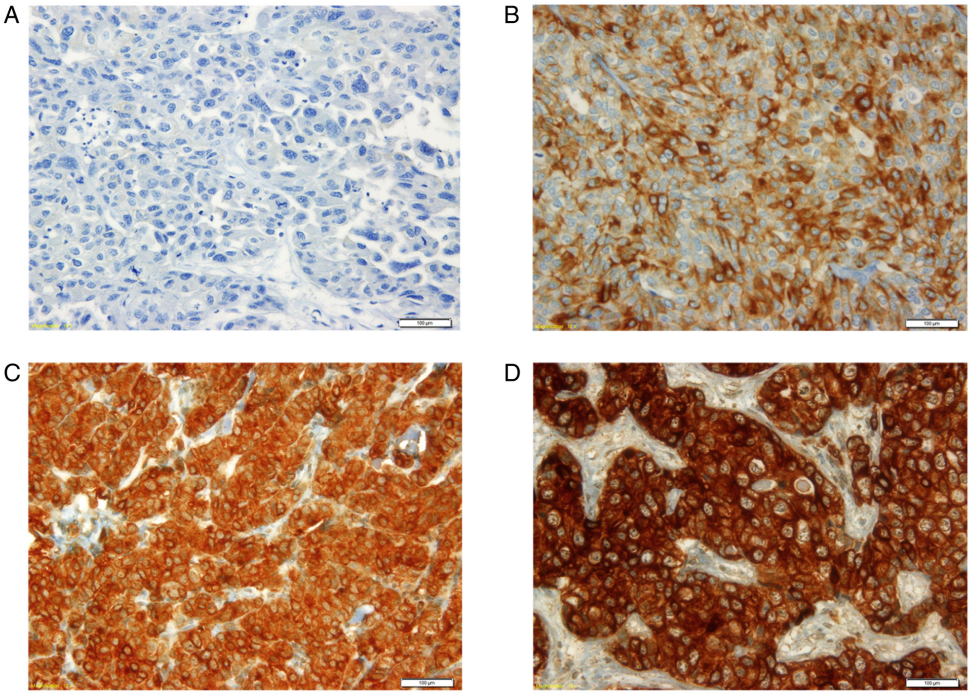

Figure 1. Frizzled receptor 8 protein expression pattern in patients with breast cancer with invasive ductal carcinoma. Different cytoplasmic intensity profiles ranging from level 0 to 3+ were observed. (A) Level 0, no expression; (B) level 1+, weak expression; (C) level 2+, moderate expression; (D) level 3+, strong expression. Magnification, x40.

When the cohort was classified and analyzed according to TP and TN molecular subtypes (ER^+, PR^+, $HER2^+$ vs. ER^-, PR^-, $HER2^-$), the prognostic value of FZD8 protein expression was reserved for patients with TPBC, whereas it was completely lost in those with TN tumors. In that regard, patients with TPBC and lower FZD8 expression patterns survived for longer than those with higher FZD8 expression patterns (Fig. 4). Following a 5-year follow-up period, 20% of the patients with BC and a high FZD8 protein expression were deceased, whereas none of the patients with a low FZD8 expression were deceased, suggesting that TP patients exhibiting a reduced FZD8 expression had a better prognosis and a longer survival (log-rank test, $P<0.04$).

It is worth mentioning that DSS was calculated as the time from the diagnosis of the disease to mortality (due to cancer) or to the date patients were last recorded alive. In general, patients were followed-up at 3- to 6-month intervals until they succumbed or did not pursue medical follow-up (i.e., who were not admitted for hospitalization). When follow-up was not available (patients censored or when the exact death time of the patient was not known/not appeared), the patients were excluded from the statistical survival analysis since the outcome (deceased or alive) was not disclosed. Overall, the present study revealed a stable trend toward the worse survival of patients with BC exhibiting an increased FZD8 expression, as compared with that of those with a decreased FZD8 expression pattern.

Cox regression analysis. According to a multivariate Cox regression analysis, a reduced FZD8 cytoplasmic protein expression pattern was found to be an independent predictor of a poor survival, along with age, lymph node status, tumor grade, and vascular and tumor invasion ($P<0.008$). Tumor invasion is one of the most considerable histopathological

Table II. Associations between FZD8 protein expression patterns and the clinicopathological characteristics of patients with BC.

		FZD8 protein expression pattern (%)		
Features	No. of cases (%)	Low expression (0, 1+) (%)	High expression (2+, 3+) (%)	P-value
Age (years)				0.41
<50	353 (63)	193 (64)	110 (36)	
>50	209 (37)	160 (62)	99 (38)	
Tumor invasion				0.11
Positive	15 (3)	6 (40)	9 (60)	
Negative	499 (97)	315 (63)	184 (37)	
Lymph node status				0.23
Positive	183 (39)	122 (67)	61 (33)	
Negative	289 (61)	183 (63)	106 (37)	
Vascular invasion				**0.03**
Positive	236 (59)	160 (68)	76 (32)	
Negative	164 (41)	103 (63)	61 (37)	
Tumor size (cm)				**0.06**
0-3	189 (39)	116 (61)	73 (39)	
3-6	231 (48)	150 (65)	81 (35)	
>7	62 (13)	45 (73)	17 (27)	
Tumor grade				**0.003**
Grade 1	88 (18)	44 (50)	44 (50)	
Grade 2	239 (50)	151 (63)	88 (37)	
Grade 3	151 (32)	101 (67)	50 (33)	
Histopathological type				0.26
Ductal carcinoma	500 (90)	309 (62)	191 (38)	
Others	56 (10)	39 (70)	17 (30)	
Molecular subtypes				**0.001**
Triple-positive	62 (41)	57 (92)	5 (8)	
Triple-negative	88 (59)	42 (48)	46 (52)	

FZD8, frizzled receptor 8; BC, breast cancer. Values in bold font indicate statistically significant differences (P<0.05).

variables and is often used as a potential prognostic indicator. The association of FZD8 expression with tumor invasion may reflect the tumorigenic effects of the FZD protein family in general, including FZD8 in particular. In addition, Cox regression analysis revealed that patients with BC with a high expression of FZD8 exhibited an ~5-fold higher risk of cancer-related mortality compared with those with a low expression of FZD8 (Table III).

miRNA target prediction analysis. In this target prediction analysis, 3,923 DEGs were identified from a total of 20,150 genes whose expression had been measured. These were identified using a statistical significance (P-value) threshold of 0.05 and a log-fold change in expression with an absolute value of a minimum of 1.5. The analysis of upstream regulatory miRNAs using iPathwayGuide revealed 374 miRNAs predicted to regulate DEGs in TNBC (Table SI). Further filtering based on the association between regulatory upstream miRNAs and FZD8 yielded 29/374 miRNAs (Fig. 5). Filtering of the 29 miRNAs based on the prediction score revealed that eight miRNAs, (hsa-miR-124-3p, hsa-miR-506-3p, hsa-miR-495-3p, hsa-miR-410-3p, hsa-miR-208b-3p, hsa-miR-208a-3p, hsa-miR-99b-5p and hsa-miR-99a-5p) were significantly associated with FZD8 signaling in TNBC (Table IV). Of note, the same sets of the aforementioned miRNAs were found when the TNBCs were compared with the non-TNBCs, and similar to the TNBCs compared with healthy tissues. The DEGs potentially regulated by these miRNAs are listed in Table SII.

Discussion

BC accounts of at least 53% of all female patient cancer cases in Saudi Arabia, rendering it one of the most frequent malignancies among Saudi women (4). Such increased incidence of BC in Saudi Arabia may be mainly attributed to the lack of early, frequent and effective BC screening programs (51). Therefore, the development of such programs and the identification of BC biomarkers are critical for the early detection of the disease. BC is controlled by crucial complexities of signaling cascades (52), which, following decades of focused research are not yet fully

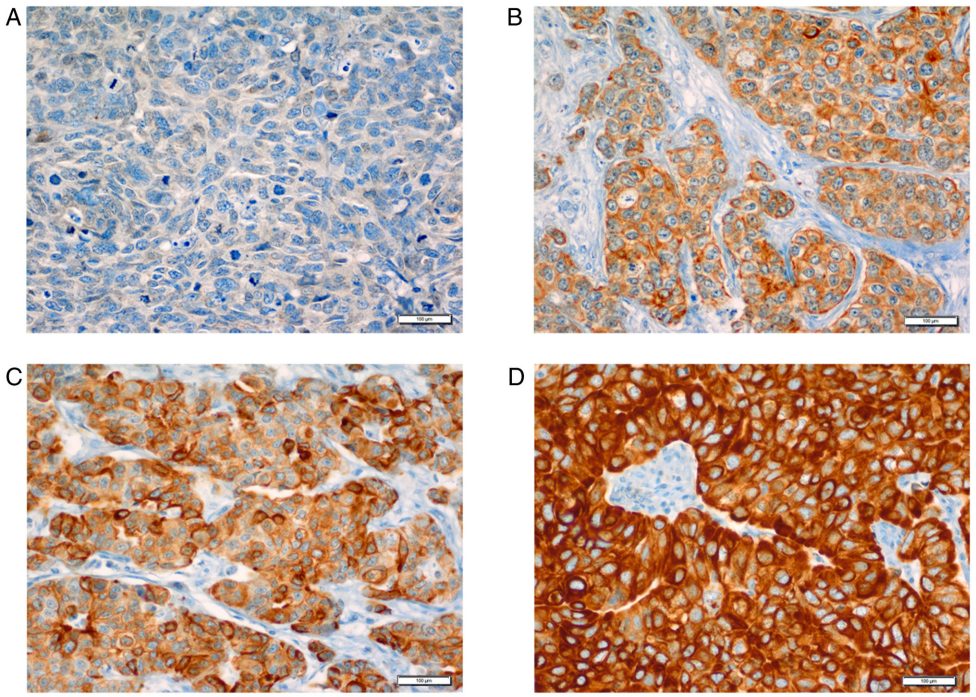

Figure 2. Frizzled receptor 8 protein expression pattern in the lymph nodes of patients with breast cancer. The cytoplasmic intensity profiles ranging from levels 0 to 3+ were detected. (A) Level 0, no expression; (B) level 1+, weak expression; (C) level 2+, moderate expression; (D) level 3+, strong expression. Magnification, x40.

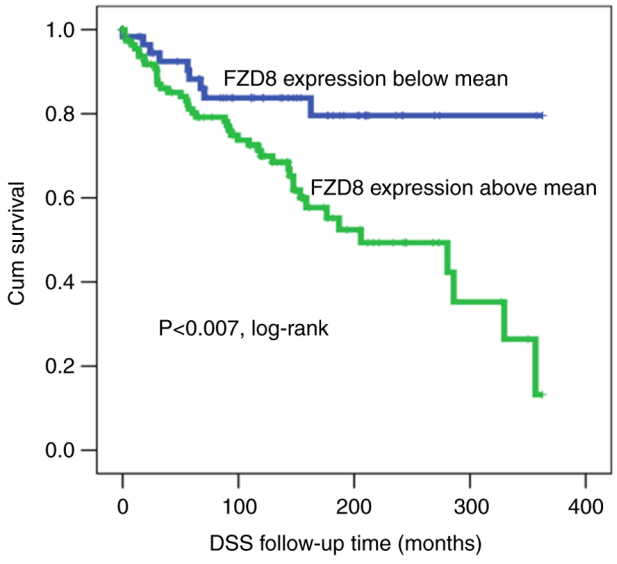

Figure 3. FZD8 protein expression status (below mean vs. above mean) as a determinant of the DSS of the overall breast cancer cohort in univariate Kaplan-Meier analysis. FZD8, frizzled receptor 8; DSS, disease-specific survival.

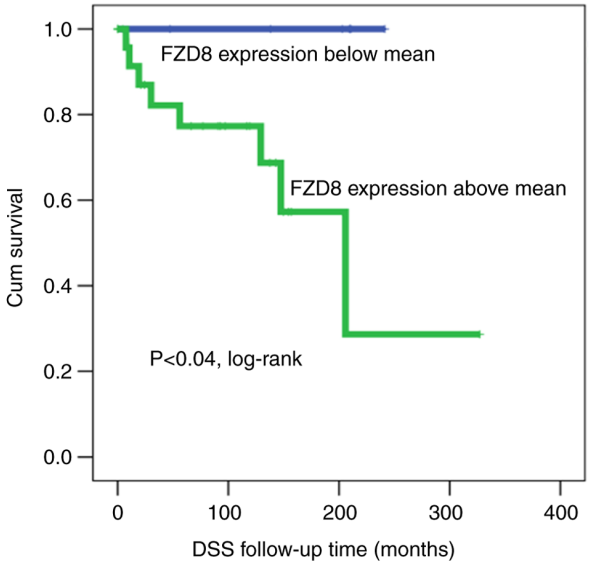

Figure 4. FZD8 protein expression status (below mean vs. above mean) as a determinant of the DSS of patients with triple-positive breast cancer in univariate Kaplan-Meier analysis. FZD8, frizzled receptor 8; DSS, disease-specific survival.

understood. Wnt/FZD signaling has been demonstrated to regulate numerous cellular events during embryonic development and cancer. Despite having a significant impact on embryonic development and disease, FZD8 is a critical WNT receptor that has received minimal attention in BC investigations. Of note, FZD8 expression has been detected during mammary stem cell development (53-55). In the present study, the clinicopathological characteristics of Saudi patients with BC and their association with the FZD8 expression pattern were examined. The majority of the patients exhibited medium-to-high expression levels, either in BC or lymph node tissues. A previous study (33) revealed that increased expression levels of FZD8 were detected in the breast squamous cell carcinoma-derived TNBC cell line. Elevated FZD8 expression levels have also been observed in various other malignancies, including lung cancer (56), medulloblastoma (57), renal cancer (58) and osteosarcoma of the spine (59). Taken together, the findings of the present study suggest that targeting FZD8 expression in BC may be an effective therapeutic approach.

Table III. Cox regression analysis of the prognostic values of FZD8, age at diagnosis, tumor grade and lymph node status.

Feature	P-value	Standard error value	Relative risk	95% CI
FZD 8 expression (low vs. high)	0.008	0.614	5.13	0.058-0.649
Tumor grade (low vs. high)	0.05	0.627	0.29	0.994-11.614
Age at diagnosis (<50 vs. >50 years)	0.12	0.389	1.70	0.274-1.260
Lymph node status (Neg vs. Pos)	0.47	0.409	1.20	0.372-1.848

FZD8, frizzled receptor 8; CI, confidence interval; Neg, negative; Pos, positive.

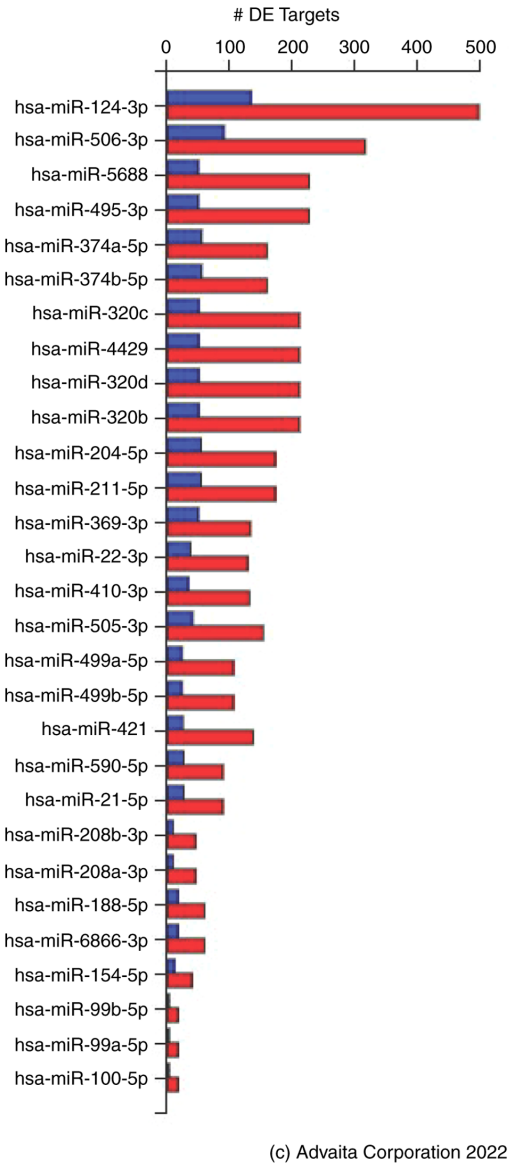

Figure 5. Analysis of upstream regulatory miRNAs in triple-negative breast cancer in the context of FZD8 signaling and their differentially expressed target genes. miRNA/miR, microRNA; FZD8, frizzled receptor 8.

Herein, Cox regression analysis results revealed that FZD8 was associated with tumor invasion. An increased expression of FZD8 has been reported to be associated with other significant cellular events during carcinogenesis in different types of cancer. For instance, in spinal osteosarcoma, the downregulation of FZD8 has been shown to suppress the invasion, migration and proliferation of osteosarcoma cells (59). Increased FZD8 expression has been linked to proliferation and metastasis in renal cell cancer (58). Similarly, in prostate (35) and colorectal (60) cancer, an increased expression of FZD8 has been revealed to promote metastasis.

The results of the present study revealed a significant association between low expression levels of FZD8 and tumor aggressiveness, including vascular invasion, tumor size and grade, molecular subtype and survival outcomes in the BC cohort studied. A higher proportion of patients with grade III cancer and lymphovascular invasion also exhibited a decreased FZD8 expression. Notably, these clinicopathological features were mainly observed in the TP molecular subtype. It is known that the TPBC subtype is associated with a good prognosis, as compared with the TN molecular subtype, mainly due to the multimodality of treatment options, which leads to a better prognosis and longer survival (61,62). Overall, these results suggest a powerful prognostic value of FZD8 expression in BC. To the best of our knowledge, this is the first report revealing the association between an increased expression of FZD8 and these clinicopathological characteristics of patients with BC. In other types of cancer, including gastric cancer, an increased expression of FZD8 has been reported and demonstrated to indicate a poor prognosis (34,63). One of the limitations of the survival analysis performed during the present study was the limited sample size of the patient cohort. However, in future studies, the authors aim to expand the sample size, launching a national sample network termed Saudi Cancer Tissue Array-based Network (SCTAN) that could aim towards the collection of additional tumor samples.

The present study attempted to unravel several complex signaling mechanisms through which FZD8 may function. miRNAs have been shown to mediate BC tumorigenesis through WNT signaling (64). In the present study, microarray datasets were analyzed in TNBC patient samples from the GEO database and compared with healthy BC samples, using the iPathwayGuide high-throughput knowledge discovery platform. A total of 29 miRNAs were predicted to target FZD8 expression in BC, eight of which exhibited significant prediction scores. Previous reports have revealed that several of these eight miRNAs, including hsa-miR-124-3p, hsa-miR-506-3p, hsa-miR-410-3p and hsa-miR-99a-5p function as tumor suppressors (65-68). Additionally, hsa-miR-495-3p has been reported to be a predictor of a poor prognosis of patients with TNBC (69), hsa-miR-208a-3p has been found to be a TNBC promoter (70), while hsa-miR-99b-5p has been found to be a promoter of tumor

Table IV. miRNA target-prediction for FZD8 expression, highlighting predicted WNT ligands for each miRNA.

MicroRNA	No. of differentially expressed targets (-/all)	No. of targets (-/all)	P-value	Prediction score	Predicted WNT ligands for the microRNA
hsa-miR-124-3p	134/631	1065/2110	1	**0.010**	**WNT2B, 4, 5A, 5B, 7B, 9A, 9B, 11, 16**
hsa-miR-506-3p	91/407	622/1262	1	**0.005**	**WNT2B, 4, 5A, 5B, 7B, 9A, 9B, 10B, 11, 16**
hsa-miR-374a-5p	55/214	396/717	1	0.581	WNT2, 2B, 3, 3A, 5A, 5B, 11, 16
hsa-miR-374b-5p	55/214	396/717	1	0.105	WNT2, 2B, 3, 3A, 5A, 5B, 11, 16
hsa-miR-204-5p	54/227	391/744	1	0.211	WNT2, 2B, 3, 3A, 4, 5A, 8B, 9B, 10B, 11, 16
hsa-miR-211-5p	54/227	391/744	1	0.202	WNT2, 2B, 3, 3A, 4, 5A, 8B, 9B, 10B, 11
hsa-miR-320c	51/262	394/803	1	0.994	WNT2, 2B, 5A, 8A, 8B, 9A, 9B, 10B, 11, 16
hsa-miR-4429	51/262	394/803	1	0.999	WNT2B, 8A, 8B, 9A, 9B, 10B
hsa-miR-320d	51/262	394/803	1	0.994	WNT2, 2B, 8A, 10B, 11, 5A, 8B, 9A, 9B, 16
hsa-miR-320b	51/262	394/803	1	0.994	WNT2, 2B, 5A, 8A, 8B, 9A, 9B, 10B, 11, 16
hsa-miR-5688	50/276	411/831	1	0.980	WNT2B
hsa-miR-495-3p	50/276	411/831	1	**0.040**	**WNT2B, 4, 5B, 8A, 8B, 9B, 11, 16**
hsa-miR-369-3p	50/183	338/600	1	0.807	WNT2, 2B, 3, 5A, 5B, 16
hsa-miR-505-3p	41/194	282/586	1	0.851	WNT2B, 4, 5A, 7A, 7B, 8B, 16
hsa-miR-22-3p	37/166	323/584	1	0.991	WNT1, 2B, 3, 3A, 4, 5A, 8A, 9B, 10B, 11
hsa-miR-410-3p	34/165	295/581	1	**0.013**	**WNT2, 2B, 3A, 4, 5A, 5B, 7B, 9B, 11, 16**
hsa-miR-21-5p	26/115	187/365	1	0.989	WNT2B, 4, 3A, 5A, 9B
hsa-miR-590-5p	26/115	188/366	1	0.989	
hsa-miR-421	25/162	196/436	1	0.597	WNT2B, 5A, 7A, 7B, 8B, 9B, 16

miRNA, microRNA; FZD8, frizzled receptor 8. Values and text in bold font indicate statistically significant differences (P<0.05).

aggressiveness (71). The miRNA prediction analysis of the present study suggested that these miRNAs could target FZD8 expression and hence, they may play a pivotal role in the miRNA silencing of WNT signaling in BC. However, validation analysis of the miRNAs with a significantly high prediction score was not performed in the present study. *In vivo* and/or *in vitro* validation assays would further elucidate the mechanisms through which this silencing functions. This could include the use of total RNA from BC biopsies to investigate whether the expression of the predicted miRNAs was simultaneously increased with FZD8 expression, using RT-qPCR. Another approach could include using miRNA antagomirs to knock down the predicted miRNA function in BC cell line(s) and also investigate the increase in FZD8 expression.

One important miRNA, hsa-miR-100, has been reported to inhibit migration and invasion, and regulate apoptosis and metastasis in BC (38,72,73). The present study investigated whether FZD8 was a predicted target for this miRNA, and it was revealed that FZD8 could be a target for hsa-miR-100 with a prediction score of 0.075. In addition, two other miRNAs expressed in BC were investigated in the present study that were previously reported to target FZD8 in other types of cancer, including hsa-miR-375 [colorectal cancer (60)] and hsa-miR-520b [spinal osteosarcoma (59)]. The miRNA prediction analysis performed herein revealed a prediction score of 0.017 for hsa-miR-375 and 0.974 for hsa-miR-520. However, these results need to be further investigated in future validation research in BC experimental models.

In conjunction to the miRNA prediction analysis carried out in the present study, the expression of another WNT receptor (FZD6) in BC was previously analyzed by the authors (74). It was demonstrated that the FZD6 prognostic value was more potent in younger patients with BC, which indeed is not the case for FZD8 expression. In addition, at least 18 miRNAs were predicted to silence FZD6 expression, presented with a high prediction score. Specifically, the miRNAs were hsa-miR-101-3p, hsa-miR-302b-3p, hsa-miR-302d-3p, hsa-miR-372-3p, hsa-miR-373-3p, hsa-miR-520c-3p, hsa-miR-519a-3p, hsa-miR-519b-3p, hsa-miR-568, hsa-miR-545-3p, hsa-miR-130a-3p, hsa-miR-130b-3p, hsa-miR-301a-3p, hsa-miR-301b-3p, hsa-miR-454-3p, hsa-miR-3121-3p, hsa-miR-19a-3p, and hsa-miR-19b-3p (74). In this previous study by the authors, the predicted miRNAs that could target FZD6 expression were compared with those predicted to target FZD8 expression in BC. The comparison analysis revealed that there were no common miRNAs to target both FZD6 and FZD8, indicating that the prediction database tools used in both studies and the analysis carried out were gene specific.

The therapeutic potential of FZD8 in TNBC has been previously reported to play a crucial role in mediating chemotherapy resistance through WNT signaling (33). The biological mechanism through which FZD8 exerts this function remains unclear. It is suggested that FZD8 expression may be either downregulated/fine-tuned or silenced by one or more of the predicted miRNAs revealed in the analysis of the present study to activate this chemotherapy resistance process. Overall, the

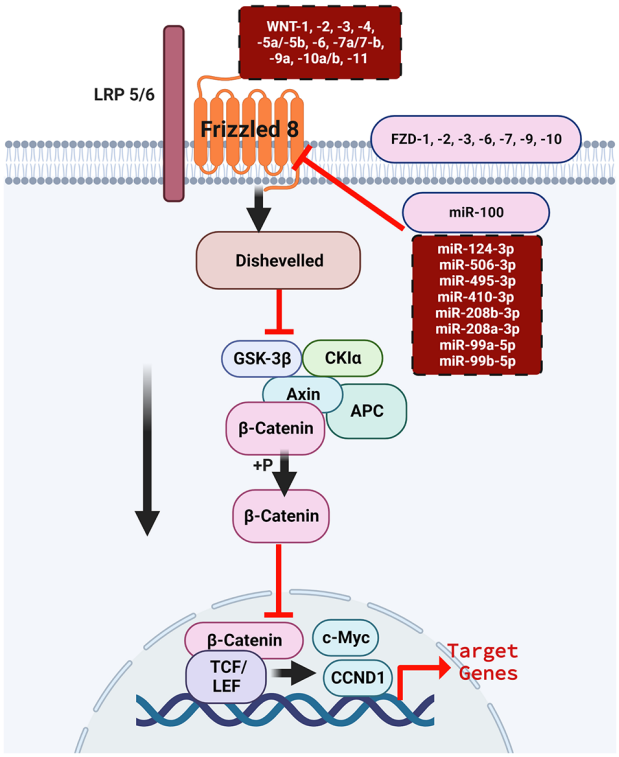

Figure 6. Predicted WNT ligands and miRNA regulation of FZD8 expression in breast cancer through the canonical (β-catenin-dependent) pathway. Potential WNT ligands, including WNT1, WNT2, WNT3, WNT4, WNT5a/b, WNT6, WNT7a/b, WNT9a, WNT10a/b and WNT11 (top brown dotted box), bind to the FZD receptors, including FZD1, FZD2, FZD3, FZD6, FZD7, FZD8, FZD9 and FZD10. Several potential miRNAs (lower brown dotted brown box, right), including hsa-miR-100, hsa-miR-124-3p, hsa-miR-506-3p, hsa-miR-495-3p, hsa-miR-410-3p, hsa-miR-208a/b-3p and hsa-miR-99a/b-5p silence FZD8 expression to recruit Dvl, which in turn suppresses protein complex containing β-catenin, GSK-3β, Axin, APC and CKIa. Upon phosphorylation, β-catenin translocates to the nucleus and binds to the TCF/LEF complex to drive the transcription of the target genes. LRP5/6 are the FZD co-receptors. miRNA, microRNA; FZD8, frizzled receptor 8; Dvl, Dishevelled; TCF/LEF, T-cell factor/lymphoid enhancer factor; LRP, low-density lipoprotein receptor-related protein; FZD, FZD8, frizzled receptor; GSK-3β, glycogen synthase kinase-3 β; APC, adenomatous polyposis coli; CKIα, cyclin-dependent kinase inhibitory protein α.

miRNAs identified in the present study, particularly those with the top prediction scores, are highly recommended for further validation analyses (Fig. 6).

The ability of FZD8 to activate the non-canonical or canonical WNT/β-catenin pathways during tumorigenesis in various cancer types such as renal cell carcinoma (58) and BC (75) has been previously reported. In BC, FZD8 has been revealed to play a crucial role in TNBC drug resistance through canonical WNT/β-catenin (75). The miRNA analysis performed herein predicted several WNT ligands to be involved in FZD8 silencing in BC. These included WNT1, WNT2, WNT3, WNT4, WNT5a/b, WNT6, WNT7a/b, WNT9a, WNT10a/b and WNT11 (Fig. 6). These potential ligands could bind to several FZD receptors, including FZD1, FZD2, FZD3, FZD6, FZD7, FZD8, FZD9 and FZD10. This activates the downstream targets of the canonical pathways of Dishevelled, which in turn blocks several downstream protein targets (β-catenin, glycogen synthase kinase-3, Axin, adenomatous polyposis coli and cyclin-dependent kinase inhibitory protein a; Fig. 6). Once β-catenin is phosphorylated, it translocates to the nucleus to bind to the T-cell factor/lymphoid enhancer factor complex, following which the transcription of the target genes is activated (Fig. 6). The miRNAs identified in the present study could play a role in fine-tuning the expression of FZDs, including that of FZD8, to activate the WNT downstream targets (Fig. 6). These results support those of previous research, demonstrating that these WNT ligands are highly expressed and play critical functional roles in the development of BC through the canonical WNT/β-catenin pathway, as previously reviewed by Xu *et al* (10). It is also suggested that FZD8 in the BC cohort of the present study was more likely to function through the canonical WNT/β-catenin pathway than the non-canonical pathway; however, further validation studies are required in the future.

In conclusion, female BC has been identified in multiple studies and in the Saudi Cancer Registry as the most frequent type of cancer among female patients in Saudi Arabia. The lack of early BC biomarkers to aid the early detection of the disease further aggravates the existing difficulties of the health system and individual patients. Herein, the expression of the WNT signaling receptor, FZD8, was investigated in a Saudi BC cohort. The majority of the patients of the cohort exhibited moderate-to-high FZD8b cytoplasmic expression. Patients exhibiting an increased expression of FZD8 had a low survival rate, and vice versa. Increased levels of FZD8 expression were consistently associated with a poor prognosis. This refers to several clinicopathological features, including tumor vascular invasion, size, grade, molecular subtypes and survival outcomes. miRNA target prediction analysis using microarray TNBC datasets revealed that FZD8 was a target for 29 miRNAs expressed in BC, among which eight miRNAs exhibited significant prediction scores. This miRNA analysis would benefit from further validation assays. The results reported in the present study suggest the necessity of future functional analyses, particularly by applying *in vivo* and *in vitro* gain- and loss-of-function approaches to further decipher FZD8 biological functions in BC. The results of the present study suggest that FZD8 may be a powerful prognostic BC marker and partially elucidate the mechanistic complexity of the involvement of WNT/FZD8/miRNA silencing in BC.

Acknowledgements

The authors would like to express their gratitude to Dr Habiba Abu-Elmagd from Bristol Dental School, University of Bristol, UK for proofreading the manuscript.

Funding

The authors extend their appreciation to the Deputyship for Research and Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number (IFPRC-088-247-2020) and King Abdulaziz University, Deanship of Scientific Research, DSR, Jeddah, Saudi Arabia.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

MAE, AB, JAM and MHAZ designed the study. MAE, AB, MA, AA and PNP performed the histopathological, statistical and bioinformatics analyses. MAE, AB, JAM and AZ were responsible for collecting breast cancer tumor samples and the preparation of the tissue microarray slides. MAE, AB, MA, AA and PNP conducted data curation. MAE, AB and MA drafted the manuscript. MAE, PNP and AZ reviewed and edited the manuscript. MAE was responsible for study supervision and project administration. All authors read and approved the final manuscript. AB and PNP confirm the authenticity of all the raw data.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Center of Excellence in Genomic Medicine Research, King Abdulaziz University (Approval no. 012-CEGMR-ETH), and was conducted in accordance with The Declaration of Helsinki. Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Abu-Elmagd M, Assidi M, Alrefaei AF and Rebai A: Editorial: Advances in genomic and genetic tools, and their applications for understanding embryonic development and human diseases. Front Cell Dev Biol 10: 1016400, 2022.
2. World Health Organization: Global status report on noncommunicable diseases 2010, 2010. https://www.who.int/nmh/publications/ncd_report_full_en.pdf.
3. World Health Organization: Fact sheets 2018, 2018. https://www.who.int/news-room/factsheets/detail/cancer.
4. Alqahtani WS, Almufareh NA, Domiaty DM, Albasher G, Alduwish MA, Alkhalaf H, Almuzzaini B, Al-Marshidy SS, Alfraihi R, Elasbali AM, *et al*: Epidemiology of cancer in Saudi Arabia thru 2010-2019: A systematic review with constrained meta-analysis. AIMS Public Health 7: 679-696, 2020.
5. Unger-Saldaña K: Challenges to the early diagnosis and treatment of breast cancer in developing countries. World J Clin Oncol 5: 465-477, 2014.
6. Barrios CH: Global challenges in breast cancer detection and treatment. Breast 62 (Suppl 1): S3-S6, 2022.
7. World Health Organization: Cancer control: Knowledge into action: WHO guide for effective programmes: Module 3: Early detection. Geneva: World Health Organization, 2007.
8. Song K and Farzaneh M: Signaling pathways governing breast cancer stem cells behavior. Stem Cell Res Ther 12: 245, 2021.
9. Yousefnia S, Seyed Forootan F, Seyed Forootan S, Nasr Esfahani MH, Gure AO and Ghaedi K: Mechanistic pathways of malignancy in breast cancer stem cells. Front Oncol 10: 452, 2020.
10. Xu X, Zhang M, Xu F and Jiang S: Wnt signaling in breast cancer: Biological mechanisms, challenges and opportunities. Mol Cancer 19: 165, 2020.
11. Kontomanolis EN, Kalagasidou S, Pouliliou S, Anthoulaki X, Georgiou N, Papamanolis V and Fasoulakis ZN: The notch pathway in breast cancer progression. TScientificWorldJournal 2018: 2415489, 2018.
12. Arnold SF, Tims E and McGrath BE: Identification of bone morphogenetic proteins and their receptors in human breast cancer cell lines: Importance of BMP2 . Cytokine 11: 1031-1037, 1999.
13. Furth PA: STAT signaling in different breast cancer sub-types. Mol Cell Endocrinol 382: 612-615, 2014.
14. Yip CH and Rhodes A: Estrogen and progesterone receptors in breast cancer. Future Oncol 10: 2293-2301, 2014.
15. Krishnamurti U and Silverman JF: HER2 in breast cancer: A review and update. Adv Anat Pathol 21: 100-107, 2014.
16. Mirzoeva OK, Das D, Heiser LM, Bhattacharya S, Siwak D, Gendelman R, Bayani N, Wang NJ, Neve RM, Guan Y, *et al*: Basal subtype and MAPK/ERK kinase (MEK)-phosphoinositide 3-kinase feedback signaling determine susceptibility of breast cancer cells to MEK inhibition. Cancer Res 69: 565-572, 2009.
17. Mohapatra P, Preet R, Das D, Satapathy SR, Siddharth S, Choudhuri T, Wyatt MD and Kundu CN: The contribution of heavy metals in cigarette smoke condensate to malignant transformation of breast epithelial cells and in vivo initiation of neoplasia through induction of a PI3K-AKT-NFκB cascade. Toxicol Appl Pharmacol 274: 168-179, 2014.
18. Xu X, Zhang L, He X, Zhang P, Sun C, Xu X, Lu Y and Li F: TGF-β plays a vital role in triple-negative breast cancer (TNBC) drug-resistance through regulating stemness, EMT and apoptosis. Biochem Biophys Res Commun 502: 160-165, 2018.
19. Bhateja P, Cherian M, Majumder S and Ramaswamy B: The Hedgehog signaling pathway: A viable target in breast cancer? Cancers (Basel) 11: 1126, 2019.
20. Wu L and Yang X: Targeting the hippo pathway for breast cancer therapy. Cancers (Basel) 10: 422, 2018.
21. Bhatelia K, Singh K and Singh R: TLRs: Linking inflammation and breast cancer. Cell Signal 26: 2350-2357, 2014.
22. González-Reyes S, Marín L, González L, González LO, del Casar JM, Lamelas ML, González-Quintana JM and Vizoso FJ: Study of TLR3, TLR4 and TLR9 in breast carcinomas and their association with metastasis. BMC Cancer 10: 665, 2010.
23. Roychowdhury A, Jondhale M, Saldanha E, Ghosh D, Kumar Panda C, Chandrani P and Mukherjee N: Landscape of toll-like receptors expression in tumor microenvironment of triple negative breast cancer (TNBC): Distinct roles of TLR4 and TLR8. Gene 792: 145728, 2021.
24. Niu T, Zhang W and Xiao W: MicroRNA regulation of cancer stem cells in the pathogenesis of breast cancer. Cancer Cell Int 21: 31, 2021.
25. Taciak B, Pruszynska I, Kiraga L, Bialasek M and Krol M: Wnt signaling pathway in development and cancer. J Physiol Pharmacol 69, 2018.
26. Klaus A and Birchmeier W: Wnt signalling and its impact on development and cancer. Nat Rev Cancer 8: 387-398, 2008.
27. Clevers H: Wnt/beta-catenin signaling in development and disease. Cell 127: 469-480, 2006.
28. Yin P, Wang W, Zhang Z, Bai Y, Gao J and Zhao C: Wnt signaling in human and mouse breast cancer: Focusing on Wnt ligands, receptors and antagonists. Cancer Sci 109: 3368-3375, 2018.
29. Zhang X, Zhang R, Hou C, He R, Wang QS, Zhou TH, Li XQ, Zhai QL and Feng YM: FOXF2 oppositely regulates stemness in luminal and basal-like breast cancer cells through the Wnt/beta-catenin pathway. J Biol Chem 298: 102082, 2022.
30. Huang C, Ye Z, Wan J, Liang J, Liu M, Xu X and Li L: Secreted frizzled-related protein 2 is associated with disease progression and poor prognosis in breast cancer. Dis Markers 2019: 6149381, 2019.
31. Klopocki E, Kristiansen G, Wild PJ, Klaman I, Castanos-Velez E, Singer G, Stöhr R, Simon R, Sauter G, Leibiger H, *et al*: Loss of SFRP1 is associated with breast cancer progression and poor prognosis in early stage tumors. Int J Oncol 25: 641-649, 2004.
32. Saitoh T, Hirai M and Katoh M: Molecular cloning and characterization of human frizzled-8 gene on chromosome 10p11.2. Int J Oncol 18: 991-996, 2001.
33. Yin S, Xu L, Bonfil RD, Banerjee S, Sarkar FH, Sethi S and Reddy KB: Tumor-initiating cells and FZD8 play a major role in drug resistance in triple-negative breast cancer. Mol Cancer Ther 12: 491-498, 2013.
34. Chen W, Liu Z, Mai W, Xiao Y, You X and Qin L: FZD8 indicates a poor prognosis and promotes gastric cancer invasion and metastasis via B-catenin signaling pathway. Ann Clin Lab Sci 50: 13-23, 2020.
35. Li Q, Ye L, Zhang X, Wang M, Lin C, Huang S, Guo W, Lai Y, Du H, Li J, *et al*: FZD8, a target of p53, promotes bone metastasis in prostate cancer by activating canonical Wnt/β-catenin signaling. Cancer Lett 402: 166-176, 2017.
36. Murillo-Garzón V, Gorroño-Etxebarria I, Åkerfelt M, Puustinen MC, Sistonen L, Nees M, Carton J, Waxman J andf Kypta RM: Frizzled-8 integrates Wnt-11 and transforming growth factor-β signaling in prostate cancer. Nat Commun 9: 1747, 2018.

37. Wang K, Li Y, Wang J, Chen R and Li J: A novel 12-gene signature as independent prognostic model in stage IA and IB lung squamous cell carcinoma patients. Clin Transl Oncol 23: 2368-2381, 2021.
38. Jiang Q, He M, Guan S, Ma M, Wu H, Yu Z, Jiang L, Wang Y, Zong X, Jin F and Wei M: MicroRNA-100 suppresses the migration and invasion of breast cancer cells by targeting FZD-8 and inhibiting Wnt/β-catenin signaling pathway. Tumour Biol 37: 5001-5011, 2016.
39. World Medical Association: World medical association declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA 310: 2191-2194, 2013.
40. Schauer A, Korabiowska M, Kellner S, Schumacher M, Sauer R, Bojar H and Rauschecker H: Grading system for breast cancer. Anticancer Res 18: 2139-2144, 1998.
41. Al-Maghrabi J, Emam E, Gomaa W, Saggaf M, Buhmeida A, Al-Qahtani M and Al-Ahwal M: c-MET immunostaining in colorectal carcinoma is associated with local disease recurrence. BMC Cancer 15: 676, 2015.
42. Nedjadi T, Al-Maghrabi J, Assidi M, Dallol A, Al-Kattabi H, Chaudhary A, Al-Sayyad A, Al-Ammari A, Abuzenadah A, Buhmeida A and Al-Qahtani M: Prognostic value of HER2 status in bladder transitional cell carcinoma revealed by both IHC and BDISH techniques. BMC Cancer 16: 653, 2016.
43. Lipponen PK and Collan Y: Simple quantitation of immunohistochemical staining positivity in microscopy for histopathology routine. Acta Stereol 11: 125-132, 1992.
44. Buhmeida A, Elzagheid A, Algars A, Collan Y, Syrjänen K and Pyrhönen S: Expression of the cell-cell adhesion molecule beta-catenin in colorectal carcinomas and their metastases. APMIS 116: 1-9, 2008.
45. Lipponen P and Collan Y: Simple quantitation of immuno-histochemical staining positivity in microscopy. Acta Stereol 11: 125-132, 1992.
46. Maubant S, Tesson B, Maire V, Ye M, Rigaill G, Gentien D, Cruzalegui F, Tucker GC, Roman-Roman S and Dubois T: Transcriptome analysis of Wnt3a-treated triple-negative breast cancer cells. PLoS One 10: e0122333, 2015.
47. Maire V, Baldeyron C, Richardson M, Tesson B, Vincent-Salomon A, Gravier E, Marty-Prouvost B, De Koning L, Rigaill G, Dumont A, *et al*: TTK/hMPS1 is an attractive therapeutic target for triple-negative breast cancer. PLoS One 8: e63712, 2013.
48. Pushparaj PN, Kalamegam G, Wali Sait KH and Rasool M: Decoding the role of astrocytes in the entorhinal cortex in Alzheimer's disease using high-dimensional single-nucleus RNA sequencing data and next-generation knowledge discovery methodologies: Focus on drugs and natural product remedies for dementia. Front Pharmacol 12: 720170, 2022.
49. Lewis BP, Burge CB and Bartel DP: Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120: 15-20, 2005.
50. Quillet A, Saad C, Ferry G, Anouar Y, Vergne N, Lecroq T and Dubessy C: Improving bioinformatics prediction of microRNA targets by ranks aggregation. Front Genet 10: 1330, 2020.
51. AlSaleh KA: Efficacy of breast cancer screening program in Kingdom of Saudi Arabia. Saudi Med J 43: 428-430, 2022.
52. Katoh M: Networking of WNT, FGF, Notch, BMP, and Hedgehog signaling pathways during carcinogenesis. Stem Cell Rev 3: 30-38, 2007.
53. Oakes SR, Gallego-Ortega D and Ormandy CJ: The mammary cellular hierarchy and breast cancer. Cell Mol Life Sci 71: 4301-4324, 2014.
54. Kendrick H, Regan JL, Magnay FA, Grigoriadis A, Mitsopoulos C, Zvelebil M and Smalley MJ: Transcriptome analysis of mammary epithelial subpopulations identifies novel determinants of lineage commitment and cell fate. BMC Genomics 9: 591, 2008.
55. Alexander CM, Goel S, Fakhraldeen SA and Kim S: Wnt signaling in mammary glands: Plastic cell fates and combinatorial signaling. Cold Spring Harb Perspect Biol 4: a008037, 2012.
56. Wang HQ, Xu ML, Ma J, Zhang Y and Xie CH: Frizzled-8 as a putative therapeutic target in human lung cancer. Biochem Biophys Res Commun 417: 62-66, 2012.
57. Salsano E, Paterra R, Figus M, Menghi F, Maderna E, Pollo B, Solero CL, Massimi L and Finocchiaro G: Expression profile of frizzled receptors in human medulloblastomas. J Neurooncol 106: 271-280, 2012.
58. Yang Q, Wang Y, Pan X, Ye J, Gan S, Qu F, Chen L, Chu C, Gao Y and Cui X: Frizzled 8 promotes the cell proliferation and metastasis of renal cell carcinoma. Oncotarget 8: 78989-79002, 2017.
59. Wang J, Pang W, Zuo Z, Zhang W and He W: MicroRNA-520b suppresses proliferation, migration, and invasion of spinal osteosarcoma cells via downregulation of frizzled-8. Oncol Res 25: 1297-1304, 2017.
60. Xu L, Wen T, Liu Z, Xu F, Yang L, Liu J, Feng G and An G: MicroRNA-375 suppresses human colorectal cancer metastasis by targeting frizzled 8. Oncotarget 7: 40644-40656, 2016.
61. Waks AG and Winer EP: Breast cancer treatment: A review. JAMA 321: 288-300, 2019.
62. Bianchini G, De Angelis C, Licata L and Gianni L: Treatment landscape of triple-negative breast cancer-expanded options, evolving needs. Nat Rev Clin Oncol 19: 91-113, 2022.
63. Kirikoshi H, Sekihara H and Katoh M: Expression profiles of 10 members of frizzled gene family in human gastric cancer. Int J Oncol 19: 767-771, 2001.
64. Hu X, Zhang Q, Xing W and Wang W: Role of microRNA/lncRNA intertwined with the Wnt/β-catenin axis in regulating the pathogenesis of triple-negative breast cancer. Front Pharmacol 13: 814971, 2022.
65. Wang Y, Chen L, Wu Z, Wang M, Jin F, Wang N, Hu X, Liu Z, Zhang CY, Zen K, *et al*: miR-124-3p functions as a tumor suppressor in breast cancer by targeting CBL. BMC Cancer 16: 826, 2016.
66. Zhao X, Bai X, Li W, Gao X, Wang X and Li B: microRNA-506-3p suppresses the proliferation of triple negative breast cancer cells via targeting SNAI2. Mol Cell Toxicol 17: 513-522, 2021.
67. Zhang YF, Yu Y, Song WZ, Zhang RM, Jin S, Bai JW, Kang HB, Wang X and Cao XC: miR-410-3p suppresses breast cancer progression by targeting Snail. Oncol Rep 36: 480-486, 2016.
68. Long X, Shi Y, Ye P, Guo J, Zhou Q and Tang Y: MicroRNA-99a suppresses breast cancer progression by targeting FGFR3. Front Oncol 9: 1473, 2020.
69. Lin S, Zhao M, Lv Y, Mao G, Ding S and Peng F: The lncRNA GATA3-AS1/miR-495-3p/CENPU axis predicts poor prognosis of breast cancer via the PLK1 signaling pathway. Aging (Albany NY) 13: 13663-13679, 2021.
70. Zou Y, Zheng S, Xiao W, Xie X, Yang A, Gao G, Xiong Z, Xue Z, Tang H and Xie X: circRAD18 sponges miR-208a/3164 to promote triple-negative breast cancer progression through regulating IGF1 and FGF2 expression. Carcinogenesis 40: 1469-1479, 2019.
71. Gujrati H, Ha S, Waseem M and Wang BD: Downregulation of miR-99b-5p and upregulation of nuclear mTOR cooperatively promotes the tumor aggressiveness and drug resistance in African American prostate cancer. Int J Mol Sci 23: 9643, 2022.
72. Gong Y, He T, Yang L, Yang G, Chen Y and Zhang X: The role of miR-100 in regulating apoptosis of breast cancer cells. Sci Rep 5: 11650, 2015.
73. Wang W, Liu Y, Guo J, He H, Mi X, Chen C, Xie J, Wang S, Wu P, Cao F, *et al*: miR-100 maintains phenotype of tumor-associated macrophages by targeting mTOR to promote tumor metastasis via Stat5a/IL-1ra pathway in mouse breast cancer. Oncogenesis 7: 97, 2018.
74. Assidi M, Buhmeida A, Al-Zahrani MH, Al-Maghrabi J, Rasool M, Naseer MI, Alkhatabi H, Alrefaei AF, Zari A, Elkhatib R, *et al*: The prognostic value of the developmental gene FZD6 in young Saudi breast cancer patients: A biomarkers discovery and cancer inducers OncoScreen approach. Front Mol Biosci 9: 783735, 2022.
75. Zeng CM, Chen Z and Fu L: Frizzled receptors as potential therapeutic targets in human cancers. Int J Mol Sci 19: 1543, 2018.