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Impact of Water-Pipe Smoking on Gene Expression Linked to Breast Cancer Progression and Prognosis

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Abstract

Water-pipe smoking (WPS), a common form of tobacco use, is particularly prevalent among young women in the Middle East. The smoke produced by WPS contains harmful substances similar to those found in cigarettes and is often associated with an increased risk of various cancers, including breast cancer. However, the specific genes affected by WPS and the mechanisms driving cancer initiation and progression, particularly in breast cancer, remain largely unknown. This study investigated the effects of chronic WPS exposure on normal human mammary epithelial cells. We analyzed the differential expression of genes using the NanoString nCounter PanCancer pathways panel, which includes 770 gene transcripts, and supplemented this with quantitative real-time polymerase chain reaction (PCR) analysis. The NanoString analysis revealed that 13 genes were significantly dysregulated due to WPS exposure. These genes are involved in various cellular processes, including signal transduction, cell cycle regulation, cell motility, proliferation, migration, invasion, and inflammation. Further, in silico analysis revealed that several of these genes were associated with breast cancer prognosis and were upregulated in breast cancer tissues compared to normal tissues. Notably, Kaplan-Meier survival analysis highlighted a strong correlation between dysregulation of WPS-related genes (MX1, CCL8, GNGT1, and MMP9) and relapse-free survival in breast cancer patients. The findings suggest that WPS exposure can significantly alter the expression of critical genes involved in breast cancer development and prognosis, suggesting its potential role in influencing breast cancer outcomes.

Keywords: Breast cancer, Gene dysregulation, Mammary epithelial cells, Smoking, Water-pipe

Introduction

Smoking tobacco is a modifiable risk factor for numerous chronic diseases, such as respiratory conditions, diabetes, cardiovascular diseases, and various cancers, and is a significant contributor to increasing mortality rates globally [1, 2]. There are several forms of tobacco consumption, including water-pipe smoking (WPS) [3, 4], cigarettes, cigars, and electronic cigarettes (e-cigarettes). Recently, WPS and e-cigarettes have gained popularity worldwide, especially among youth and women [5], due to their social appeal and recreational use [3, 6], with WPS emerging as a particularly prevalent choice [7]. Approximately 100 million individuals globally regularly engage in water-pipe smoking, contributing to nearly 5 million deaths annually [8].

Water-pipe smoking is especially popular in the Middle East and among Middle Eastern communities in Western countries [5], where it is deeply woven into cultural practices, further fueling its rise in these regions. In WPS, air heated by charcoal passes through flavored tobacco, producing smoke that contains toxic substances similar to those found in cigarette smoke, including carbon

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monoxide, nicotine, tar, and other harmful compounds [9-11]. Notably, the plasma nicotine levels in individuals who smoke water pipes daily are comparable to those of individuals who smoke ten cigarettes a day [12, 13]. While some believe that WPS is less harmful than cigarette smoking, research shows that both forms of tobacco use share severe health risks, such as nicotine dependence and an increased likelihood of developing several serious diseases [14-18]. Furthermore, WPS has been shown to cause significant harm to embryonic development, leading to complications during early pregnancy [19].

Previous studies have demonstrated that exposure to water-pipe smoking (WPS) plays a significant role in the development of different cancers [20-23], including those of the head, neck, oral cavity, and breast. Long-term WPS exposure has been shown to cause changes in genes responsible for DNA repair [24, 25], stability, detoxification, and xenobiotic metabolism, which collectively increase cancer risk. Additionally, WPS exposure can trigger the epithelial-to-mesenchymal transition, enhancing the invasive potential of breast cancer cells through Erk1/Erk2 signaling pathways. However, it is crucial to note that the impact of WPS on gene alterations in normal mammary tissue, which could contribute to the initiation and progression of breast cancer, remains unexplored [26-30]. In this study, we aimed to investigate the effects of chronic WPS exposure on a range of well-established carcinogenesis-related genes and associated molecular pathways in normal mammary epithelial cells [22].

Materials and Methods

Protocol for water-pipe smoking and solution preparation

For this study, the Aleppo method, a well-established water-pipe smoking procedure, was employed. Briefly, 10 g of a tobacco mixture was placed into the water-pipe head and covered with aluminum foil to facilitate airflow. Quick-lighting charcoal blocks were ignited and positioned above the tobacco mixture to begin the smoking process. After 60 minutes of smoking, the condensate was collected using laboratory-grade filter paper attached to the mouthpiece. The filters were dried and weighed both before and after collection. The collected smoke particles were then dissolved in phosphate-buffered saline (PBS) or keratinocyte serum-free medium (KSFM) (1×) (Gibco®, Life Technologies, Burlington, ON, Canada) at a concentration of 20 mg/mL. The smoking particulates were filtered using 0.45 µm filters (Costar, Washington, DC, USA) to produce the final WPS extract.

Cell culture

Human normal mammary epithelial (HNME) cells were cultured in KSFM (1×) (Gibco®, Life Technologies, Burlington, ON, Canada) with the addition of heregulin (5 ng/mL), bovine pituitary extract (BPE) (5 mg/100 mL) (Life Technologies, Burlington, ON, Canada), and penicillin-streptomycin (100 µg/mL) (Invitrogen, Life Technologies, Burlington, ON, Canada). These cells were exposed to 150 µg/mL of WPS, dissolved in either PBS or KSFM, for 48 hours. The cultures were maintained in a controlled environment at 37 °C with 5% CO2 and high humidity.

NanoString gene expression profiling

To assess gene expression, we utilized the NanoString PanCancer Pathways Panel (NanoString Technologies, Seattle, WA, USA), which targets 770 genes involved in cancer ways extracted from The Cancer Genome Atlas. The data (RCC files) generated from NanoString were normalized using the established protocols from the nSolver User Manual. The data was first normalized using the mean of housekeeping genes and then log2-transformed for further analysis, which was conducted using Microsoft Excel.

To identify differentially expressed genes (DEGs), we applied a fold-change threshold of 1.5 or 2 and a significance level of $P < 0.05$, in line with standards used in previous studies. Genes that met these criteria were selected for further examination.

RNA extraction and real-time pcr analysis

Total RNA from both WPS-exposed and non-exposed HNME cells was extracted using the RNeasy Mini Kit following the methods we previously described. First-strand cDNA synthesis was made using the 5X All-In-One MasterMix (MasterMix-LR, Diamed, Mississauga, Ontario, Canada), following the manufacturer's instructions. Quantitative reverse transcriptase real-time PCR (qRT-PCR) was performed with iTaq Universal SYBR Green Supermix (BioRad, Hercules, CA, USA). The primers used for PCR amplification were carried out

using Primer ExpressTM Software v3.0.1 (ThermoFisher Scientific, Franklin, MA, USA) as shown in **Table 1**.

Table 1. List of primer sequences used for reverse transcriptase real-time polymerase chain reaction

Gene	Forward (5'-3')	Reverse (3'-5')
CCL5	GGTGCCAGCAAGATAACCCT	GCTTGCCTGACTTCCTCCTT
MXI	AGGTTCCAGTAGGGCATGTG	TTGGAAAGAAGGTGCTTGCT
CCL21	CTGGACAAGACACCATCCCC	TGTACTGGGGAGCCGTATCA
IFNγ	CTCATGTAAGCCCCCAGAAA	GCCCAGTTCCTGCAGAGTAG
ALOX5	ACTTCGCCGACTTTGAGAAA	CAAGGGTGACCACAGTGATG
CCL8	GCCGCAGAGTTCAATAGAGG	CACGTTAAAGCAGCAGGTGA
GNGT1	CAGGCACCTTCAAAACCAAT	CCAGGAAGCATTTGTCAGGT
MMP9	GTCTTGTGGAGGCTTTGAGC	CAGGGATCTCCCCTCCTTAG
TNFSF14	CTGCAAAGCAGGGATAAAGC	GTAGAGGTGGGGGTCTCACA
PTGR1	GAAAGTCAGGTAGGGCCACA	TCCCTCTCTTTTGCCTCTCA
CCL4	GCTAAATCCAGTGGGTGGAA	GCTTGCTTCTTTTGGTTTGG
IL3	GTAGAGACGGGGTTTCACCA	GGCACAGGCCTAGAAGTGAG
TLR9	CAGCAGCTCTGCAGTACGTC	AAGGCCAGGTAATTGTCACG

In silico analysis and gene profiling

The differentially expressed genes (DEGs) identified from the NanoString analysis were further validated using in silico methods. For this purpose, we accessed the Oncomine TM database accessed November 14, 2020), a comprehensive public resource that includes approximately 65 gene expression datasets [31]. We focused on the TCGA Finak and Zhao datasets to examine the mRNA expression of the found DEGs in normal versus malignant tissues. Additionally, the Bittner breast cancer dataset was utilized to compare the log2 median intensity between smoker and non-smoker breast cancer patients. The expression levels were determined by adjusting parameters, and the program provided the respective data for each dataset. Genes with significant differential expression were selected based on statistical criteria.

To assess the clinical relevance of the DEGs, we utilized the PanCancer RNA-seq dataset from the Kaplan–Meier plotter database to investigate the correlation between gene expression and patient outcomes in breast cancer [32].

Additionally, we leveraged the GOBO database [33], which includes data from 1,881 breast cancer samples categorized according to PAM50 or Hu subtypes, to study the association between WPS-dysregulated genes and specific breast cancer molecular subtypes. Expression levels were visualized using boxplots, where the median expression is indicated by a line within the box, and the top and bottom of the box represent the interquartile range. Outliers were marked as individual circles. Statistical significance for gene expression differences across subtypes was determined using an ANOVA test.

Network interaction

To explore the interactions and networks between the WPS-dysregulated genes and their biological functions, we utilized the search tool for the retrieval of interacting genes (STRING v9.1) accessed November 10, 2020 [23]. This database allowed us to identify potential networks linking the altered genes, providing insight into the mechanisms driving breast cancer progression under the influence of water-pipe smoking.

Statistical analysis

All in vitro tests were conducted in triplicate with a minimum of three independent trials. The results are presented as mean values ± standard error of the mean. Statistical significance was assessed using the Student's t-test. Statistical analyses were performed using GraphPad Prism (Version 8.4.3) and nSolver analysis software. To evaluate the relationship between WPS-

dysregulated genes and patient survival, Kaplan–Meier survival analysis was conducted, including relapse-free survival (RFS) and overall survival (OS). A P-value < 0.05 (log-rank test) was considered statistically significant.

Results

Exploring the impact of water-pipe smoking (WPS) on human breast carcinogenesis, we assessed how WPS exposure affects human normal mammary epithelial (HNME) cells. Our results indicated that WPS exposure led to a mild induction of Epithelial-Mesenchymal Transition (EMT), with HNME cells adopting a more mesenchymal phenotype compared to the controls that were not exposed to WPS. As displayed in Figure 1, the exposed cells appeared more elongated and exhibited reduced cell-to-cell contact compared to the unexposed ones. When HNME cells were exposed to 100 µg/mL of WPS solution for forty-eight hours, the regulation of cell proliferation and cell cycle progression were disrupted in comparison to the untreated cells (data not shown).

Further investigation revealed that WPS exposure caused significant gene deregulation linked to breast cancer development. A differential gene expression analysis using the NanoString nCounter PanCancer Pathways Panel, which targets 770 genes associated with cancer pathways, identified 13 differentially expressed genes (DEGs) in the WPS-exposed HNME cells versus the unexposed controls. These genes were CCL5, MX1, CCL21, IFNγ, ALOX5, CCL8, GNGT1, MMP9, TNFSF14, PTGR1, CCL4, IL3, and TLR9, all showing a fold-change of 1.5 or higher with a statistical significance of $P < 0.05$.

To validate these findings, we performed a quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analysis, which confirmed the upregulation of the same 13 genes identified in the NanoString analysis. The gene expression changes ranged from a 1.6-fold to a 24-fold increase in the exposed cells, as depicted in **Figure 2.**

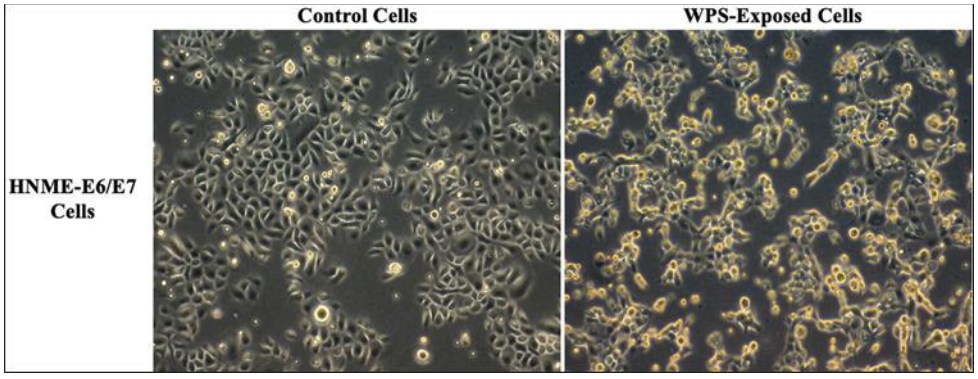


Figure 1. Water-pipe smoking triggers epithelial-mesenchymal transition (EMT) in a human normal mammary epithelial cell line. Exposure to a 100 µg/mL water-pipe smoking solution for 48 hours causes a transformation in cell morphology from an epithelial to a mesenchymal phenotype, indicating the induction of EMT.

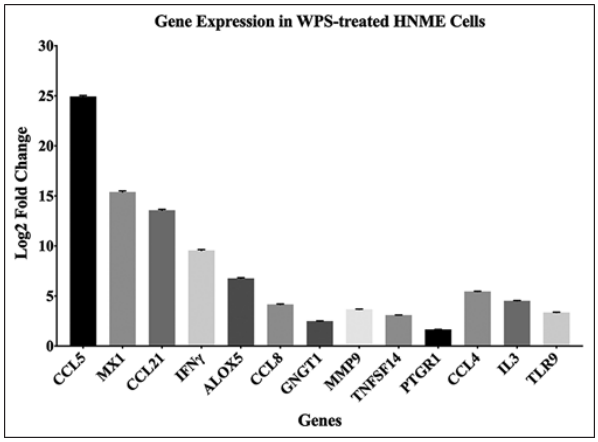


Figure 2. Differentially expressed genes identified by the NanoString PanCancer Pathways Panel. The threshold values used were a 1.5-fold change or higher amongst the different groups and ranged from 1.6 to 24-fold

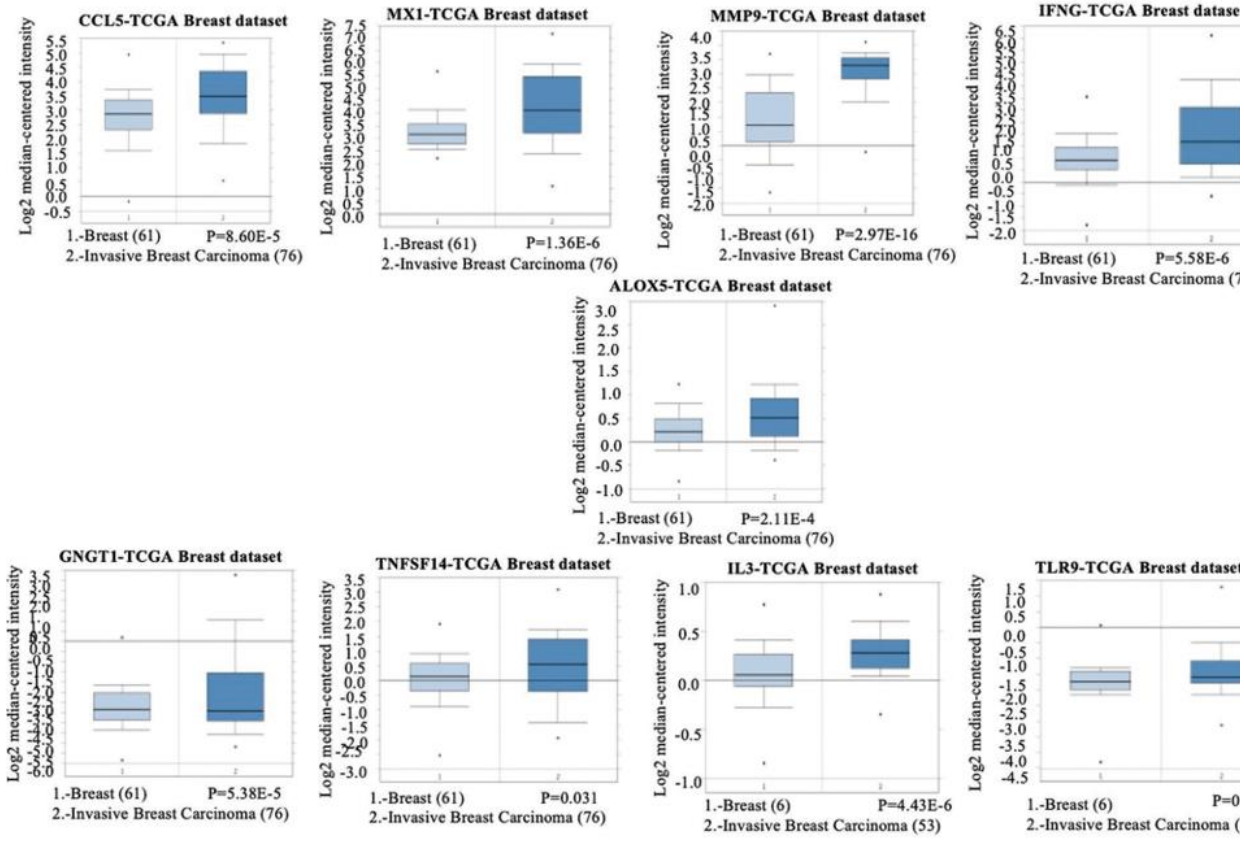
Additionally, through functional annotations and an analysis of the molecular pathways involved in carcinogenesis, we determined that the 13 differentially expressed genes (DEGs) play a direct role in regulating processes such as cell proliferation, cell cycle, survival, migration/invasion, apoptosis, signal transduction, and the inflammatory response **(Table 2)**.

Table 2. Classification of deregulated genes based on their functional annotations

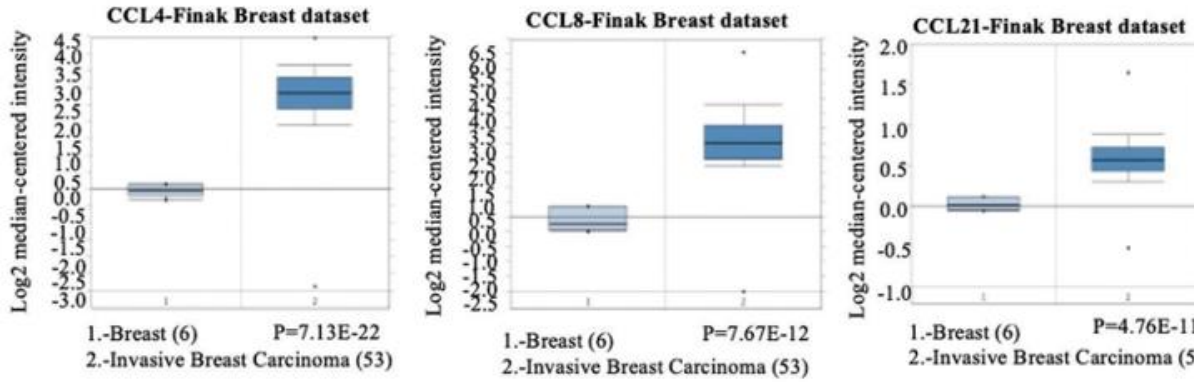
Molecular and cellular functions	Genes
Cellular processes (cell cycle, cell proliferation, cell migration, cell invasion, cell apoptosis and angiogenesis)	IL3, MMP9, TNFSF14
Signal transduction (NF-κB signaling, TLR signaling, cytosolic DNA-sensing, GPCR signaling, Erk1/2 signaling, Ras signaling and PI3K-Akt signaling pathways)	CCL4, CCL5, CCL8, CCL21, GNGT1, IFNγ, MX1, PTGR1, TLR9, TNFSF14
Inflammatory response	ALOX5, CCL4, CCL5, CCL8, IFNγ

To further investigate the relevance of the top differentially expressed genes (DEGs) affected by WPS in our study, we sought to validate their expression in patient samples using an in silico approach. We examined the expression profiles of these DEGs in both normal and invasive breast cancer tissues, utilizing multiple databases from the publicly accessible Oncomine platform.

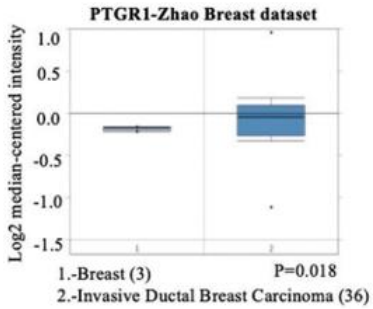
The TCGA dataset, which included 137 patient samples, showed that the use of CCL5 ($P < 0.001$), MX1 ($P < 0.001$), MMP9 ($P < 0.001$), IFNγ ($P < 0.001$), ALOX5 ($P < 0.001$), GNGT1 ($P < 0.001$), TNFSF14 ($P = 0.031$), IL3 ($P < 0.001$), and TLR9 ($P = 0.004$) was significantly elevated in invasive breast carcinoma compared to normal tissue. Similarly, the Finak dataset, comprising 59 patient samples, identified upregulation of CCL4 ($P < 0.001$), CCL8 ($P < 0.001$), and CCL21 ($P < 0.001$) in invasive breast carcinoma. Furthermore, the Zhao dataset, with 39 patient samples, showed that PTGR1 ($P = 0.018$) was expressed in invasive breast cancers **(Supplementary Figure 1c)**.


CCL5-TCGA Breast dataset
Log2 median-centered intensity
1.-Breast (61) P=8.60E-5
2.-Invasive Breast Carcinoma (76)
MX1-TCGA Breast dataset
Log2 median-centered intensity
1.-Breast (61) P=1.36E-6
2.-Invasive Breast Carcinoma (76)
MMP9-TCGA Breast dataset
Log2 median-centered intensity
1.-Breast (61) P=2.97E-16
2.-Invasive Breast Carcinoma (76)
IFNG-TCGA Breast datase
Log2 median-centered intensity
1.-Breast (61) P=5.58E-6
2.-Invasive Breast Carcinoma (7
ALOX5-TCGA Breast dataset
Log2 median-centered intensity
1.-Breast (61) P=2.11E-4
2.-Invasive Breast Carcinoma (76)
GNGT1-TCGA Breast dataset
Log2 median-centered intensity
1.-Breast (61) P=5.38E-5
2.-Invasive Breast Carcinoma (76)
TNFSF14-TCGA Breast dataset
Log2 median-centered intensity
1.-Breast (61) P=0.031
2.-Invasive Breast Carcinoma (76)
IL3-TCGA Breast dataset
Log2 median-centered intensity
1.-Breast (6) P=4.43E-6
2.-Invasive Breast Carcinoma (53)
TLR9-TCGA Breast dataset
Log2 median-centered intensity
1.-Breast (6) P=0
2.-Invasive Breast Carcinoma (

a)


CCL4-Finak Breast dataset
Log2 median-centered intensity
1.-Breast (6) P=7.13E-22
2.-Invasive Breast Carcinoma (53)
CCL8-Finak Breast dataset
Log2 median-centered intensity
1.-Breast (6) P=7.67E-12
2.-Invasive Breast Carcinoma (53)
CCL21-Finak Breast dataset
Log2 median-centered intensity
1.-Breast (6) P=4.76E-1
2.-Invasive Breast Carcinoma (5

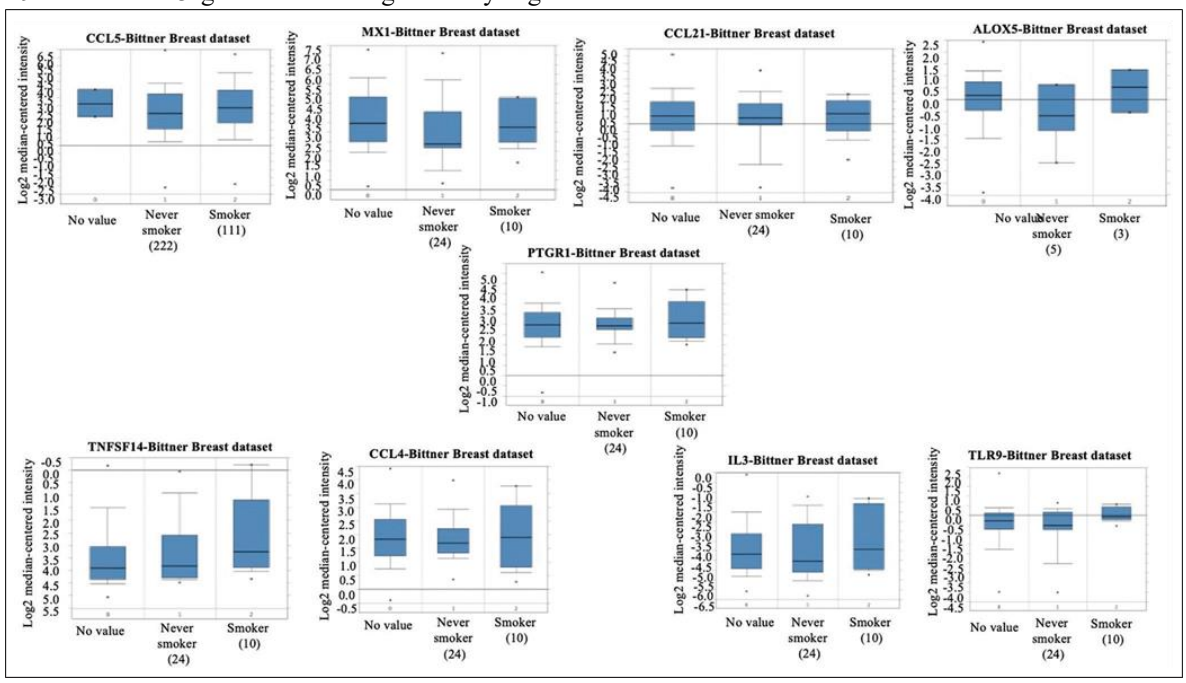
b)


PTGR1-Zhao Breast dataset
Log2 median-centered intensity
1.-Breast (3) P=0.018
2.-Invasive Ductal Breast Carcinoma (36)

c)

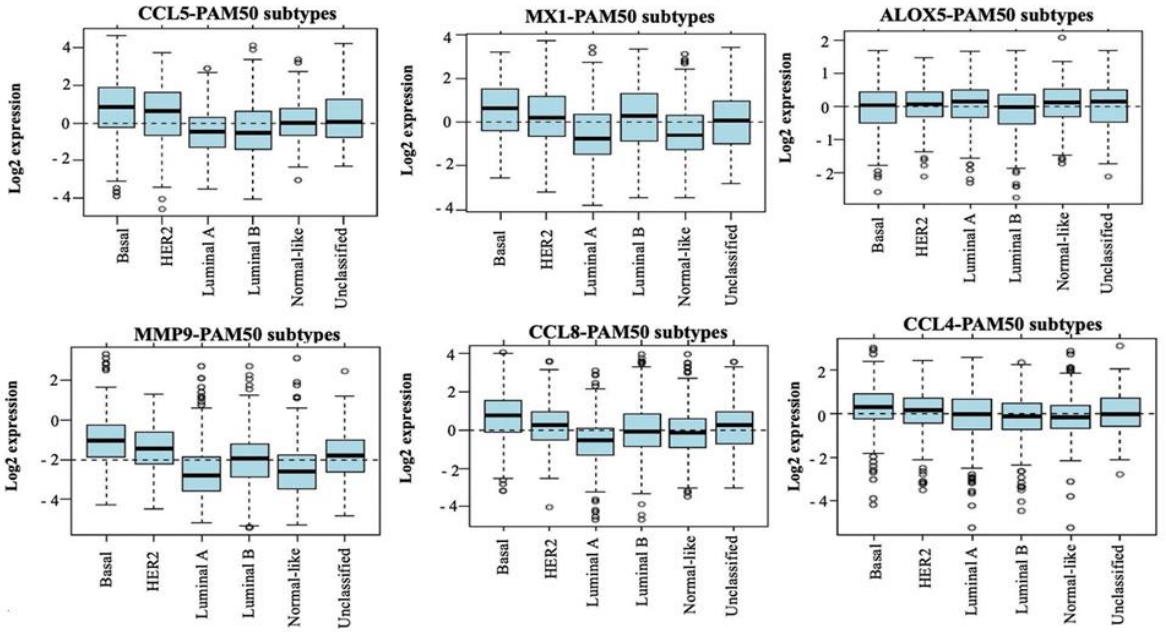
Supplementary Figure 1. mRNA expression levels of water-pipe smoking-deregulated differentially expressed genes in normal tissue compared to invasive breast cancer. Data is presented using (a) the TCGA dataset, (b) the Finak dataset, and (c) the Zhao dataset, all sourced from the Oncomine database.

To further investigate the link between smoking and the expression of genes affected by water-pipe smoking (WPS) in breast cancer, we assessed the levels of thirteen deregulated genes in breast cancer samples from smokers and non-smokers using data from the Bittner breast dataset in the Oncomine database. Our analysis revealed that 9 out of the 13 genes showed significantly higher expression in smokers with breast cancer compared to non-smokers. These upregulated genes included CCL5, MX1, CCL21, ALOX5, PTGR1, TNFSF14, CCL4, IL3, and TLR9 ($P \leq 0.05$). However, data on smoking status for MMP9, IFNγ, GNGT1, and CCL8 were not available in the dataset **(Supplementary Figure 2)**.

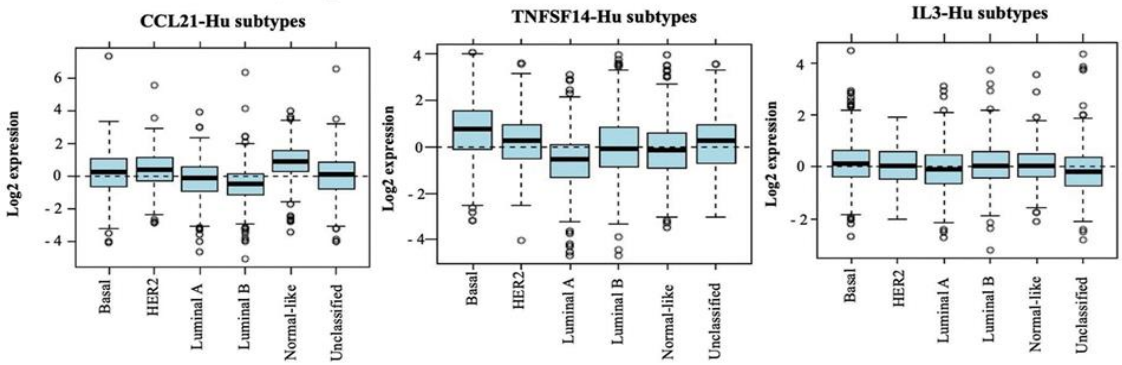


Supplementary Figure 2. DNA copy number of the top water-pipe smoking-deregulated differentially expressed genes in smokers versus never-smoked breast cancer patients, analyzed using the Bittner Breast dataset from the Oncomine database. The central band within the box indicates the median DNA copy number, while the upper and lower edges of the box represent the range between quartile 1 and quartile 3, along with 1.5 times the interquartile range.

To explore the relationship between water-pipe smoking (WPS) deregulated genes and cancer molecular subtypes, we examined the expression of these genes across different subtypes of breast cancer. Using clinical data from the GOBO database, which includes samples from 1,881 patients, we categorized breast cancer into five subtypes: Luminal A, Luminal B, HER2-positive, normal-like, and basal-type. Analysis revealed that several WPS-deregulated genes, such as CCL5, MX1, MMP9, CCL8, and CCL4, were most highly expressed in the basal subtype, known for its aggressive behavior, as shown by the PAM50 classification [Figure 3a]. Additionally, CCL21, TNFSF14, and IL3 also exhibited elevated in the basal subtype based on the Hu classification **(Figure 3b)**.



a)



b)

Figure 3. Expression levels of water-pipe smoking -deregulated genes and their correlation with the molecular subtypes of breast cancer. (a) Water-pipe smoking -deregulated genes and their association with the molecular subtypes of breast cancer according to the PAM50 classification using 1881 breast cancer cases from the GOBO database ($P < 0.0001$). (b) Water-pipe smoking -deregulated genes and their correlation with the molecular subtypes of breast cancer according to the Hu classification using 1881 breast cancer cases from the GOBO database ($P < 0.0001$). The GSA-Tumor tool of the GOBO database automatically stratifies the 1881 cases into equal quantiles based on each gene expression. The band in the middle of the box represents the gene expression median, while the top and bottom of each box represent the distance between quartile 1, and quartile 3 as well as 1.5 times the interquartile range

We then investigated the potential impact of WPS-deregulated genes on the prognosis of cancer patients. Using data from the Kaplan-Meier plotter database, which includes 1,764 breast cancer cases, we examined the relationship between the expression levels of these genes and relapse-free survival (RFS). Our analysis revealed mixed results concerning the connection between gene expression and patient survival. Specifically, genes like MXI ($P = 0.0049$), CCL8 ($P < 0.001$), GNGT1 ($P = 0.012$), and MMP9 ($P = 0.0039$) were significantly linked to a shorter RFS, suggesting a poor prognosis. Conversely, other genes were associated with better survival outcomes, indicating a prolonged survival time **(Figure 4)**. These results underscore the influence of WPS on breast cancer cells, potentially contributing to a more complex tumor phenotype and

poorer prognosis. However, no significant association was found between these genes and overall survival (OS) **(Supplementary Figure 3)**.

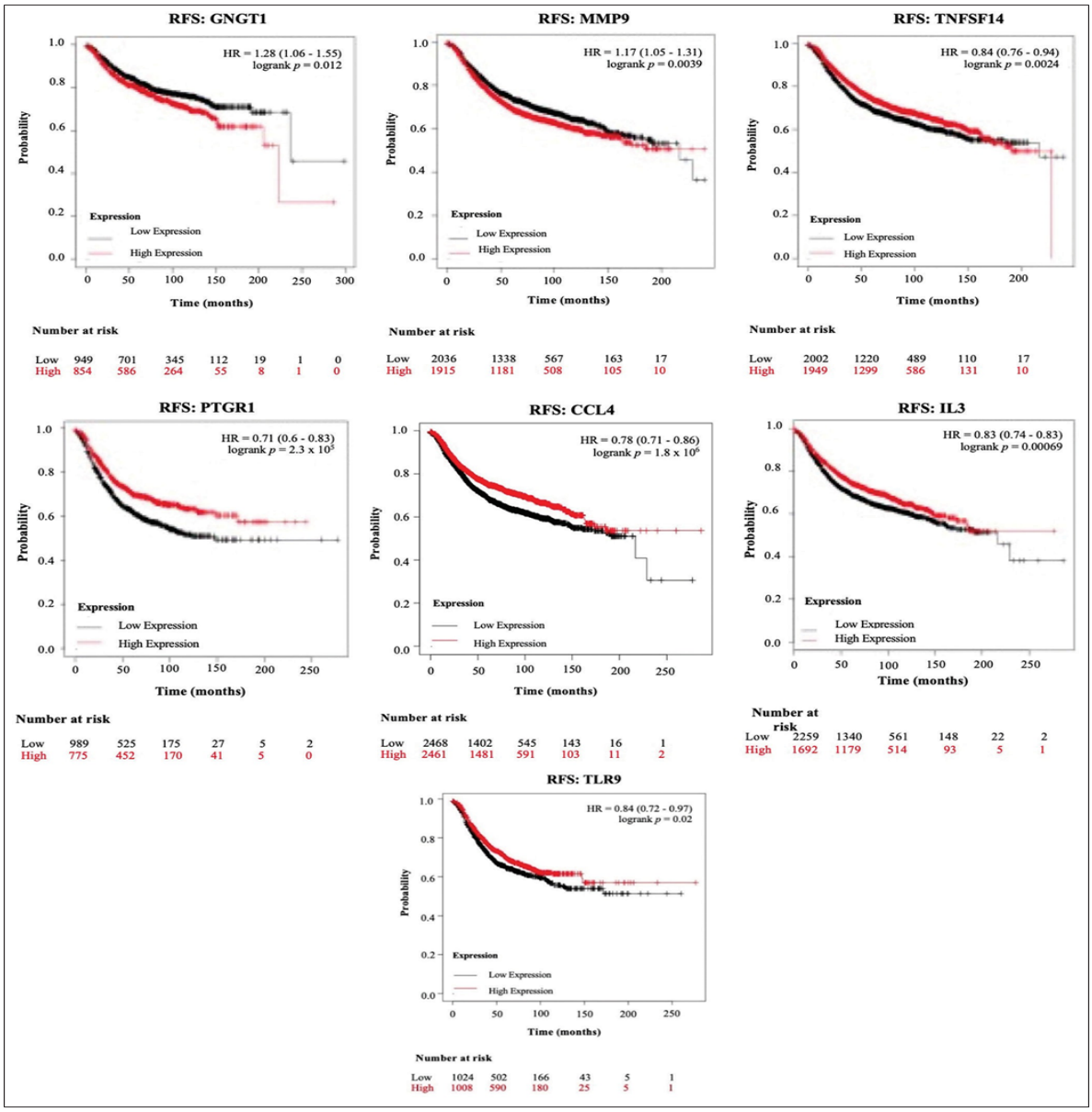
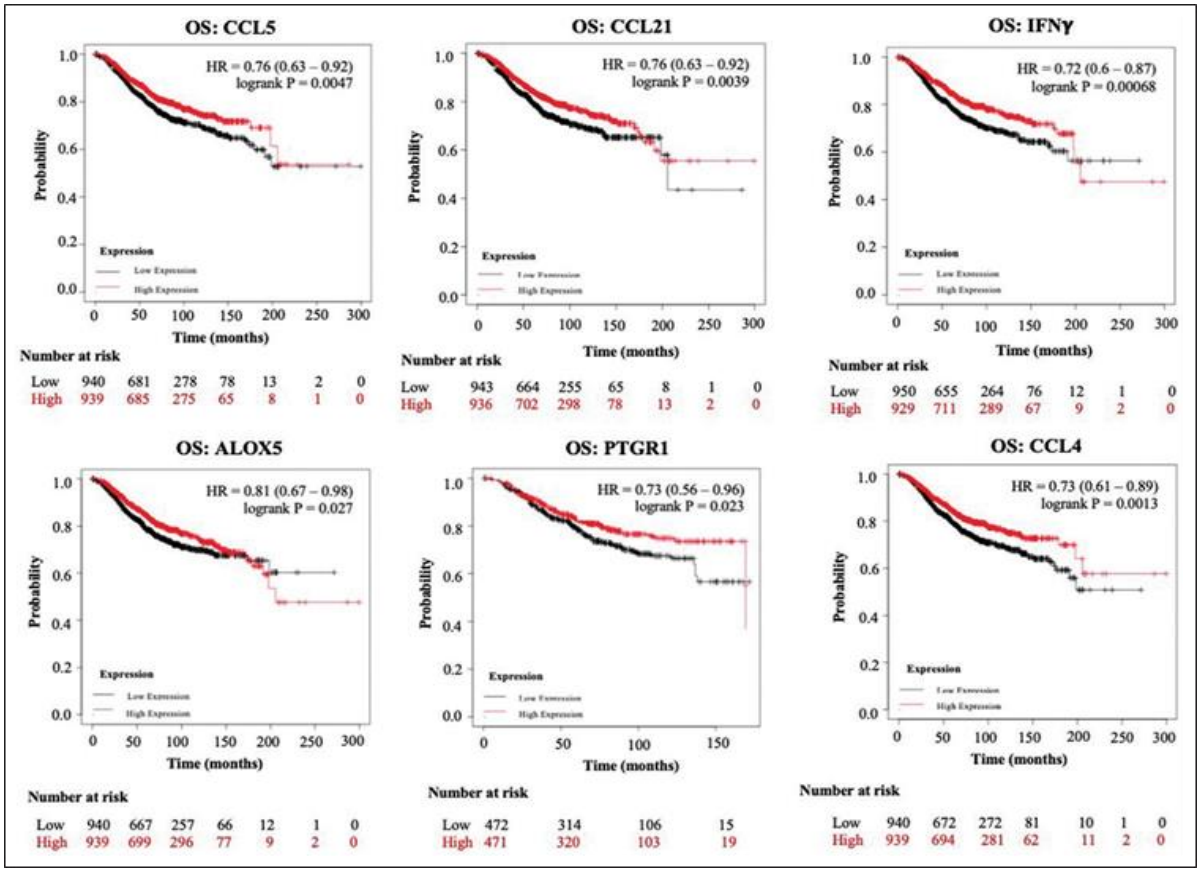


Figure 4. Association between deregulated genes under the effect of water-pipe smoking and prognosis in breast cancer patients using the Kaplan–Meier plotter database expressed by relapse-free survival



Supplementary Figure 3. Association between deregulated genes under the effect of water-pipe smoking and prognosis in breast cancer patients using the Kaplan–Meier plotter database expressed by overall survival

We then explored the potential interactions among the WPS-deregulated DEGs and their involvement in various biological pathways **(Figure 5)**. Our analysis revealed that these genes participated in critical pathways related to signal transduction, ligand binding, and the production of molecules such as lipoxins, leukotrienes, interleukins, and interferons **(Table 3)**. Additionally, these DEGs were implicated in molecular functions, including chemokine and cytokine receptor binding, as well as activities related to phospholipases, phosphotransferases, and kinases with catalytic roles **(Table 3)**.

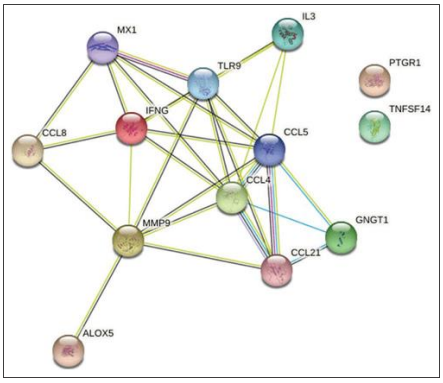


Figure 5. Schematic protein interaction analysis of water-pipe smoking-deregulated genes using the Search Tool for the Retrieval of Interacting Genes (STRING v9.1). The enriched biological process and molecular functions of those proteins are included.

Table 3. Functional annotations of the differentially expressed genes

Reactome Pathways[a]				
Pathway	**Description**	**Count in the network**	**Strength**	**P-value**
HAS-2142700	Synthesis of Lipoxins	2 of 6	2.7	0.00036
HAS-2142691	Synthesis of Leukotriens	2 of 21	2.16	0.0020
HAS-6783783	Interleukin-10 signaling	2 of 45	1.83	0.00068
HAS-380108	Chemokine receptor bind	2 of 48	1.8	0.0068
HAS-6785807	Interleukin-4 and 13 signaling	2 of 106	1.45	0.0257
HAS-449147	Signaling by interleukins	6 of 439	1.31	1.25×10^{-5}
HAS-1280215	Cytokine signaling in the immune system	8 of 654	1.27	2.27×10^{-7}
HAS-913531	Interferon signaling	2 of 189	1.2	0.0478
HAS-418594	G alpha (i) signaling	4 of 387	1.19	0.0020
HAS-500792	GPCR ligand binding	3 of 443	1.01	0.0295
HAS-162582	Signal transduction (NF-κB signaling, TLR signaling, cytosolic DNA-sensing, GPCR signaling, Erk1/2 signaling, Ras signaling, and PI3K-Akt signaling pathways)	6 of 2605	0.54	0.0398
Molecular function[b]				
GO term	**Description**	**Count in the network**	**Strength**	**P-value**
GO: 0031726	CCR1 chemokine receptor binding	2 of 6	2.7	0.00017
GO: 0031730	CCR5 chemokine receptor binding	2 of 7	2.63	0.00020
GO: 0016004	Phospholipase activator activity	2 of 11	2.44	0.00036
GO: 0005149	Inteleukine-1 receptor binding	2 of 18	2.22	0.00071
GO: 0048020	CCR chemokine receptor binding	4 of 41	2.17	4.73×10^{-7}
GO: 0008009	Chemokine activity	4 of 48	2.1	7.15×10^{-7}
GO: 0005125	Cytokine activity	7 of 216	1.69	2.53×10^{-9}
GO: 0005126	Cytokine receptor binding	8 of 272	1.65	2.64×10^{-10}
GO: 0016773	Phosphotransferase activity	4 of 767	0.89	0.0104
GO: 0016301	Kinase activity	4 of 835	0.086	0.0135
GO: 0004672	Protein kinase activity	3 of 635	0.85	0.0463
GO: 0042802	Identical protein binding	6 of 1754	0.71	0.0044
GO: 0003824	Catalytic activity	9 of 5592	0.38	0.0193
GO: 0005515	Protein binding	10 of 6607	0.36	0.0135

[a]Enlisted Reactome pathways involved in the interaction. In the table count network represents how many proteins in the network from the total are annotated with a particular term. Strength represents how large the enrichment effect is (Log10 (observed/expected), [b]Enlisted molecular functions of the network interaction.

Discussion

This research represents the first known investigation into cancer gene expression profiling induced by WPS in HNME cells. Our results are in line with previous studies, which have shown that WPS promotes EMT progression and enhances the invasive potential of breast cancer cells via the Erk1/2 signaling pathway, while also causing changes in E-cadherin and FAK gene expression in breast cancer cells [22]. Additionally, studies on cigarette smoking have revealed its ability to trigger EMT in various carcinoma cell types [34-40], making smoking a major factor in the initiation and advancement of numerous cancers, including breast cancer [22, 41-44].

Our findings suggest that WPS exposure could be a key contributor to the onset and potential progression of breast cancer.

We employed the NanoString nCounter PanCancer Pathways Panel, which examines 770 gene transcripts across 13 biological pathways, to identify gene targets in HNME cells exposed to WPS. This analysis uncovered significant alterations in the expression of 13 genes, with further confirmation via qRT-PCR and the Oncomine TM database. We also explored the prognostic significance of these WPS-deregulated genes in breast cancer using the PanCancer RNA-seq dataset from the Kaplan–Meier plotter database. Notably, this study is the first to identify these genes as targets of WPS exposure in human normal mammary cells. The genes identified are involved in critical biological processes such as cell cycle control, proliferation, migration, apoptosis, signal transduction, and inflammation. These processes are essential for the transformation of normal mammary cells, potentially leading to the development of breast cancer.

Out of the 13 differentially expressed genes identified, 5 (CCL5, CCL4, CCL8, CCL21, and TNFSF14) belong to the chemokine family. Elevated levels of CCL5 have been strongly associated with breast cancer progression, metastasis, and recurrence [45, 46], as well as with drug resistance [47], highlighting its crucial role in cancer development [48]. Previous studies have reported increased CCL5 expression in breast cancer tissues compared to normal tissues [49], and its higher levels in the tumor microenvironment help recruit monocytes, which further promotes tumor progression [50]. CCL5 has also been found to accelerate breast cancer progression through a p53-dependent mechanism via CCR5 [51]. Consistent with our findings from the PAM50 classification analysis, elevated CCL5 expression has been observed in triple-negative breast cancer (TNBC) [48, 49, 52], which suggests a potential link between CCL5 and aggressive breast cancer upon exposure to WPS.

This connection between CCL5 expression and aggressive, non-remissive breast cancer may stem from its role in triggering the release of matrix metalloproteinases (MMPs), such as MMP9, a gene identified in our study. Prior research has shown that MMP9 overexpression is linked to the transition from dysplasia to breast cancer [53] and is correlated with poorer prognosis [54]. Elevated CCL5 expression has also been found to contribute to tumor tolerance and poor prognosis in breast cancer [55], particularly in advanced stages [46, 56]. This could be attributed to CCL5's role in enhancing MMP9 activity and monocyte migration, which promote angiogenesis and tumor growth [57]. Several studies have shown that high MMP9 levels are linked to shorter relapse-free survival (RFS) and worsened breast cancer-related survival [58, 59]. Our results further confirm that both CCL5 and MMP9 are WPS-regulated genes in normal human mammary cells.

Interestingly, ALOX5, another key gene identified in this study, is implicated in tumor invasion through the stimulation of MMP9. Increased ALOX5 expression has been linked to tumor progression [60], and ALOX5-associated tumor-initiating genes contribute to mitogenesis, mutagenesis, angiogenesis, cell survival, immunosuppression, and metastasis in breast cancer [61]. A study by Wculek *et al.* [62] found that neutrophils facilitated ALOX5-dependent lung metastasis in breast cancer. Furthermore, the ALOX5 inhibitor Zileuton significantly reduced breast cancer metastasis [62], supporting our observation that ALOX5 may play a significant role in both the initiation and progression of breast cancer. Recent studies have also indicated that ALOX5 activation is linked with HER2 expression, which not only regulates ALOX5 but also promotes breast cancer growth and migration [63]. In line with these findings, we observed that upregulated ALOX5 was mostly correlated with the HER2-positive subtype of breast cancer in our study.

Similar to CCL5, CCL4 plays an important role in cancer, particularly in breast cancer metastasis [64]. A recent study highlighted that smoking, in combination with a CCL4 polymorphism, could significantly increase the risk of breast cancer [65]. In line with these findings, our study showed that WPS exposure elevated CCL4 expression, thereby enhancing the inflammatory response and promoting tumor progression. Additionally, CCL8, also known as monocyte chemoattractant protein-2, deregulates several cellular functions, including proliferation, apoptosis, and differentiation, while also facilitating EMT progression [66, 67]. CCL8 can stimulate fibroblasts, creating a pro-tumor microenvironment, particularly in the stroma of triple-negative breast cancer (TNBC), thus promoting metastasis [49, 68]. Our results align with previous studies that found higher levels of CCL8 in breast cancer tissues were mostly associated with negative hormone receptor status, TNBC, basal-like subtypes, higher-grade cancers, and poorer prognosis [49, 69].

CCL21, another chemokine identified in our study, is known to regulate cellular proliferation, invasion, apoptosis, and metastasis [70, 71]. Smoking has been shown to elevate levels of CCR7 ligands, including CCL19 and CCL21, in blood and bronchioalveolar lavage fluid, contributing to the migration of lung cancer cells via the EMT and ERK1/2 signaling pathways [51, 55, 72]. Several studies have reported that CCL21 plays a role in the migration of breast cancer cells [73], and in our study, we found that high levels of CCL21 were significantly correlated with the basal-like breast cancer subtype, which is consistent with findings by Chen *et al.* [69]. Interestingly, previous research has suggested a cross-talk between various CC chemokines in breast cancer, particularly between CCL8 and CCL21, which plays a role in cancer development and progression, correlating with patient prognosis [69]. In agreement with these studies, our results showed that WPS exposure in normal mammary epithelial cells led to the expression of both CCL8 and CCL21, indicating their involvement in the transition to cancerous states.

Tumor necrosis factor superfamily member 14 (TNFSF14), also known as LIGHT, is an inflammatory cytokine that contributes to anti-tumor immune responses [74]. An earlier study by Ganstev *et al.* [75] found that TNFSF14 was upregulated in recently formed lymph nodes in breast cancer, which aligns with the data and suggests a role for TNFSF14 in the initiation and progression of the disease. Moreover, research has shown that smoking increases TNFSF14 expression [76, 77], with higher levels observed in female smokers when male smokers show little to no TNFSF14 expression [76]. This finding further supports our data, where TNFSF14 expression was found to be upregulated in breast cancer following WPS exposure [78, 79].

In the study, we acknowledged TLR9, a gene crucial to the innate immune system. Previous research has linked increased TLR9 expression with higher tumor grades in breast cancer [80-82]. Research by Merrell *et al.* [81] demonstrated that the TNBC cell line MDA-MB-231 exhibited upregulated TLR9 expression, suggesting its involvement in tumor growth, metastasis, and progression. Notably, various studies have reported that cigarette and e-cigarette smoke can induce TLR9 expression [83-85], which aligns with our findings showing WPS exposure leads to increased TLR9 in breast cancer cells.

Additionally, we found prostaglandin reductase 1 (PTGR1), an enzyme that suppresses the chemotactic factor leukotriene B4. A previous study found PTGR1 to be highly expressed in many breast cancer cell lines, particularly in HER2-positive and TNBC cell lines, with the highest expression in the TNBC cell line HCC1937 [86]. Another study further supported its role in TNBC pathogenicity, demonstrating that silencing PTGR1 using licochalcone A led to a decrease in TNBC progression [87], reinforcing our data.

On the other hand, our study also highlighted IL3, a cytokine that acts as a selective growth factor released by certain tumor-infiltrating T cells in breast cancer, stimulating tumor angiogenesis [88]. Our results showed that WPS exposure led to increased IL3 expression in human mammary epithelial cells. Notably, previous studies have linked IL3 overexpression to breast cancer bone metastasis [89], further supporting the association between WPS-induced gene upregulation and tumor progression.

Furthermore, our study identified interferon-gamma (IFNG), another cytokine, which was significantly upregulated in mammary epithelial cells exposed to WPS. Prior studies have shown that breast cancer cells exhibit increased IFNG expression [90], which facilitates cancer invasion and angiogenesis [91]. IFNG signaling is a key component of the immune response pathway associated with prolonged RFS in breast cancer patients [92].

In addition, we observed upregulation of MX1, an interferon-related gene, in mammary epithelial cells following WPS exposure. Elevated MX1 expression has been documented in breast cancer [93], consistent with our findings. Notably, MX1 mRNA and protein levels have been found to increase in both in vivo and in vitro models of tamoxifen and fulvestrant resistance [94-96], indicating its role in RFS and poor prognosis. A recent study also linked MX1 overexpression to the PIK3/AKT pathway, which enhances MX1 expression and stimulates growth signaling pathways in relapsing breast cancer patients [97].

In our study, we found the G protein subunit gamma transducin 1 (GNGT1) gene, which shows a guanine nucleotide-binding protein (G protein), as a target of WPS exposure. While previous research has reported increased GNGT1 expression in head and neck squamous cell carcinoma [98], lung cancer [99, 100], and liver cancer [101], this is the first study to highlight its overexpression in breast cancer. Given that smoking is a well-established risk factor for lung cancer [102], we propose that GNGT1 may also contribute to WPS-

induced breast cancer. Additionally, previous studies have linked GNGT1 expression with poor overall and progression-free survival in serous ovarian cancer [103, 104], further reinforcing our findings.

Smoking is widely recognized as a major risk factor for various cancers, including those of the lung, oral cavity, and breast [22, 39-41, 44, 105, 106]. A prior study demonstrated that exposure to WPS can enhance the invasive potential of breast cancer cells [22]. As WPS consumption continues to rise, the associated intake of toxic substances also increases. It is suggested that WPS may have carcinogenic properties, potentially playing a role in the initiation and progression of multiple human cancers and contributing to cancer-related mortality at a level comparable to, or even greater than, that of smoking cigarettes.

However, in our study, we found differentially expressed genes (DEGs) that may serve as potential therapeutic targets. However, further research is necessary to validate these findings and to explore the underlying mechanisms of WPS-induced breast carcinogenesis.

Conclusion

This study provides novel insights into the potential role of WPS in driving EMT in HNME cells, accompanied by the deregulation of critical genes implicated in the initiation and progression of human breast cancer, as well as RFS. Our findings suggest that WPS exposure may contribute to breast cancer development and advancement, primarily through its influence on key regulatory genes associated with carcinogenesis, which could directly affect patient outcomes. However, additional research is essential to further explore and clarify the mechanisms underlying WPS-induced breast cancer development.

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References

1. World Health Organization. (ꝛ2018)ꝛ. WHO global report on trends in prevalence of tobacco smoking 2000-2025, 2nded. World Health Organization. https://apps.who.int/iris/handle/10665/272694. License: CC BY-NC-SA 3.0 IGO.
2. Onor IO, Stirling DL, Williams SR, Bediako D, Borghol A, Harris MB, et al. Clinical effects of cigarette smoking: Epidemiologic impact and review of pharmacotherapy options. Int J Environ Res Public Health 2017;14:E1147.
3. Maziak W, Taleb ZB, Bahelah R, Islam F, Jaber R, Auf R, et al. The global epidemiology of waterpipe smoking. Tob Control 2015;24 Suppl 1:i3-12.
4. Jawad M, McEwen A, McNeill A, Shahab L. To what extent should waterpipe tobacco smoking become a public health priority? Addiction 2013;108:1873-84.
5. Akl EA, Gunukula SK, Aleem S, Obeid R, Jaoude PA, Honeine R, et al. The prevalence of waterpipe tobacco smoking among the general and specific populations: A systematic review. BMC Public Health 2011;11:244.
6. Maziak W, Nakkash R, Bahelah R, Husseini A, Fanous N, Eissenberg T. Tobacco in the Arab world: Old and new epidemics amidst policy paralysis. Health Policy Plan 2014;29:784-94.
7. Wolfram RM, Chehne F, Oguogho A, Sinzinger H. Narghile (water pipe) smoking influences platelet function and (iso-) eicosanoids. Life Sci 2003;74:47-53.
8. Neergaard J, Singh P, Job J, Montgomery S. Waterpipe smoking and nicotine exposure: A review of the current evidence. Nicotine Tob Res 2007;9:987-94.
9. Javed F, ALHarthi SS, BinShabaib MS, Gajendra S, Romanos GE, Rahman I. Toxicological impact of waterpipe smoking and flavorings in the oral cavity and respiratory system. Inhal Toxicol 2017;29:389-96.
10. Eissenberg T, Shihadeh A. Waterpipe tobacco and cigarette smoking: Direct comparison of toxicant exposure. Am J Prev Med 2009;37:518-23.
11. Cobb CO, Shihadeh A, Weaver MF, Eissenberg T. Waterpipe tobacco smoking and cigarette smoking: A direct comparison of toxicant exposure and subjective effects. Nicotine Tob Res 2011;13:78-87.
12. Maziak W, Ward KD, Eissenberg T. Factors related to frequency of narghile (waterpipe) use: The first insights on tobacco dependence in narghile users. Drug Alcohol Depend 2004;76:101-6.

13. Rastam S, Eissenberg T, Ibrahim I, Ward KD, Khalil R, Maziak W. Comparative analysis of waterpipe and cigarette suppression of abstinence and craving symptoms. Addict Behav 2011;36:555-9.
14. Joseph S, Pascale S, Georges K, Mirna W. Cigarette and waterpipe smoking decrease respiratory quality of life in adults: Results from a national cross-sectional study. Pulm Med 2012;2012:868294.
15. Radwan G, Hecht SS, Carmella SG, Loffredo CA. Tobacco-specific nitrosamine exposures in smokers and nonsmokers exposed to cigarette or waterpipe tobacco smoke. Nicotine Tob Res 2013;15:130-8.
16. Ali M, Jawad M. Health effects of waterpipe tobacco use: Getting the public health message just right. Tob Use Insights 2017;10:1179173X17696055.
17. Layoun N, Saleh N, Barbour B, Awada S, Rachidi S, Al-Hajje A, et al. Waterpipe effects on pulmonary function and cardiovascular indices: A comparison to cigarette smoking in real life situation. Inhal Toxicol 2014;26:620-7.
18. Javed F, Al-Kheraif AA, Rahman I, Millan-Luongo LT, Feng C, Yunker M, et al. Comparison of clinical and radiographic periodontal status between habitual water-pipe smokers and cigarette smokers. J Periodontol 2016;87:142-7.
19. Ashour AA, Haik MY, Sadek KW, Yalcin HC, Bitharas J, Aboulkassim T, et al. Substantial toxic effect of water-pipe smoking on the early stage of embryonic development. Nicotine Tob Res 2018;20:502-7.
20. Fouad H, Awa FE, Naga RA, Emam AH, Labib S, Palipudi KM, et al. Prevalence of tobacco use among adults in Egypt, 2009. Glob Health Promot 2016;23:38-47.
21. Rastam S, Li FM, Fouad FM, Al Kamal HM, Akil N, Al Moustafa AE. Water pipe smoking and human oral cancers. Med Hypotheses 2010;74:457-9.
22. Sadek KW, Haik MY, Ashour AA, Baloch T, Aboulkassim T, Yasmeen A, et al. Water-pipe smoking promotes epithelial-mesenchymal transition and invasion of human breast cancer cells via ERK1/ERK2 pathways. Cancer Cell Int 2018;18:180.
23. López-Ozuna VM, Gupta I, Kiow RL, Matanes E, Kheraldine H, Yasmeen A, et al. Water-pipe smoking exposure deregulates a set of genes associated with human head and neck cancer development and prognosis. Toxics 2020;8:E73.
24. Waziry R, Jawad M, Ballout RA, Al Akel M, Akl EA. The effects of waterpipe tobacco smoking on health outcomes: An updated systematic review and meta-analysis. Int J Epidemiol 2017;46:32-43.
25. Montazeri Z, Nyiraneza C, El-Katerji H, Little J. Waterpipe smoking and cancer: Systematic review and meta-analysis. Tob Control 2017;26:92-7.
26. Bou Fakhreddine HM, Kanj AN, Kanj NA. The growing epidemic of water pipe smoking: Health effects and future needs. Respir Med 2014;108:1241-53.
27. Kim KH, Kabir E, Jahan SA. Waterpipe tobacco smoking and its human health impacts. J Hazard Mater 2016;317:229-36.
28. Yasmeen A, Alachkar A, Dekhil H, Gambacorti-Passerini C, Al Moustafa AE. Locking Src/Abl tyrosine kinase activities regulate cell differentiation and invasion of human cervical cancer cells expressing E6/E7 oncoproteins of high-risk HPV. J Oncol 2010;2010:530130.
29. Zhao B, Erwin A, Xue B. How many differentially expressed genes: A perspective from the comparison of genotypic and phenotypic distances. Genomics 2018;110:67-73.
30. Thomas JG, Olson JM, Tapscott SJ, Zhao LP. An efficient and robust statistical modeling approach to discover differentially expressed genes using genomic expression profiles. Genome Res 2001;11:1227-36.
31. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, et al. Oncomine 3.0: Genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia 2007;9:166-80.
32. Győrffy B, Lanczky A, Eklund AC, Denkert C, Budczies J, Li Q, et al. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. Breast Cancer Res Treat 2010;123:725-31.
33. Ringnér M, Fredlund E, Häkkinen J, Borg Å, Staaf J. GOBO: Gene expression-based outcome for breast cancer online. PLoS One 2011;6:e17911.
34. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature 2000;406:747-52.
35. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with

clinical implications. Proc Natl Acad Sci U S A 2001;98:10869-74.
36. Sun X, Deng Q, Liang Z, Liu Z, Geng H, Zhao L, et al. Cigarette smoke extract induces epithelial-mesenchymal transition of human bladder cancer T24 cells through activation of ERK1/2 pathway. Biomed Pharmacother 2017;86:457-65.
37. Pillai S, Trevino J, Rawal B, Singh S, Kovacs M, Li X, et al. β-arrestin-1 mediates nicotine-induced metastasis through E2F1 target genes that modulate epithelial-mesenchymal transition. Cancer Res 2015;75:1009-20.
38. Liu Y, Luo F, Xu Y, Wang B, Zhao Y, Xu W, et al. Epithelial-mesenchymal transition and cancer stem cells, mediated by a long non-coding RNA, HOTAIR, are involved in cell malignant transformation induced by cigarette smoke extract. Toxicol Appl Pharmacol 2015;282:9-19.
39. Dinicola S, Masiello MG, Proietti S, Coluccia P, Fabrizi G, Catizone A, et al. Nicotine increases colon cancer cell migration and invasion through epithelial to mesenchymal transition (EMT): COX-2 involvement. J Cell Physiol 2018;233:4935-48.
40. Chen PC, Lee WY, Ling HH, Cheng CH, Chen KC, Lin CW. Activation of fibroblasts by nicotine promotes the epithelial-mesenchymal transition and motility of breast cancer cells. J Cell Physiol 2018;233:4972-80.
41. Andersen ZJ, Jørgensen JT, Grøn R, Brauner EV, Lynge E. Active smoking and risk of breast cancer in a Danish nurse cohort study. BMC Cancer 2017;17:556.
42. Inoue-Choi M, Hartge P, Liao LM, Caporaso N, Freedman ND. Association between long-term low-intensity cigarette smoking and incidence of smoking-related cancer in the national institutes of health-AARP cohort. Int J Cancer 2018;142:271-80.
43. Lee PN, Thornton AJ, Hamling JS. Epidemiological evidence on environmental tobacco smoke and cancers other than lung or breast. Regul Toxicol Pharmacol 2016;80:134-63.
44. Liu M, Zhou C, Zheng J. Cigarette smoking impairs the response of EGFR-TKIs therapy in lung adenocarcinoma patients by promoting EGFR signaling and epithelial-mesenchymal transition. Am J Transl Res 2015;7:2026-35.
45. Bièche I, Lerebours F, Tozlu S, Espie M, Marty M, Lidereau R. Molecular profiling of inflammatory breast cancer: Identification of a poor-prognosis gene expression signature. Clin Cancer Res 2004;10:6789-95.
46. Niwa Y, Akamatsu H, Niwa H, Sumi H, Ozaki Y, Abe A. Correlation of tissue and plasma RANTES levels with disease course in patients with breast or cervical cancer. Clin Cancer Res 2001;7:285-9.
47. Yi EH, Lee CS, Lee JK, Lee YJ, Shin MK, Cho CH, et al. STAT3-RANTES autocrine signaling is essential for tamoxifen resistance in human breast cancer cells. Mol Cancer Res 2013;11:31-42.
48. Lv D, Zhang Y, Kim HJ, Zhang L, Ma X. CCL5 as a potential immunotherapeutic target in triple-negative breast cancer. Cell Mol Immunol 2013;10:303-10.
49. Thomas JK, Mir H, Kapur N, Bae S, Singh S. CC chemokines are differentially expressed in Breast Cancer and are associated with disparity in overall survival. Sci Rep 2019;9:4014.
50. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature 2007;449:557-63.
51. Mañes S, Mira E, Colomer R, Montero S, Real LM, Gómez-Moutón C, et al. CCR5 expression influences the progression of human breast cancer in a p53-dependent manner. J Exp Med 2003;198:1381-9.
52. Swamydas M, Ricci K, Rego SL, Dréau D. Mesenchymal stem cell-derived CCL-9 and CCL-5 promote mammary tumor cell invasion and the activation of matrix metalloproteinases. Cell Adh Migr 2013;7:315-24.
53. Ha HY, Moon HB, Nam MS, Lee JW, Ryoo ZY, Lee TH, et al. Overexpression of membrane-type matrix metalloproteinase-1 gene induces mammary gland abnormalities and adenocarcinoma in transgenic mice. Cancer Res 2001;61:984-90.
54. Vilen ST, Salo T, Sorsa T, Nyberg P. Fluctuating roles of matrix metalloproteinase-9 in oral squamous cell carcinoma. ScientificWorldJournal 2013;2013:920595.
55. Zhang JF, Li Y, Zhang AZ, He QQ, Du YC, Cao W. Expression and pathological significance of CC chemokine receptor 7 and its ligands in the airway of asthmatic rats exposed to cigarette smoke. J Thorac Dis 2018;10:5459-67.
56. Luboshits G, Shina S, Kaplan O, Engelberg S, Nass D, Lifshitz-Mercer B, et al. Elevated expression of the CC chemokine regulated on activation, normal T

cell expressed and secreted (RANTES) in advanced breast carcinoma. Cancer Res 1999;59:4681-7.
57. Azenshtein E, Luboshits G, Shina S, Neumark E, Shahbazian D, Weil M, et al. The CC chemokine RANTES in breast carcinoma progression: Regulation of expression and potential mechanisms of promalignant activity. Cancer Res 2002;62:1093-102.
58. Li HC, Cao DC, Liu Y, Hou YF, Wu J, Lu JS, et al. Prognostic value of matrix metalloproteinases (MMP-2 and MMP-9) in patients with lymph node-negative breast carcinoma. Breast Cancer Res Treat 2004;88:75-85.
59. Pellikainen JM, Ropponen KM, Kataja VV, Kellokoski JK, Eskelinen MJ, Kosma VM. Expression of matrix metalloproteinase (MMP)-2 and MMP-9 in breast cancer with a special reference to activator protein-2, HER2, and prognosis. Clin Cancer Res 2004;10:7621-8.
60. Kummer NT, Nowicki TS, Azzi JP, Reyes I, Iacob C, Xie S, et al. Arachidonate 5 lipoxygenase expression in papillary thyroid carcinoma promotes invasion via MMP-9 induction. J Cell Biochem 2012;113:1998-2008.
61. Kennedy BM, Harris RE. Cyclooxygenase and lipoxygenase gene expression in the inflammogenesis of breast cancer. Inflammopharmacology 2018;26:909-23.
62. Wculek SK, Malanchi I. Neutrophils support lung colonization of metastasis-initiating breast cancer cells. Nature 2015;528:413-7.
63. Zhou X, Jiang Y, Li Q, Huang Z, Yang H, Wei C. Aberrant ALOX5 activation correlates with HER2 status and mediates breast cancer biological activities through multiple mechanisms. Biomed Res Int 2020;2020:1703531.
64. Sasaki S, Baba T, Nishimura T, Hayakawa Y, Hashimoto S, Gotoh N, et al. Essential roles of the interaction between cancer cell-derived chemokine, CCL4, and intra-bone CCR5-expressing fibroblasts in breast cancer bone metastasis. Cancer Lett 2016;378:23-32.
65. Hu GN, Tzeng HE, Chen PC, Wang CQ, Zhao YM, Wang Y, et al. Correlation between CCL4 gene polymorphisms and clinical aspects of breast cancer. Int J Med Sci 2018;15:1179-86.
66. Zhou J, Zheng S, Liu T, Liu Q, Chen Y, Tan D, et al. MCP2 activates NF-κB signaling pathway promoting the migration and invasion of ESCC cells. Cell Biol Int 2018;42:365-72.
67. Bryja A, Dyszkiewicz-Konwińska M, Huang Y, Celichowski P, Nawrocki MJ, Jankowski M, et al. Genes involved in regulation of cellular metabolic processes, signaling and adhesion are the markers of porcine buccal pouch mucosal cells long-term primary cultured in vitro. J Biol Regul Homeost Agents 2018;32:1129-41.
68. Farmaki E, Chatzistamou I, Kaza V, Kiaris H. A CCL8 gradient drives breast cancer cell dissemination. Oncogene 2016;35:6309-18.
69. Chen B, Zhang S, Li Q, Wu S, He H, Huang J. Bioinformatics identification of CCL8/21 as potential prognostic biomarkers in breast cancer microenvironment. Biosci Rep 2020;40:BSR20202042.
70. Hwang TL, Lee LY, Wang CC, Liang Y, Huang SF, Wu CM. CCL7 and CCL21 overexpression in gastric cancer is associated with lymph node metastasis and poor prognosis. World J Gastroenterol 2012;18:1249-56.
71. Xiong Y, Huang F, Li X, Chen Z, Feng D, Jiang H, et al. CCL21/CCR7 interaction promotes cellular migration and invasion via modulation of the MEK/ERK1/2 signaling pathway and correlates with lymphatic metastatic spread and poor prognosis in urinary bladder cancer. Int J Oncol 2017;51:75-90.
72. Kuźnar-Kamińska B, Mikuła-Pietrasik J, Sosińska P, Książek K, Batura-Gabryel H. COPD promotes migration of A549 lung cancer cells: The role of chemokine CCL21. Int J Chron Obstruct Pulmon Dis 2016;11:1061-6.
73. Tutunea-Fatan E, Majumder M, Xin X, Lala PK. The role of CCL21/CCR7 chemokine axis in breast cancer-induced lymphangiogenesis. Mol Cancer 2015;14:35.
74. Mauri DN, Ebner R, Montgomery RI, Kochel KD, Cheung TC, Yu GL, et al. LIGHT, a new member of the TNF superfamily, and lymphotoxin alpha are ligands for herpesvirus entry mediator. Immunity 1998;8:21-30.
75. Gantsev SK, Umezawa K, Islamgulov DV, Khusnutdinova EK, Ishmuratova RS, Frolova VY, et al. The role of inflammatory chemokines in lymphoid neoorganogenesis in breast cancer. Biomed Pharmacother 2013;67:363-6.

76. Faner R, Gonzalez N, Cruz T, Kalko SG, Agustí A. Systemic inflammatory response to smoking in chronic obstructive pulmonary disease: Evidence of a gender effect. PLoS One 2014;9:e97491.
77. Yun JH, Morrow J, Owen CA, Qiu W, Glass K, Lao T, et al. Transcriptomic analysis of lung tissue from cigarette smoke-induced emphysema murine models and human chronic obstructive Pulmonary disease show shared and distinct pathways. Am J Respir Cell Mol Biol 2017;57:47-58.
78. Maker AV, Ito H, Mo Q, Weisenberg E, Qin LX, Turcotte S, et al. Genetic evidence that intratumoral T-cell proliferation and activation are associated with recurrence and survival in patients with resected colorectal liver metastases. Cancer Immunol Res 2015;3:380-8.
79. Maker AV. Precise identification of immunotherapeutic targets for solid malignancies using clues within the tumor microenvironment-evidence to turn on the LIGHT. Oncoimmunology 2016;5:e1069937.
80. González-Reyes S, Marín L, González L, González LO, del Casar JM, Lamelas ML, et al. Study of TLR3, TLR4 and TLR9 in breast carcinomas and their association with metastasis. BMC Cancer 2010;10:665.
81. Merrell MA, Ilvesaro JM, Lehtonen N, Sorsa T, Gehrs B, Rosenthal E, et al. Toll-like receptor 9 agonists promote cellular invasion by increasing matrix metalloproteinase activity. Mol Cancer Res 2006;4:437-47.
82. Berger R, Fiegl H, Goebel G, Obexer P, Ausserlechner M, Doppler W, et al. Toll-like receptor 9 expression in breast and ovarian cancer is associated with poorly differentiated tumors. Cancer Sci 2010;101:1059-66.
83. Foronjy RF, Salathe MA, Dabo AJ, Baumlin N, Cummins N, Eden E, et al. TLR9 expression is required for the development of cigarette smoke-induced emphysema in mice. Am J Physiol Lung Cell Mol Physiol 2016;311:L154-66.
84. Nadigel J, Préfontaine D, Baglole CJ, Maltais F, Bourbeau J, Eidelman DH, et al. Cigarette smoke increases TLR4 and TLR9 expression and induces cytokine production from CD8(+) T cells in chronic obstructive pulmonary disease. Respir Res 2011;12:149.
85. Li J, Huynh DL, Tang MS, Simborio H, Huang J, Kosmider B, et al. Electronic cigarettes induce mitochondrial DNA damage and trigger toll-like receptor 9-mediated atherosclerosis. bioRxiv Arterioscler Thromb Vasc Biol; 2021;41:839-853.
86. Liu M, Liu Y, Deng L, Wang D, He X, Zhou L, et al. Transcriptional profiles of different states of cancer stem cells in triple-negative breast cancer. Mol Cancer 2018;17:65.
87. Roberts LS, Yan P, Bateman LA, Nomura DK. Mapping novel metabolic nodes targeted by anti-cancer drugs that impair triple-negative breast cancer pathogenicity. ACS Chem Biol 2017;12:1133-40.
88. Dentelli P, Rosso A, Calvi C, Ghiringhello B, Garbarino G, Camussi G, et al. IL-3 affects endothelial cell-mediated smooth muscle cell recruitment by increasing TGF beta activity: Potential role in tumor vessel stabilization. Oncogene 2004;23:1681-92.
89. Mora EM, Torres D, Tari AM. Inhibition of the interleukin-3 receptor decreases the growth of bone-metastatic breast carcinoma cells. Cancer Res 2006;66:386.
90. Yaghoobi H, Azizi H, Oskooei VK, Taheri M, Ghafouri-Fard S. Assessment of expression of interferon γ (IFN-G) gene and its antisense (IFNG-AS1) in breast cancer. World J Surg Oncol 2018;16:211.
91. Ni L, Lu J. Interferon gamma in cancer immunotherapy. Cancer Med 2018;7:4509-16.
92. Bedognetti D, Hendrickx W, Marincola FM, Miller LD. Prognostic and predictive immune gene signatures in breast cancer. Curr Opin Oncol 2015;27:433-44.
93. Liu YP, Suksanpaisan L, Steele MB, Russell SJ, Peng KW. Induction of antiviral genes by the tumor microenvironment confers resistance to virotherapy. Sci Rep 2013;3:2375.
94. Johansson HJ, Sanchez BC, Forshed J, Stål O, Fohlin H, Lewensohn R, et al. Proteomics profiling identify CAPS as a potential predictive marker of tamoxifen resistance in estrogen receptor positive breast cancer. Clin Proteomics 2015;12:8.
95. Becker M, Sommer A, Krätzschmar JR, Seidel H, Pohlenz HD, Fichtner I. Distinct gene expression patterns in a tamoxifen-sensitive human mammary carcinoma xenograft and its tamoxifen-resistant subline MaCa 3366/TAM. Mol Cancer Ther 2005;4:151-68.

96. Huber M, Bahr I, Krätzschmar JR, Becker A, Müller EC, Donner P, et al. Comparison of proteomic and genomic analyses of the human breast cancer cell line T47D and the antiestrogen-resistant derivative T47D-r. Mol Cell Proteomics 2004;3:43-55.
97. Chai Y, Huang HL, Hu DJ, Luo X, Tao QS, Zhang XL, et al. IL-29 and IFN-α regulate the expression of MxA, 2',5'-OAS and PKR genes in association with the activation of Raf-MEK-ERK and PI3K-AKT signal pathways in HepG2.2.15 cells. Mol Biol Rep 2011;38:139-43.
98. Ghias K, Rehmani SS, Razzak SA, Madhani S, Azim MK, Ahmed R, et al. Mutational landscape of head and neck squamous cell carcinomas in a South Asian population. Genet Mol Biol 2019;4242:526-42.
99. Zhang JJ, Hong J, Ma YS, Shi Y, Zhang DD, Yang XL, et al. Identified GNGT1 and NMU as combined diagnosis biomarker of non-small-cell lung cancer utilizing bioinformatics and logistic regression. Dis Markers 2021;2021:6696198.
100. Juarez-Flores A JM. Squamous cell carcinoma of the lung: Gene expression and network analysis during carcinogenesis. Int J Clin Exp Med 2019;12:6671-83.
101. Qian Z, Zhang G, Song G, Shi J, Gong L, Mou Y, et al. Integrated analysis of genes associated with poor prognosis of patients with colorectal cancer liver metastasis. Oncotarget 2017;8:25500-12.
102. O'Keeffe LM, Taylor G, Huxley RR, Mitchell P, Woodward M, Peters SAE. Smoking as a risk factor for lung cancer in women and men: A systematic review and meta-analysis. BMJ Open 2018;8:e021611.
103. Zhang YB, Jiang Y, Wang J, Ma J, Han S. Evaluation of core serous epithelial ovarian cancer genes as potential prognostic markers and indicators of the underlying molecular mechanisms using an integrated bioinformatics analysis. Oncol Lett 2019;18:5508-22.
104. Mucaki EJ, Zhao JZ, Lizotte DJ, Rogan PK. Predicting responses to platin chemotherapy agents with biochemically-inspired machine learning. Signal Transduct Target Ther 2019;4:1.
105. Strumylaite L, Kregzdyte R, Poskiene L, Bogusevicius A, Pranys D, Norkute R. Association between lifetime exposure to passive smoking and risk of breast cancer subtypes defined by hormone receptor status among non-smoking Caucasian women. PLoS One 2017;12:e0171198.
106. White AJ, D'Aloisio AA, Nichols HB, DeRoo LA, Sandler DP. Breast cancer and exposure to tobacco smoke during potential windows of susceptibility. Cancer Causes Control 2017;28:667-75.