# To Explore the Mechanism of Muskone in the Treatment of Breast Cancer based on Network Pharmacology

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Keywords: Muskone, Breast Cancer, Network Pharmacology, Mechanism of Action.

Abstract: To investigate the possible mechanism of Muskone in the treatment of Breast cancer through network pharmacology. First, through Pubchem compound structure and Canonical SMILES, then use Swiss Target Prediction and Targetnet database query targets of musk ketone, through Genecards and OMIM database query targets for breast cancer disease, use the Target of muskone and breast cancer disease targets draw the VENN diagram, using the STRING database and Cytosacpe3.7.1 software for network topology parameters selection and muskone core targets for the treatment of breast cancer. Finally, the potential core targets were analyzed through the DAVID platform for GO biological process and enrichment analysis of KEGG signaling pathway. 135 targets of muskone and 1166 targets of breast cancer were obtained. There were 27 intersecting targets between muskone and breast cancer. Cytosacpe3.7.1 software was used to analyze network topology parameters, and 13 core targets of muskone in the treatment of breast cancer were obtained. A total of 78 GO enrichment results were obtained. KEGG enrichment analysis revealed 9 major signaling pathways, including cancer signaling pathway, prolactin signaling pathway, estrogen signaling pathway, etc. Muskone may be used to treat breast cancer through multiple targets and different therapeutic approaches, which provides reference for further study of pharmacodynamic substances and mechanism of action.



## 1 INTRODUCTION

At present, breast cancer has jumped to the first place among female malignant tumors in China, especially in urban areas. The mortality rate has increased by 96% compared with 30 years ago, breast cancer is becoming the most threatening tumor to women (Wang 2013). Breast cancer is A systemic disease with strong clinical heterogeneity and complex etiology. According to the origin or molecular characteristics of cancer cells, it can be divided into four molecular subtypes, namely luminal A/B, HER-2 expression and triple-negative type. Meanwhile, breast cancer is also the result of the combined effects of environmental and genetic susceptibility factors (Xin 2021). Western medicine has many methods to treat breast cancer, such as surgical resection, endocrine therapy, radiotherapy and chemotherapy, but its side effects are obvious, increasing the uncertainty of patients during the treatment. A large number of studies have shown that traditional Chinese medicine has great advantages in the treatment of breast cancer. Traditional Chinese medicine can be treated by dredging liver, regulating qi and other syndromes, and it can significantly relieve clinical symptoms and enhance patients' confidence in the treatment of breast cancer.

Musk is the dry secretion from the mature male musk capsule of forest musk deer, horse musk deer or former musk deer, which has the functions of activating the orifice and awakening the mind, promoting blood circulation and menstruation, reducing swelling and relieving pain (Li 1963). Muskone is the main active component of musk, and research results show that muskone has an important function in malignant tumors (Qi 2020). However, systematic and comprehensive studies on how muskone plays a role in the treatment of breast cancer from the cellular and molecular levels have rarely been reported, and need to be further strengthened.

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Chen, H. and Wen, Z.
To Explore the Mechanism of Muskone in the Treatment of Breast Cancer based on Network Pharmacology.
DOI: 10.5220/0011316000003444
In *Proceedings of the 2nd Conference on Artificial Intelligence and Healthcare (CAIH 2021)*, pages 249-254
ISBN: 978-989-758-594-4

Based on this, this study explored the role and mechanism of muskone in the treatment of breast cancer through network pharmacology, as to provide a reference for further study of the role of muskone in the treatment of breast cancer.

# 2 METHODS AND RESULTS

## 2.1 Establishment of Chemical Composition

Compound structure is obtained through the Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) (see Fig.1) and Canonical SMILES (CC1CCCCCCCCCCCCC (= NO) C1).

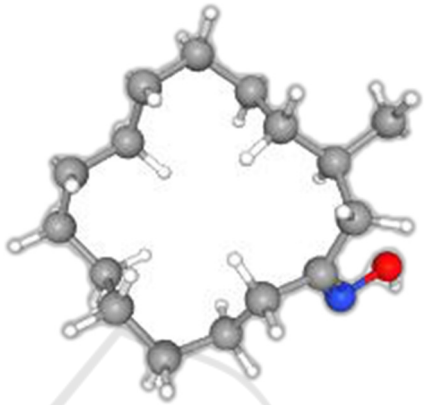


Figure 1: 3D structure of musk ketone.

## 2.2 Target Prediction of Muskone

The Canonical SMILES by Swiss Target Prediction database of musk ketone gene targets, according to the size of aim-listed Probability screen in the top 15 targets (see table 1), then Canonical SMILES input Targetnet database (http://targetnet.scbdd.com/) of muskone gene targets, selection targets which Prob > 0.The targets obtained from Swiss Target Prediction database and Targetnet database were combined, and the gene names were converted through Uniprot (http://uniprot.org/) database, and the duplicates were removed. A total of 135 related targets were obtained.

Table 1: Target of muskone.

| Target | Common name | Probability |
|---|---|---|
| Cytochrome P450 19A1 | CYP19A1 | 0.184 |
| Carbonic anhydrase II | CA2 | 0.112 |
| Nuclear receptor subfamily 1 group I member 3 | NR1I3 | 0.0940 |
| Acyl coenzyme A: cholesterol acyltransferase | CES1 | 0.0850 |
| Carboxylesterase 2 | CES2 | 0.0850 |
| Carbonic anhydrase I | CA1 | 0.0850 |
| Carbonic anhydrase IV | CA4 | 0.0760 |
| Androgen Receptor | AR | 0.0490 |
| P2X purinoceptor 7 | P2RX7 | 0.0490 |
| Poly [ADP-ribose] polymerase-1 | PARP1 | 0.0490 |
| Epoxide hydratase | EPHX2 | 0.0490 |
| Epoxide hydrolase 1 | EPHX1 | 0.0490 |
| Melatonin receptor 1A | MTNR1A | 0.0490 |
| Melatonin receptor 1B | MTNR1B | 0.0490 |
| Arachidonate 5-lipoxygenase | ALOX5 | 0.0490 |

## 2.3 Establishment of Breast Cancer Related Targets

Enter the keyword Breast Cancer through Genecards database (HTTP://www.genecards.org) and OMIM database (https://www.omim.org/), search for gene targets that have been reported and are related to Breast Cancer. Gene targets with Score>20 were selected from the data obtained from Genecards database, according to the size of the Score value, while the data obtained from OMIM database was not selected because of the small number of data. Finally, the data obtained from Genecards database and OMIM database were combined, and the Uniprot (http://uniprot.org/) database was used to convert gene names and remove duplications. A total of 1166 disease targets were obtained.

## 2.4 Screening of Drug and Disease Intersection Targets

Venn diagrams of drug and disease targets are plotted (see Fig.2), 135 muskone targets with 1166 targets for breast cancer disease input Venn platform (https://bioinfogp.cnb.csic.es/tools/venny/). A total of 27 potential targets of muskone active components against breast cancer were obtained: CYP19A1, AR, PARP1, ABCB1, AHR, CA9, CASP9, CDK1, CDK2, CDK4, CYP17A1, CYP1A2, CYP2D6, etc.

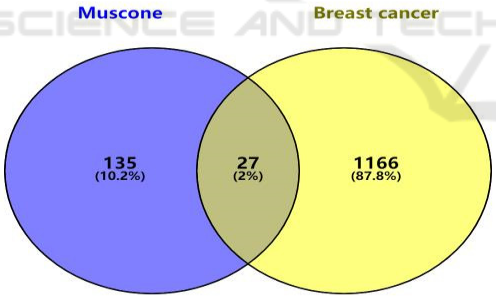



Figure 2: VENN diagram of the intersection target of muskone and breast cancer target.

## 2.5 Screening of PPI Protein Interaction Network and Core Targets

The PPI network map was obtained by STING database (https://string-db.org/) for 27 potential targets of muskone active ingredients against breast cancer. Import all relevant data directly obtained in STING network database into Cytoscape3.7.1 software. Each node represents a target, and the edge represents the interaction between the two targets. The more connected node lines, the more critical the target is, and the key proteins are CYP19A1, AR, PARP1, ABCB1, AHR, CA9, CASP9, CDK1, CDK2, CDK4, CYP17A1, CYP1A2, CYP2D6, DNMT1, and ESR1 (see Fig.3). The muskone active substances obtained in STING database and 27 potential action targets effective against breast cancer were imported into Cytoscape3.7.1 software to get the potential core target, and the median of Degree value was 9.48, median of Betweenness Centrality value was 0.0078, and median of So-called Centrality value was 0.604. Degree value, Betweenness Centrality, Closeness Centrality values were greater than the value of target, the target can be thought of as potential core targets 13 muskone in the treatment of breast cancer, such as ESR1, HSP90AA1, AR, that the above targets may is the core of muskone is used to treat breast cancer gene (see Table 2).

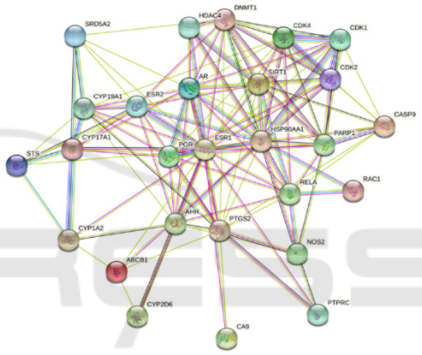


Figure 3: Network diagram of PPI protein interaction.

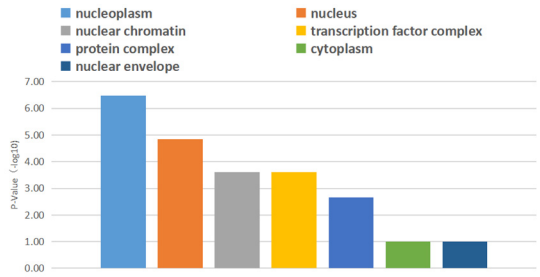
Table 2: Topological parameters of potential core targets of muskone in the treatment of breast cancer.

| Name | Betweenness Centrality | Closeness Centrality | Degree |
|---|---|---|---|
| ESR1 | 0.281 | 0.930 | 24 |
| HSP90A | 0.102 | 0.761 | 18 |
| AR | 0.0620 | 0.740 | 17 |
| PTGS2 | 0.0700 | 0.702 | 15 |
| SIRT1 | 0.0140 | 0.671 | 13 |
| RELA | 0.0450 | 0.650 | 12 |
| CDK4 | 0.0100 | 0.650 | 12 |
| DNMT1 | 0.0170 | 0.650 | 12 |
| PARP1 | 0.0130 | 0.651 | 12 |
| AHR | 0.0360 | 0.630 | 11 |
| PGR | 0.0140 | 0.629 | 11 |
| ESR2 | 0.0280 | 0.620 | 10 |
| CYP19 A1 | 0.0120 | 0.600 | 10 |

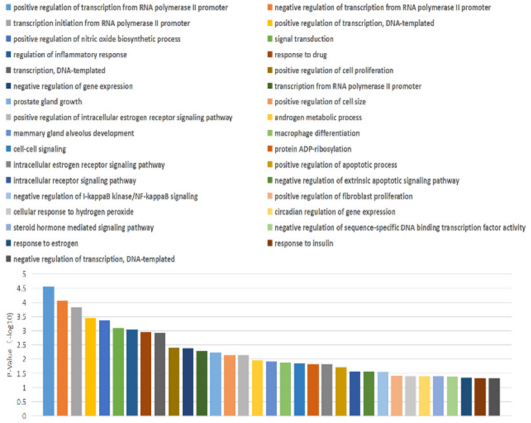
## 2.6 GO Biological Process Analysis

13 potential core targets were imported into DAVID database for GO enrichment analysis. A total of 78 results were obtained from GO enrichment analysis. There were 44 Biological processes (BP), 27 Molecular functions (MF) and 7 Cellular components (CC). The top biological functions included core promoter sequence specific DNA binding, enzyme binding, protein binding, regulation of human transcription binding factor activity complex, positive and negative regulation of human cell function growth, transcriptional regulation process, positive regulation process of NF-κB transcription factor activity, DNA binding and other processes. The results with P value less than 0.05 were screened out from the obtained data to draw a bar chart (see Fig.4). The color of the column represents the GO biological process information entry. The column height from low to high means that the P value decreases from large to small. The smaller the P value, the higher the significance. This suggests that muskone may play a therapeutic role in the treatment of breast cancer by regulating these biological processes.


A. molecular function




B. cell component




C. biological process

Figure 4: GO enrichment analysis of muskone in the treatment of breast cancer.

## 2.7 Enrichment Analysis of KEGG Pathway

13 potential core targets were imported into DAVID database for Enrichment analysis of KEGG pathway. Results show that There were 9 main enrichment pathways of key target genes in the treatment of breast cancer by muskone. Five key signaling pathways, namely pathways in cancer, Prolactin signaling Pathway, Small cell Lung cancer, prostate cancer, and estrogen signaling Pathway, are used to play the anti-cancer role (see Table 3). The signal conduction pathway with P value less than 0.05 was taken as the bar chart. This is the main enrichment pathway of key target genes in the mechanism of action of muskone in the treatment of breast cancer, indicating that muskone may treat breast cancer through cancer transduction pathway, prolactin signaling pathway, estrogen signaling pathway (See Fig.5). In the figure, the height of the column represents p-value, and the column height from low to high represents the P value from large to small, and the smaller the P value is, the stronger the significance is.

Table 3: Pathway enrichment analysis table.

| Term | Count | P-Value |
|---|---|---|
| Pathways in cancer | 5 | 0.00250 |
| Prolactin signaling pathway | 3 | 0.00540 |
| Small cell lung cancer | 3 | 0.00770 |
| Prostate cancer | 3 | 0.00830 |
| Estrogen signaling pathway | 3 | 0.0100 |

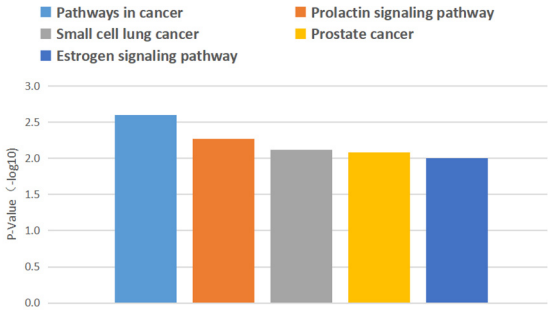



Figure 5: Enrichment analysis of KEGG metabolic pathway in the treatment of breast cancer with muskone.

## 3 DISCUSSION

Traditional Chinese medicine thinks, cancer etiology and pathogenesis of blood stasis block, musk can begin to understand wake up, invigorate the circulation of menstruation, acetanilide detumescence, conforms to the principle of TCM anticancer treatment, has antitumor effect, the study also showed that musk can inhibit the growth of cancer cells proliferation, muskone as main ingredients, inhibit breast cancer tissue fibroblast growth factor, bFGF and VEGF expression antitumor the formation of new blood vessels, thereby potentially anticancer(Meng 1998). However, there are few reports on the molecular mechanism of musk's anti-breast cancer and anti-drug resistance and its therapeutic effect on breast cancer. Therefore, to explore the molecular mechanism of musk ketone in the treatment of breast cancer is conducive to further research, promote the development of Traditional Chinese medicine, and enhance the cultural confidence of traditional Chinese medicine.

Researchers in this study by using the way of network pharmacology, by constructing PPI network discovery ESR1, HSP90AA1, AR, PTGS2, SIRT1, RELA, CDK4, DNMT1, PARP1, AHR, PGR, ESR2, CYP19A1 as the core target of muskone breast cancer treatment, illustrates the muskone can through a variety of target gene therapy of breast cancer, consistent with modern research.

ESR can specifically bind with estrogen and participate in the occurrence and development of breast cancer by activating SRC-Ras-PI3K-Akt and MAPK/ERK. AR can combine with target genes and activate them, thus activating the downstream cell proliferation signaling pathway and participating in the proliferation of mammary epithelial cells (Filardo 2002).

As a molecular protein family, heat shock protein family is involved in the whole process of tumor occurrence and development. HSP90α protein is increased in breast cancer patients. Studies have shown that HSP90 encoded by HSP90AA1 gene can inhibit apoptosis, regulate cell division and promote angiogenesis (Chatterjee 1978). Through TCGA data analysis found that HSP90AA1 mRNA was highly expressed in breast cancer tissues and was related to patient survival (Jia 2020). PGR is a target of endocrine therapy for breast cancer and has good reference value for prognosis of breast cancer. Aryl hydrocarbon receptor (AHR) is a ligand-dependent activated transcription factor, which is associated with the occurrence of breast cancer and can regulate the proliferation and apoptosis of tumor cells.

Abnormal expression of PARP1 gene is closely related to triple-negative breast cancer (TNBC), and PARP1 may be involved in the treatment of breast cancer by inhibiting angiogenesis (Feng 2020). DNMT 1 regulates the occurrence and development of breast cancer by regulating the migration and proliferation of breast cancer cells (Li 2020). Cyclin-dependent kinase CDK4 is a key regulator of cell cycle. In estrogen receptor positive (ER+) breast cancer, CDK4 is overexpressed and cell proliferation is uncontrolled. RELA is a member of NF-κB family. NF-κB gene in breast cancer cells affects the invasion and migration ability of breast cancer cells by inhibiting the epithelial-mesenchymal transformation of tumor cells (Zhao 2019). Sirtuins family proteins are a class of NAD+ dependent deacetylases involved in the development and Multidrug resistance (MDR) of breast cancer (Li 2021). PTGS 2 gene variation is associated with breast cancer susceptibility (Uwe 2006, Laure 2010). The expression of aromatase gene (CYP19A1) has a certain effect on the biological behavior of tumor cells (Luo 2014).

In order to predict and muskone key targets for therapy of breast cancer in gene function and the role of signaling pathways, this study analyzes the GO biological function of enrichment, found that the core PPI gene may be through the core promoter sequence specific dna-binding proteins, enzyme combination, combined with activity, as well as the combination of human transcription factor complex regulation, to the human body cell function positive negative regulation of growth, transcriptional regulation, the nf-kappa B the activity of transcription factors are regulation and DNA binding have the effect of breast cancer.

Through KEGG enrichment analysis, the results showed that the target genes of breast cancer treated by muskone mainly involved pathways in cancer, Prolactin signaling pathway, Small cell lung cancer,

prostate cancer, estrogen signaling pathway. These pathways are closely related to the formation and development of cancer. The prolactin signal transduction pathway and lactin signal transduction pathway mainly control and treat breast cancer by regulating human hormones. This study suggests that muskone may be mainly used in the treatment of breast cancer through the regulation of human transcription binding factor activity complex, positive regulation process of NF-κB transcription factor activity, cancer pathway, prolactin pathway and estrogen signal transduction pathway.

This study adopts the network pharmacology method to predict the mechanism of muskone in the treatment of breast cancer, which involves a variety of biological processes and multiple pathways, reflecting the multi-target-multi-pathway action characteristics of Chinese medicine components. At the same time, it was found that multiple targets interact to regulate a signal pathway and affect the biological response, reflecting the characteristics of muskone as a Chinese medicine ingredient in the treatment of breast cancer. However, the experimental results still need further verification and further development. With the deepening of the research on the pharmacological action and mechanism of natural musk and muskone, the medicinal value and market prospect of musk will be better displayed.

## 4 CONCLUSIONS

Based on the results and discussions presented above, the conclusions are obtained as below:

(1) It is shown that 135 targets of muskone and 1166 targets of breast cancer were obtained. There were 27 intersecting targets between muskone and breast cancer. Cytosacpe3.7.1 software was used to analyze network topology parameters, and 13 core targets of muskone in the treatment of breast cancer were obtained. A total of 78 GO enrichment results were obtained. KEGG enrichment analysis revealed 9 major signaling pathways, including cancer signaling pathway, prolactin signaling pathway, estrogen signaling pathway.

(2) Muskone may be used to treat breast cancer through multiple targets and different therapeutic approaches, which provides reference for further study of pharmacodynamic substances and mechanism of action.

## REFERENCES


Chatterjee S, Burns TF.. (2017) Targeting heat shock proteins in cancer: apromising therapeutic approach. Int J Mol Sci., 9:1978.

Filardo E J, Quinn J A, Frackelton Ar Jr,. (2002) Estrogen action via the G protein-coupled receptor, GPR30: stimulation ofadenylyl cyclase and cAMP-mediated attenuation of theepidermal growth factor receptor-to-MAPK signaling axi. Mol Endocrinol, 1: 70-84

Feng, H.Y., Chen, Q.Q., Yuan, M.. (2020). PARP1 and VEGF expression in breast cancer. International Medical and health report., 13:1873-1877.

Hao, J.L., Guang, L. Y., Teng, H.Y.. (2014) GPER-mediated proliferation and estradiol production in breast cancer-associated fibroblasts. Endocr Relat Cancer., 2: 355-369.

Jia, Z., YU, L.F., Wen, Q.. (2020) Expression and prognosis of HSP90AA1 in breast cancer based on TCGA data. Advances in anatomical science., 3:347-349+353.

Li, S.Z., (1963). Compendium of Materia medica. Shanghai, The Commercial Press. pp. 1560.

Li, H. H.. (2020) McL-1/Mir-142-5p /DNMT1/ Maspin signaling regulates migration and proliferation of breast cancer cells. Wuhan University of Science and Technology.

Li, Y. Y., Zhang, Y.. (2021) Advances in the mechanism of Sirtuins in breast cancer. Journal of Practical Oncology., 3:277-281.

Laure Dossus, Rudolf Kaaks, Federico Canzian. (2010) PTGS2 and IL6 genetic variation and risk of breast and prostate cancer: results from the Breast and Prostate Cancer Cohort Consortium (BPC3). Carcinogenesis., 3:455-461.

Meng, Z.H., Shan, L.C., Zeng, J.X.. (1998) Effects of subcutaneous muskiness on the growth of BALB/ C pure mice. Chinese Oncology Clinic., 11:58-60.

Qi, N., Duan, W.J., Li, Y.J.. (2020) Research progress of pharmacological action of musk ketone. World Science and Technology-Modernization of Traditional Chinese Medicine., 8:3042-3047.

Uwe Langsenlehner, Babak Yazdani-Biuki, Tanja Eder. (2006) The homozygous cyclooxygenase-2(PTGS2)8473CC genotype is associated with breast cancer risk and estrogen receptor positivity. ClinCanRes., 4:1392-1394.

Wang, J., Lu, W.L., Wang.Y. (2013) Analysis of current situation and optimization strategy of female breast cancer screening in China. Maternal and Child Health Care in China.,18:2864-2867.

Xin, Y., Liu, S.,Qin Y.N.. (2021) Retrospective cohort study on the treatment of triple negative breast cancer with Sanyin prescription. Shanghai Journal of Traditional Chinese Medicine., 5:63-66.

Zhao, Z., Song, Z.J., Sun, H.F.. (2019) Platelets enhance the invasion and migration of circulating tumor cells in breast cancer by up-regulating NF-κB signaling pathway. Modern Oncology Medicine., 22:3943-3949.