Molecular Comparative Study Of Janus Kinase2 Expression In Breast Cancer Of Human And Mice

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Abstract
Breast cancer is a multifactorial disease with the highest frequency in females. Genetic and environmental factors can cause mutation in several genes like tyrosine kinase, Janus kinases are a family of non-receptor tyrosine kinases involved in malignancies and other disease , In breast cancer We aimed to investigate its role at transcriptome level and its relationship with the clinical outcome of breast cancer in human and mice , This study enrolled a total of 25 samples for women with breast cancer , on the other hand we induce mammary gland carcinoma in mice by chemical carcinogenic (MNU) treated.
Twenty –five BC patients were included in this study , for these patients , 25 blood sample were taken for molecular analysis, sequenced using sanger sequencing for analyzing exon 9 of Jak2 gene , in mice tissue section of mammary gland were taken to DNA extraction for detection of mutations in jak2 gene
molecular analysis for women patient DNA extraction from blood using sanger sequencing , showed absence of SNP rs77375493 V617F , others SNPs in jak2 gene was demonstrated for the first time such as (Rs1168791735) G/A , (Rs1489598500)A/G and (Rs 755212421)C/A , in mice there was no detection of mutation by using sanger sequencing .

Introduction

The most common cancer to be diagnosed is breast cancer, which is also the second greatest cause of cancer-related death in women globally. (**Siegel et al.,2019**) Human cancer mouse models have been extremely helpful in studying carcinogenesis and providing experimental insights that other systems are unable to provide. (Walrath et al.,2010) , The human chromosome 9p24.1 region contains the gene for Janus associated kinase 2 (JAK2). Oncogenic characteristics such as tumorigenesis, invasion, metastasis, proliferation, survival, angiogenesis, anti-apoptosis, and immune evasion are promoted by deregulation of the JAK-STAT (Signal Transducer and Activator of Transcription) signalling system.(**Balko et al.,2016**) , The JAK2 mutation is of great significance as a response marker for targeted therapy of JAK kinase ,Although the JAK family members have been reported to be involved in the progression of various types of cancers, the clinical value of the entire JAK family remains poorly investigated in breast cancer. (**Kleppe et al.,2015**)
In addition ,there are no data about the jak2 gene expression in Iraqi BC population , thus our aim was to investigate the detection of Jak2 gene mutation in Iraqi breast cancer patients , In this study, we wanted to investigate whether genetic variation in the JAK2 gene regions is associated with the risk of breast cancer in human and mice , the secondary exploratory aim of this study was to compare the janus kinase mutation and expression between mice model breast cancer with human breast cancer to recommended the mice as human model for further cancer studies as cancer microenvironment or therapy strategies.

Material

Human

.Blood Sample collection

Blood sampling was carried out from Al-Hussein Teaching Hospital in karbala \ Iraq , Total twenty five blood samples of BC patients were enrolled in the current study with their permission and with the local ethics committee's blessing. blood samples were collected in 5 ml EDTA tubes, stored at 4 C until they were used for molecular analysis.

Animals

Twenty female albino mice , 8-weeks-old were obtained from College of Science,University of Kufa., randomly divided into two groups (N=10) :
Group (1): The C- group,as a normal control without any treatment received normal salin only .
Group (2): The MNU group, was received MNU only at dose 60 mg/kg for two weaks
Placed for 16 weeks, to ensure the development of mammary gland carcinoma. . All mice were euthanized by exsanguination ,under general anesthesia using ketamine and xylazine at doses of 50 mg/kg and 20 mg/kg of body weight,respectively (**Wellington et al.,2013**) after the end the experimental study mammary gland were collected from mice for molecular analysis for detection SNP(Single nucleotide polymorphisms) of janus kinase 2 via sanger sequencing.

Method

Molecular analysis

SNPs detection of jak 2 : by using sanger sequencing . The JAK2 (chromosome 9) gene promoter areas were searched for single nucleotide polymorphisms in the current investigation, and coding SNPs from the NCBI dbSNP database were utilized to determine tagging SNPs inside the gene regions. Validated SNPs whose genotypes matched the selected tagging SNPs by more than 80% were also employed.

DNA extraction from human

DNA extraction from blood were extracted by using G-spinTM Total DNA Extraction Kit from Blood. Korea, done according to company instructions

DNA extraction from mice

DNA extraction from tissue were extracted by using G-spinTM Total DNA Extraction Kit from tissue . Korea, done according to company instructions

Agarose gel electrophoresis of DNA

When the PCR program is complete, the electrophoresis is used to assess the quality of the DNA extractions and to see the size of the PCR product. . Depending on the product kind, the gels' concentration varied. The agarose gel was generally 0.7% for DNA quality, compared to 1-2% for pcr Based results.

Preparation of primers

The primers were originally lyophilized, and as per the primer synthesiser company's instructions, they were dissolved in free ddH2O to a final concentration of 100 M/l, which served as a stock solution that could be kept at -20 oC. To be employed as a work primer, a concentration of 10 M/l of the stock primers was created.

Primers used in this study

Table: (3.3): SNP rs77375493 V617F

Species	Gene	Primer name	5'-3'	Product	Accession number	Designer
Mus mucullus	JAK2	M93F	GACACACGCTGGTTCTCTTA	423 bp	NC_000085.7	UGene lab
		M93R	CATCCATCACCTGGGCTTTA			
Homo sapiens	JAK2	H93F	GCTCTCTCTCACTTTGATCTCC	529 bp	NG_009904.1	UGene lab
		H93R	TGGGCATTGTAACCTTCTACTT			

GoTaq® G2 Green Master Mix

GoTaq® G2 Green Master Mix is a high-quality Taq DNA Polymerase, deoxynucleotides, and reaction buffer solution that is prepared for use in a 2X concentration. It includes all of the tools required for DNA amplification. An inert green dye and a stabiliser are included in the GoTaq® G2 Green Master Mix to enable direct loading of the finished products onto a gel for analysis.

Table (3.4): Preparation of PCR solutions

Components	Concentration	Volume (50 µl)
GoTaq® G2 Green Master Mix	1X	25 µl
Forward primer	10 µM/µl	4 µl
Reverse primer	10 µM/µl	4 µl
ddH_2O	-	13 µl
DNA	40 ng	4 µl

Result

SNPS detection results

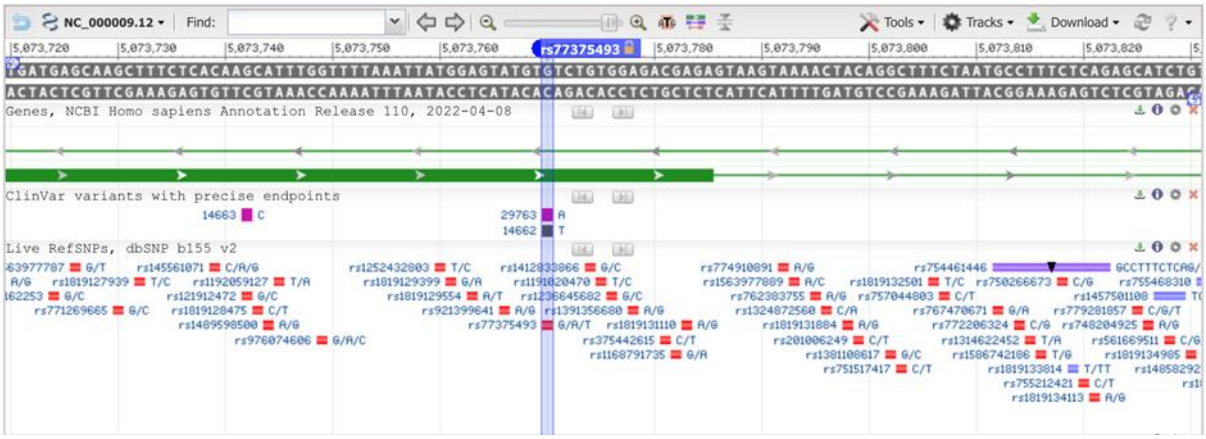
The present case-control study was performed to clarify whether genetic variations in the exon 14 of gene JAK2 of humans are associated with the risk of BC,in a small set of 25 BC samples

Table (4.1): SNPs detected in the tested samples of human

	SNPs			Affected samples
	Code of SNP	Nucleotide change	Amino acid change	
1	**rs1586741726**	**C/A**		21
3	**rs1296290861**	**A/C**		21
4	**rs1020069103**	**T/C**		21
5	**rs545677910**	**A/T**		21
9	**rs1489598500**	**A/G**		1,4,5,8,9,10,13,15,16,17,22,23,24
10	**rs1168791735**	**G/A**		1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,22,23,24
12	**rs755212421**	**C/A**		1,9,15,16,17,22

rs: Reference SNP

the table (4.1): there is 13 SNPs mutation in jak2 of breast cancer in human blood sample
The SNP (Rs1168791735) G/A snp was detected in 23 samples out of 25 samples
The SNP (Rs1489598500)A/G snp was detected in 13 samples out of 25 samples
The SNP(Rs 755212421)C/A snp was detected in 6 samples out of 25 samples
The SNPs : rs1586741726(C /A),rs1296290861(A /C),rs1020069103 (T/C),rs545677910 (A/T) was detected in one sample out of 25 samples



Fig(4.23): Some SNPs of gene JAK2 of Homo sapiens according to the SNP database of NCBI. The highlighted SNP is the tested mutation (rs77375493 G/T/A).

Homo sapiens Janus kinase 2 (JAK2), RefSeqGene (LRG_612) on chromosome 9

Sequence ID: NG_009904.1 **Length:** 149939 **Number of Matches:** 1

Range 1: 93268 to 93724 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
798 bits(432)	0.0	449/457(98%)	1/457(0%)	Plus/Plus

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Query  1      GGGGTTTCCTCAGAACGTTGATGGCAGTTGCAGGTCCATAT-AAGGGACCAAAGCACATT  59
              ||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  93268  GGGGTTTCCTCAGAACGTTGATGGCAGTTGCAGGTCCATATAAAGGGACCAAAGCACATT  93327

Query  60     GTATCCTCATCTATAGTCATGCTGAAAGTAGGAGAAAGTGCATCTTTATTATGGCAGAGA  119
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  93328  GTATCCTCATCTATAGTCATGCTGAAAGTAGGAGAAAGTGCATCTTTATTATGGCAGAGA  93387

Query  120    GAATTTTCTGAACAATTTATGGACAACAGTCAAACAACAATTCTTTGTACtttttttttt  179
              |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  93388  GAATTTTCTGAACTATTTATGGACAACAGTCAAACAACAATTCTTTGTACTTTTTTTTTT  93447

Query  180    CCTTAGTCTTTCTTTGAAGCAGCAGGTATAATGAGCAAGCTTTCTCACAAGCTTTTGGTT  239
              ||||||||||||||||||||||| |||| |||||||||||||||||||||| |||||||
Sbjct  93448  CCTTAGTCTTTCTTTGAAGCAGCAAGTATGATGAGCAAGCTTTCTCACAAGCATTTGGTT  93507

Query  240    TTAAATTATGGAGTATGTGTCTGTGGAGACAAGAGTAAGTAAAACTACGGGCTTTCTAAT  299
              |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  93508  TTAAATTATGGAGTATGTGTCTGTGGAGACGAGAGTAAGTAAAACTACAGGCTTTCTAAT  93567

Query  300    GCCTTTCTCAAAGCATCTGTTTTTGTTTATATAGAAAATTCAGTTTCAGGATCACAGCTA  359
              |||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  93568  GCCTTTCTCAGAGCATCTGTTTTTGTTTATATAGAAAATTCAGTTTCAGGATCACAGCTA  93627

Query  360    GGTGTCAGTGTAAACTATAATTTAACAGGAGTTAAGTATTTTTGAAACTGAAAACACTGT  419
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  93628  GGTGTCAGTGTAAACTATAATTTAACAGGAGTTAAGTATTTTTGAAACTGAAAACACTGT  93687

Query  420    AGGACTATTCAGTTATATCTTGTGAAAAAGGAAAGCA  456
              |||||||||||||||||||||||||||||||||||||
Sbjct  93688  AGGACTATTCAGTTATATCTTGTGAAAAAGGAAAGCA  93724
```

Fig (4.24): Blast of DNA sequence of a PCR product of sample AH04 against a RefSeqGene of JAK2 deposited in the NCBI (Accession number: NG_009904.1). This sample was showing high SNPs ratio among other samples.

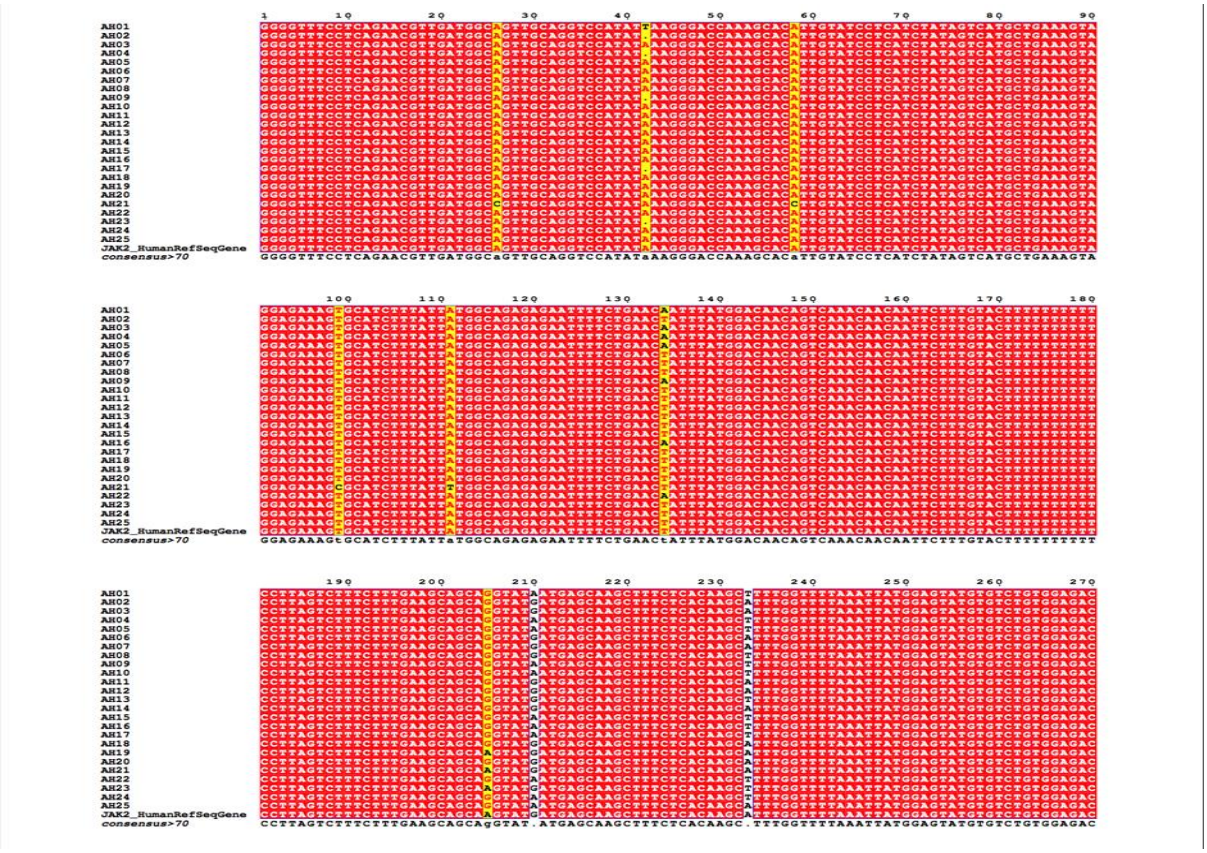
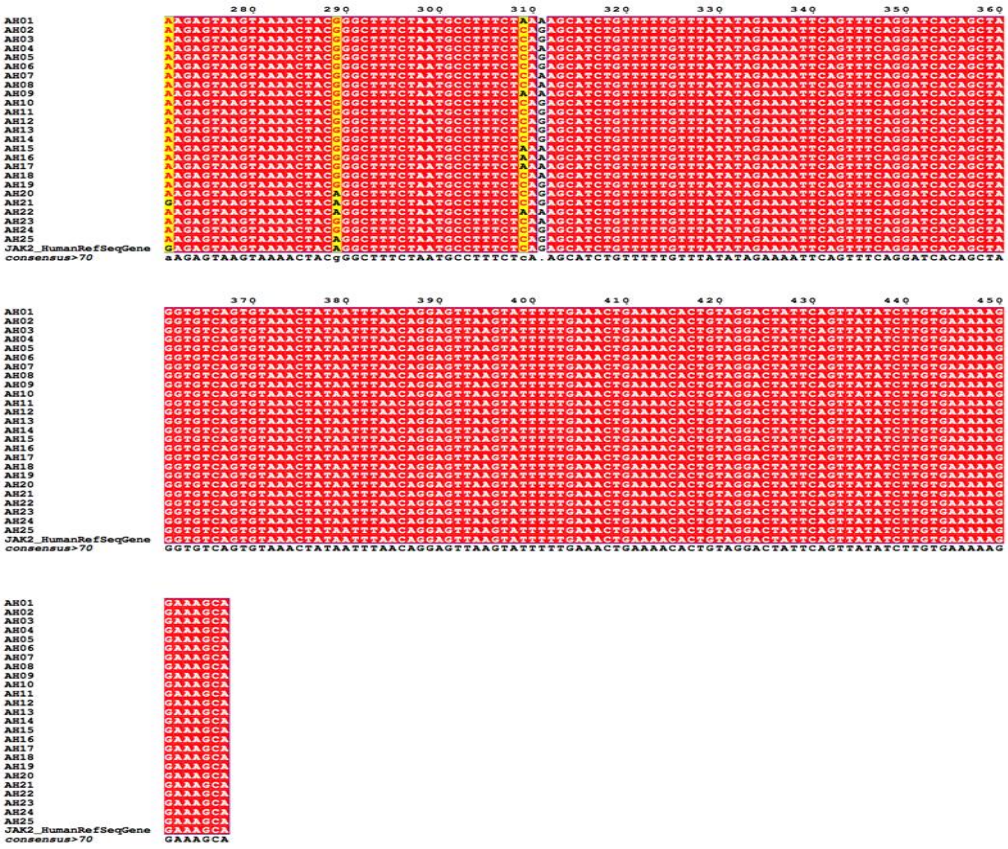
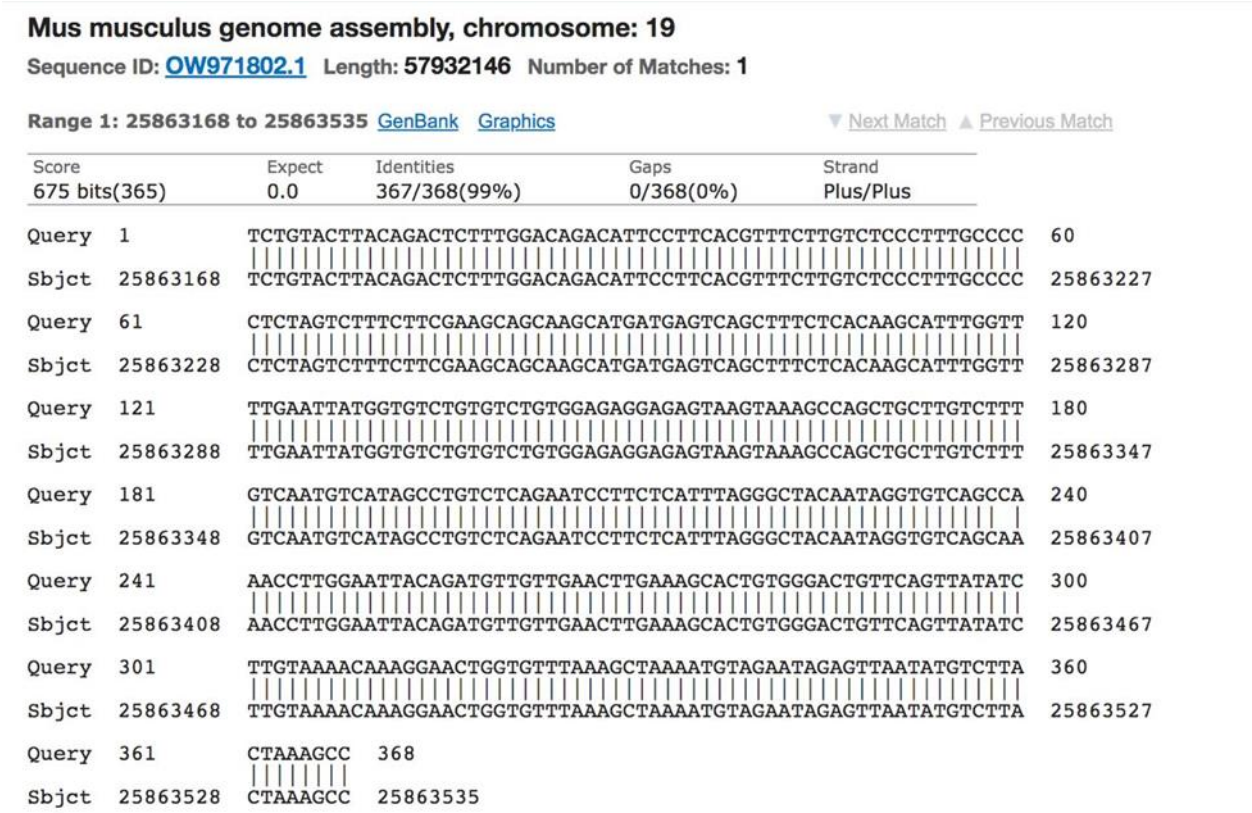


Fig4.25 (Part-1): Alignment of the sequences of partial region of gene JAK2 of 25 human samples against a RefSeqGene of JAK2 deposited in the NCBI (Accession number: NG_009904.1).
Detected SNPs are highlighted vertically with yellow or white lines.



Fig(4.25) (Part-2): Alignment of the sequences of partial region of gene JAK2 of 25 human samples against a RefSeqGene of JAK2 deposited in the NCBI (Accession number: NG_009904.1).
Detected SNPs are highlighted vertically with yellow or white lines.



Fig(4.26): Blast of DNA sequence of a PCR product of Mus musculus sample against a RefSeqGene of JAK2 deposited in the NCBI

No mutation is detected in the mice gene

Discussion

Breast cancer (BC) is the most frequently diagnosed cancer in women and the second leading cause of death from cancer in women.(**Siegel et al.,2017**)

According to reports, JAKs play a role in the pathophysiology of immunological and inflammatory diseases as well as cancerous tumors.

(**Schoknecht et al.,2015: Ciobanu et al.,2020**)

JAK2, a non-receptor tyrosine kinase, is essential for cancer cell growth, angiogenesis, immune evasion, and anti-apoptosis.(**Qian et al,.2011**)

A number of cancers have been found to have constitutive activation of the JAK signaling pathway. including human breast cancer . (**Yu et al.,2004:Behera et al.,2010**) ,

N-methyl-N-nitrosourea is a typical carcinogen that is used in rodent models to cause the development of breast cancer (MNU). (**Gal et al.,2020**)

Because the mammaries of rodents and humans are so similar, it is usually easy to predict the course of mammary carcinogenesis in both species when rats or mice are chosen as the primary animal species in breast cancer experimental investigations.(**Cardiff & Wellings,. 1999**)

In mice with mammary glands produced by MNU, the results of gross pathology revealed a variance in mammary gland cancer. Around 8 weeks after MNU treatment, mice that have been induced by the chemical grow breast nodules of various sizes in compared to the control group.(**Roomi et al,.2005**)

Moreover, the MNU group mice displayed a mammary gland cancer, whereas the control group is thought to be normal. These findings concur with (**Faustino-Rocha et al,.2017**)

One of these nitroso-compounds is methyl-nitrosourea (MNU). A large range of chemical compounds known as N-nitroso compounds can be produced by the reaction of nitrogen oxides. In numerous experimental animal models, nitroso compounds encourage the development of tumors and are carcinogenic substances that may also contribute to the emergence of a variety of human cancers. (**Saffhill et al.,1985**)

The MNU-only treated mice developed invasive ductal carcinoma (IDC), which is characterized by massive proliferation of undifferetiated pleomorphic neoplastic epithelial cells. These neoplastic cells formed a large mass that occupied the majority of the fatty tissue beneath the nipples area and had severe angiogenesis in comparison to other groups. this result supported by(**Manral et al.,2016**)

In molecular analysis result our study investigate the influence of genetic variation in the JAK2 genes on BC risk , we observed several associations between SNPs and risk of breast cancer.

JAK2 (V617F) exon 14 is a most probable mutation in occurring in pseudo kinase domain with loss of intrinsic auto inhibitory activity and can result in malignant transformation and uncontrolled proliferation leading to breast cancer. It is because of this reason for particularly choosing this mutation for study .(Lips et al.,2015), Recent studies suggest that an amplification of JAK2 gene on somatic chromosome 9p24.1 region in patients with breast cancer .(**Gupta et al.,2019**)

(**Karim et al,.2019**) Suggest the Allelic variation of JAK2 V617F point mutation could provide a better understanding of BC progression and pathogenesis. In-depth structural analysis of JAK2-V617F has potential to pave way for targeted therapies using small rationally designed selective inhibitors.

In the result if this mutation is found, it may have a role in the pathogenesis of breast cancer, but if we do not find it, then we believe that it has no role in the disease,We screening JAK2 exon 14 Mutation V617F (rs77375493) in sample of breast cancer by DNAsequencing the result appeared wild type , We believe that this mutation is not associated with breast cancer in our result ,We did another analysis by DNA sequencing for additional accompanying mutations in jak2 gene ,results show another possible SNPs candidates in breast cancer pathogenesis .

The highest SNPs (Rs1168791735) G/A , (Rs1489598500)A/G and (Rs 755212421)C/A , We believe this snps may have a role in breast cancer ,The information about these snp still un known , There should be studies on this snps .

In mice the results show wild type and not foud mutations , the reason could be the mutation may be need more time to happen and the chemical carcinogene make another oncogene to cause carcinogenesis .

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ResponseJAK–STAT Pathway Activation in Malignant and Nonmalignant Cells in MPN. Cancer discovery, 5(3), 316-331.