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Appendices

APPENDICES

Appendix I

Isolation of DNA from primary breast tumor tissue

DNA isolation is done to obtain pure and intact DNA molecules from a biological sample, such as tumor tissue.

Principle:

The principle of DNA isolation is to selectively disrupt the cells, remove unwanted components, and purify the DNA, resulting in a pure and concentrated DNA sample suitable for genetic analysis techniques.

Reagents:

1. QIAamp DNA Mini Kit (CA, USA).
2. Buffer ATL
3. Buffer AL
4. Buffer AW1
5. Buffer AW2
6. Buffer AE
7. Proteinase K
8. Ethanol

Procedure:

The tumor tissue, weighing less than 25 mg, was fragmented into small pieces and transferred into a 1.5 ml micro-centrifuge tube. 180 µl of buffer ATL and 20 µl of proteinase K were added to the centrifuge tube. The mixture was vortexed and incubated at 56°C in a water bath for 3 hours until complete tissue lysis occurred. Following this, 200 µl of buffer AL was added, and the tube was incubated at 70°C in a water bath for 10 minutes. Subsequently, 200 µl of 96% ethanol was added, and the solution was

centrifuged. The resulting mixture was pipetted onto a QIAamp mini spin column (2 ml collection tube) and centrifuged at 8000 rpm for 1 minute. After centrifugation, the flow-through and collection tubes were discarded. The mini spin column was then placed in a new 2 ml collection tube containing 500 µl of buffer AW1, followed by centrifugation at 8000 rpm for 1 minute. Again, the flow-through and collection tube was discarded, and the mini spin column was transferred to a new 2 ml collection tube containing 500 µl of buffer AW2. Centrifugation was performed at 14,000 rpm for 3 minutes. After removing the flow-through and collection tube, the mini spin column was placed in a new 1.5 ml micro-centrifuge tube, and 200 µl of buffer AE was added. The mixture was incubated at room temperature for 1 minute and then centrifuged at 8000 rpm for 1 minute to elute the DNA.

Appendix II

Agarose gel electrophoresis

Agarose gel electrophoresis separated DNA fragments based on size and charge. This technique allows for the analysis and characterization of DNA samples, such as determining fragment sizes, assessing DNA quality, detecting genetic variations, and confirming the presence of specific DNA sequences.

Principle:

The principle of agarose gel electrophoresis is based on the movement of charged DNA molecules through a gel matrix in the presence of an electric field.

Reagents:

1. 50X TAE buffer (pH-8.0)
   - Tris base
   - Glacial acetic acid
   - EDTA (0.5M)
   - Distilled water
2. Gel loading dye
   - Bromophenol blue
   - Xylene cyanol
   - Glycerol
   - Distilled water
3. Agarose
4. Ethidium bromide

Procedure:

100 ml of 1X electrophoretic buffer was prepared from 50X TAE stock. 1% of agarose was suspended in the electrophoretic buffer and heated in a microwave until completely dissolved. The agarose solution was cooled, and 0.6 µl of ethidium bromide was added. The agarose solution was poured into a gel boat, bound on two sides by cellophane tape, and a comb was placed to create wells. After solidification, the gel was placed in a horizontal electrophoretic tank. The 2 µl DNA sample and DNA ladder were prepared with the appropriate concentration and mixed with 2 µl 6X loading dye, which was loaded into the wells. Electrophoresis was conducted at 120V for 30 minutes. Finally, the banding pattern was observed and recorded using a digital gel documentation system.

Appendix III

Whole exome sequencing of isolated DNA from breast cancer tissue

Whole exome sequencing identifies and analyses genetic variants within the Genome's protein-coding regions (exome). Focusing on the exome helps to investigate the functional areas of the Genome most likely to harbour disease-causing variants. This approach enables the identification of potential genetic causes of breast cancer.

Principle:

The principle of whole exome sequencing involves selectively capturing and sequencing the exonic regions of the Genome. This is achieved using specialized capture probes or baits targeting and capturing the exons. These probes are designed based on known exome sequences and are synthesized with short DNA or RNA molecules complementary to the exonic regions of interest.

Reagents:

1. NovaSeq 6000 S4 Reagent Kit v1.5

EDTA removal from DNA samples

1. DNA Cleanup Beads
2. Wash buffer
3. Elution buffer

DNA fragmentation and addition of dA tails

1. 10 mM Tris-HCI, pH 7.5–8.5
2. 10X Fragmentation and dA-Tailing buffer (blue)
3. 5X Fragmentation and dA-Tailing Enzyme Mix (colorless)

Post-ligation double-sided size selection

1. DNA Cleanup Beads
2. Wash buffer (diluted with 96% ethanol)
3. Elution buffer

Amplified DNA library purification

1. Ethanol
2. Guanidine Salts
3. Silica Membrane
4. Elution buffer

Target enrichment

Name	Kit Composition
Twist Hybridization and Wash Kit (2 boxes), 96 reactions	**Twist hybridization reagents**
	Hybridization mix
	Hybridization enhancer
	Amplification primers
	Twist wash buffers
	Binding buffer
	Wash buffer 1
	Wash buffer 2
Twist Comprehensive Exome, 96 reactions	Comprehensive panel
Twist Comprehensive Exome, 12 reactions	Comprehensive panel
Twist Comprehensive Exome, 2 reactions	Comprehensive panel
Twist Binding and Purification Beads (96 reactions/12 reactions)	Streptavidin binding beads
	DNA purification beads
Twist Universal Blockers (96 reactions/12 reactions)	Universal blockers
	Blocker solution

Post-capture library amplification with Library Amplification Master Mix

1. Enriched library from the Target Enrichment and Amplification Beads slurry (brown)
2. 2X Collibri Library Amplification Master Mix (blue)
3. Colibri Primer Mix

Procedure:

Sample

An aliquot of 50 ng human genomic DNA (gDNA) samples obtained from the breast tumor tissue was used for whole-exome library preparation.

Removal of EDTA from DNA samples

The sample DNA was mixed with twice the volume of DNA cleanup beads and vortexed until a homogeneous suspension was obtained. The mixture was then briefly centrifuged to collect all the droplets at the bottom of the tube. It was incubated for 5 minutes at room temperature. The tube was placed in the magnetic rack for 2 minutes, and the supernatant was carefully removed and discarded using a pipette. On a magnetic rack, 200 µl of wash buffer (pre-mixed with ethanol) was added to the tube and incubated for 30 seconds at room temperature. Again, the supernatant was carefully removed and discarded using a pipette. To remove the remaining ethanol, the tube was briefly centrifuged and placed back on the magnetic rack, and any remaining supernatant was carefully removed with a pipette without disturbing the pellet. The magnetic particles were air-dried for 1 minute at room temperature. The tube was removed from the magnetic rack, and 10 µl of elution buffer was added directly to the pellet to disperse the beads. The suspension was mixed thoroughly by vortexing. The tube was briefly centrifuged and then placed back on the magnetic rack for 2 minutes. The eluted DNA supernatant was collected and transferred to a new 1.5-mL Eppendorf™ LoBind™ tube without disturbing the pellet**.** The DNA was stored at -20°C.

Library generation

The gDNA samples were converted into sequencing-ready libraries via enzymatic shearing (ES) for Fragmentation using the Collibri ES DNA Library Prep Kit for Illumina

Systems with UDIs. The standard protocol of the Collibri ES DNA Library Prep Kit for Illumina Systems was used with the following modifications: 31 µl of EDTA-free double-stranded DNA. A buffer/DNA mixture (36 µl) was prepared by adding 5 µl of 10X Fragmentation and dA-Tailing buffer to the DNA mixture. The 5X Fragmentation and dA-Tailing Enzyme Mix (14 µl) was added to the buffer/DNA mixture for a 50 µl total volume.

Fragmentation was performed for 20 min at 37°C. The adaptor-ligated library was purified using a customized cleanup protocol (Pub. No. MAN0025489) designed explicitly for the Collibri ES DNA Library Prep Kit for Illumina Systems.

Post-ligation cleanup

1. Bind the library

The Dual Index Adaptor-ligated DNA library (70 µl) was mixed with 56 µl of DNA Cleanup Beads by vortexing until a homogeneous suspension was obtained. The tube was briefly centrifuged to collect all the droplets at the bottom and incubated for 5 minutes at room temperature. After that, the tube was briefly centrifuged again to collect all the droplets at the bottom and then placed in a magnetic rack for 2 minutes or until the beads formed a tight pellet. The reaction tube was kept in the magnetic rack, and the supernatant was carefully removed and discarded using a pipette.

2. Wash the magnetic bead pellet

The reaction tube was kept in the magnetic rack, and 200 µl of wash buffer (pre-mixed with ethanol) was added. It was incubated for 30 seconds at room temperature. After that, the supernatant was carefully removed and discarded using a pipette. The reaction tube was briefly centrifuged and placed back in the magnetic rack. Any remaining supernatant and residual ethanol were carefully removed without disturbing the pellet. The reaction tube was kept in the magnetic rack, and the magnetic beads were air-dried for 1 minute at room temperature.

3. Bind the library for second time

The tube was removed from the magnetic rack, and 70 µl of elution buffer was added, followed by thorough mixing by vortexing. The tube was briefly centrifuged to

collect all the droplets at the bottom and then incubated for 1 minute at room temperature. Subsequently, 70 µl of fresh DNA Cleanup Beads was added directly to the bead suspension in an elution buffer, and the mixture was homogenized by vortexing. The tube was briefly centrifuged to collect all the droplets at the bottom and then incubated for 5 minutes at room temperature. The tube was kept in the magnetic rack, and the supernatant was carefully removed and discarded using a pipette. Next, the reaction tube was kept in the magnetic rack, and 200 µl of wash buffer (pre-mixed with ethanol) was added, followed by 30 seconds of incubation at room temperature. The supernatant was carefully removed and discarded using a pipette. The reaction tube was briefly centrifuged and placed back in the magnetic rack. Any remaining supernatant and residual ethanol were carefully removed without disturbing the pellet.

Finally, the reaction tube was kept in the magnetic rack, and the magnetic beads were air-dried for 1 minute at room temperature.

4. Elute the library

The tube was removed from the magnetic rack, and 25 µl of elution buffer was added, followed by thorough mixing by vortexing. The tube was briefly centrifuged to collect all the droplets at the bottom and then incubated for 1 minute at room temperature. The tube was placed in the magnetic rack for 2 minutes or until the beads formed a tight pellet. The solution was allowed to clear. Without removing the tube from the magnetic rack, 22–23 µl of the supernatant was transferred to a new sterile tube for storage. After purification, the eluted DNA library could be used for library amplification or stored at 4°C for up to 1–2 weeks. For longer-term storage, the library could be stored at –20°C.

The library was then amplified for 8 PCR cycles before the enrichment step.

Target enrichment

The WGS libraries were quantified using the Invitrogen™ Qubit™ Fluorometer and pooled. The exome targets were then enriched using a synthetic probe panel (Twist Human Core Exome Panel, from Twist Bioscience). “Long” hybridization reactions were performed using the Twist Standard Hybridization and Wash Kit

overnight (~16 hr) target enrichment. The enriched libraries were amplified for 8 PCR cycles using the 2X Library Amplification Master Mix from the Collibri ES DNA Library Prep Kit for Illumina Systems.

Library quantification and sequencing

The final concentrations of the sequencing libraries were determined using the Invitrogen™ Collibri™ Library Quantification Kit. Libraries were sequenced on the Illumina™ NovaSeq™ 6000 Sequencing System with a depth of 100X coverage by paired-end sequencing of 2 x 150 bp.

Whole exome sequencing analysis

The Illumina™ BaseSpace™ Enrichment App v3.1.0 aligned a subsample of 30M passing filter (PF) clusters (60M reads) from each sample to the hg39 reference genome. The enrichment parameters were calculated using the Picard CollectHsMetrics tool. The target regions limited variant calling in the Variant Calling Assessment Tool v4.0.2. Gold-standard data for NA12878 from the Genome in a Bottle (GIAB) consortium hosted by NIST determined the precision and recall parameters.

Bioinformatics and statistical analysis

The Genome in a Bottle (GIAB) consortium provides reference materials and data to evaluate and benchmark for various bioinformatics pipelines used for genome analysis.

1. **Read Alignment:**

   BWA-MEM: Burrows-Wheeler Aligner was used for mapping sequencing reads to a reference genome.

   Bowtie: An ultrafast and memory-efficient aligner for short DNA sequences.

2. **Variant Calling:**

   GATK: Genome Analysis Toolkit is used for variant discovery and genotyping.

   FreeBayes: A Bayesian variant caller was used to detect genetic variants in population-scale sequencing data.

SAMtools: A suite of tools for manipulating sequence alignment data, including variant calling.

VarScan: Variant detection software that identifies SNPs and indels in NGS data.

3. **Structural Variant Calling:**

DELLY: A tool for detecting genomic structural variants from paired-end and split-read sequencing data.

Lumpy: A tool for discovering structural variants using read-pair, split-read, and read-depth information.

4. **Quality Control and Metrics:**

Picard: A set of command-line tools for manipulating high-throughput sequencing data and metrics generation.

Qualimap: A tool for assessing the quality of mapping and sequencing data, including coverage and GC bias.

FastQC: A quality control tool for high-throughput sequence data, providing QC metrics and visualization.

5. **Haplotype Phasing:**

ShapeIt: A statistical phasing algorithm for estimating haplotypes using genotype data.

Additionally, to evaluate the functional effects of the annotated variants, the following filters were applied to identify rare variants:

Allele frequency: Variants with allele frequencies ≤ 0.05 were selected, focusing on rare variants.

SIFT (Sorting Intolerance From Tolerant): Variants were filtered based on SIFT predictions, with a preference for Deleterious (D) and Tolerated (T).

Polyphen2: Variants were prioritized based on Polyphen2 predictions, particularly Damaging (D), Possibly Damaging (P) and Benign (B) predictions.

Mutation Taster: Variants were assessed for their disease-causing potential using mutation taster, focusing on Disease-Causing (D) predictions.

CLINSIG (Clinical Significance): Variants were filtered based on their clinical significance, such as Benign, Benign/Likely_benign, Likely_benign, Pathogenic/ Likely_pathogenic, Uncertain_significance

Statistical differences among the enrichment protocol metrics were assessed in the R 4.0.2 environment considering pairwise comparisons against SureSelectQXT V6, which was regarded as the reference, based on the nonparametric Mann–Whitney U-test.

Appendix IV

Touchdown polymerase chain reaction

Touchdown PCR is a modified version of PCR that introduces a gradual decrease in the annealing temperature during the initial cycles of the reaction. Touchdown PCR enhances specificity and reduces nonspecific amplification in isolated DNA samples.

Principle:

The principle behind touchdown PCR involves gradually decreasing the annealing temperature during the initial cycles of the reaction. The touchdown improves specificity by allowing primers to bind more specifically to their target sequences. The initial annealing temperature is set higher than the primers' calculated melting temperature (Tm), and it is gradually lowered in subsequent cycles.

Reagents:

1. DNA template
2. Primers
3. Taq DNA polymerase 2X master mix red 1.5mM $MgCl_2$ (Deoxynucleotide Triphosphates (dNTPs), Taq polymerase, Buffer solutions, Mg^{2+} ions)
4. Nuclease free water

Procedure:

The reaction mixture contains 1 µl of forward primer, 1 µl of reverse primer, 1.5 µl of template DNA, 5 µl of master mix and 1.5 µl of nuclease free water, were added into a PCR tube. The solutions were mixed by gentle vortexing, and the tubes were placed into the preheated thermal cycler. The PCR process involves the following steps,

Stage	Temperature (°C)	Time	Cycle
Stage 1 (Initial denaturation)	95	3 minute	20
Stage 2 (High-temperature denaturation and primer annealing)	95 (Denaturation)	15 seconds	13
	68 (Annealing)	10 seconds	
	72 (Extension)	15 seconds	
Stage 3 (Gradual reduction of annealing temperature)	95 (Denaturation)	15 seconds	20
	55 (Annealing)	10 seconds	
	72 (Extension)	20 seconds	
Stage 4 (Final extension)	72	2 minute	1
Stage 5 (Final hold)	4	∞	

Stage 1 initiated the PCR reaction by denaturing the DNA template, separating the double-stranded DNA into single strands. In Stage 2, high-temperature denaturation separated the DNA strands, followed by primer annealing at a relatively high temperature (68°C). The extension step occurred at 72°C. In Stage 3, the "touchdown" aspect was introduced. The annealing temperature started at 55°C, allowing for more specific primer binding, and decreased gradually during the initial cycles. The extension step occurred at 72°C. Stage 4 provided a final extension step to ensure the complete synthesis of the DNA strands. Stage 5 allowed for the indefinite cooling of the reaction. Repeating these denaturation, annealing, and extension steps in cycles exponentially amplifies the target DNA sequence with each cycle, leading to a rapid accumulation of the desired DNA product. The PCR took place for 3 hours 23 minutes 58 seconds. After completing the process of PCR amplification, a 2 µl aliquot of the PCR products was taken for 1% agarose gel electrophoresis.

Gel electrophoresis

1% of agarose was dissolved in 100 ml of TAE buffer. The mixture was heated in an oven for 1-2 minutes and then cooled to a bearable temperature. 0.6 µl of ethidium bromide was added to the agarose solution, and the solution was gently shaken to mix

without creating bubbles. A cleanly wiped comb was placed on the gel boat, and the prepared agarose gel was poured into the boat. The gel was left to solidify for 30-45 minutes. The gel boat with the solidified agarose gel was placed in a tank filled with TAE buffer. A volume of 2 µl of DNA sample mixed with 2 µl of gel-loading dye was loaded into the well, along with a 2 µl volume of ladder in a nearby well. The gel was allowed to run at 50V initially, and after 15 minutes, the voltage was increased to 90-100V. The gel was allowed to run for 45-60 minutes. After completion of the electrophoresis, the gel was placed in a UV transilluminator or gel documentation system. The DNA bands were observed under UV light to visualize the separated fragments.

Appendix V

Sanger sequencing

Sanger sequencing is a widely used method for validating and confirming genetic variants, including novel variants, in DNA samples. It is mainly used for validating genetic variants identified through next-generation sequencing (NGS) in breast tumor samples.

Principle:

Sanger sequencing relies on incorporating chain-terminating dideoxynucleotides (ddNTPs) during DNA synthesis. The ddNTPs lack the 3' hydroxyl (-OH) group necessary for forming the phosphodiester bond between nucleotides, resulting in chain termination when incorporated into the growing DNA strand. A series of DNA fragments of varying lengths can be generated using a mixture of normal deoxynucleotides (dNTPs) and low concentrations of fluorescently labelled ddNTPs. These fragments are separated by size using capillary electrophoresis, and the sequence is determined by detecting the fluorescence signal corresponding to each terminating ddNTP.

Reagents:

Clean up

1. ExoSAP IT™ reagent
2. PCR product

Cyclic PCR

1. BigDye® Direct Cycle Sequencing Kit
2. RR 100 (Fluorescent dye)
3. Sequencing buffer
4. Nuclease free water
5. Primers

Ethanol purification

1. 125mM EDTA
2. 3M Sodium Acetate
3. Ethanol

Denaturation

1. Hidi formamide

Procedure:

Clean up for unpurified PCR product

The 1 µl of PCR product was mixed with 1 µl of ExoSAP IT™ reagent in a 96-well plate. The sample was added twice separately for both forward and reverse primers in a 96-well plate. The mixture was kept in PCR for 7 minutes. Hotstart was performed at 37°C for 4 minutes, followed by 8°C for 1 minute and 4°C for 2 minutes. After PCR, the mixture was kept in a 4°C cooling centrifuge for 1 minute. The reaction mix was used for further process.

Cyclic PCR

0.5 µl of RR 100, 2 µl of sequencing dilution buffer, 0.5 µl of primer, 5 µl of nuclease-free water, and 2 µl of DNA template + ExoSAP were mixed in the 96-well plate and kept in PCR for 2 hours and 4 minutes. The PCR process is given below.

Stage	Temperature (°C)	Time	Cycle
Stage 1 (Initial denaturation)	96	1 minute	25
Stage 2 (Cycle denature)	96	10 seconds	
Stage 3 (Cycle anneal)	50	5 seconds	
Stage 4 (Cycle extension)	60	4 minutes	
Stage 5 (Final hold)	4	∞	

Ethanol purification of post-cyclic PCR products

1 µl of 125 mM EDTA, 1 µl of 3M sodium acetate, and 25 µl of 100% ethanol were mixed thoroughly to the reaction in the 96-well plate. The well plate was kept in a room temperature for 15 minutes. The mixture was centrifuge at 2500 RCF for 40 minutes. The well plate was inverted on a paper towel and spin at 100 RCF for 1 minute. 35 µl of 70% ethanol was added and centrifuged at 1700 RCF for 15 minutes. Again, the 96-well plate was inverted on a paper towel and centrifuged at 100 RCF for 1 minute. The mixture was allowed to air dry in dark conditions for 1 hour.

Denaturation

10 µl of Hidi formamide was added to the reaction mixture. The plate was kept in PCR for denaturation for 2 minutes at 90°C. After PCR, the reaction is allowed to snap chill for 2 minutes.

Sequencing analysis

After denaturation, the sequencing reaction products were loaded onto a capillary array of the 3500 genetic analyzer instrument by Applied Biosystems. The sequencing took place at 60°C for 2 hours and 4 minutes. The instrument separated the labelled sequencing fragments based on their sizes. The Bioedit software was used to analyze the sequenced data. The sequencing traces and the base calls corresponding to each fluorescent peak were identified. The sequencing data was compared to a reference sequence (human Genome) to identify any variations or mutations in the breast tumor DNA sample. The obtained sequence was also analyzed in nucleotide BLAST to check the similarity. The variants were validated in both forward and reverse sequencing reads. The presence of the identified variant was confirmed by visual inspection of the sequencing traces. The quality of the peaks and the reliability of the base calls supporting the variants were assessed according to the nucleotide change and position.

Appendix VI

Mitosis detection - Algorithm

The mitosis count in breast cancer plays a crucial role in assessing tumor aggressiveness. Automated mitosis detection systems based on image analysis techniques are developed to assist pathologists in quantifying mitotic figures more objectively and efficiently. These systems employ algorithms to identify and count mitotic figures, potentially reducing inter-observer variability and increasing accuracy**.**

CNN code

```
import keras
from keras.models import Sequential, Model
from keras.layers import Dense, Dropout, Flatten, Conv2D, MaxPool2D
from keras import layers
from keras.optimizers import Adam
from keras import backend as K
from keras.wrappers.scikit_learn import KerasClassifier

import cv2
import pandas as pd
import glob
import numpy as np
from PIL import Image

read = lambda imname: np.asarray(Image.open(imname).convert("RGB"))

IMG_Mitosis1 = []
IMG_Mitosis2 = []

# data folder path
path = 'C:/Users/acer/Downloads/data'

# interested only in csv files to calculate the average of nuclear atypia scores
filenames = glob.glob(path + "\*.csv")
len1 = len(filenames)
print("Files No", len1)

for i in range(len1):

    data = pd.read_csv(filenames[i], header=None)

    avg=sum(data[data.columns[2]])/(len(data[data.columns[2]]))
```

```
    # If the block is for mitosis stage low, which has an average nuclear atypia score of
more than 0.8

    if(avg > 0.80):
        print("Mitosis Low ", filenames[i].split("\\")[-1])
        print(sum(data[data.columns[2]])/(len(data[data.columns[2]])))

        img=cv2.resize(read(path+"/"+filenames[i].split("\\")[-1][:-3]+"jpg"), (240,240))

        IMG_Mitosis2.append(np.array(img*avg))
        print("\n")

     # If block is for mitosis stage very low which has average nuclear atypia score less tha
       n 0.8
    else:
        print("Mitosis very low", filenames[i].split("\\")[-1])
        print(sum(data[data.columns[2]])/(len(data[data.columns[2]])))

        img=cv2.resize(read(path+"/"+filenames[i].split("\\")[-1][:-3]+"jpg"), (240,240))

        IMG_Mitosis1.append(np.array(img*avg))
        print("\n")

mitosis1_label = np.zeros(len(IMG_Mitosis1))
mitosis2_label = np.ones(len(IMG_Mitosis2))

X_train = np.concatenate((IMG_Mitosis1, IMG_Mitosis2), axis = 0)
Y_train = np.concatenate((mitosis1_label, mitosis2_label), axis = 0)

from sklearn.model_selection import train_test_split
%matplotlib inline
import matplotlib.pyplot as plt

x_train, x_val, y_train, y_val = train_test_split(X_train/255, Y_train, test_size=0.2, rando
m_state=1)

w=60
h=40
fig=plt.figure(figsize=(16, 16))
columns = 4
rows = 4

for i in range(1, columns*rows +1):
    ax = fig.add_subplot(rows, columns, i)
    #if np.argmax(y_train[i]) == 0:
    if (y_train[i] == 0):
```

```
        ax.title.set_text('Mitosis very Low nuclear atypia score')
    else:
        ax.title.set_text('Mitosis Low nuclear atypia scores')
    plt.imshow(x_train[i], interpolation='nearest')
plt.show()
```

The main CNN model code

```
model = Sequential()
model.add(Conv2D(32,3,padding="same", activation="relu", input_shape=(240,240,3)))
model.add(MaxPool2D())

model.add(Conv2D(32, 3, padding="same", activation="relu"))
model.add(MaxPool2D())

model.add(Conv2D(64, 3, padding="same", activation="relu"))
model.add(MaxPool2D())
model.add(Dropout(0.4))

model.add(Flatten())
model.add(Dense(128,activation="relu"))
model.add(Dense(1, activation="sigmoid"))

model.summary()
```

Parameters that were trained

```
opt = Adam(learning_rate=0.001)

model.compile(optimizer = opt , loss = 'binary_crossentropy' , metrics = ['accuracy'])

history = model.fit(x_train,y_train,epochs = 25, validation_data = (x_val, y_val))
```

Output of the CNN model

```
plt.figure(figsize=(30,5))
plt.plot(history.history['loss'])
plt.plot(history.history['val_loss'])
plt.xlabel('epochs')
plt.ylabel('loss')
plt.legend(['train_data','test_data'])
plt.title('loss analysis')
```

```
plt.show()

plt.figure(figsize=(30,5))
plt.plot(history.history['accuracy'])
plt.plot(history.history['val_accuracy'])
plt.xlabel('epochs')
plt.ylabel('Accuracy')
plt.legend(['train_data','test_data'])
plt.title('Accuracy Improvement')
plt.show()
```

Supplementary Tables

SUPPLEMENTARY TABLES

Supplementary Tables

All the Supplementary Tables are provided as soft copy in the enclosed CD-ROM.

Supplementary Table S1: Exonic variants of six breast cancer patients

Supplementary Table S2: Clinical significance and associated disease of significant variants

Supplementary Table S3: Intronic variants of breast cancer patients

Supplementary Table S4: *BRCA* gene variants in breast cancer patients

Annexures

ANNEXURES

Annexure I

Ethical Clearance - Sri Ramakrishna Hospital, Coimbatore

Sri Ramakrishna Hospital

Medical Service : M/s. S.N.R. SONS CHARITABLE TRUST

SRI RAMAKRISHNA HOSPITAL ETHICAL COMMITTEE

395, SAROJINI NAIDU ROAD, SIDHAPUDUR, COIMBATORE - 641 044.

Phone : 0422 - 4500000, E-mail : ec@sriramakrishnahospital.co.in website : sriramakrishnahospital.com

Ethics Committee Registration No. ECR/690/Inst/TN/2014/RR-18

Ethics Committee Chairman
Dr. Murali. P. M. M.Sc.,Ph.D.,D.Sc.,

Ethics Committee Vice Chairman
Dr. Vimal Veereshwarayya, Ph.D.,RAC.,

Ethics Committee Member Secretary
Dr. Isaac Christian Moses., MD.,FICP.,FACP.,

Ethics Committee Basic Scientist
Dr. Paramasivam. N, MD(Pharm),DA.,

Ethics Committee Clinical Scientist
Dr. Booma. V, MD(Paediatrics)
Dr. Karthikesh. K, MS.,FRCS.,DNB.,M.Ch.,
Dr. Loganathan. N, MBBS.,MD(GM)., DM,
Dr. S. Lokeshwaran, MBBS.,MD., DNB,EDIC, PDCC.,

Ethics Committee Social Scientist
Dr. Nagalingam. M, MSW, Ph.D.,

Ethics Committee Legal Expert
Mr. Sivakumar. V, B.Sc., B.L.,

Ethics Committee Layperson
Mr. Subramanian. V, B.A.,

EC/2019/ 0411/CR/57 17.11.2019

ETHICAL CLEARANCE CERTIFICATE

Project title: "ASSESSMENT OF ANTICANCER ACTIVITY OF RUTIN IN TRIPLE NEGATIVE BREAST CANCER TUMOR SAMPLES AND EXPLORING NEW TARGETS BY GENOME PROFILING."

Researcher: Ms. K.Suganya
Research Scholar
Avinashilingam Institute for Home science and Higher Education for Women, Coimbatore

The following members of the Ethics Committee were present at the meeting held on 04.11.2019 at 2.30 pm in Auditorium, Sri Ramakrishna Hospital Campus, Coimbatore.

S. No	Members Name	Qualification	Designation	Address	Affiliation to the Institution (Yes/ No)
1.	Dr. Murali.P.M	M.Sc., Ph.D., D.Sc.,	Chairperson	Jananom Private Limited, 26-1 Natesar Nagar, Kovaipudur, Coimbatore	No
2.	Dr. Karthikesh	MS.,FRCS.,DNB.,M.Ch	Active Member Secretary/ Clinical Scientist	Consultant - Oncologist, Sri Ramakrishna Hospital, No. 395, Sarojini Naidu Road, Siddhapudur,CBE	Yes
3.	Dr. Loganathan.N	MBBS., MD (GM)., DM	Clinical Scientist	Consultant Pulmonologist, Sri Ramakrishna Hospital, No. 395, Sarojini Naidu Road, Siddhapudur, cBE	Yes
4.	Mr. Sivakumar.V	B.Sc., B.L.,	Legal Expert	9 Ground Floor, Parsn Trade Plaza, 156, Dr.Nanjappa Road, CBE	No
5.	Mr. Subramanian.V	BA	Layperson	Supreme mills, ERA Mohan Nagar, Kalapatti Road, Coimbatore	No
6.	Dr. Subramanya Sharma	MDS	Subject Expert	Director, Sri Ramakrishna Dental College and Hospital,	Yes

Sri Ramakrishna Hospital

Medical Service : M/s. S.N.R. SONS CHARITABLE TRUST

SRI RAMAKRISHNA HOSPITAL ETHICAL COMMITTEE

395, SAROJINI NAIDU ROAD, SIDHAPUDUR, COIMBATORE - 641 044.
Phone : 0422 - 4500000, E-mail : ec@sriramakrishnahospital.co.in website : sriramakrishnahospital.com

Ethics Committee Registration No. ECR/690/Inst/TN/2014/RR-18

7.	Dr. Subramanian.K	M.Sc., M.Phil., PhD.,	Statistician	Associate Professor & Head- Department of Statistics PSG College of Arts & Science, Coimbatore	No
8.	Dr. T.K.Ravi,	M.Pharm, Ph.D,FAGE	Subject expert	Principal Sri Ramakrishna College of Pharmacy, 395, Sarojini Naidu road, Sidhapudur, Coimbatore	Yes
9.	Dr. Vasanth Raj	Mpharm,Phd	Subject expert	Research Officer Sri Ramakrishna Hospital, 395, Sarojini Naidu road, Sidhapudur, Coimbatore	Yes
10.	Dr. Ananth S	MBBS, Ph.D, MD, ABPN	Subject expert	Consultant – Psychiatry Sri Ramakrishna Hospital, 395, Sarojini Naidu road, Sidhapudur, Coimbatore	Yes
11.	Dr. Baskar Rao Pandian	M.D , D.M	Subject expert	Consultant- Oncology Sri Ramakrishna Hospital, 395, Sarojini Naidu road, Sidhapudur, Coimbatore	Yes
12.	Dr. Madhushankar	M.B.B.S & M.D	Subject expert	Consultant- Nephrology Sri Ramakrishna Hospital, 395, Sarojini Naidu road, Sidhapudur, Coimbatore	Yes

This is to certify that the research work entitled **"ASSESSMENT OF ANTICANCER ACTIVITY OF RUTIN IN TRIPLE NEGATIVE BREAST CANCER TUMOR SAMPLES AND EXPLORING NEW TARGETS BY GENOME PROFILING."** placed before the Institutional Ethical Committee and has been approved as there is no objection to hold this research work.

The Ethics committee expects to be informed about the progress of the study, any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report.

The Ethics committee wishes for the research.

Yours truly,

Member Secretary,

Institutional Ethics Committee

MEMBER SECRETARY
SRI RAMAKRISHNA HOSPITAL ETHICAL COMMITTEE
No: 395, SAROJINI NAIDU ROAD,
SIDDHAPUDUR, COIMBATORE - 641 044

Annexure II

Ethical Clearance - Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore

INSTITUTIONAL HUMAN ETHICS COMMITTEE

Avinashilingam
Institute for Home Science and Higher Education for Women
(Deemed to be university under Category 'A' by MHRD, Estd. u/s 3 of UGC Act 1956) Re-accredited with 'A^{++}' Grade by NAAC.
Recognised by UGC Under Section 12 B
Coimbatore- 641043, Tamil Nadu, India

06.02.2023

Chairman
Dr. Sudha Ramalingam
Director – Research and Innovation
Professor- Community Medicine,
PSG Institute of Medical Sciences
&Research, Coimbatore

Member Secretary
Dr. A Thirumani Devi
Professor
Department of Food Science and Nutrition

Members
Mr. K. Arulmoli (Legal Expert)
Dr. Subashini K. Sripathi
Dr. A Saraswathy(Medical Officer)
Ms. D. Kavitha
Dr. A R Sudamani Ramasamy
Dr. G. Victoria Naomi
Dr. Judith Justin
Dr. Anitha Subash
Dr. K. Sambath Rani

To
Ms. K. Suganya
Department of Biotechnology
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore- 641043

Dear Suganya,

Ref: Your proposal No. IHEC/22-23/BT-02 entitled "Identification of Diagnostic Biomarkers in Breast Cancer'' submitted for approval of IHEC on 19.11.2022.

The Institutional Human Ethics Committee of our University hereby grants approval to your research proposal No.IHEC/22-23/BT-02 entitled "Identification of Diagnostic Biomarkers in Breast Cancer" submitted by you. The Approval number for the same is AUW/IHEC/BT-22-23/FHP-02.

We wish you all the best in your research endeavours.

Regards

6-2-23

Dr. A Thirumani Devi
Member Secretary

Annexure III

Questionnaire and Consent form

PERSONAL INFORMATION

Name (பெயர்)		No. of siblings (உடன் பிறப்புகள்)	Male(ஆண்)___ Female(பெண்)____
Age (வயது)		Migration history (இடம் பெயர்வு வரலாறு)	
Male/Female (ஆண்/பெண்)		Blood group (இரத்த வகை)	
Language (மொழி)		Height (உயரம்)	
Marital status (திருமண நிலை)		Weight (எடை)	
Occupation (தொழில்) Education		Phone number (தொலைபேசி எண்)	
Present Address (முகவரி)			

S.No	HABITS, MEDICAL AND FAMILY HISTORY	
	HABITS	
1.	How often do you exercise? (எத்தனை முறை நீங்கள் உடற்பயிற்சி செய்கிறீர்கள்?)	➢ Rarely/never(அரிதாக/ஒருபோதும் இல்லை) ➢ Less than once a week (வாரத்திற்கு ஒரு முறை குறைவாக) ➢ Once a week (வாரத்திற்கு ஒரு முறை) ➢ 2-3 times a week (வாரத்திற்கு 2-3 முறை) ➢ 4-6 times a week (வாரத்திற்கு 4-6 முறை) ➢ Every day (ஒவ்வொரு நாளும்)
2.	How many hours of sleep do you get? (உங்களுக்கு எத்தனை மணி நேரம் உறக்கம் வருகிறது?)	
3.	Is your job stressful or do you perform shift work (night duty)? (உங்கள் வேலை மன அழுத்தத்தைத் தருகிறதா அல்லது ஷிப்ட் முறை பின்பற்றுகிறீர்களா? இரவு நேரங்களில் பணி செய்கிறீர்களா?)	➢ Yes (ஆம்) ➢ No (இல்லை)

4.	How many days a week do you eat each of the following food? (பின்வரும் ஒவ்வொரு உணவையும் வாரத்தில் எத்தனை நாட்கள் எடுத்துக் கொள்கிறீர்கள்?)	➢ Fruit /Fruit juices(பழம்/பழச்சாறுகள்): ➢ Vegetables (காய்கறிகள்): Eggs (முட்டை): ➢ Fried foods (வறுத்த உணவுகள்): ➢ Smoked meat (புகைபதனிட்ட இறைச்சி): ➢ Smoked Vegetables (புகைபிடித்த காய்கறிகள்): ➢ Extra Salt intake(கூடுதல் உப்பு உட்கொள்ளல்): Meat consumption per week (வாரத்திற்குஇறைச்சிநுகர்வு) ➢ Pork(பன்றிஇறைச்சி): ➢ Fish(மீன்): ➢ Mutton(ஆட்டிறைச்சி): ➢ Chicken(கோழி): ➢ Others(மற்றவைகள்):
5.	Water intake per day (ஒரு நாளைக்கு நீர் உட்கொள்ளல் அளவு?)	
	TOBACCO AND ALCOHOL HISTORY	
6.	Do you smoke? If yes, how often? (நீங்கள் புகைபிடிப்பவரா? ஆம் என்றால், எத்தனை முறை?)	
7.	Do you consume alcohol? If yes, how often? (நீங்கள் மது அருந்துகிறீர்களா? ஆம் என்றால், எவ்வளவு உட்கொள்கிறீர்கள்?)	
	REPRODUCTIVE HISTORY	
8.	Age at Menarche (பூப்படைந்த வயது) No. of children (குழந்தைகளின் எண்ணிக்கை) Age at first delivery (முதல் பிரசவத்தின் போது தங்களின் வயது என்ன?) Duration of Breast feeding (தாய்ப்பால் கொடுத்த காலம்)	

	Birth control pills (பிறப்பு கட்டுப்பாடு மாத்திரைகள் எடுத்துக் கொண்டீர்களா?) Abortions (கருக்கலைப்பு ஏற்பட்டுள்ளதா?) Age at menopause (மாதவிடாய் நின்ற வயது)	
MEDICAL HISTORY		
9.	Have you ever been diagnosed with any other type of cancer? If yes, what type of cancer? (நீங்கள் வேறு ஏதாவது புற்று நோயை கண்டறிந்துள்ளீர்களா? ஆம் என்றால், எவ்வகை புற்றுநோய்?)	
10.	Do you have any major illnesses? If yes, what type of illness? (உங்களுக்கு ஏதேனும் நோய்கள் இருக்கிறதா? ஆம் என்றால், என்ன வகை நோய்?)	
11.	Have you done X-ray or CT scan? If yes, why? (நீங்கள் எக்ஸ்ரே அல்லது சி.டிஸ்கேன் செய்திருக்கிறீர்களா? ஆம் என்றால், எதற்காக?)	
12.	Have you ever had a surgical operation? If yes, mention. (நீங்கள் எப்போதாவது ஒரு அறுவைசிகிச்சை செய்திருக்கிறீர்களா? ஆம் எனில், குறிப்பிடவும்?)	
13.	When was your cancer diagnosed? (உங்கள் புற்றுநோய் எப்போது கண்டறியப்பட்டது?)	
14.	Which side of the breast was tumor found? (மார்பகத்தின் எந்தப் பக்கத்தில் கட்டிக் காணப்பட்டது?)	➢ Right (வலது) ➢ Left (இடது) ➢ Both sides (இருபுறமும்)

15.	After diagnosed with breast cancer, what type of treatment did you take? (நீங்கள் மார்பக புற்றுநோயைக் கண்டறிந்த பிறகு எந்த வகையான சிகிச்சையை மேற்கொண்டீர்கள்?)	➢ Surgery (அறுவை சிகிச்சை) ➢ Chemotherapy (கீமோதெரபி) ➢ Radiation (கதிர்வீச்சு) ➢ Hormone Therapy (ஹார்மோன் சிகிச்சை) ➢ Any other (வேறு எதாவது)
16.	Have you ever worked with or used the following: (பின்வருவனவற்றுள் நீங்கள் பணிபுரிகிறீர்களா?)	➢ Radiation (e.g. in a factory, laboratory or medical setting) (கதிர்வீச்சு (எ.கா. ஒரு தொழிற்சாலை, ஆய்வகம் அல்லது மருத்துவ அமைப்பில்)) ➢ Plastics (பிளாஸ்டிக்) ➢ Agriculture(Pesticides/Pest) விவசாயம் (பூச்சிக்கொல்லிகள் / பூச்சி)) ➢ Control/Mosquito Repellant Chemicals/Dyes (கட்டுப்பாடு / கொசு விரட்டும் இரசாயனங்கள் / சாயங்கள்) ➢ Any Other Exposure (வேறு எந்த வெளிப்பாடு)
	FAMILY DETAILS	
17.	Do you have any first-degree relatives - mother, sisters, daughters – with breast cancer? If yes mention their age (தாய், சகோதரிகள், மகள்கள் என மார்பக புற்றுநோய் உள்ளவர்களுடன் உங்களுக்கு ஏதேனும் முதல்நிலை உறவு இருக்கிறதா? ஆம் என்றால் அவர்களின் வயது)	
18.	Do you have any second degree relatives diagnosed with breast cancer and age? If yes mention their age (மார்பக புற்றுநோய் கண்டறியப்பட்ட இரண்டாம் நிலை உறவினர்கள் யாராவது உண்டா? ஆம் என்றால் அவர்களின் வயது)	

19.	Do you have any first or second degree relatives diagnosed with any other types of cancer? If yes mention their age (வேறு ஏதேனும் புற்றுநோய் கண்டறியப்பட்ட முதல் அல்லது இரண்டாம் நிலை உறவினர்கள் இருக்கிறார்களா? ஆம் என்றால் அவர்களின் வயது)	
20.	Do you have a twin sister/brother? (உங்களுக்கு இரட்டை சகோதரி / சகோதரர் இருக்கிறாரா?)	➢ Yes (ஆம்) ➢ No (இல்லை)
21.	Any other type of major inheritable diseases in the family? (குடும்பத்தில் வேறு ஏதேனும் பரம்பரை நோய்கள் உள்ளதா?)	

CONSENT

The information provided above was given with my full consent and I do not have any objection in providing my biological sample for research purposes. I have read and understood the consent information.

மேற்கண்ட தகவல்கள் அனைத்தும் எனது முழு ஒப்புதலுடன் வழங்கப்பட்டதாகும், மேலும் எனது உயிரியல் மாதிரிகளை ஆராய்ச்சி நோக்கத்திற்காக வழங்குவதில் எனக்கு எந்தவித மாற்றுக் கருத்தும் கிடையாது. நான் முழுமையாக படித்து புரிந்த பிறகே தகவல் அளிக்கிறேன்.

Place (இடம்):
Date (தேதி):

Signature (கையொப்பம்):
Name (பெயர்):

Publications

PUBLICATIONS

- **Suganya, K**., Sudha, B., Poornima, A., Senthil Kumar, N., and Sumathi, S. (2022) Reduced Expression of *SFRP1* is Associated with Poor Prognosis and Promotes Cell Proliferation in Breast Cancer: An Integrated Bioinformatics Approach. Indian J. Gynecol. Oncol., 20, 4. https://doi.org/10.1007/s40944-022-00650-z

- **Suganya, K**., and Sumathi, S. (2022) A Comprehensive Review on Role of Nutrition in Management of Breast Cancer. Indian. J. Nutr. Diet., 59, 506-523. https://doi.org/10.21048/IJND.2022.59.4.30271

- **Suganya, K**., Sumathi, S., Karthikesh, K., Bhargavi, S., and Sethumadhavan, T. (2023) Performance Analysis of Various Filters for Denoising Breast Cancer Histopathology Images. Indian J. Gynecol. Oncol., 21, 4. https://doi.org/10.1007/s40944-023-00761-1

Avinashilingam Institute for Home Science and Higher Education for Women
(Deemed to be University Estd. u/s 3 of UGC Act 1956, Category 'A' by MHRD
Re-accredited with A++ Grade by NAAC. CGPA 3.65/4, Category I by UGC
Coimbatore - 641 043, Tamil Nadu, India

Appendix L2
(Item No 5 of Check List)
Details of Research Publications

S.No	Article	Journal	Other Details Vol No/ Page No/ Year	Published in UGC CARE/ Scopus Indexed/ Web of Science
1.	Reduced Expression of *SFRP1* is Associated with Poor Prognosis and Promotes Cell Proliferation in Breast Cancer: An Integrated Bioinformatics Approach	Indian Journal of Gynecologic Oncology	20,4,2022	Scopus
2.	A Comprehensive Review on Role of Nutrition in Management of Breast Cancer	The Indian Journal of Nutrition and Dietetics	59,4,2022	UGC-CARE
3.	Performance Analysis of Various Filters for Denoising Breast Cancer Histopathology Images	Indian Journal of Gynecologic Oncology	21,4,2023	Scopus

*Proof of list of Journals from Internet to be attached along with copies of reprints.

Scholar : Suganya 10.05.2024

Supervisor : 10/5/2024

Checked By:

HoD 10/5/24

Dean of Respective School 10/5/2024

The Scholar miss. Suganya, K (20PHBTF001) has published her research articles in the following journals:
1. Indian Journal of Gynecologic oncology - indexed in Scopus from 2016 to present.
This may be considered.

10.05.24

Indian Journal of Gynecologic Oncology (2022)20:46
https://doi.org/10.1007/s40944-022-00650-z

ORIGINAL ARTICLE

Reduced Expression of *SFRP1* is Associated with Poor Prognosis and Promotes Cell Proliferation in Breast Cancer: An Integrated Bioinformatics Approach

Kanagaraj Suganya[1] · Balraj Sudha[1] · Arumugam Poornima[1] · Nachimuthu Senthil Kumar[2] · Sundaravadivelu Sumathi[1]

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Abstract

Purpose Breast carcinoma is the most frequent form of malignancy in women globally. Exploration of the breast cancer genome tiled the way for the validation of novel cancer biomarkers and to explore various mechanisms involved in the progression of carcinogenesis. The purpose of the research is to find an identification of potential gene linked to breast cancer (BC) progression and prognosis.

Methods Three datasets (GSE71053, GSE61724, and GSE36295) were downloaded from the Gene Expression Omnibus (GEO) database. An integrated analysis of several gene expression profile datasets was used to find differentially expressed genes (DEGs) in BC and normal breast tissue samples. Protein–protein interaction (PPI) network was used to verify hub genes associated with the pathogenesis and prognosis of BC. The functional enrichment and pathway analysis was performed by FunRich and cBioPortal. The expression pattern was assessed using COSMIC, GEPIA2, and BC-GenExMiner.

Results The results revealed that among the hub genes, Secreted Frizzled-related protein 1 (*SFRP1*) was a negative regulator of the Wnt pathway in breast cancer. Loss of *SFRP1* may result in abnormal cellular proliferation, migration, and invasion, which may trigger cancer cells, leading to progression of the disease, poor prognosis, and therapy resistance. Lastly, the Kaplan–Meier plotter online database demonstrated that expression levels of the *SFRP1* gene were related to lower survival.

Conclusion The findings of this research would provide some directive significance for further investigating the diagnostic and prognostic biomarker to facilitate the molecular targeting therapy of breast cancer; *SFRP1* expression may be effective as a novel prognostic biomarker in early breast cancer.

Keywords Breast cancer · Integrative analysis · Hub genes · *SFRP1* · Prognostic biomarker

Abbreviations

BC	Breast cancer
ER	Estrogen receptor
PR	Progesterone receptor
HER-2	Human epidermal growth factor receptor-2
GEO	Gene expression omnibus
DEG	Differentially expressed genes
PPI	Protein–protein interaction
STRING	Search tool for the retrieval of interacting genes/proteins
TCGA	The cancer genome atlas
cBioportal	Cancer genomics portal
COSMIC	Catalogue of somatic mutations in cancer
GEPIA	Gene expression profiling interactive analysis
KM Plotter	Kaplan–Meier plotter
RFS	Relapse-free survival
OS	Overall survival
HR	Hazard ratio
EGA	European genome-phenome archive
GO	Gene ontology

✉ Sundaravadivelu Sumathi
sumathi_bc@avinuty.ac.in

[1] Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu 641 043, India

[2] Department of Biotechnology, Mizoram University, Aizawl, Mizoram 796 004, India

Published online: 28 October 2022

BP	Biological process
MF	Molecular function
SFRP1	Secreted frizzled related protein 1

Introduction

Breast cancer (BC) is the most frequent cancer in women and the second leading cause of cancer death. Breast cancer is associated with several risk factors, including long-term fertility, hormonal contraceptive usage, lack of physical activity, and alcohol intake; nonetheless, its etiology and pathophysiology are not fully determined. Several genes and cellular processes are implicated in the genesis and progression of BC [1].

The molecular mechanisms underlying the development and progression of BC tumors are unclear. As a result, determining the development of disease and key signaling pathways is vital for developing more efficient diagnostic and treatment strategies. Bioinformatics analysis has been used to promote oncology research in recent years, providing a basis for better disease prevention, early detection, and therapy [2]. Using bioinformatics tools, we can now screen and identify essential genes comprehensively. Identification of potential genes and pathways linked to BC carcinogenesis and disease prognosis will not only to the discovery of new diagnostic biomarkers and treatment targets but also helps in elucidating the underlying molecular mechanisms [3].

Microarrays are especially useful for detecting differentially expressed genes (DEGs) since they can detect gene expression on a global level rapidly. Gene chips are a type of microarray that allows for high-throughput gene expression studies with excellent sensitivity, selectivity, and reliability [4]. Microarrays have produced a huge amount of data, and the majority of that data have already been uploaded and preserved in publicly available databases searches. This insight could help researchers understand better the molecular pathways causing BC [5].

In this study, we tried to identify novel indicators for prognosis in BC patients as well as the prospective therapeutic targets for this disease. An integrated analysis of DEGs involved in BC will reveal more information about the BC mechanism. These findings could serve as the basis for the development of future BC diagnostic and therapeutic tools.

Methods

Gene Expression Profile Data

GSE71053, GSE61724, and GSE36295 gene expression datasets were screened out based on gene expression omnibus (GEO) datasets, a public repository for data storage containing microarray data (http://www.ncbi.nlm.nih.gov/geo/). The DEGs in BC samples were compared with normal samples using the Limma tool in R language [6]. DEGs were calculated using the following criteria: jlog2FCj ≥ 1 and adjust P value < 0.05.

Functional Enrichment Analysis of DEGs

FunRich was used for functional enrichment and interaction network analysis of genes and proteins to elucidate the underlying biological processes and molecular activities of DEGs [7]. Meanwhile, P-value < 0.05 was defined as the cut-off criterion.

Protein–Protein Interaction (PPI) Analysis

To construct a PPI network, DEG protein products were matched to the search engine for retrieving the interacting genes database (STRING, https://stringdb.org/cgi/input.pl), using a confidence score ≥ 0.9 as the cut-off criterion. The PPI network was visualized using the Cytoscape software [8].

Pathway Analysis

Breast invasive carcinoma datasets (TCGA, PanCancer Atlas) encompassing 1084 samples were chosen from the Cancer Genomics Portal (cBioportal) (http:// www.cbioportal.org) to investigate gene alterations and activities of hub genes in breast cancer. We developed a group using cBioportal to display hub genes in the context of biological interactions derived from public pathway databases [9].

Analysis of Genetic Alterations of Hub Genes

Somatic mutation information from COSMIC (https://cancer.sanger.ac.uk/cosmic) was utilized to examine hub gene alterations in breast cancer [10].

Analysis of Expression Level and Correlation Analysis

The gene expression profiling interactive analysis (GEPIA, http://gepia.cancer-pku.cn/index.html) was used to analyze the hub gene's expression level and correlation. It

Table 1 Characteristics of three datasets in this study

Expression profile dataset	Platform	Number of samples	
		Breast cancer	Normal
GSE71053	GPL570[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	6	12
GSE61724	GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]	64	4
GSE36295	GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]	45	5

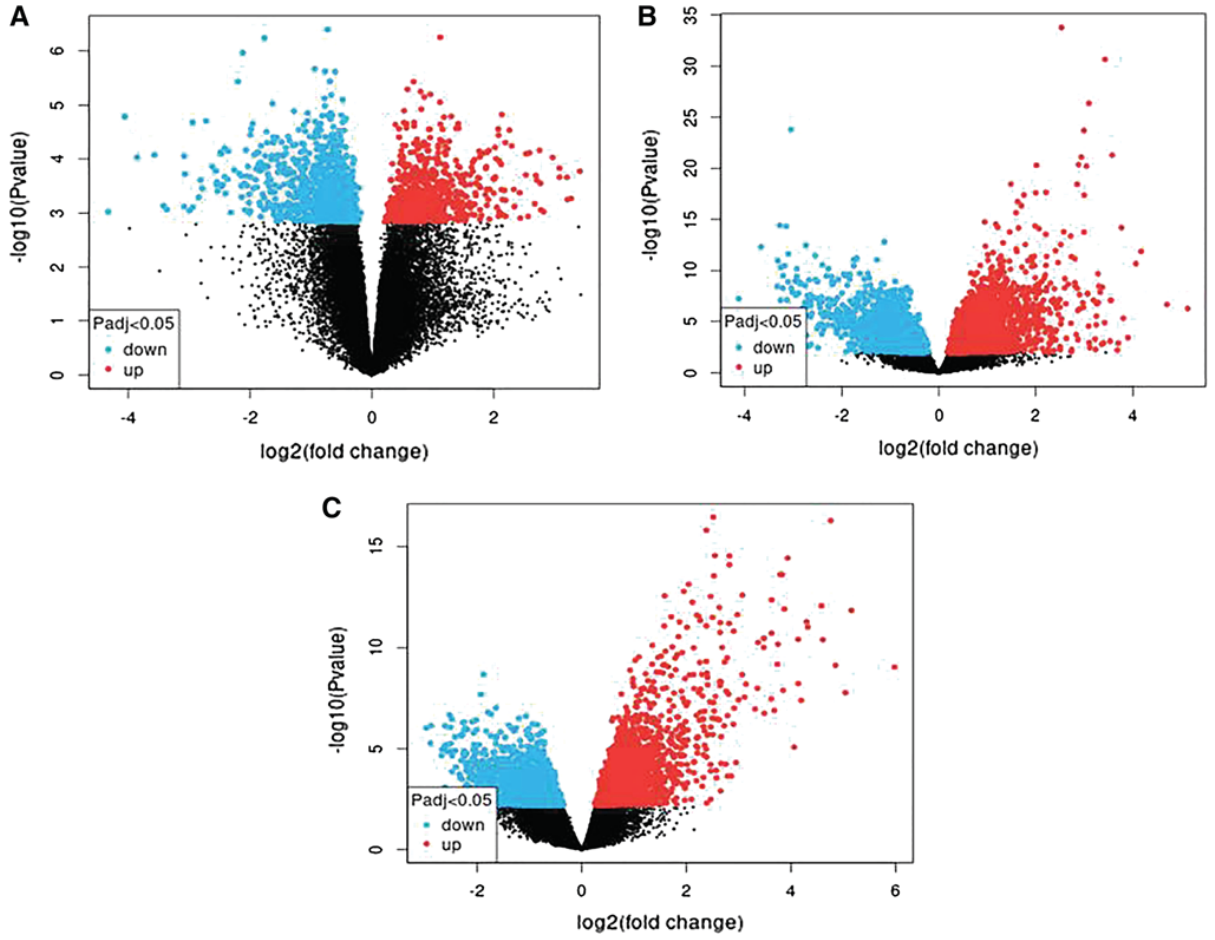

Fig. 1 Volcano plot of gene expression profile data in breast cancer samples and normal tissues of DEGs. **a** Volcano plot of GSE71053; **b** Volcano plot of GSE61724; **c** Volcano plot of GSE36295

examines tumor and normal differential expression and was used to show the expression of hub genes in BC and normal tissues. The link between these hub genes was then shown using a boxplot [11].

Assessment of Clinicopathological Parameters

The Breast Cancer Gene Expression Miner v4.4 (https://bcgenex.centregauducheau.fr/BC-GEM/GEM-Accueil.php?js=1), a DNA microarray and RNA-seq database were used to analyze prognosis based on gene expression.

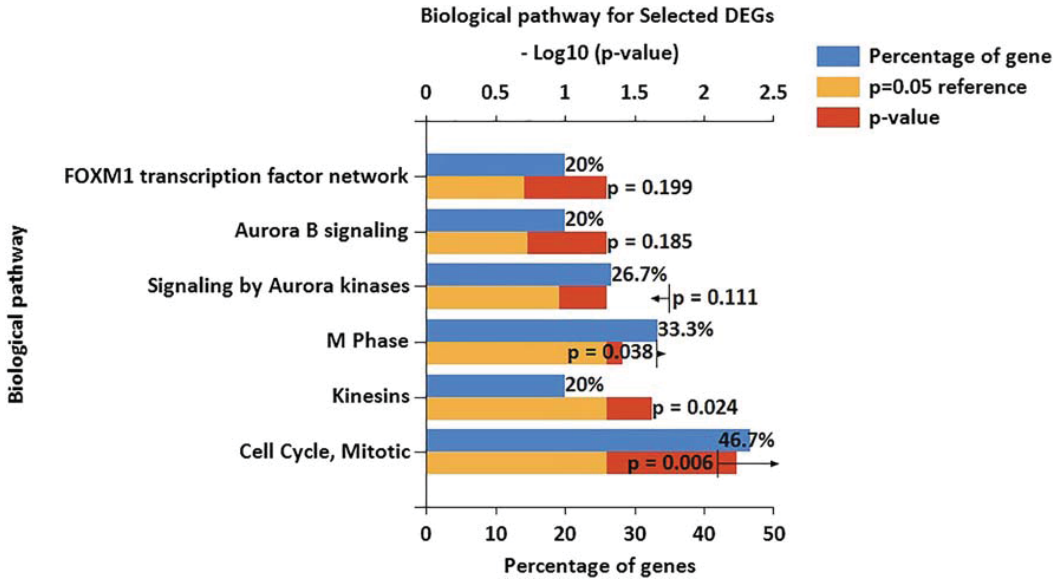

Fig. 2 Biological pathway for differentially expressed genes

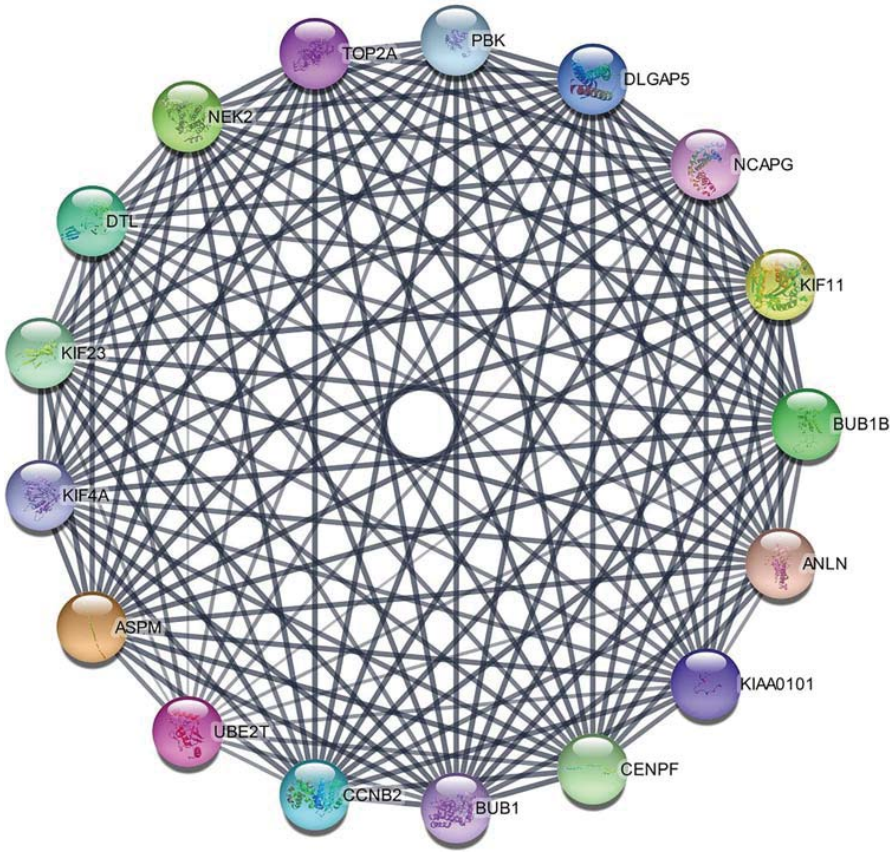

Fig. 3 PPI network analysis of DEGs

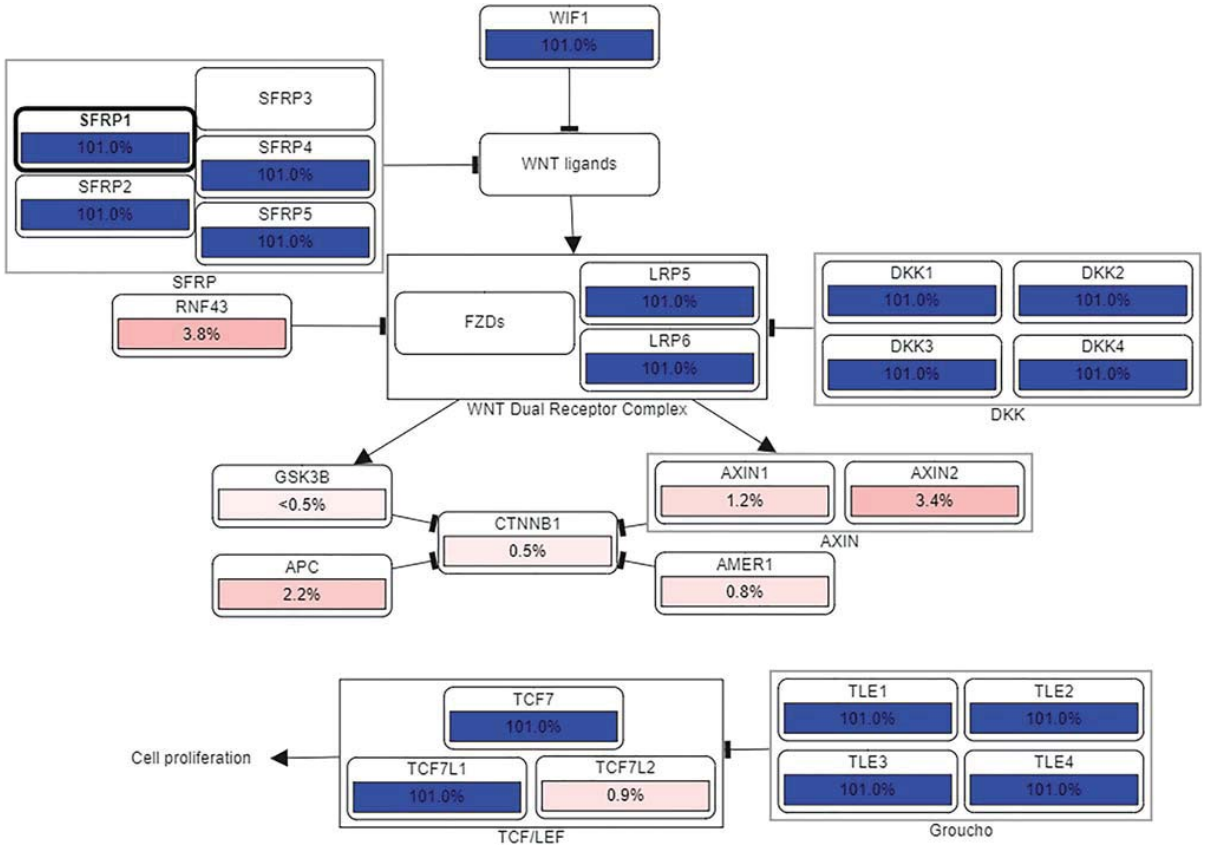

Fig. 4 The Wnt pathway analysis and the loss of *SFRP1* lead to cell proliferation in breast cancer

Clinicopathological parameters, such as ER, PR, and HER-2, were evaluated [12].

Survival Analysis

The hub gene's prognosis values were calculated using the Kaplan–Meier plotter (KM plotter, http://kmplot.com/analysis/) mRNA BC database. According to this software, the relapse-free survival (RFS) and overall survival (OS) information were based on GEO, TCGA, and EGA database. To assess the relationship between gene expression and survival, the hazard ratio (HR) with 95% confidence intervals and log-rank *P* value were calculated and plotted [13].

Results

Identification of Differentially Expressed Genes (DEGs)

Three gene expression profiles were selected, and Table 1 shows the comprehensive information about 115 breast cancer and 21 normal tissue samples in the included datasets. A total of 24 DEGs comprising 19 down-regulated and 5 up-regulated genes were retrieved after the integrated analysis of three GEO datasets. Figure 1 shows the volcano plot of the DEGs.

Functional Enrichment Analysis of DEGs

FunRich software was used to perform enrichment analysis for up- and down-regulated DEGs after gene integration. Cell division, mitotic nuclear division, kinesins, aurora B signaling, FOXM1 transcription network, signaling by aurora kinases, and M phase signaling were found to be notably abundant in up-regulated DEGs and down-regulated DEGs (Fig. 2).

PPI Network Construction and Analysis of Interrelations Between Pathways

The STRING database was used to construct a PPI network. A total of 23 nodes and 136 edges were mapped in the PPI network with a local clustering coefficient of 0.739

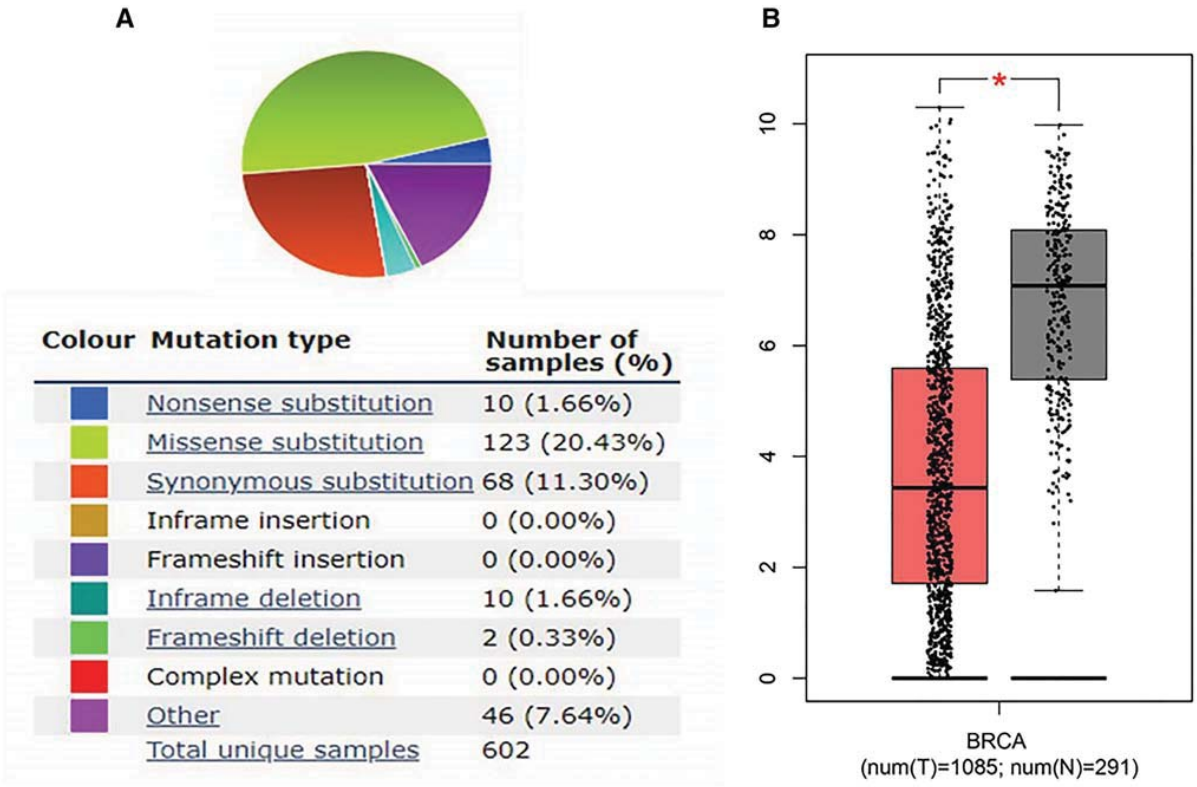

Fig. 5 Mutation and expression analysis of *SFRP1*. **a** Breast cancer mutations observed in *SFRP1* using a COSMIC database; **b** Expression of *SFRP1* in breast cancer patients. Down-regulated expression of *SFRP1* in breast cancer patients when compared with adjacent normal tissues

and a PPI enrichment *P*-value $< 1.0e^{-16}$. Figure 3 depicts the information for the PPI network constructed in string and visualized using cytoscape. Gene ontology (GO) analysis showed that the DEGs are involved in the biological process (BPs) and cellular components such as meiotic sister chromatid cohesion and centromeric, actomyosin contractile ring assembly, centrosome separation, mitotic spindle midzome assembly, and regulation of mitotic centrosome separation. Moreover, the GO molecular function (MFs) analysis showed that the DEGs are mainly involved in ATP binding, carbohydrate derivative binding, and anion binding. The DEGs were mainly enriched in pathways such as mitotic prometaphase, M phase, cell cycle, mitotic, and resolution of sister chromatid cohesion.

Pathway Analysis of DEGs Generated by cBioPortal

We analyzed the influence of DEGs on biological pathways in the breast cancer dataset containing 1918 samples. Among the DEGs, *SFRP1* involved in the WNT signaling pathway is the down-regulated gene. The Wnt signaling pathway's abnormal activation is linked to the formation of solid tumors including breast cancer. Analysis of breast carcinoma revealed similar frequencies of *SFRP1* loss in breast cancer (101%, respectively). Figure 4 depicts the loss of *SFRP1* gene activity and its modulation of the Wnt pathway.

Mutational Analysis of SFRP1

The *SFRP1* mutations were evaluated in 602 samples from patients with breast cancer. Out of 602 samples, the major types of mutation were found to be a missense substitution (123 samples), synonymous substitution (68 samples), nonsense substitution (10 samples), inframe deletion (10 samples) followed by frameshift deletion (2 samples) (Fig. 5a).

Validation of Down-Regulation of SFRP1 mRNA in Breast Cancer Tissues in TCGA Database

Using the GEPIA (Gene Expression Profiling Interactive Analysis) tool, we compared the mRNA expression of *SFRP1* between breast cancer and breast tissues. The

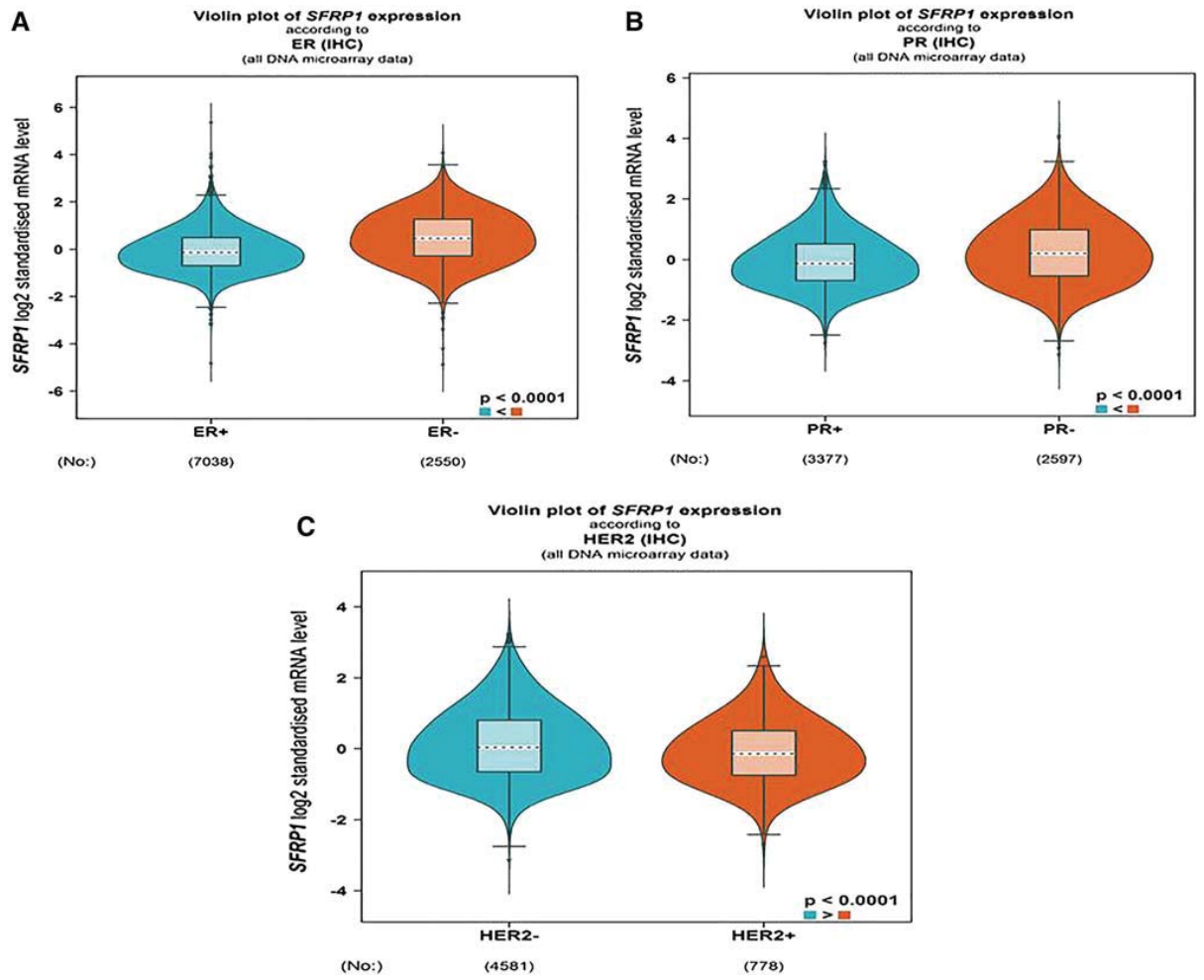

Fig. 6 Violin plot depicting *SFRP1* expression among groups of patients expressing receptor status. **a** ER; **b** PR; **c** HER-2 receptors

results indicated that the expression level of *SFRP1* was down-regulated in breast cancer tissues than in normal tissues and the difference was statistically significant as shown in Fig. 5b

Clinicopathological Relevance of SFRP1 Expression in Breast Cancer Patients

Next, the bc-GenExMiner database was used to determine the association between *SFRP1* expression and clinicopathological variables in patients with BC. The results demonstrated that *SFRP1* mRNA expression was negatively associated with estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER-2) status as shown in Fig. 6.

Survival Analysis and the Prognostic Value of SFRP1

To investigate the prognostic value of *SFRP1*, the survival analysis was conducted by the K–M plotter platform. Figure 7 shows the K–M survival curves for the *SFRP1* gene (HR = 0.91, $P = 0.069$). It was found that *the SFRP1* gene was the risky gene for prognosis with HR > 1 and $P < 0.01$. Higher expression of *SFRP1* predicts shorter survival times for BC patients.

Discussion

Despite advances in treatment, breast cancer remains the most common malignant tumor in women worldwide, with the highest rate of increase in prevalence. The understanding of breast cancer's molecular pathways is critical

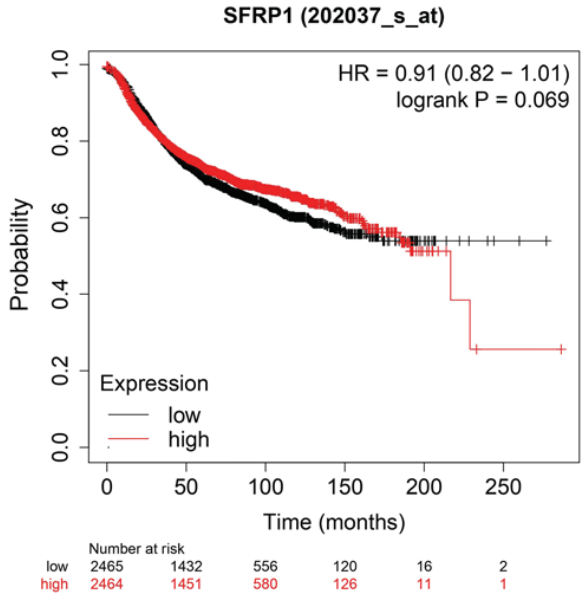

Fig. 7 Survival curves of *SFRP1*

for its diagnosis, treatment, and prognosis. The use of DNA microarray gene expression profiles to investigate DEGs involved in cancer has yielded useful diagnostic and medical applications [14].

In the present study, three gene expression profile datasets (GSE71053, GSE61724, and GSE36295) from the GEO database were retrieved and analyzed. The DEGs were identified using the 'limma' R package. The common DEGs were filtered out, and 24 hub genes were identified. GO function and pathway enrichment analysis was performed to further analyze the mechanisms of action of these DEGs. These DEGs were associated with the GO BP terms such as cell division, mitotic nuclear division, kinesins, aurora B signaling, FOXM1 transcription network, signaling by aurora kinases, M phase, meiotic sister chromatid cohesion and centromeric, actomyosin contractile ring assembly, centrosome separation, mitotic spindle midzome assembly, regulation of mitotic centrosome separation and response to ATP binding, carbohydrate derivative binding, and anion binding as molecular functions terms. Furthermore, the pathways of DEGs were mainly enriched in mitotic prometaphase, M phase, cell cycle, mitotic, and resolution of sister chromatid cohesion.

Of the 24 genes, *SFRP1* (Secreted Frizzled Related Protein 1) gene that is closely associated with breast cancer Wnt signaling pathway was identified. One of the most essential mechanisms controlling cell physiologic activities such as division, multiplication, and adhesion is the Wnt/β-catenin signaling pathway [15]. Wnt ligand bind to Frizzled proteins and lipoprotein receptor-related proteins 5 and 6 receptors initiates signaling in normal circumstances. Then, as a transcription cofactor with T-cell factor/lymphoid enhancer factor, β-catenin aggregates and modulates the transcription of genes involved such as c-myc and cyclin D1 [16]. Abnormal activation of the Wnt/β-catenin signaling pathway is a common occurrence in malignancy, and also the abnormal methylation state of Wnt antagonists including such Dickkopf proteins, Wnt inhibitory factor1, and SFRPs may contribute to it [17]. *SFRP1*, a member of the SFRP family, can inhibit Wnt/-catenin signaling by interfering with Wnt–receptor associations via an N-terminal cysteine-rich domain similar to Frizzled proteins. *SFRP1* is hypermethylated and down-regulated in breast cancer [18]. *SFRP1* hypermethylation and down-regulation are also associated with poor prognosis in breast tumors [19]. Moreover, *SFRP1* is associated with tumor chemotherapy, and some antitumor drugs inhibit cell growth through the re-expression of *SFRP1*. However, new research has revealed that *SFRP1* may also be strongly expressed in carcinomas and enhance tumor development or migration, in breast cancer [20].

Our study demonstrates that *SFRP1* is down-regulated in breast cancer patients which were analyzed in GEPIA and bc-GenExMiner database. Kaplan–Meier analysis showed that patient's low *SFRP1* expression had significantly poorer survival rates. These findings imply that the level of *SFRP1* expression can predict patient prognosis and could be used as a novel therapy target for personalized patient treatment. As a result, *SFRP1* may be linked to breast cancer pathogenesis and could be used as a diagnostic biomarker for the disease.

Conclusion

Using an integrated analysis approach of different cohort profile datasets, the current study discovered possible candidate gene *SFRP1* and the pathway involved in BC progression. These findings could contribute to a greater understanding of the biological mechanisms behind BC and the development of a possible biomarker. As a result, more research with higher patient cohorts is needed to validate the findings of this study. To characterize the precise roles of the identified gene, in vivo and in vitro examination of gene and pathway interaction is required, which might help to confirm gene functions and reveal the mechanisms behind BC.

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Author Contributions KS: Processing and writing the original draft of the manuscript. BS: Formal analysis. AP: Methodology. NSK:

Review and editing the manuscript. SS: Conceptualization, supervision, and editing the manuscript. All authors have read and approved the manuscript.

Funding Not applicable.

Data Availability Datasets analyzed in this study were retrieved from NCBI database (URL: https://www.ncbi.nlm.nih.gov/gds).

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

Consent for Publication Not applicable.

Ethical Approval Not applicable.

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A Comprehensive Review on Role of Nutrition in Management of Breast Cancer

Suganya, K. and Sumathi, S.

(Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore - 641 043, Tamil Nadu, India)

e-mail: sumathi_bc@avinuty.ac.in

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Abstract

Breast cancer (BC) is the most frequent malignancy in women and the second most common cause of cancer worldwide. There's a lot of evidence that lifestyle factors including food, body weight, and physical activity are linked to a higher risk of breast cancer. Several bioactive food ingredients, including both essential and non-essential nutrients, can change gene expression profiles. Consequently, nutrigenomics provides information on the effects of consumed nutrients and other food components on gene regulation and transcription factors, i.e., diet-gene interaction, to find dietetic components that are beneficial or damaging to one's health. Biological processes such as epigenetics, transcriptomics, and proteomics influence nutritional genomics (nutrigenomics), which is the junction of health, food, and genomics. As a result, it will help to determine unique nutritional requirements based on a person's genetic composition (personalized diet), and also the link between diet and chronic diseases such as cancer, opening up new avenues for a better understanding of the impacts of breast cancer and its management. Chemotherapy or radiotherapy patients with BC experience a variety of symptoms that influence their quality of life. According to research studies on nutritional therapy during BC treatment, nutritional counseling and supplementation with certain dietary elements may be useful in reducing drug-induced side effects and increasing therapeutic efficacy. As a result, nutritional control in BC patients may be considered a critical component of a multimodal treatment strategy. The goal of this review is to give a summary of the existing research on the association between dietary variables and BC.

Keywords: *Breast cancer, lifestyle, diet, nutrigenomics, personalized medicine*

Introduction

Breast Cancer (BC) is the most frequent cancer in women and the leading cause of mortality worldwide. Patients are divided into three groups depending on the presence or absence of Estrogen Receptors (ER), Progesterone Receptors (PR) and Human Epidermal growth factor Receptor 2 (HER2) in their tumor cells, and patients with Triple-Negative Breast Cancer (TNBC) having none of this receptors[1]. Only 5-10% of all malignancies are caused by hereditary predispositions, while environmental variables or poor lifestyles, such as alcohol consumption, unhealthy eating patterns, and obesity, cause 90-95% of tumor pathogenicity. Reproductive characteristics like the patient's age at menarche, age at first birth, breastfeeding status, and age at menopause have all been linked to an increased risk of breast cancer. These reproductive factors are known as non-modifiable risk factors because they cannot be managed or changed by public policies[2]. On the other hand, unbalanced diets, lack of physical activity, a high Body Mass Index (BMI), and excessive alcohol and/or tobacco consumption are all controllable lifestyle factors. Older age (>65 *vs* 55 years), age at the first childbirth more than 30 years, reduced fertility and not having children, contraceptive methods use, hormonal treatment after menopause, and lack of breastfeeding experience are all non-modifiable risks factors. Dietary decisions and being overweight or obese are linked to various risks of BC development and recurrence, among modifiable lifestyle factors. In recent decades, several researchers have aimed to investigate the relationship between various diets (such as fruits, vegetables, meat, and soy protein) and BC development. Nonetheless, it has been suggested that food may play a role in BC outcomes[3].

Adopting a diet that is low in red meat and high in fruits, vegetables, healthy grains, chicken, fish, processed foods, sweets, and high-fat dairy products may help women with early-stage BC to improve their overall prognosis and lifespan. Additionally, research has contributed to a correlation between physical exercise and an increased probability of surviving BC patients. The World Cancer Research Fund and American Institute for Cancer Research (WCRF/AICR) developed lifestyle guidelines based on appropriate research[4]. These guidelines suggest that maintaining a healthy body mass, being physically healthy, eating a diet high in fiber and soy, and reducing fat intake (especially saturated fatty acids) may all help to enhance overall survival after a BC diagnosis. Nutritional therapy in cancer patients undergoing chemotherapy has been proven to improve responsiveness and reduce the side effects of pharmaceutical cancer treatments[5]. In addition, altering one's lifestyle through food and exercise may lessen the negative

side effects of treatment plans in the long-term and enhance general health by lowering BC comorbidities (e.g., obesity, diabetes mellitus hypertension, and hyperlipidemia). Dietary variables have long been recognized as risk modifiers in carcinogenesis, impacting cancer initiation and progression[6].

Nutrigenomics is the study of the link between diet and chronic disease, as well as the assessment of individual nutritional requirements based on genetic makeup. Nutrigenomics is part of a rising trend toward personalized treatment that places a strong emphasis on food. Nutrigenomics is connected to nutrigenetics, which studies the genetic basis of people's reactions to various dietary stimuli[7]. Gene polymorphism is to blame for this occurrence. As a result, while genes play a part in determining a function, nutrition can change the level of gene expression. When comparing Western diets to Mediterranean or native Mexican cuisines, the risk of certain malignancies such as breast cancer increases. As a result, nutritional food should be provided to neutralize carcinogenic substances, target and eliminate premalignant lesions at an early stage and prevent cell proliferation (*i.e.,* induce apoptosis)[8].

As a result, the objective of this paper is to highlight the essential role of dietary variables and nutrition in breast cancer risk, prevention and therapy.

The connection between food and cancer

Carbohydrates

Insulin and Insulin-like Growth Factor 1 (IGF-1) levels in the blood have been connected to type 2 diabetes and have been implicated in breast cancer tumor development and progression. A range of dietary factors can influence insulin and IGF-I levels in the blood. Glycemic Index (GI) and Glycemic Load (GL) are the major determinants of postprandial glucose levels, and circulating insulin levels[9]. A high-GI or high-GL meal boosts blood glucose and insulin levels after eating, which may impact breast cancer risk by triggering insulin receptors in breast tissues and/or raising IGF-1 bioactivity, which stimulates cell proliferation. Strong evidence from cohort and meta-analytic studies has shown a strong link between a high-GI or GL diet and the risk of breast cancer. Furthermore, elevated insulin and blood glucose levels were connected to poor outcomes in women with breast cancer[10].

Red and processed meats

Red and processed meat consumption has been associated with a higher risk of total, cardiovascular, and cancer death. Potential human carcinogens found in red and processed meats include heterocyclic amines, n-nitroso compounds and polycyclic aromatic hydrocarbons. Meat also includes animal lipids and saturated fats, which have both been related to an

increased risk of breast cancer, especially the ER+/ER and HER2 subtypes[11].

Trans-fatty acids

Trans-fatty acids are typically found in industrially processed sweet and salty foods such as chocolates, candies, brownies, industrial bread, and packaged foods. Trans-fatty acids can cause changes in metabolic and signaling pathways, increased lipid levels in the blood, inflammatory processes, vascular dysfunction, and possibly increased visceral adiposity, body weight, and insulin resistance. According to a study, trans-fatty acids may be connected to an increased risk of breast cancer. Breast cancer has been linked to alcohol intake in both premenopausal and postmenopausal women[12].

Omega-6 fatty acids

Pro-inflammatory omega-6 (n-6) Polyunsaturated Fatty Acids (PUFAs) can be present in industrialized and fast foods, as well as poultry, egg, maize, and also most vegetable oils. They're thought to be an anti-inflammatory complement to omega-3 Polyunsaturated Fatty Acids (n-3) (PUFA). PUFAs are critical for metabolism, inflammation, cell communication, and gene expression regulation[13]. Evidence suggests that having a greater n-6/n-3 PUFA ratio increases inflammation and increases the risk of chronic inflammatory diseases such as obesity, non-alcoholic fatty liver disease, and cardiovascular problems. In preclinical studies, N-6 PUFAs appear to have a tumor-enhancing impact.N-6 PUFA intake was linked to an increased risk of breast cancer in a recent Japanese cohort study involving 38,200 women, with the strongest connection identified in ER+/PR+ tumors[14].

Foods with tumor-preventive potential

Fruits and vegetables

Inflammatory and metabolic processes, as well as endothelial function, have been demonstrated to benefit from anti-inflammatory and detoxifying components contained in fruits and vegetables. Fruit and vegetable consumption has been linked to a lower risk of cancer and overall mortality[15]. Furthermore, eating fruits and vegetables daily helped obese breast cancer survivors lose weight. Fruits contain antioxidant vitamins and dietary fiber, which both protect DNA from oxidative stress and may thus protect against breast cancer[16].

Polyphenols and fiber, both of which have been related to cancer protection, are abundant in the Mediterranean diet due to their high consumption of vegetables and fruits. Polyphenols' ability to combat oxidative stress and inflammation may be one of their potential mechanisms of action. For instance, Interleukin (IL)-6, which can limit BC development and metastatic activity, is regulated by polyphenols in blueberry powder. Additionally, lipoxygenase (LOX), cyclooxygenase (COX), and the

transcription factor NF-B, which are over expressed in tumor cells and necessary for the release of pro-inflammatory cytokines such tumor necrosis factor and IL-117, can be inhibited by polyphenols. Finally, numerous polyphenols have already been discovered to destroy estrogen signaling by binding the ER receptor or blocking aromatase, the enzyme responsible for estrogen synthesis. This inhibits tumor cell development. Fiber can prevent tumorigenesis by binding estrogen and lowering its levels in the blood, or by improving insulin sensitivity and reducing weight gain by a similar mechanism. Despite these results, a significant meta-analysis of 15 observational trials discovered a connection between eating vegetables and fruits which lower the risk of BC[18]. Higher consumption of greens and fruity vegetables was found to be inversely correlated with a lower incidence of BC in the Italian trial of the European Prospective Investigation into Cancer and Nutrition (EPIC). Fruit and vegetable consumption, particularly cruciferous and yellow/orange vegetables, has been linked to a decreased risk of BC[19].

Vitamins and minerals

Blood levels of carotenoids and vitamin C are good indications of vegetable and fruit consumption. Because of their antioxidant properties and ability to limit cell proliferation while maintaining DNA methylation and hormone metabolism, carotenoids, and vitamin C are thought to be linked to lower cancer risk. Increased total carotenoid and vitamin C concentrations have been linked to a lower risk of breast cancer in case-control studies, however, this link may be statistically stronger for ER-than ER+ breast cancer tumors[20]. Vitamin D is obtained mostly from UVB (ultraviolet B) radiation and is essential for several physiological functions. Calcium homeostasis, bone health, and anti-cancer activities have all been linked to it. In multiple studies, vitamin D has been associated with a reduced risk of breast cancer. Additionally, higher vitamin D levels may be correlated to a better chance of survival[21].

Targets for prevention and intervention

A proper diet, weight management, and physical activity are all critical for breast cancer prevention and recurrence treatment. Adolescence is thought to be the period in a woman's life when she is most exposed to breast cancer. In premenopausal women, red meat and fat consumption throughout adolescence were both associated with an increased risk of breast cancer. Premenopausal breast cancer risk may be increased by adolescent dietary patterns linked to inflammation, such as high intake of sugar-sweetened and diet soft drinks, refined grains, red and processed meats, and margarine, as well as low intake of green leafy vegetables, cruciferous vegetables, and coffee. As a

result, a healthy adolescent diet, similar to a sensible eating pattern, may reduce the incidence of breast cancer. Weight gain during breast cancer treatment is related to a poor prognosis and may increase the risk of recurrence. 'It has been shown that adopting a healthy lifestyle after cancer treatment, which includes eating nutritious foods, exercising regularly, and maintaining a healthy weight, improves cancer survivors' quality of life and overall outcome[23].

On the other hand, changing long-term dietary patterns is challenging. Even though the time when cancer is first diagnosed is often referred to as a "teachable moment," and many cancer patients actively seek advice on efficient strategies to enhance treatment-related outcomes, cancer patients are no more likely than healthy people of similar age, gender, ethnicity and socioeconomic status to maintain a healthy diet. As a result, to maintain dietary modifications, personalized support is required over time[24]. Weight loss interventions and dietary education can help patients improve their diet quality, lose weight, improve cardiorespiratory fitness, and improve their overall quality of life, fatigue, and body image after a breast cancer diagnosis, as well as decrease the occurrence of comorbidities and prefer modifications in biomarkers associated to breast cancer risk and prognosis[25].

Furthermore, physical activity helps patients to manage treatment adverse effects, minimize cancer-related fatigue, improve the quality of life and functional capacity, promote biomarker changes, and potentially improve the overall prognosis and longevity of breast cancer survivors[26]. Overall, these findings suggest that dietary and lifestyle interventions can improve nutrition in breast cancer survivors, and they could lead to weight loss and nutritional counseling as part of breast cancer treatment and care[27].

Many of the treatments employed in BC therapy have been demonstrated to have long-term negative consequences. Different chemotherapy medications, as well as radiation, surgery (mastectomy or lumpectomy), and hormone therapy, are employed depending on the stage. Surgery and radiation therapy are commonly used to treat BC in stages I to III, sometimes in combination with chemo or other pharmaceutical regimens before or after surgery. Systemic therapy is the standard treatment for stage IV breast cancer with distant recurrence (chemotherapy, hormone therapy, and antibody therapy).The most standard chemotherapy treatment regimens are CMF (cyclophosphamide, methotrexate, 5-fluorouracil) or anthracyclines (epirubicin or doxorubicin), which have been known to reduce mortality by 35%[28]. Nausea, vomiting, lack of appetite, dry mouth, and variations in taste or smell perception are all common side effects of the three-to six-

month treatment. The most prevalent side effect of chemotherapy for women is weight gain, which is correlated to a lower quality of life and a decreased likelihood of survival. According to the Women's Healthy Eating and Living (WHEL) study, women who get cytotoxic therapies had a 65 percent higher chance of weight gain during therapy than women who receive alternative treatments such as radiotherapy or hormone therapy (tamoxifen or aromatase inhibitors). A rise in body weight of 1 to 5 kg after chemotherapy is frequent, and it may be related to changes in body composition, such as an increase in abdominal fat and a loss of muscle mass (sarcopenic obesity)[29].

Because it might affect other medical diseases including diabetes, heart disease, hypertension, and hypercholesterolemia, being overweight or obese during chemotherapy could harm BC prognosis and overall survival. When energy intake exceeds energy expenditure, weight gain occurs. However, during the first year after diagnosis, calorie consumption decreases in BC patients receiving chemotherapy; thus, weight gain may be attributable to decreased physical activity and resting metabolic rate rather than overeating. Women following chemotherapy, surgery, or radiation had a 50% reduction in activity level due to continuous fatigue or a lack of energy[30]. Chemotherapy can cause early menopause and impact weight gain and tumor growth pathways in BC patients by decreasing glucose metabolism. The main evidence that physical activity-induced weight loss is connected to improved results for BC patients comes from the After Breast Cancer Pooling Project (AFCPP), large-scale research that examined post-diagnosis lifestyle factors and consequences in four prospective cohorts studies of BC survivors[31]. The study found that the risk of death was reduced by 27% in women who walked for at least 10 Metabolic Equivalent per Task (MET)-hours per week, or 3-5 hours per week. Furthermore, cohort analysis and small randomized studies have shown that lifestyle changes (such as changing one's dietary habits or increasing physical exercise) dramatically lower insulin, estrogens, IGF-1, and inflammatory markers secretion. Increasing physical exercise and lowering body fat in BC women to maintain a healthy weight may thus be a suitable intervention for improving prognosis[32].

Nutrigenomics

The ultimate objective of nutrigenomics is to develop genomics-based biomarkers to aid in the early detection and prevention of diet-related diseases like breast cancer. To achieve this goal, tissue-specific nutritional responses must be developed, which can be used as signatures or fingerprints to evaluate risk. Nutritional indicators that are available early in the disease process (e.g., initiation) can be used as prognostic tools[33]. The fact

is that the food comprises a vast number of substances and that each nutrient has different gene targets and affinities. The interaction of estrogens and isoflavones with estrogen receptors, for example, may have an impact on the development and prevention of breast cancer[34].

Nutritional genomics (nutrigenomics) acts as an intermediary between health, food, and genomics by combining molecular nutrition and genomics. Nutrigenomics will aid researchers in better understanding how nutrition influences metabolic processes and homeostatic regulation, how this regulation is influenced in the early stages of diet-related disease, and how specific sensitivity genotypes contribute to such disorders[35]. Nutrigenomics will also identify genes implicated in physiological effects on diet, as well as genes in which minor variations, known as polymorphisms, can have significant nutritional consequences, and the influence of environmental factors on gene regulation. To create personalized nutrition plans for improved prevention of diseases, the combined effect of genomic data and high-throughput "omic" technologies enables the development of new knowledge. This new knowledge is designed to improve our understanding of nutrient-gene interactions based on genotype[36]. Figure 1 shows how nutritional variables might influence cancer growth by influencing essential cellular processes.

In sporadic breast cancer research, fruits and vegetables, vitamin D, calcium,

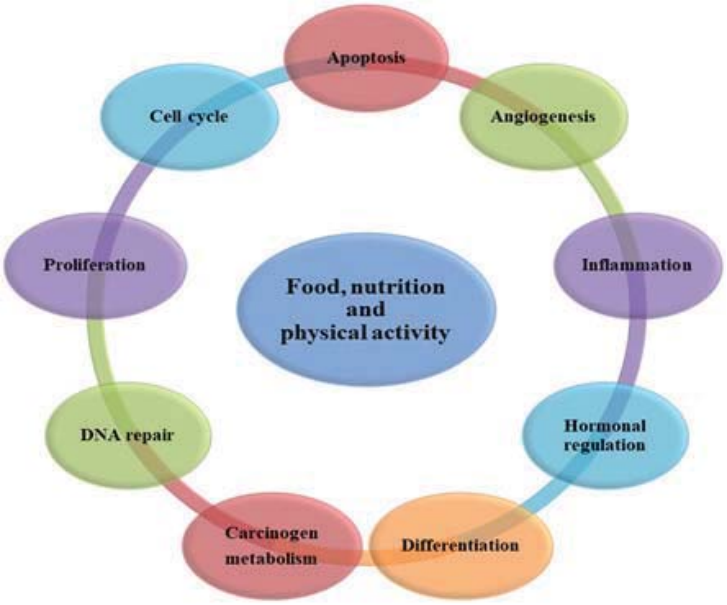

Figure 1
Food, nutrition, physical activity and the cellular processes linked to cancer

phytoestrogens, fish, and monounsaturated and polyunsaturated fatty acids, have all been implicated in a decreased risk of breast cancer. Breast cancer risk has been associated with a high intake of meat, poultry, total calories, fat intake, and saturated fatty acids[37]. Dietary Folate Equivalents (DFE) and breast cancer were the subjects of research by the Malmo Diet and Cancer cohort in people who had two MTHFR gene polymorphisms (MTHFR 677C/T and MTHFR 1298A/C). In MTHFR 677CT/ TT-1298AA carriers, a correlation between DFE and breast cancer was discovered. Furthermore, the connection between MTHFR genetic variations and breast cancer risk may be influenced by vitamin B intake. The risk of MTHFR breast cancer was shown to be highest in people who consumed the least dietary folate and vitamin B6[38].

The Singapore Chinese Health Study's nested case-control investigation found an inverse relationship between the risk of breast cancer and low folate consumption and weekly/daily green tea consumption compared to less green tea consumption. Women with elevated MTHFR/TYMS genotypes and weekly/daily consumption of green tea, especially those with inadequate folate intake, had a decreased breast cancer risk. These findings suggest that one of the ways green tea can protect against breast cancer is through folate modulation[39]. Epigenetic modulation may be involved in the anticancer effect of EGCG (tea polyphenol -epigallocatechin3-gallate). By modifying histone acetylation and methylation status, as well as affecting the chromatin structure of the ER promoter, EGCG has been shown to cause ER reactivation in ER-negative breast cancer cell lines. Furthermore, EGCG has been demonstrated to inhibit telomerase in MCF-7 cell lines by reducing hTERT promoter methylation and removing histone H3 Lys9 acetylation. Another study found a negative correlation between drinking green tea and breast cancer incidence in Asian-American women based on their catechol-O-methyltransferase (COMT) genotype. The degradation of tea polyphenols is known to be aided by this enzyme. Only tea drinkers (both green and black) with at least one low-activity COMT allele had a decreased risk of breast cancer. These research results of lowered risk of breast cancer with tea catechins, especially in women with reduced COMT alleles, recommend that these individual people have been less efficient in eliminating tea catechins, hence maximizing the benefits of the tea and its associated bioactive ingredient[40]. The Shanghai Breast Cancer Study looked at the relationship between the risk of developing breast cancer, the genetic variations in GSTP1, and other dietary factors such as green leafy vegetables. Breast cancer risk was associated with the GSTP1 Val/Val genotype, especially

in premenopausal women who ate little cruciferous vegetables. As a result, eating high-isothiocyanate cruciferous vegetables may reduce breast cancer risk while simultaneously altering the effect of the GSTP1 genotype. Moreover, genetics may have an impact on the relationship between marine n-3 fatty acids and breast cancer risk. The risk of breast cancer from marine n-3 fatty acids was 30% lower in those with genetic variations that encode decreased or no enzymatic activity of GSTT1 compared to women with high activity genotypes. This evidence suggests that n-3 fatty acid peroxidation products could help prevent breast cancer[41].

Watercress has anti-cancer capabilities due to its high concentration of Phenethyl Isothiocyanates (PEITC). By lowering the phosphorylation of the translation regulator 4E binding protein 1 (4E-BP1), a crude watercress extract was discovered to reduce angiogenesis, Hypoxia-inducible Factor (HIF) activity, and cancer cell proliferation[42].A breast case-control research found that women with two variant alleles for the ALOX5AP 4900 A/G who consumed a lot of linoleic acids had a greater chance of developing breast cancer than those with the AG or GG genotype. These findings suggest that when investigating the relationship between dietary fat and breast cancer risk, genetic predisposition related to n-6 polyunsaturated fatty acid metabolisms should be considered[43]. Additionally, the growth of the Polycomb Group (PcG) protein, a Zeste Homolog 2 (EZH2) enhancer, in breast cancer cells is decreased by dietary omega-3 polyunsaturated fatty acids. EZH2's histone 3 lysine 27 trimethylation (H3K27me3) activities were reduced in this study, while E-cadherin and insulin-like growth factor binding protein 3 were elevated. Treatment with omega-3 PUFAs reduced the ability of breast cancer cells to invade[44]. In addition, oxidative stress-related genes like catalase (CAT) C262T, myeloperoxidase (MPO) G463A, endothelial nitric oxide synthase (NOS3) G894T, and heme oxygenase-1 (HO-1) GT (n) dinucleotide length polymorphism were examined in a nested case-control study of postmenopausal women. Women who ate fewer vegetables and fruits, as well as those who had four or more low-risk alleles, were shown to have a higher risk of breast cancer, implying that both endogenous and exogenous antioxidants have a role in breast carcinogenesis[45].

Four SNPs in the genes 17 beta-hydroxysteroid dehydrogenase type I (17beta-HSD1), aromatase (CYP19), cytochrome P450c17alpha (CYP17), and sex hormone-binding globulin (SHBG) were shown to influence the link among isoflavone intake and risk of breast cancer. In postmenopausal Japanese women with the GG genotype for the SHBG gene and women with at least one variant allele

for the 17 beta-HSD1 polymorphism, the scientists found an inverse relationship between isoflavone intake and breast cancer risk, indicating that genetic variations of the 17 beta-HSD1 and SHBG genes may change the relationship among isoflavone intake and breast cancer risk[46]. Isoflavones' antiestrogenic properties, as well as their effects on DNA methylation, may reveal a link between isoflavones and breast cancer risk. It was discovered that consistently giving isoflavones to healthy premenopausal women generated dose-dependent methylation changes in the RARbeta2 and CCND2 gene promoters. Additionally, a negative correlation between genistein and the estrogenic marker complement C3 was found, suggesting an antiestrogenic action. Furthermore, genistein has been associated with epigenetic changes. After being exposed to genistein for a long time, Acetylated histone 3 (H3) expressions were shown to be down regulated in MCF-7 breast cancer cell lines. Changes in mitogenic factor and histone deacetylase inhibitor growth responses were also connected to this exposure. Although there is a wide range of responses to eating berries, it is thought that they may influence the risk of breast cancer (AICR Report). Preclinical studies suggest that E2-metabolizing enzyme levels reduce tumor formation during the early phases of E2 (Estradiol) carcinogenesis[47].

Diet has an impact on the BRCA gene, which has been related to breast cancer. A diet rich in vegetables serves to protect a woman's BRCA gene against activation. New research indicates that olive oil, a staple of the "Mediterranean diet," may reduce cancer risk in several different ways. Olive oil consumption has been linked to a lower risk of cancer in several epidemiological studies, and scientists are actively investigating this link in laboratory trials[48]. One of the ways by which the oil's cancer-preventive benefits are related may be the potential of the monounsaturated fatty acid (MUFA) oleic acid (OA; 18:1n-9) in olive oil to particularly control cancer-associated oncogenes. The well-studied oncogene HER2 (Her-2/neu, erbB-2) is implicated in the development, progression, and response to chemotherapy and endocrine therapy in about 20% of breast cancers. Exogenous supplementation of cultured breast cancer cells with physiological amounts of OA reduced HER2 over expression. Trastuzumab (Herceptin), a humanized monoclonal antibody that binds with high affinity to the ectodomain (ECD) of the Her2-coded p185 (HER2) oncoprotein, was likewise made more effective by OA treatment. Additionally, OA exposure decreased the proteolytic cleavage of the ECD of HER2 and, as a result, its activation state, a key molecular event that determines the aggressiveness of Her2-overexpressing

breast carcinomas and their response to trastuzumab. Upregulation of the ETS (Erythroblast transformation specific) protein PEA3, a DNA-binding protein that preferentially suppresses HER2 promoter activity in breast, stomach, and ovarian cancer cell lines, may similarly lower HER2 transcription[49]. The anti-HER2 effect of olive oil discloses a previously unknown molecular mechanism through which it can limit cancer cell malignancy. From a clinical standpoint, it could be an effective strategy to impact the outcome of Her-2/neu-over expressing human carcinomas with a poor outcome. Indeed, because it appears to investigate a variety of Her-2/neu-related carcinomas, OA-induced transcriptional suppression of the HER2 oncogene could represent a potential genetic link between olive oil and cancer. In another study, OA treatment elevated the Ets protein polyomavirus (PEA3) enhancer activator 3 in cancer cells that were over expressing Her-2/neu, a transcriptional regulator of the Her-2/neu promoter. For OA-induced gene suppression, a functioning PEA3 DNA-binding site at the endogenous Her-2/ neu gene promoter was also required. Her-2/neu protein levels in MCF-7/Her2-18 transfectants, which express full-length human Her-2/neu cDNA under the control of an SV40 viral promoter, were unaffected by OA treatment. The PEA3 protein's activity at the promoter level contributes to the OA-induced transcriptional inhibition of Her-2/neu50[50].

Various diet components and their cellular/ molecular effects on breast cancer

Every major signaling system in cancer is disturbed, including carcinogen metabolism, inflammation, cell proliferation, apoptosis, DNA repair, immunity, differentiation and angiogenesis. Each of these is increasingly being identified as a cancer-prevention molecular target. Because many of these sites appear to be customized by a variety of dietary components, separating nutrient–nutrient interactions and so determining what constitutes an ideal diet for health promotion becomes difficult[51].For instance, dietary bioactive substances such as genistein, curcumin, resveratrol, luteolin, lupeol, indole-3-carbinol, and others may target the intrinsic, mitochondrial-mediated pathway or the extrinsic, death receptor-mediated pathway of apoptosis, or programmed cell death, which is crucial in the management of cancer. Dietary components can influence apoptosis on numerous levels, resulting in changes in gene expression[52].

The anticancer, disease-prevention, and health-promoting qualities of Japanese knotweed, resveratrol, ginger, gingerols, rosemary carnosic acid, rosemarinic acid, ursolic acid and others have been demonstrated. The fact that numerous civilizations around the world regularly use these compound-rich foods, spices and herbs is another significant characteristic of them. According to cancer

prevention studies, nutrients affect all of the key signaling pathways that are uncontrolled in many types of cancer[53]. Carcinogen metabolism, DNA repair, inflammation, cell proliferation/apoptosis, differentiation, oxidant/antioxidant balance, and angiogenesis were some of the systems examined. More than a thousand phytochemicals with anti-cancer properties have so far been discovered. Breast cancer risk has been demonstrated to be reduced by dietary fiber. The physiological functions of growth, neural development, lean and fat mass accumulation, reproduction, innate and acquired immunity, viral, bacterial, and parasitic infectious pathologies, and the prevalence and severity of almost all chronic and degenerative illnesses, such as cancer, arthritis, diabetes, osteoporosis, atherosclerosis, stroke, neurodegenerative, inflammatory, and skin conditions are enhanced by long-chain polyunsaturated fatty acids (LC-PUFA). Fish oil, which is high in omega-3 fatty acids, inhibits the development of malignant tumors in both in vitro and in vivo settings[54].

Bioactive components in vegetables and fruits can assist to prevent cancer by avoiding metabolic activation and enhancing detoxification, among other factors. Flavonoids, phenols, isothiocyanates, allyl sulfur compounds, indoles, and selenium are examples of detoxifying enzymes that may be affected by a plant-based diet[55]. Covalent conformational alterations with specific DNA or RNA nucleic acids are brought on by the activation of carcinogens. Reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl radicals can harm DNA bases, which can result in a mistranslation of the nucleotide sequence. By interfering with DNA replication, such disturbances can generate changes in oncogenes and tumor-suppressor genes.ROS can also cause DNA strands to break, resulting in genetic mutations or deletions. Many plant-derived natural substances stimulate the generation of reactive oxygen species (ROS), which triggers cell death in cancer cells via apoptotic pathways[56].

Dietary guidelines

The World Cancer Research Fund/ American Institute for Cancer Research (WCRF) affords cancer health promotion and prevention guidelines that highlight individual lifestyle suggestions, such as (1) achieving and maintaining a healthy weight throughout life, (2) leading a physically active lifestyle, (3) eating a healthy diet rich in plant-based foods, and (4) limiting tobacco and alcohol consumption. Dietary guidelines for cancer prevention are based on nutrition-related aspects that are either clearly or probably linked to cancer risk[57]. According to the WCRF, a healthy cancer-prevention diet is one that (1) allows someone to be as healthy as possible without becoming underweight; (2) is rich in vegetables, fruits,

whole grains, and pulses; (3) includes only a small amount of red meat; (4) contains no processed foods; (5) contains limited salt; (6) reduces sugary drinks; (7) limits calorie-rich foods; and (8) prevents alcohol and tobacco consumption[58]. Women, who eat an anti-inflammatory diet consisting primarily of fruits, vegetables, seafood, and olive/sunflower oil, while avoiding many western-style foods, may be able to minimize their breast cancer risk. Finally, throughout and after cancer-targeted treatment, all breast cancer patients should be offered personalized, patient-centered and comprehensive nutritional interventions and weight management/ lifestyle programs[59].

Conclusion

Breast cancer biology is a prolonged process, and patients' dietary choices may have an impact on their risk of developing the disease as well as their post-diagnosis results. Dietary interventions can be used as a supplement to other therapies or as a secondary/tertiary prevention method. Future research should concentrate on factors such as race/ethnicity, tumor subtype, receptor status, reproductive characteristics, and lifestyle factors that influence breast cancer risk. Based on the finding, BC patients should be urged to improve their lifestyle rather than asserting the impact of certain meals or food constituents, and dietary habits before, during, and after treatment to improve their long-term survival and quality of life. The recognition that genetic history, gender, and lifestyle can all have an impact on dietary requirements is increasing, and we're edging closer to the individualized diet approach. As a result, nutrigenomic research has become a major source of hope for effective breast cancer treatment.

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ORIGINAL ARTICLE

Performance Analysis of Various Filters for Denoising Breast Cancer Histopathology Images

Kanagaraj Suganya[1] · Sundaravadivelu Sumathi[1] · Kuttiappan Karthikesh[2] · Swaminathan Bhargavi[2] · Thanikasalam Sethumadhavan[2]

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Abstract

Purpose Medical imaging plays a crucial role in healthcare, particularly in breast cancer diagnosis, which remains a leading cause of cancer-related deaths in women worldwide. Histopathology image analysis is the current gold standard for cancer diagnosis. However, the presence of noise in histopathology images poses a significant challenge and may lead to misdiagnosis. To ensure accurate diagnoses and preserve image features, this study aims to effectively remove noise from medical histological images for improved breast cancer diagnosis.

Methods The study utilizes Python-based filtering techniques, including Median, Gaussian, and Mean filters, to process both the MITOS-ATYPIA-14 dataset and real-time histopathology images. The performance of these filtering approaches is assessed using statistical measures such as mean squared error (MSE) and peak signal-to-noise ratio (PSNR).

Results The study findings reveal that both the Median and Gaussian filters exhibit exceptional performance on both the dataset and real-time histopathology images. These filters successfully reduce noise levels and enhance overall image quality.

Conclusion Based on the results, the Median and Gaussian filters demonstrate remarkable effectiveness in denoising histopathology images. As a result, these filters can be used for clinical research in breast cancer diagnosis, enabling more accurate and reliable assessments in medical practice.

Keywords Histopathology image · Denoising · Median filter · Gaussian filter · Mean filter

Introduction

Early detection and timely treatment of breast cancer are crucial for preventing its progression to an advanced stage. Various medical imaging techniques, such as mammograms, ultrasounds, MRI, and biopsies, are utilized for breast cancer detection. Among these, histopathology is the method used to definitively confirm the presence of cancer [1]. Histopathology slides are created by carefully processing tissue samples. The tissue is first submerged in formalin solution and then embedded in paraffin wax and stained with Hematoxylin and Eosin (H&E). From these tissue biopsy slides, histopathology images are captured, which play a vital role in improving the accuracy of breast cancer classification [2].

Noise can significantly alter the quality of a digital image. Noise refers to random variations in pixel values or unwanted artifacts that can be introduced during the image acquisition, processing, or transmission. The effects of noise on image quality can vary depending on the severity and type of noise present, as well as the specific image processing techniques used to mitigate noise [3]. However, it is essential to strike a balance between noise reduction and preserving important image details, because excessive noise can lead to loss of sharpness and detail in the final image [4].

Image processing encompasses various essential stages, including noise removal, feature extraction, and segmentation extraction. Among these stages, noise removal is considered the most critical and fundamental step [5]. Noise in digital images is primarily caused by factors like insufficient lighting and temperature for the imaging sensor.

✉ Sundaravadivelu Sumathi
sumathi_bc@avinuty.ac.in

[1] Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India

[2] Department of Oncology, Sri Ramakrishna Hospital, Coimbatore, Tamil Nadu, India

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Additionally, environmental factors, such as the presence of dust particles on the screen or interruptions during image transmission, can also impact the image sensor and introduce noise [6]. To achieve accurate and precise medical imaging diagnosis, it is crucial to obtain images devoid of blur, noise, and artifacts. To reduce noise and improve image quality, various noise reduction algorithms are employed in image processing software [7].

Several filtering methods commonly used to denoise histopathology images. Each method for denoising filter depends on the characteristics of the noise present in the images and the desired outcome. Popular filtering methods used for denoising histopathology images are Gaussian filter, Median filter, Mean filter, Wiener filter, Anisotropic diffusion filter, Wavelet denoising, Total variation (TV) denoising, Adaptive bilateral filter, and Contourlet transform denoising [8]. Noise techniques aid in smoothing out intensity values over the entire image, which codes for the capacity to remove noise. Image denoising is very significant and that directly correlates to the image's overall quality in further processing [9]. Removal of noise from an observed histopathological image is a more challenging aspect of medical image processing. The size and structure of histological images of breast cancer are quite large. Doctors require clear images for diagnosing the diseases of a patient. [10]. The examination of histology slides has been simpler with the development of digital scanners and image processing-capable computers, and the various pre-processing and segmentation techniques aid in the quick identification and characterization of malignant tissue. The difficulty of digital imaging has prompted advancements in image analysis methods, which have improved the opportunities available to pathologists for treatment [11].

In the present study, we have used three different filtering methods to remove noise from histopathological images. A comparative analysis is carried out to find the best filter by evaluating the MSE and PSNR values.

Methods

Collection of Breast Cancer Images

MITOS-ATYPIA-14, a publicly available database and real-time images collected from Sri Ramakrishna Hospital, Coimbatore. A total of 96 MITOS-ATYPIA-14 dataset images and 31 real-time images with a magnification factor of 40X were used for further processing.

Platform

The experiments were conducted on Python, specifically using Spyder as the integrated development environment (IDE). Spyder provides a user-friendly interface and essential tools for implementing and evaluating various denoising techniques on histopathology images.

Image Filtering

Median Filter

One of the nonlinear techniques used in the study was the Median filter, which was highly effective in removing various types of noise and restoring the sharpness of the images. The Median filter applied a 3X3 mask across the entire image, replacing each pixel value with its median value. For each pixel in the image, the Median was computed by sorting the elements within the mask in ascending order. The mask was then moved to the next pixel, and the process was repeated for all image components [12]. The Median is calculated as follows:

$$\hat{f}(x,y) = \underset{(x.t)\in S_{xy}}{\text{median}} \{g(s,t)\}$$

Gaussian Filter

The edges of the input image were preserved by utilizing the 2D distribution of the Gaussian filter. This involved combining the image with the 2D Gaussian distribution function. When applied to an image, the Gaussian filter operates in two steps. In the first step, the filter was centred on each horizontal pixel's values, and at each filter point, the weights were multiplied with the respective pixel values to obtain new pixel values. This process was carried out for all the horizontally processed pixels of the image. In the second step, all the horizontally processed pixels were filtered vertically, resulting in the final image where the edges were effectively preserved [13]. The Gaussian G(x,y) of an image was calculated as per the equation given below:

$$G(x,y) = \frac{1}{2\pi\sigma^2} \exp \frac{-x^2 + y^2}{2\sigma^2}$$

Mean Filter

The Mean filter, being a linear filter, was utilized to eliminate image noise caused by grain. This technique aimed to reduce the intensity difference between neighbouring pixels, resulting in smoother images. The process involved pixel-by-pixel scanning of the image, where each pixel's value was replaced with the average of its neighbouring pixels. The Mean filter is operated by substituting each image pixel's value with the average of its adjacent and neighboring pixels [14]. For arithmetic Mean filter,

$$f(x,y) = \frac{1}{mn} \sum_{(s,t)\in S_{x,y}} g(s,t)$$

Performance Analysis of the Filters

Mean Squared Error (MSE)

The MSE algorithm was employed to assess visible picture distortions. It involved calculating the difference between an image's estimated and actual pixel values . Images with high MSE values exhibited higher distortion compared than with low MSE values [15]. To calculate the MSE, the algorithm considered two monochrome images (X, Y) of size axb, where one image served as a noisy approximation of the other.

$$\mathrm{MSE} = \frac{1}{ab} \sum_{i=0}^{a-1} \sum_{j=0}^{b-1} \left[X(i,j) - Y(i,j)\right]^2$$

Peak Signal-to-Noise Ratio (PSNR)

The PSNR value served as a comparison of the quality between the original and noisy images. It was calculated as the ratio of the image's MSE value to its highest pixel value (Maxi) [16]. Before estimating the PSNR value, the MSE value was computed. A high PSNR value indicated a higher quality image, reflecting reduced distortion and noise in the image. This value was determined using the appropriate formula,

$$\mathrm{PSNR} = 10 \log_{10} \left[\frac{\mathrm{Max}_i^2}{\mathrm{MSE}}\right]$$

Results and Discussion

The noise removal filter techniques such as Median, Gaussian, and Mean filters were used for the images obtained from the MITOS-ATYPIA-14 dataset and real-time images from Sri Ramakrishna Hospital, Coimbatore. Figures 1 and 2 show the processed histopathology images using different filters. In the Median filtered image, the borders of the objects were significantly preserved, and image background information was also removed to project the actual image data. High-frequency noise signals were removed from the image while using the Gaussian filter. Compared to the original image, the image information was decreased in the case of the Mean filter. The visual observation concludes that the Median and Gaussian-filtered images included accurate information and were noise-free. We are the first to apply these filters in real-time breast cancer histopathology images.

The selection of an efficient filter for histological noise was aided by evaluating the mean squared error (MSE) and peak signal-to-noise ratio (PSNR) outcomes. The chosen

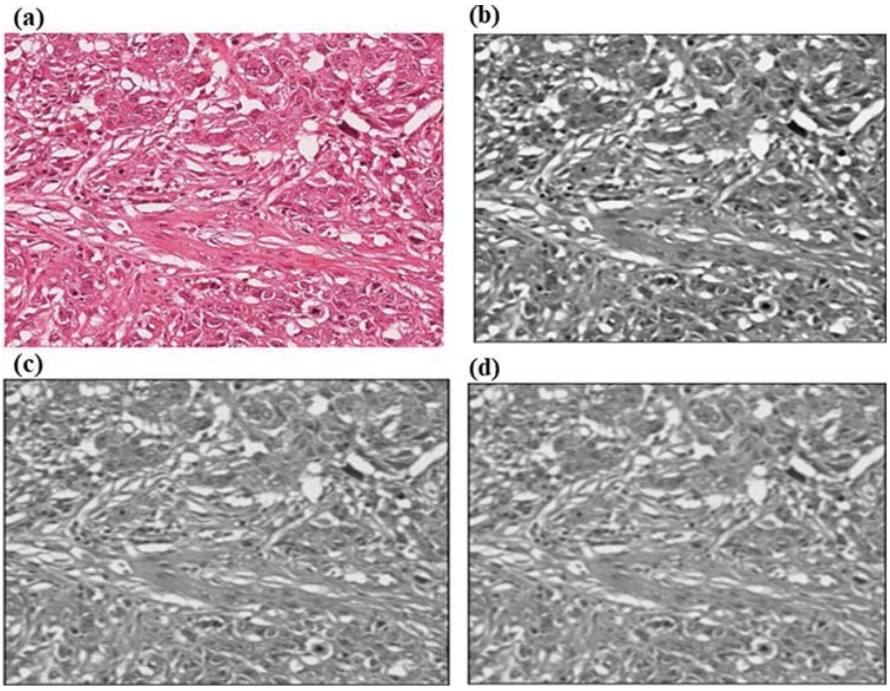

Fig. 1 MITOS-ATYPIA-14 dataset breast cancer histopathology image **a** Original image, **b** Median filter, **c** Gaussian filter, **d** Mean filter

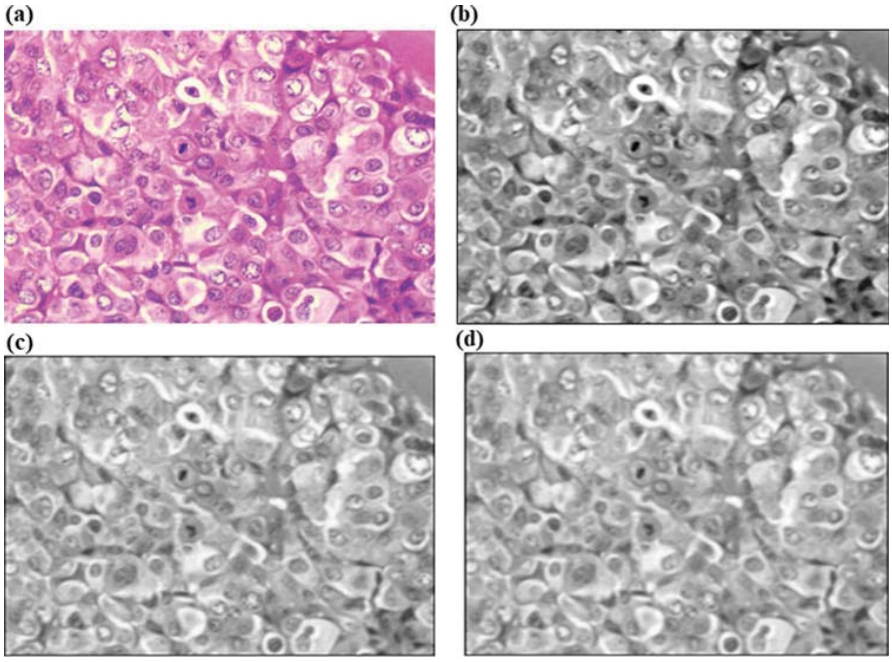

Fig. 2 Real-time breast cancer histopathology image **a** Original image, **b** Median filter, **c** Gaussian filter, **d** Mean filter

filter needed to exhibit low MSE and high PSNR values to achieve the highest image quality. These evaluation criteria played a crucial role in identifying the most effective noise removal technique, ensuring improved image quality and enhanced diagnostic capabilities for histopathology analysis. Overall, the dataset and real-time images were well-performed by the Median and, Gaussian filters. The MSE of both dataset and real-time images were employed, Median has a low MSE value of 0.90 and 0.41 and Gaussian found to be 0.89 and 0.40. The Mean filter MSE is 0.95 and 0.53. According to the performance study of the dataset and real-time images, the Median filter had a PSNR of 28.60 and 32.1, whereas the Gaussian filter had a better PSNR of 28.63 and 32.14. Low PSNR of 28.34 and 30.97 was maintained using the Mean filter. Tables 1 and 2 display the results of the filters performance analysis.

Histopathology of digital tissue is currently a significant application of machine learning and computational image analysis [17]. A different pre-processing technique was applied to improve performance on pathological and cytological slide images. To achieve smoothing of image features, Gaussian filter is used [18, 19]. All denoising techniques preserved the structures after removing the artifacts from a digital image [20, 21]. Two color models were used to blur and decouple the edges' sharpness while recovering the signal to reduce noise [22]. The "Wavelet Image Denoising" technique has been used to recover the original signal from the noisy one by eliminating the noise in the Bayesian model. This technique is based on a filter designed based on the minimum mean square error (MMSE). The filters in multistage convolution network architecture enhance the recognition and detection of a histopathological slide picture. It also reported that the Gaussian filter effectively removed the speckle noise of images [23].

In medical images, Gaussian, Pepper, Speckle, and Poisson noises are among the most commonly encountered types of noise. A Median filter was used to remove the salt and pepper noise in the whole slide images. [24]. The Median filter, known for its nonlinear filtering capability, has been employed in the pre-processing step. Its use is specifically crucial in psychological pictures of breast cancer as it effectively preserves the edges of cells. This preservation of edges is of utmost importance for accurate and reliable analysis in breast cancer imaging [25].

An effective Gaussian filtering technique was employed during the pre-processing stage for noise removal in breast cancer cytology images. This technique helps to enhance the image quality by effectively reducing noise, ensuring more accurate and reliable analysis for breast cancer detection and diagnosis [26]. In a study, 630 histopathology images were subjected to denoising using Gaussian filtering. This denoising technique was applied to enhance the interpretability of the images in the context of HCCANet, thereby facilitating the visualization of regions of interest. The utilization of Gaussian filtering aimed to improve the clarity and accuracy of the images, leading to better insights and understanding in the HCCANet model for detecting and analyzing carcinoma [27].

Noise removal techniques play a crucial role in histopathology image analysis, especially in the context of

Table 1 Performance analysis of different filters on MITOS-ATYPIA-14 dataset breast cancer histopathology images

S. No.	Image	Median filter		Gaussian filter		Mean filter	
		MSE	PSNR	MSE	PSNR	MSE	PSNR
1	A03_00Aa	0.87	28.71	0.88	28.70	0.94	28.40
2	A03_00Ab	0.88	28.68	0.88	28.68	0.94	28.38
3	5.A03_00Ac	0.89	28.62	0.89	28.66	0.95	28.35
4	A03_00Ad	0.88	28.67	0.87	28.72	0.94	28.41
5	A03_00Ba	0.92	28.51	0.91	28.52	0.97	28.26
6	A03_00Bb	0.89	28.64	0.89	28.63	0.95	28.35
7	A03_00Bc	0.87	28.74	0.86	28.79	0.93	28.46
8	A03_00Bd	0.90	28.62	0.89	28.62	0.95	28.35
9	A03_00Ca	0.90	28.59	0.90	28.61	0.96	28.33
10	A03_00Cb	0.88	28.68	0.89	28.69	0.94	28.39
11	A03_00Cc	0.93	28.43	0.93	28.46	0.98	28.22
12	A03_00Cd	0.90	28.59	0.90	28.60	0.96	28.32
13	A03_00Da	0.91	28.56	0.90	28.57	0.96	28.31
14	A03_00Db	0.90	28.60	0.89	28.62	0.95	28.36
15	A03_00Dc	0.90	28.58	0.89	28.64	0.95	28.34
16	A03_00Dd	0.92	28.50	0.92	28.49	0.98	28.23
17	A03_01Aa	0.92	28.49	0.91	28.53	0.97	28.25
18	A03_01Ab	0.89	28.62	0.89	28.65	0.94	28.38
19	A03_01Ac	0.92	28.48	0.91	28.52	0.97	28.24
20	A03_01Ad	0.89	28.66	0.88	28.68	0.94	28.40
21	A03_01Ba	0.86	28.76	0.84	28.87	0.91	28.52
22	A03_01Bb	0.81	29.02	0.80	29.11	0.88	28.70
23	A03_01Bc	0.86	28.80	0.84	28.84	0.92	28.51
24	A03_01Bd	0.82	28.98	0.81	29.03	0.89	28.64
25	A03_01Ca	0.77	29.26	0.77	29.25	0.83	28.96
26	A03_01Cb	0.90	28.58	0.90	28.63	0.95	28.34
27	A03_01Cc	0.90	28.58	0.90	28.59	0.96	28.33
28	A03_01Cd	0.90	28.59	0.89	28.62	0.95	28.33
29	A03_01Da	0.92	28.47	0.91	28.53	0.97	28.25
30	A03_01Db	0.83	28.96	0.81	29.06	0.88	28.67
31	A03_01Dc	0.92	28.50	0.91	28.54	0.97	28.27
32	A03_01Dd	0.86	28.77	0.85	28.83	0.92	28.49
33	A03_02Aa	0.90	28.59	0.88	28.68	0.94	28.38
34	A03_02Ab	0.92	28.51	0.90	28.58	0.96	28.29
35	A03_02Ac	0.94	28.39	0.91	28.46	0.98	28.21
36	A03_02Ad	0.92	28.49	0.90	28.57	0.96	28.29
37	A03_02Ba	0.89	28.66	0.89	28.64	0.96	28.33
38	A03_02Bb	0.88	28.70	0.88	28.69	0.94	28.39
39	A03_02Bc	0.90	28.57	0.89	28.62	0.96	28.33
40	A03_02Bd	0.86	28.79	0.86	28.76	0.94	28.42
41	A03_02Ca	0.90	28.60	0.88	28.67	0.95	28.36
42	A03_02Cb	0.92	28.51	0.91	28.55	0.97	28.27
43	A03_02Cc	0.86	28.79	0.84	28.88	0.91	28.52
44	A03_02Cd	0.92	28.50	0.90	28.57	0.97	28.28
45	A03_02Da	0.94	28.38	0.93	28.45	0.98	28.19
46	A03_02Db	0.89	28.64	0.88	28.67	0.95	28.34
47	A03_02Dc	0.92	28.51	0.91	28.56	0.97	28.28
48	A03_02Dd	0.90	28.59	0.89	28.63	0.96	28.31
49	A03_03Aa	0.95	28.33	0.95	28.38	1.00	28.15

Table 1 (continued)

S. No.	Image	Median filter		Gaussian filter		Mean filter	
		MSE	PSNR	MSE	PSNR	MSE	PSNR
50	A03_03Ab	0.91	28.52	0.91	28.56	0.97	28.28
51	A03_03Ac	0.93	28.45	0.92	28.48	0.97	28.24
52	A03_03Ad	0.88	28.66	0.87	28.72	0.94	28.40
53	A03_03Ba	0.96	28.29	0.96	28.33	1.00	28.12
54	A03_03Bb	0.90	28.58	0.90	28.57	0.96	28.31
55	A03_03Bc	0.93	28.45	0.92	28.48	0.98	28.22
56	A03_03Bd	0.90	28.60	0.90	28.61	0.95	28.34
57	A03_03Ca	0.89	28.65	0.89	28.64	0.95	28.36
58	A03_03Cb	0.94	28.42	0.93	28.45	0.98	28.21
59	A03_03Cc	0.95	28.36	0.95	28.35	1.00	28.14
60	A03_03Cd	0.95	28.34	0.95	28.36	1.00	28.14
61	A03_03Da	0.92	28.49	0.92	28.50	0.97	28.26
62	A03_03Db	0.87	28.71	0.95	28.37	1.00	28.15
63	A03_03Dc	0.91	28.55	0.90	28.58	0.96	28.32
64	A03_03Dd	0.87	28.71	0.87	28.73	0.94	28.41
65	A03_04Aa	0.93	28.45	0.92	28.49	0.98	28.21
66	A03_04Ab	0.93	28.45	0.92	28.49	0.98	28.23
67	A03_04Ac	0.91	28.56	0.90	28.60	0.96	28.31
68	A03_04Ad	0.92	28.50	0.91	28.54	0.97	28.27
69	A03_04Ba	0.89	28.62	0.88	28.70	0.95	28.37
70	A03_04Bb	0.88	28.66	0.87	28.73	0.94	28.38
71	A03_04Bc	0.86	28.79	0.86	28.80	0.92	28.50
72	A03_04Bd	0.88	28.71	0.87	28.73	0.94	28.40
73	A03_04Ca	0.92	28.50	0.91	28.54	0.97	28.26
74	A03_04Cb	0.91	28.52	0.90	28.57	0.96	28.29
75	A03_04Cc	0.90	28.57	0.90	28.62	0.95	28.33
76	A03_04Cd	0.92	28.48	0.91	28.53	0.97	28.26
77	A03_04Da	0.89	28.64	0.88	28.67	0.95	28.37
78	A03_04Db	0.88	28.68	0.88	28.68	0.95	28.37
79	A03_04Dc	0.91	28.56	0.90	28.60	0.96	28.32
80	A03_04Dd	0.89	28.64	0.89	28.66	0.95	28.35
81	A03_05Aa	0.89	28.64	0.88	28.67	0.94	28.36
82	A03_05Ab	0.89	28.64	0.89	28.64	0.95	28.34
83	A03_05Ac	0.88	28.67	0.88	28.70	0.94	28.38
84	A03_05Ad	0.88	28.70	0.88	28.67	0.92	28.41
85	A03_05Ba	0.86	28.77	0.86	28.79	0.92	28.47
86	A03_05Bb	0.89	28.62	0.92	28.51	0.97	28.25
87	A03_05Bc	0.89	28.65	0.89	28.65	0.95	28.37
88	A03_05Bd	0.89	28.62	0.89	28.65	0.95	28.38
89	A03_05Ca	0.89	28.64	0.89	28.64	0.95	28.35
90	A03_05Cb	0.91	28.53	0.91	28.52	0.97	28.28
91	A03_05Cc	0.88	28.69	0.88	28.69	0.94	28.39
92	A03_05Cd	0.90	28.59	0.90	28.61	0.96	28.33
93	A03_05Da	0.90	28.59	0.90	28.59	0.96	28.32
94	A03_05Db	0.90	28.58	0.90	28.59	0.96	28.33
95	A03_05Dc	0.89	28.62	0.89	28.63	0.95	28.37
96	A03_05Dd	0.90	28.57	0.90	28.59	0.95	28.33
Average		**0.90**	**28.60**	**0.89**	**28.63**	**0.95**	**28.34**

Table 2 Performance analysis of different filters on real-time breast cancer histopathology images

S. No.	Image	Median filter		Gaussian filter		Mean filter	
		MSE	PSNR	MSE	PSNR	MSE	PSNR
1	A656110_a	0.33	32.94	0.28	33.63	0.43	31.76
2	A656110_b	0.35	32.74	0.29	33.45	0.44	31.67
3	A656110_c	0.36	32.50	0.31	33.19	0.46	31.45
4	A656110_d	0.38	32.34	0.31	33.26	0.46	31.45
5	A685126_a	0.38	32.27	0.38	32.30	0.55	30.71
6	A685126_b	0.44	31.65	0.41	32.01	0.58	30.46
7	A685126_c	0.45	31.57	0.46	31.48	0.62	30.19
8	A685126_d	0.46	31.49	0.48	31.34	0.63	30.11
9	A491190_a	0.45	31.60	0.43	31.76	0.59	30.40
10	A491190_b	0.46	31.45	0.47	31.42	0.62	30.18
11	A491190_c	0.48	31.28	0.49	31.22	0.65	30.01
12	A491190_d	0.54	30.81	0.53	30.89	0.67	29.87
13	A684624_a	0.43	31.77	0.41	32.01	0.56	30.62
14	A684624_b	0.47	31.37	0.45	31.57	0.60	30.36
15	A684624_c	0.35	32.67	0.33	32.88	0.49	31.25
16	A684624_d	0.50	31.15	0.53	30.86	0.65	29.99
17	A688502_a	0.43	31.75	0.39	32.17	0.56	30.63
18	A688502_b	0.42	31.88	0.39	32.19	0.57	30.53
19	A688502_c	0.44	31.71	0.41	32.03	0.58	30.52
20	A688502_d	0.53	30.89	0.47	31.43	0.64	30.07
21	A688744_a	0.32	33.09	0.27	33.77	0.41	31.96
22	A688744_b	0.26	33.94	0.21	34.80	0.33	32.98
23	A688744_c	0.41	31.95	0.36	32.51	0.51	31.07
24	A688744_d	0.37	32.41	0.31	33.13	0.46	31.53
25	A116311_a	0.38	32.35	0.35	32.70	0.49	31.22
26	A116311_b	0.35	32.63	0.34	32.84	0.48	31.30
27	A116311_c	0.28	33.70	0.25	34.11	0.38	32.36
28	A703241_a	0.56	30.62	0.58	30.49	0.72	29.52
29	A703241_b	0.47	31.41	0.46	31.53	0.60	30.34
30	A703241_c	0.39	32.21	0.42	31.87	0.56	30.62
31	A703241_d	0.39	32.18	0.39	31.19	0.53	30.89
Average		**0.41**	**32.1**	**0.40**	**32.14**	**0.53**	**30.97**

breast cancer diagnosis and research. Histopathology involves the examination of tissue samples under a microscope to study the presence and characteristics of diseases like breast cancer. Histopathology image analysis often involves extracting meaningful features from the images for disease diagnosis or classification. Noise can interfere with accurately detecting tissue structures, cellular patterns, and other relevant features. Reducing noise makes the extracted features more reliable, leading to better classification and diagnosis outcomes. Identifying regions of interest, such as tumor regions or specific tissue structures, is vital in breast cancer diagnosis. Noise removal can aid in segmenting these regions more accurately and effectively. Cleaned-up images allow segmentation algorithms to better distinguish between different tissue components and identify abnormalities associated with breast cancer. Noise-free histopathology images provide more precise visualizations to pathologists, enabling them to make more accurate diagnosis. Noise removal ensures that important cellular details are not obscured or misinterpreted due to artifacts. Noise-free images are crucial for training deep learning models and other artificial intelligence (AI) algorithms in computer-aided diagnosis (CAD) systems. These models rely on clean and consistent data to learn and generalize effectively. Noise removal helps prepare the dataset for training and validation, leading to more robust and accurate AI-based analysis. Noise removal helps achieve more precise and reliable quantitative measurements from histopathology images. These measurements can include cell counts, nuclear features, and tissue characteristics, essential for quantifying disease progression and treatment response in breast cancer.

Based on the comprehensive performance analysis, our findings demonstrated that both the Median and Gaussian filters proved to be the most effective for denoising histological images. These results align with previously reported studies, further supporting the efficacy of these filters in addressing image histology noise. These filters can significantly improve the quality and interpretability of histopathology images, contributing to more accurate and reliable diagnostic outcomes in medical imaging applications.

Conclusion

In the medical domain, achieving a noiseless image is of utmost importance, as the accuracy of detection heavily depends on the quality of the image. Denoising techniques play a critical role in this process by effectively separating the noisy components from the image. Among the filters tested,the Median and Gaussian filters have shown remarkable success and yielded promising outcomes in noise removal from medical imaging. Their effectiveness in enhancing the image quality is particularly crucial for histopathology images. By denoising these images, a complete and accurate diagnosis, detection, and treatment of diseases are facilitated, empowering medical professionals to make more informed decisions and improve patient care.

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Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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