Review Article
Running Title: Phytochemicals in TNBC
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Insight into the Role of Phytochemicals in the Treatment of Triple-Negative Breast Cancer

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Abstract

Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer which is characterized by the absence of progesterone receptor, estrogen receptor, and human epidermal growth factor receptor 2, thus, TNBC patient tumour does not respond to the endocrine therapy. TNBC is highly invasive, highly metastatic, and shows poor prognosis, recurrence, and short survival rate. Surgery, chemotherapy, and radiotherapy are now used as treatments. Despite the wide range of treatment choices, the main drawbacks of current therapies include drug resistance, decreased effectiveness, recurrence within 5 years, and a variety of side effects. A unique targeted approach is therefore desperately required for the treatment of TNBC. Researchers now have fresh perspectives on the tailored strategy for treating TNBC thanks to phytochemicals. Phytochemicals have shown antiproliferative properties in TNBC and also overcome the drawbacks like recurrence, toxicity, adverse effect, and quality of life. This review highlights different phytochemicals and their potential to target signalling pathways and gene expression to induce apoptosis, cell cycle arrest, inhibition of metastasis, and angiogenesis.
Keywords: Triple-negative breast neoplasms, Genetics, Phytochemicals, Chemotherapy, Signalling

Introduction

Every year 2.09 million people are diagnosed with breast cancer worldwide.[1] Breast cancers are the principal cause of mortality in women. Triple-negative breast cancer (TNBC) is a subtype of breast cancer which comprises approximately 10%-15% of breast cancers.[2,3] TNBC is distinguished from other breast cancer types by the absence of progesterone receptor (PR), estrogen receptor (ER), and human epidermal growth factor receptor 2 (HER2).[4] Among the other types of breast cancers, TNBCs are highly metastatic, more aggressive, and show poor prognosis.[5] The mortality rate of TNBC is higher than any other subtype of breast cancer.[6] According to studies, the average TNBC patient survives for 5 years following diagnosis.[7] TNBC is further split into six molecular subtypes, including luminal androgen receptor (LAR), immunomodulatory (IM),

mesenchymal (M), basal-like (BL1 and BL2), mesenchymal stem-like, and another unidentified group (UNS).[8] TNBC is a more heterogeneous kind of cancer due to its variety in markers. African-American women are more likely than those of European and Asian heritage to develop the TNBC tumor subtype.[9] Hormonal therapy, sedimentary lifestyle, obesity, exposure to carcinogens and germline mutation in the BRCA1 gene are the cause of TNBC.[10,11,12]

Over the past few years, advancement in the research and technologies of breast cancers has given better treatment options which improved survival rates of TNBC patients, but some adverse effects affect the overall quality of life.[13] TNBC is diagnosed using imaging methods and a sample, which is then subjected to morphological analysis, biomarker analysis, and immunohistochemical characterisation of the tumor.[14,15] Certain endocrine treatments may slow tumor development in certain forms of normal breast cancer, but standard hormonal targeted therapies are ineffective because they lack drug target receptors..[16] Current treatments include surgery, radiation therapy and chemotherapy.[17] Combined chemotherapy treatment with anthracyclines or taxanes exhibits good tumour regression rates but also carried out recurrence during the first 5 years after therapy.[18] Radiation therapy is a preferred option depending on certain characteristics of the tumour.[19] Radiation therapy is given post-surgery to prevent recurrence or along with chemotherapy. These conventional treatments are somewhat effective, but they still have several downsides, including medication toxicity, drug resistance, tumor recurrence, and a variety of adverse effects. 20 Because there is no one conventional therapy for treating all diseases due to their variability, combination therapies are often advised.[21,22]

Understanding the limitations of available treatment options in TNBC, the need for new targeted treatment options which are less toxic and cause minimum side effects are critically needed. Plant-based secondary metabolites (phytochemicals) have drawn the attention of researchers as a natural candidate for the TNBC treatment which may overcome the issue of recurrence, drug resistance, metastasis inhibition and major adverse effects in the patient.[23,24]

Genetics of TNBC

Human normal cells have controls on their growth rate, frequency of division, and lifespan. Cells need proteins involved in cell cycle control to operate correctly in order for them to carry out these tasks. Any mutation in DNA repair, tumor suppressor, or oncogene genes results in aberrant proteins, which ultimately cause unchecked cell division.[25,26] Germline mutations in these genes lead to inheritable cancer[27] which were enlisted in table 1. Among those, important genes regarding their role in TNBC cancer are discussed below and their distribution among TNBC patients was shown in figure 1.

BRCA1 and BRCA2

BRCA1 and BRCA2 are autosomal dominant genes, commonly associated with inheritable TNBC.[29] BRCA1 and BRCA2 are located on chromosome number 17 and 13 respectively.[30] BRCA1&2 are involved in double-stranded DNA break repair mechanism, transcription, cell cycle regulation, and DNA damage response to repair mechanism.[31] Therefore, there is a relationship between increased TNBC risk and BRCA1 and BRCA2 gene mutation. According to studies, triple-negative disease affects around 71 percent of people with BRCA1 gene mutations, compared to just 25 percent of patients with BRCA2 mutations.[32] In general, TNBC is nearly three times more likely associated with inherited BRCA1

gene compared to the BRCA2 gene. Both genes suppress tumour growth thus called Tumour suppressor genes or anti-oncogenes.[33] BRCA1 mutations associated with the patients include a younger age woman population with a median age of 39 years that shows higher grade tumour and higher stage tumour.[34,35]

Checkpoint kinase 2 (CHEK2)

CHEK2 gene encodes an enzyme serine-threonine kinase, that acts as a cell cycle checkpoint regulator and tumour suppressor gene by DNA repair activity.[36] Mutations in CHEK2 will affect the DNA repair mechanism negatively which may induce tumour formation. Germline CHEk2 mutation leads to hereditary breast cancer.[37] CHEK2 mutation is vulnerable to chemotherapy drugs because it induces resistance to drugs.[38]

Ataxia telangiectasia mutated (ATM)

An enzyme called phosphatidylinositol (3,4,5)-trisphosphate (PIP3)-kinase, which is encoded by the ataxia telangiectasia mutation, activates downstream proteins involved in DNA repair and cell cycle control.[39] A mutation in ATM changes how the cell cycle and DNA repair mechanisms are regulated. ATM is recruited when a double-stranded break in DNA is sensed. In one study, it is found that one woman out of 158 with TNBC harboured a mutation in ATM.[40]

PALB2

A protein known as a partner and colocalize with BRCA2 and promotes recombination repair and checkpoint functions by stabilization and localization within nuclear matrix and chromatin.[41] PALB2 mutation accounts small percentage of TNBC. PALB2 mutation carrying tumours presented TNBC phenotype more often than other familial or sporadic breast cancer patients and are a more aggressive type of tumour.[42]

RAD51

RAD51 has a compensatory function for BRCA1.[43] RAD51 gene encodes proteins involved in homologous recombination (HR) in TNBC.[44] Mutation in RAD51 is associated with TNBC by altered homologous recombination. According to one research, polymorphisms in the RAD51 gene may be used to predict the development of TNBC since they statistically significantly enhance the risk of TNBC in a group that has the C allele for the variation 135C allele of the gene.[45]

Other genes

The mutation of many other genes like tumour protein 53 (TP53), phosphatase and tensin homolog (PTEN), STK11, B cell lymphoma 2 (BCL2), MSH2, PIK3CA, BARD1, epidermal growth factor receptor (EGFR), fibroblast growth factor (FGFR) and vascular endothelial growth factor receptor (VEGFR) are involved in triple-negative breast cancer by inhibiting apoptosis, inhibiting tumour suppressor genes, absence of DNA damage repair mechanism and inducing oncogenes.

Signalling pathways in TNBC

Unregulated cell proliferation or the inhibition of apoptotic pathways may be caused by uncontrolled and incorrect signal transduction, ineffective signal response, or both. These conditions may lead to the development of tumors. The following discussion covers many signalling pathways connected to the development of TNBC.

Wingless-related integration site (Wnt)/β-catenin signalling

The role of Wnt/β-catenin signalling pathway was first identified in mouse mammary tumour virus-mediated oncogenic transformation in human cells.[63] Wnt ligands binding to a Frizzled receptor (FZD) as well as the co-receptor low-density lipoproteins 5/6 (LRP5/6) initiates Wnt/β-catenin signalling.[64] It leads to activation of FZD,

which allows binding of Dishevelled (Dvl) protein, and phosphorylation of cytoplasmic motifs of LRP5/6 receptor.[64] Phosphorylation in a single motif is adequate to activate Wnt signalling.[65] LRP5/6 that has been phosphorylated may interact with Axin, the motif of β-catenin destruction complex. Thus, β-catenin stays intact and accumulates in the cytoplasm before entering the nucleus, where it binds to transcription factor/lymphoid enhancer-binding factor (TCF/LEF) transcription factors and displaces transcriptional repressor Groucho, resulting in transcription of Wnt target genes.[66]

When the Wnt signal is absent β-catenin continuously undergoes the cycle of synthesis and degradation by the polyubiquitin mediated proteasomal degradation recruited by the β-catenin destruction complex. Wnt target genes translate proteins involved in cell proliferation and differentiation.[67] Upregulation of Wnt/β-catenin signalling leads to the tumour development, thus, this pathway is highly active in cancer cells.[68] Inhibiting or downregulating the Wnt/β-catenin pathway may give new hope in TNBC tumour suppression.

Notch signalling

Notch signalling is a type of juxtacrine signalling in which transmembrane receptors of one cell interact with neighbouring cell ligands.[69] Notch signalling is involved in multiple spheres of tumour progression, including regulation of cell proliferation, apoptosis, stem cells, angiogenesis and transition from epithelial-to-mesenchymal cell type.[70] When a ligand binds to the Notch receptor, γ-secretase is recruited and cleaves the Notch intracytoplasmic domain (NICD). Multiple enzymes further modify NICD. NICD penetrates the nucleus and encourages transcription of genes that encourage cell growth. [71] For patients with TNBC, inhibiting γ-secretase activity and controlling aberrant Notch signalling are potential treatments.[72]

Hedgehog Signalling

Hh signalling induces tumour growth, metastasis, and chemotherapeutic drug resistance.[73] In absence of Hedgehog signal smoothened protein is inactive, thus, downstream effector GLI1 is inactivated in the cytoplasm by suppressor of fused protein (SUFU) protein by phosphorylation followed by degradation. Degradation products of GLI1 protein, GLI2 and GLI3 act as repressors after modification processing.[74] When the Hedgehog signal (Hh) binds to the patched receptor, smoothened protein becomes active and stops the degradation of the GLI1 protein.[74] GLI1 separates from SUFU and functions as a transcription factor for genes involved in cell motility, invasion, proliferation, and angiogenesis.[75,76] It is investigated in a preclinical study that unregulated Hh signalling leads to a more aggressive and highly metastatic tumour phenotype in the TNBC subtype.[77] Higher level of signal Sonic hedgehog (SHH) is correlated with overall poor survival of patients compared to patients whose tumour expressed a lower SHH level.[78] Downregulating the Hedgehog pathway and prolonging suppression of GLI protein may control the proliferation of tumours (Figure 2).

Phosphoinositide 3-kinase (PI3K)/AKT/ mammalian target of rapamycin (mTOR)

PI3K/AKT/mTOR signalling pathway is involved in the regulation of cell proliferation, angiogenesis and apoptosis.[79] PI3K, AKT kinase and mTOR pathways are inhibitors of apoptosis.[80] Protein kinase B, commonly known as AKT kinase, is a serine/threonine kinase that serves as the major mediator of PI3K-initiated signalling.[80] PIP3 kinase phosphorylates AKT kinase. AKT kinase inhibits apoptosis by inhibiting apoptosis-inducing proteins like BCL2 associated agonists of cell death

(BAD), Bim, P53 and Mcl1.[81] Thus, apoptosis can be induced by inhibition of the PI3K/AKT/mTOR pathway and promote cell cycle arrest.[82] Inhibition of PI3K/AKT/mTOR pathway can be a promising approach in the treatment of TNBC.

Mitogen-activated protein kinases (MAPK)

MAPK pathways play an important role in proliferation, development, differentiation, apoptosis and transformation.[83] MAPK signalling has a very important role in the development of TNBC.[84] Within protein kinase cascades, three conserved active enzymes are present in series: MAPK kinase (MAPKKK), a MAPK kinase (MAPKK), and a MAP kinase (MAPK).[85] Stimulation of receptor Ras, rapidly accelerated fibrosarcoma (RAF), MEK, and extracellular signal-regulated kinase (Erk) is phosphorylated in a cascade manner.[86] RAF-MEK-ERK cascade is responsible for the control of the G1/S progression in the cell cycle, since Cyclin D transcription is a result of signal transduction.[83] Unrestrained MAPK may result in the perpetuation of TNBC cell proliferation.[87] TNBC patients with overexpression of the MAPK pathway exhibit anthracycline resistance and a higher risk of disease recurrence.[88] TNBC patients with higher levels of ERK protein expression had a worse survival rate.[89]

The interplay between Wnt/β-catenin signalling pathway, Notch pathway, hedgehog pathway, and other oncogenic signalling pathways like PI3K, Akt, mTOR, transforming growth factor-beta (TGF-β) signalling pathway, Ras androgen receptor (AR), EGFR, were thought to play important role in tumour development.

Phytochemicals in TNBC

Phytochemicals are chemical compounds produced by the plant from the primary or secondary metabolic pathway. Phytochemicals are known for their antioxidant, anti-inflammatory and anti-cancer and antiviral properties.[90] Mostly being secondary metabolites, they are produced in a very low amount from a plant. Based on their chemical makeup and features, several well-known phytochemicals have been grouped into six broad groups. Alkaloids, phenolics, terpenoids, lipids, carbohydrates, and other nitrogen-containing molecules are some of these categories. Phytochemicals have been studied for the treatment of breast cancer during the last 20 years, with promising results as a natural contender for TNBC treatment.

Gossypol

Gossypol (GOSS) is a polyphenol compound, present in Gossypium hirsutum L (cotton) seeds in minor concentrations.[91] In China, Gossypol is traditionally used to cure viral infections. Gossypol is suggested to be an effective anticancer agent against TNBC.[92] In many studies, gossypol exhibited anti-metastatic and antiproliferative effects in many human cancer types.[93] The antiproliferative property of gossypol is achieved by inducing apoptosis in TNBC cells.[92]

Messeha *et al.* examined the antitumour effects of gossypol in both MM-468 and MM-231 cell lines in in-vitro. Real-time quantitative reverse transcription PCR (qRT-PCR) test of gossypol treated cell lines was performed to study the genes associated with gossypol induced apoptosis.[94] The expression of apoptosis-related genes was significantly upregulated by the compound. In both cell lines gossypol remarkably increases the expression of proapoptotic genes TNFRSF9, BNIP3, and growth arrest and DNA damage-inducible alpha (GADD45A) genes. More than 90% inhibition in BIRC5 (inhibitor of apoptosis) was noticed in MM-468 as well as an MM-231 cell line.[94] In another study, it was found that (-) enantiomer of gossypol exhibit

more therapeutic effect in TNBC compared to the racemic mixture of gossypol.[95]

Lycopene

In tomatoes and many other red and pink foods, lycopene—a significant carotenoid—is naturally present in higher amounts. It has antioxidant properties and prevents reactive oxygen species from damaging DNA in cells.[96] Lycopene's primary function in cancer cells is to stop the cell cycle and start apoptosis.[97] AKT kinase inhibits the production of proteins that cause apoptosis when it is phosphorylated.[81] Lycopene prevents Akt kinase and the downstream protein mTOR from being phosphorylated.[98] It results in the upregulation of pro-apoptotic Bax protein to induce apoptosis in TNBC cells. Mikako Takeshima *et al.* investigated in a study that treating MDA-MB-468 cell line with lycopene for 168 h shows lower half-maximal inhibitory concentration (IC_{50}) value of 10.3 μM.[99] while shortening exposure time to 72 hours, IC_{50} values increased. Thus, a longer duration (>72 hours) of lycopene exposure is required to exhibit its true anti-proliferative activity in the MDA-MB-468 cell line.[99] FACS analysis of 50 μM lycopene treated MDA-MB-468 cells shows the declined growth mediated by cell cycle arrest in the G0/G1 phase as well as induction of apoptosis.[99]

Curcumin

Curcumin is a yellowish polyphenol compound present in turmeric extracted from the plant roots of Curcuma longa. Curcumin is a traditional Indian Ayurvedic medicine used in inflammation for centuries. Being pleiotropic in nature curcumin acts as an antioxidant, proapoptotic, antiangiogenic, and immunomodulatory by acting on multiple signalling pathways.[100] The therapeutic effects of curcumin on the MDA-MB-231 TNBC cell line were studied by Xiao-Dong Sun.[101] The growth inhibition rate in a cell line treated with curcumin at various doses was substantially different from untreated cell groups in a cell line treated with 30 mol/ml of curcumin. Curcumin-treated cells had a considerably greater rate of apoptosis (26.34%) than cells from the control group (2.76%).[101] It was found in the MTT assay that curcumin concentration of 30 μmol/ml increases inhibition of MDA-MB-231 cell proliferation.[101] Flow cytometry result shows that apoptosis rates of 30 μmol/ml curcumin-treated MDA-MB-231 cells were 26.34% while control shows only 2.76%. EGFR is predominantly involved in cell proliferation in TNBC.[102] Curcumin suppressed the activation of the EGFR signalling cascade by reducing the amount of pEGFR expression in an MDA-MB-231 cell line after a 48-hour curcumin treatment.[101] Curcumin blocks Hedgehog, Notch, and Wnt/B-catenin signaling, according to a second research.[103,104] Numerous research on curcumin and its promising outcomes provide fresh perspectives on the management of TNBC.

Genistein

Genistein is a natural polyphenol compound which belongs to a class of isoflavones that is extracted from the Genista tinctoria flowering plant and Soybean.[105] For its anticancer effectiveness in TNBC, its potential for antimetastatic, apoptosis induction, cell cycle arrest, and antiangiogenetic capabilities have caught the interest of researchers.[106] According to several research, eating soy products, which are high in genistein, prevents breast cancer from spreading.[107] Hong Pan et al. studied the anticancer effect of Genistein on the MDA-MB-231 cell line.[108] It was explored that 20 μM genistein shows 60.64% apoptosis and arrest cell cycle of 30.95% cells in the G2/M phase. Genistein repressed NF-κB activation via inactivation of Notch signalling. In another study, genistein promotes BRCA1 activation by an

epigenetic factor that promotes inhibition of TNBC cell proliferation (Table 2).[109]

Quercetin

It is a plant metabolite that belongs to the flavonoid class mostly found in apples, tea, onion and broccoli.[132] Quercetin promotes apoptosis in many cancer cell lines including lung, breast, stomach and colon.[133] Ahmed S Sultan studied the effect of Quercetin in MDA-MB157 and MDA-MB-231 cell lines.[111] In both cell lines, a 48-hour quercetin administration results in a 50% viability suppression. By upregulating the activity of the caspase-3 protein and downregulating the expression of the Bcl-2 protein and β-catenin, quercetin promotes apoptosis. According to a different research, quercetin controls the -catenin pathway to stop TNBC migration.[112]

Luteolin

It is a bioflavonoid that may be found in fruits and medicinal plants. It has long been used to treat cancer and inflammation.[134] The primary apoptosis induction, anti-angiogenesis, and antiproliferative properties of luteolin are responsible for its anticancer effects. 135 Luteolin inhibits the cell cycle, survival, angiogenesis, and metastasis of TNBC cells.[113] Dan Lin looked at whether TNBC cells treated with luteolin prevented cell invasion and migration. It alters the β-catenin pathway by repressing the mRNA of β-catenin.[113] Liming Huang studied that 30 μM of luteolin treatment for 48 h triggers apoptosis in MDA-MB-231 increasing the expression of Bax protein while a decrease in the expression of BCl-2 protein.[114]

Conclusion

We tried to summarize the phytochemicals as possible future anticancer agents in targeted TNBC therapy. Being heterogeneous in nature, conventional chemotherapy has produced insignificant results in TNBC. Phytochemicals have proven effective at targeting numerous signalling pathways and gene expression in TNBC. The molecular-level understanding of TNBC has given researchers new insights into the targeted approach to treating TNBC. In addition to having antiproliferative effects on TNBC, phytochemicals have also been able to overcome problems with recurrence, toxicity, side effects, and quality of life. Apoptosis, cell cycle arrest, metastasis inhibition, and angiogenesis are just a few of the impressive anticancer properties of phytochemicals. Combination treatment is suggested by the variety of indicators. In combination with traditional chemotherapy agents, phytochemicals show synergic effects by overcoming the limitation of another agent. Thus, further investigation should focus on in vivo testing and clinical trials of phytochemicals in TNBC. Additional studies are required to examine the safety, efficacy, bioavailability, tolerance, and drug delivery options of phytochemicals in the treatment of TNBC.

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Conflict of Interest

None declared.

References

1. Mattiuzzi C, Lippi G. Current Cancer Epidemiology. *J Epidemiol Glob Health*. 2019;9(4):217-22. doi: 10.2991/jegh.k.191008.001.
2. Elsawaf Z, Sinn H-P. Triple-Negative Breast Cancer: Clinical and Histological Correlations. *Breast Care (Basel)*. 2011;6(4):273-8. doi: 10.1159/000331643.
3. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-

negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. *Cancer*. 2007;109(9):1721-8. doi: 10.1002/cncr.22618.

4. Anders C, Carey LA. Understanding and treating triple-negative breast cancer. *Oncology (Williston Park)*. 2008;22(11):1233-9; discussion 1239-40, 1243.
5. Singh J, Asad S, Zhang Y, Nock W, Adams E, Damicis A, et al. Aggressive subsets of metastatic triple negative breast cancer. *Clin Breast Cancer*. 2020;20(1):e20-6. doi: 10.1016/j.clbc.2019.06.012.
6. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res*. 2007;13(15 Pt 1):4429-34. doi: 10.1158/1078-0432.CCR-06-3045.
7. Gonçalves H, Guerra MR, Duarte Cintra JR, Fayer VA, Brum IV, Bustamante Teixeira MT. Survival study of triple-negative and non-triple-negative breast cancer in a Brazilian cohort. *Clin Med Insights Oncol*. 2018;12:1179554918790563. doi: 10.1177/1179554918790563.
8. Wang D-Y, Jiang Z, Ben-David Y, Woodgett JR, Zacksenhaus E. Molecular stratification within triple-negative breast cancer subtypes. *Sci Rep*. 2019;9(1):19107. doi: 10.1038/s41598-019-55710-w.
9. Jiagge E, Chitale D, Newman LA. Triple-negative breast cancer, stem cells, and African ancestry. *Am J Pathol*. 2018;188(2):271-9. doi: 10.1016/j.ajpath.2017.06.020.
10. Hahnen E, Hauke J, Engel C, Neidhardt G, Rhiem K, Schmutzler RK. Germline mutations in triple-negative breast cancer. *Breast Care (Basel)*. 2017;12(1):15-9. doi: 10.1159/000455999.
11. Pierobon M, Frankenfeld CL. Obesity as a risk factor for triple-negative breast cancers: a systematic review and meta-analysis. *Breast Cancer Res Treat*. 2013;137(1):307-14. doi: 10.1007/s10549-012-2339-3.
12. Rey-Vargas L, Sanabria-Salas MC, Fejerman L, Serrano-Gómez SJ. Risk factors for triple-negative breast cancer among Latina women. *Cancer Epidemiol Biomarkers Prev*. 2019;28(11):1771-83. doi: 10.1158/1055-9965.EPI-19-0035.
13. Perry S, Kowalski TL, Chang C-H. Quality of life assessment in women with breast cancer: benefits, acceptability and utilization. *Health Qual Life Outcomes*. 2007;5:24. doi: 10.1186/1477-7525-5-24.
14. Dogan BE, Turnbull LW. Imaging of triple-negative breast cancer. *Ann Oncol Off J Eur Soc Med Oncol*. 2012;23 Suppl 6(Supplement 6):vi23-9. doi: 10.1093/annonc/mds191.
15. Wong RSY. Apoptosis in cancer: from pathogenesis to treatment. *J Exp Clin Cancer Res*. 2011;30(Supplement 6):87. doi: 10.1186/1756-9966-30-87.
16. Al-Mahmood S, Sapiezynski J, Garbuzenko OB, Minko T. Metastatic and triple-negative breast cancer: challenges and treatment options. *Drug Deliv Transl Res*. 2018;8(5):1483-507. doi: 10.1007/s13346-018-0551-3.
17. Wahba HA, El-Hadaad HA. Current approaches in treatment of triple-negative breast cancer. *Cancer Biol Med*. 2015;12(2):106-16. doi: 10.7497/j.issn.2095-3941.2015.0030.
18. de Ruijter TC, Veeck J, de Hoon JPJ,

van Engeland M, Tjan-Heijnen VC. Characteristics of triple-negative breast cancer. *J Cancer Res Clin Oncol*. 2011;137(2):183-92. doi: 10.1007/s00432-010-0957-x.
19. HHe MY, Rancoule C, Rehailia-Blanchard A, Espenel S, Trone JC, Bernichon E, et al. Radiotherapy in triple-negative breast cancer: Current situation and upcoming strategies. *Crit Rev Oncol Hematol*. 2018;131:96-101. doi: 10.1016/j.critrevonc.2018.09.004.
20. Nedeljković M, Damjanović A. Mechanisms of Chemotherapy resistance in triple-negative breast cancer-how we can rise to the challenge. *Cells*. 2019; 8(9):957. doi: 10.3390/cells8090957.
21. Yagata H, Kajiura Y, Yamauchi H. Current strategy for triple-negative breast cancer: appropriate combination of surgery, radiation, and chemotherapy. *Breast Cancer*. 2011;18(3):165-73. doi: 10.1007/s12282-011-0254-9.
22. André F, Zielinski CC. Optimal strategies for the treatment of metastatic triple-negative breast cancer with currently approved agents. *Ann Oncol Off J Eur Soc Med Oncol*. 2012;23 Suppl 6(6):vi46-51. doi: 10.1093/annonc/mds195.
23. Israel BB, Tilghman SL, Parker-Lemieux K, Payton-Stewart F. Phytochemicals: Current strategies for treating breast cancer. *Oncol Lett*. 2018;15(5):7471-8. doi: 10.3892/ol.2018.8304.
24. Shahi Thakuri P, Gupta M, Singh S, Joshi R, Glasgow E, Lekan A, et al. Phytochemicals inhibit migration of triple negative breast cancer cells by targeting kinase signalling. *BMC Cancer*. 2020;20(1):4. doi: 10.1186/s12885-019-6479-2.
25. Grandér D. How do mutated oncogenes and tumour suppressor genes cause cancer? *Med Oncol*. 1998;15(1):20-6. doi: 10.1007/BF02787340.
26. Dixon K, Kopras E. Genetic alterations and DNA repair in human carcinogenesis. *Semin Cancer Biol*. 2004;14(6):441-8. doi: 10.1016/j.semcancer.2004.06.007.
27. Shimelis H, LaDuca H, Hu C, Hart SN, Na J, Thomas A, et al. Triple-negative breast cancer risk genes identified by multigene hereditary cancer panel testing. *J Natl Cancer Inst*. 2018;110(8):855-62. doi: 10.1093/jnci/djy106.
28. Buys SS, Sandbach JF, Gammon A, Patel G, Kidd J, Brown KL, et al. A study of over 35,000 women with breast cancer tested with a 25-gene panel of hereditary cancer genes. *Cancer*. 2017;123(10):1721-30. doi: 10.1002/cncr.30498.
29. Domagala P, Hybiak J, Cybulski C, Lubinski J. BRCA1/2-negative hereditary triple-negative breast cancers exhibit BRCAness. *Int J cancer*. 2017;140(7):1545-50. doi: 10.1002/ijc.30570.
30. Grimmond SM, Palmer JM, Walters MK, Scott C, Nancarrow DJ, Teh BT, et al. Confirmation of susceptibility locus on chromosome 13 in Australian breast cancer families. *Hum Genet*. 1996;98(1):80-5. doi: 10.1007/s004390050164.
31. Roy R, Chun J, Powell SN. BRCA1 and BRCA2: different roles in a common pathway of genome protection. *Nat Rev Cancer*. 2011;12(1):68-78. doi: 10.1038/nrc3181.
32. Newman LA, Reis-Filho JS, Morrow M, Carey LA, King TA. The 2014 Society of Surgical Oncology Susan

G. Komen for the Cure Symposium: triple-negative breast cancer. *Ann Surg Oncol*. 2015;22(3):874-82. doi: 10.1245/s10434-014-4279-0.

33. Mehrgou A, Akouchekian M. The importance of BRCA1 and BRCA2 genes mutations in breast cancer development. *Med J Islam Repub Iran*. 2016;30(1):369.
34. Fostira F, Tsitlaidou M, Papadimitriou C, Pertesi M, Timotheadou E, Stavropoulou AV, et al. Prevalence of BRCA1 mutations among 403 women with triple-negative breast cancer: implications for genetic screening selection criteria: a Hellenic Cooperative Oncology Group Study. *Breast Cancer Res Treat.* 2012;134(1):353-62. doi: 10.1007/s10549-012-2021-9.
35. Couch FJ, Hart SN, Sharma P, Toland AE, Wang X, Miron P, et al. Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. *J Clin Oncol*. 2015;33(4):304-11. doi: 10.1200/JCO.2014.57.1414.
36. Zannini L, Delia D, Buscemi G. CHK2 kinase in the DNA damage response and beyond. *J Mol Cell Biol*. 2014;6(6):442-57. doi: 10.1093/jmcb/mju045.
37. Kleiblová P, Stolařová L, Křížová K, Lhota F, Hojný J, Zemánková P, et al. Germline CHEK2 gene mutations in hereditary breast cancer predisposition - Mutation types and their biological and clinical relevance. *Klin Onkol*. 2019;32(Supplementum2):36-50. doi: 10.14735/amko2019S36.
38. Luo L, Gao W, Wang J, Wang D, Peng X, Jia Z, et al. Study on the mechanism of cell cycle checkpoint kinase 2 (CHEK2) gene dysfunction in chemotherapeutic drug resistance of triple negative breast cancer cells. *Med Sci Monit*. 2018;24:3176-83. doi: 10.12659/MSM.907256.
39. Maréchal A, Zou L. DNA damage sensing by the ATM and ATR kinases. *Cold Spring Harb Perspect Biol*. 2013;5(9):1-17. doi: 10.1101/cshperspect.a012716.
40. Domagala P, Jakubowska A, Jaworska-Bieniek K, Kaczmarek K, Durda K, Kurlapska A, et al. Prevalence of germline mutations in genes engaged in DNA damage repair by homologous recombination in patients with triple-negative and hereditary non-triple-negative breast cancers. *PLoS One*. 2015;10(6):e0130393. doi: 10.1371/journal.pone.0130393.
41. Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, et al. Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. *Mol Cell*. 2006;22(6):719-29. doi: 10.1016/j.molcel.2006.05.022.
42. Heikkinen T, Kärkkäinen H, Aaltonen K, Milne RL, Heikkilä P, Aittomäki K, et al. The breast cancer susceptibility mutation PALB2 1592delT is associated with an aggressive tumour phenotype. *Clin Cancer Res*. 2009;15(9):3214-22. doi: 10.1158/1078-0432.CCR-08-3128.
43. Wiegmans AP, Miranda M, Wen SW, Al-Ejeh F, Möller A. RAD51 inhibition in triple negative breast cancer cells is challenged by compensatory survival signalling and requires rational combination therapy. *Oncotarget*. 2016;7(37):60087-100. doi: 10.18632/oncotarget.11065.
44. Smolarz B, Zadrożny M, Duda-Szymańska J, Makowska M, Samulak D, Michalska MM, et al. RAD51 genotype and triple-negative breast

cancer (TNBC) risk in Polish women. *Pol J Pathol*. 2013;64(1):39-43. doi: 10.5114/pjp.2013.34602.
45. Michalska MM, Samulak D, Romanowicz H, Smolarz B. Single nucleotide polymorphisms (SNPs) of RAD51-G172T and XRCC2-41657C/T homologous recombination repair genes and the risk of triple-negative breast cancer in Polish women. *Pathol Oncol Res*. 2015;21(4):935-40. doi: 10.1007/s12253-015-9922-y.
46. Wu J, Lu LY, Yu X. The role of BRCA1 in DNA damage response. *Protein Cell*. 2010;1(2):117-23. doi: 10.1007/s13238-010-0010-5.
47. Chen H, Wu J, Zhang Z, Tang Y, Li X, Liu S, et al. Association between *BRCA* status and triple-negative breast cancer: a meta-analysis. *Front Pharmacol.* 2018;9:909. doi: 10.3389/fphar.2018.00909.
48. Lee M, Park IA, Heo SH, Kim YA, Gong G, Lee HJ. Association between p53 expression and amount of tumour-infiltrating lymphocytes in triple-negative breast cancer. *J Pathol Transl Med*. 2019;53(3):180-7. doi: 10.4132/jptm.2019.02.08.
49. Horigome E, Fujieda M, Handa T, Katayama A, Ito M, Ichihara A, et al. Mutant TP53 modulates metastasis of triple negative breast cancer through adenosine A2b receptor signalling. *Oncotarget*. 2018;9(77):34554-66. doi: 10.18632/oncotarget.26177.
50. Li JP, Zhang XM, Zhang Z, Zheng LH, Jindal S, Liu YJ. Association of p53 expression with poor prognosis in patients with triple-negative breast invasive ductal carcinoma. *Medicine (Baltimore)*. 2019;98(18):e15449. doi: 10.1097/MD.0000000000015449.
51. Na B, Yu X, Withers T, Gilleran J, Yao M, Foo TK, et al. Therapeutic targeting of *BRCA1* and *TP53* mutant breast cancer through mutant *p53* reactivation. *NPJ Breast Cancer*. 2019;5:14. doi: 10.1038/s41523-019-0110-1.
52. Khan F, Esnakula A, Ricks-Santi LJ, Zafar R, Kanaan Y, Naab T. Loss of PTEN in high grade advanced stage triple negative breast ductal cancers in African American women. *Pathol Res Pract*. 2018;214(5):673-8. doi: 10.1016/j.prp.2018.03.020.
53. Phuah SY, Looi LM, Hassan N, Rhodes A, Dean S, Taib NAM, et al. Triple-negative breast cancer and PTEN (phosphatase and tensin homologue) loss are predictors of BRCA1 germline mutations in women with early-onset and familial breast cancer, but not in women with isolated late-onset breast cancer. *Breast Cancer Res*. 2012;14(6):R142. doi: 10.1186/bcr3347.
54. Cossu-Rocca P, Orrù S, Muroni MR, Sanges F, Sotgiu G, Ena S, et al. Analysis of PIK3CA mutations and activation pathways in triple negative breast cancer. *PLoS One*. 2015;10(11):e0141763. doi: 10.1371/journal.pone.0141763.
55. Mosele F, Stefanovska B, Lusque A, Tran Dien A, Garberis I, Droin N, et al. Outcome and molecular landscape of patients with PIK3CA-mutated metastatic breast cancer. *Ann Oncol Off J Eur Soc Med Oncol*. 2020;31(3):377-86. doi: 10.1016/j.annonc.2019.11.006.
56. Ozretic P, Alvir I, Sarcevic B, Vujaskovic Z, Rendic-Miocevic Z, Roguljic A, et al. Apoptosis regulator BCL-2 is an independent prognostic marker for worse overall survival in triple-negative breast cancer patients. *Int J Biol Markers*. 2018;33(1):109-

15. doi: 10.5301/ijbm.5000291.
57. Williams MM, Cook RS. BCL-2 family proteins in breast development and cancer: could Mcl-1 targeting overcome therapeutic resistance? *Oncotarget*. 2015;6(6):3519-30. doi: 10.18632/oncotarget.2792.
58. Levva S, Kotoula V, Kostopoulos I, Manousou K, Papadimitriou C, Papadopoulou K, et al. Prognostic evaluation of epidermal growth factor receptor (EGFR) genotype and phenotype parameters in triple-negative breast cancers. *Cancer Genomics Proteomics*. 2017;14(3):181-95. doi: 10.21873/cgp.20030.
59. Hashmi AA, Naz S, Hashmi SK, Irfan M, Hussain ZF, Khan EY, et al. Epidermal growth factor receptor (EGFR) overexpression in triple-negative breast cancer: association with clinicopathologic features and prognostic parameters. *Surg Exp Pathol*. 2019;2(1):6. doi: 10.1186/s42047-018-0029-0.
60. Zhu X, Zhou W. The emerging regulation of VEGFR-2 in triple-negative breast cancer. *Front Endocrinol (Lausanne)*. 2015;6:159. doi: 10.3389/fendo.2015.00159.
61. Jafarian AH, Kooshkiforooshani M, Farzad F, Mohamadian Roshan N. The relationship between fibroblastic growth factor receptor-1 (FGFR1) gene amplification in triple negative breast carcinomas and clinicopathological prognostic factors. *Iran J Pathol*. 2019;14(4):299-304. doi: 10.30699/ijp.2019.96713.1952.
62. Erber R, Rübner M, Davenport S, Hauke S, Beckmann MW, Hartmann A, et al. Impact of fibroblast growth factor receptor 1 (FGFR1) amplification on the prognosis of breast cancer patients. *Breast Cancer Res Treat*. 2020;184(2):311-24. doi: 10.1007/s10549-020-05865-2.
63. Brown AM. Wnt signalling in breast cancer: have we come full circle? *Breast Cancer Res*. 2001;3(6):351-5. doi: 10.1186/bcr321.
64. Kucukefe Y, Kaypmaz A. Delayed feedback control as applied to active suspension of a ground vehicle. In: Yasar K, Adnan K, editors. IEEE EUROCON 2009, EUROCON 2009. St. Petersburg: IEEE; 2009. p. 916-21.
65. Tamai K, Zeng X, Liu C, Zhang X, Harada Y, Chang Z, et al. A mechanism for Wnt coreceptor activation. *Mol Cell*. 2004;13(1):149-56. doi: 10.1016/s1097-2765(03)00484-2.
66. Roose J, Molenaar M, Peterson J, Hurenkamp J, Brantjes H, Moerer P, et al. The Xenopus Wnt effector XTcf-3 interacts with Groucho-related transcriptional repressors. *Nature.* 1998;395(6702):608-12. doi: 10.1038/26989.
67. Sethi JK, Vidal-Puig A. Wnt signalling and the control of cellular metabolism. *Biochem J*. 2010;427(1):1-17. doi: 10.1042/BJ20091866.
68. Geyer FC, Lacroix-Triki M, Savage K, Arnedos M, Lambros MB, MacKay A, et al. β-Catenin pathway activation in breast cancer is associated with triple-negative phenotype but not with CTNNB1 mutation. *Mod Pathol*. 2011;24(2):209-31. doi: 10.1038/modpathol.2010.205.
69. Guo S, Liu M, Gonzalez-Perez RR. Role of Notch and its oncogenic signalling crosstalk in breast cancer. *Biochim Biophys Acta*. 2011;1815(2):197-213. doi:

10.1016/j.bbcan.2010.12.002.
70. Zhou Y, Xia L, Wang H, Oyang L, Su M, Liu Q, et al. Cancer stem cells in progression of colorectal cancer. *Oncotarget*. 2018;9(70):33403-15. doi: 10.18632/oncotarget.23607.
71. Kopan R, Ilagan MXG. The canonical Notch signalling pathway: unfolding the activation mechanism. *Cell*. 2009;137(2):216-33. doi: 10.1016/j.cell.2009.03.045.
72. Kontomanolis EN, Kalagasidou S, Pouliliou S, Anthoulaki X, Georgiou N, Papamanolis V, et al. The Notch pathway in breast cancer progression. *ScientificWorldJournal.* 2018;2018:2415489. doi: 10.1155/2018/2415489.
73. Sari IN, Phi LTH, Jun N, Wijaya YT, Lee S, Kwon HY. Hedgehog signalling in cancer: A prospective therapeutic target for eradicating cancer stem cells. *Cells*. 2018;7(11):1038. doi: 10.3390/cells7110208.
74. Habib JG, O'Shaughnessy JA. The hedgehog pathway in triple-negative breast cancer. *Cancer Med*. 2016;5(10):2989-3006. doi: 10.1002/cam4.833.
75. Bhateja P, Cherian M, Majumder S, Ramaswamy B. The Hedgehog signalling pathway: a viable target in breast cancer? *Cancers (Basel)*. 2019;11(8):1126. doi: 10.3390/cancers11081126.
76. Kwon YJ, Hurst DR, Steg AD, Yuan K, Vaidya KS, Welch DR, et al. Gli1 enhances migration and invasion via up-regulation of MMP-11 and promotes metastasis in ERα negative breast cancer cell lines. *Clin Exp Metastasis*. 2011;28(5):437-49. doi: 10.1007/s10585-011-9382-z.
77. Harris LG, Pannell LK, Singh S, Samant RS, Shevde LA. Increased vascularity and spontaneous metastasis of breast cancer by hedgehog signalling mediated upregulation of cyr61. *Oncogene*. 2012;31(28):3370-80. doi: 10.1038/onc.2011.496.
78. Noman AS, Uddin M, Rahman MZ, Nayeem MJ, Alam SS, Khatun Z, et al. Overexpression of sonic hedgehog in the triple negative breast cancer: clinicopathological characteristics of high burden breast cancer patients from Bangladesh. *Sci Rep*. 2016; 6:18830. doi: 10.1038/srep18830.
79. Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C, González-Barón M. PI3K/Akt signalling pathway and cancer. *Cancer Treat Rev*. 2004;30(2):193-204. doi: 10.1016/j.ctrv.2003.07.007.
80. Jeong SJ, Dasgupta A, Jung KJ, Um JH, Burke A, Park HU, et al. PI3K/AKT inhibition induces caspase-dependent apoptosis in HTLV-1-transformed cells. *Virology*. 2008;370(2):264-72. doi: 10.1016/j.virol.2007.09.003.
81. Bender A, Opel D, Naumann I, Kappler R, Friedman L, von Schweinitz D, et al. PI3K inhibitors prime neuroblastoma cells for chemotherapy by shifting the balance towards pro-apoptotic Bcl-2 proteins and enhanced mitochondrial apoptosis. *Oncogene*. 2011;30(4):494-503. doi: 10.1038/onc.2010.429.
82. Fend F, Quintanilla-Martínez L. Mantle cell lymphoma. Pathobiology of human disease. Elsevier; 2014. p. 1687-700. doi: 10.1016/B978-0-12-386456-7.04107-1.
83. Zhang W, Liu HT. MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Res*. 2002;12(1):9-18. doi:

10.1038/sj.cr.7290105.
84. Yin L, Duan JJ, Bian XW, Yu S. Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Res*. 2020;22(1):61. doi: 10.1186/s13058-020-01296-5.
85. Widmann C, Gibson S, Jarpe MB, Johnson GL. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol Rev*. 1999;79(1):143-80. doi: 10.1152/physrev.1999.79.1.143.
86. Lee JT, McCubrey JA. The Raf/MEK/ERK signal transduction cascade as a target for chemotherapeutic intervention in leukemia. *Leukemia*. 2002;16(4):486-507. doi: 10.1038/sj.leu.2402460.
87. Jiang W, Wang X, Zhang C, Xue L, Yang L. Expression and clinical significance of MAPK and EGFR in triple-negative breast cancer. *Oncol Lett*. 2020;19(3):1842-8. doi: 10.3892/ol.2020.11274.
88. Eralp Y, Derin D, Ozluk Y, Yavuz E, Guney N, Saip P, et al. MAPK overexpression is associated with anthracycline resistance and increased risk for recurrence in patients with triple-negative breast cancer. *Ann Oncol Off J Eur Soc Med Oncol*. 2008;19(4):669-74. doi: 10.1093/annonc/mdm522.
89. Bartholomeusz C, Gonzalez-Angulo AM, Liu P, Hayashi N, Lluch A, Ferrer-Lozano J, et al. High ERK protein expression levels correlate with shorter survival in triple-negative breast cancer patients. *Oncologist*. 2012;17(6):766-74. doi: 10.1634/theoncologist.2011-0377.
90. Gollo AL, Tanobe VOA, de Melo Pereira GV, Marin O, Bonatto SJR, Silva S, et al. Phytochemical analysis and biological activities of in vitro cultured Nidularium procerum, a bromeliad vulnerable to extinction. *Sci Rep*. 2020;10(1):7008. doi: 10.1038/s41598-020-64026-z.
91. Cao H, Sethumadhavan K, Bland JM. Isolation of cottonseed extracts that affect human cancer cell growth. *Sci Rep*. 2018;8(1):10458. doi: 10.1038/s41598-018-28773-4.
92. Messeha SS, Zarmouh NO, Mendonca P, Alwagdani H, Cotton C, Soliman KFA. Effects of gossypol on apoptosis‐related gene expression in racially distinct triple‐negative breast cancer cells. *Oncol Rep*. 2019;42(2):467-78. doi: 10.3892/or.2019.7179.
93. Gilbert NE, O'Reilly JE, Chang CJ, Lin YC, Brueggemeier RW. Antiproliferative activity of gossypol and gossypolone on human breast cancer cells. *Life Sci*. 1995;57(1):61-7. doi: 10.1016/0024-3205(95)00243-y.
94. Mishra AP, Salehi B, Sharifi-Rad M, Pezzani R, Kobarfard F, Sharifi-Rad J, et al. Programmed cell death, from a cancer perspective: an overview. *Mol Diagn Ther*. 2018;22(3):281-95. doi: 10.1007/s40291-018-0329-9.
95. Liu S, Kulp SK, Sugimoto Y, Jiang J, Chang HL, Dowd MK, et al. The (-)-enantiomer of gossypol possesses higher anticancer potency than racemic gossypol in human breast cancer. *Anticancer Res*. 2002;22(1A):33-8.
96. Kelkel M, Schumacher M, Dicato M, Diederich M. Antioxidant and anti-proliferative properties of lycopene. *Free Radic Res*. 2011;45(8):925–40. doi: 10.3109/10715762.2011.564168.
97. Gloria NF, Soares N, Brand C, Oliveira FL, Borojevic R, Teodoro AJ. Lycopene and beta-carotene

induce cell-cycle arrest and apoptosis in human breast cancer cell lines. *Anticancer Res*. 2014;34(3):1377-86.

98. Trejo-Solís C, Pedraza-Chaverrí J, Torres-Ramos M, Jiménez-Farfán D, Cruz Salgado A, Serrano-García N, et al. Multiple molecular and cellular mechanisms of action of lycopene in cancer inhibition. *Evid Based Complement Alternat Med*. 2013;2013(I):705121. doi: 10.1155/2013/705121.
99. Takeshima M, Ono M, Higuchi T, Chen C, Hara T, Nakano S. Anti-proliferative and apoptosis-inducing activity of lycopene against three subtypes of human breast cancer cell lines. *Cancer Sci*. 2014;105(3):252-7. doi: 10.1111/cas.12349.
100. Strimpakos AS, Sharma RA. Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials. *Antioxid Redox Signal*. 2008;10(3):511-45. doi: 10.1089/ars.2007.1769.
101. Sun XD, Liu XE, Huang DS. Curcumin induces apoptosis of triple-negative breast cancer cells by inhibition of EGFR expression. *Mol Med Rep*. 2012;6(6):1267-70. doi: 10.3892/mmr.2012.1103.
102. Maiello MR, D'Alessio A, Bevilacqua S, Gallo M, Normanno N, De Luca A. EGFR and MEK blockade in triple negative breast cancer cells. *J Cell Biochem*. 2015;116(12):2778-85. doi: 10.1002/jcb.25220.
103. Mimeault M, Batra SK. Potential applications of curcumin and its novel synthetic analogs and nanotechnology-based formulations in cancer prevention and therapy. *Chin Med*. 2011;6:31. doi: 10.1186/1749-8546-6-31.
104. Hajime Hirose, Hideshi Ishii, Koshi Mimori, Daisuke Ohta, Masahisa Ohkuma, Hirohiko Tsujii, et al. Notch pathway as candidate therapeutic target in Her2/Neu/ErbB2 receptor-negative breast tumours. *Oncol Rep*. 2010;23(1):35–43. doi: 10.3892/or.
105. Křížová L, Dadáková K, Kašparovská J, Kašparovský T. Isoflavones. *Molecules*. 2019;24(6):1076. doi: 10.3390/molecules24061076.
106. Banerjee S, Li Y, Wang Z, Sarkar FH. Multi-targeted therapy of cancer by genistein. *Cancer Lett*. 2008;269(2):226-42. doi: 10.1016/j.canlet.2008.03.052.
107. Wu AH, Koh WP, Wang R, Lee HP, Yu MC. Soy intake and breast cancer risk in Singapore Chinese Health Study. *Br J Cancer*. 2008;99(1):196-200. doi: 10.1038/sj.bjc.6604448.
108. Pan H, Zhou W, He W, Liu X, Ding Q, Ling L, et al. Genistein inhibits MDA-MB-231 triple-negative breast cancer cell growth by inhibiting NF-κB activity via the Notch-1 pathway. *Int J Mol Med*. 2012;30(2):337-43. doi: 10.3892/ijmm.2012.990.
109. Donovan MG, Selmin OI, Doetschman TC, Romagnolo DF. Epigenetic activation of BRCA1 by genistein in vivo and triple negative breast cancer cells linked to antagonism toward aryl hydrocarbon receptor. *Nutrients*. 2019;11(11):2559. doi: 10.3390/nu11112559.
110. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol*. 2007;35(4):495–516. doi: 10.1080/01926230701320337.
111. Sultan AS, Khalil MIM, Sami BM, Alkhuriji AF, Sadek O. Quercetin induces apoptosis in triple-negative breast cancer cells via inhibiting fatty acid synthase and ß-catenin. *Int J Clin Exp Pathol*. 2017;10(1):156-72.

112. Papaliagkas V, Anogianaki A, Anogianakis G, Ilonidis G. The proteins and the mechanisms of apoptosis: a mini-review of the fundamentals. *Hippokratia*. 2007;11(3):108-13.
113. Lin D, Kuang G, Wan J, Zhang X, Li H, Gong X, et al. Luteolin suppresses the metastasis of triple-negative breast cancer by reversing epithelial-to-mesenchymal transition via downregulation of β-catenin expression. *Oncol Rep*. 2017;37(2):895-902. doi: 10.3892/or.2016.5311.
114. Huang L, Jin K, Lan H. Luteolin inhibits cell cycle progression and induces apoptosis of breast cancer cells through downregulation of human telomerase reverse transcriptase. *Oncol Lett*. 2019;17(4):3842-50. doi: 10.3892/ol.2019.10052.
115. Li J, Gong X, Jiang R, Lin D, Zhou T, Zhang A, et al. Fisetin inhibited growth and metastasis of triple-negative breast cancer by reversing epithelial-to-mesenchymal transition via PTEN/Akt/GSK3β signal pathway. *Front Pharmacol*. 2018;9:772. doi: 10.3389/fphar.2018.00772.
116. Smith ML, Murphy K, Doucette CD, Greenshields AL, Hoskin DW. The dietary flavonoid fisetin causes cell cycle arrest, caspase-dependent apoptosis, and enhanced cytotoxicity of chemotherapeutic drugs in triple-negative breast cancer cells. *J Cell Biochem*. 2016;117(8):1913-25. doi: 10.1002/jcb.25490.
117. Shindikar A, Singh A, Nobre M, Kirolikar S. Curcumin and resveratrol as promising natural remedies with nanomedicine approach for the effective treatment of triple negative breast cancer. *J Oncol*. 2016;2016:9750785. doi: 10.1155/2016/9750785.
118. Horgan XJ, Tatum H, Brannan E, Paull DH, Rhodes L V. Resveratrol analogues surprisingly effective against triple‐negative breast cancer, independent of ERα. *Oncol Rep*. 2019;41(6):3517-26. doi: 10.3892/or.2019.7122.
119. Alasmari MM, Alshaeri HK, Alashari RA, Böhlke M, Maher T, Pino-Figueroa A. Study the effects of capsaicin on triple negative breast cancer cells. *J Clin Exp Pathol*. 2018;8.
120. De Santi M, Galluzzi L, Lucarini S, Paoletti MF, Fraternale A, Duranti A, et al. The indole-3-carbinol cyclic tetrameric derivative CTet inhibits cell proliferation via overexpression of p21/CDKN1A in both estrogen receptor-positive and triple-negative breast cancer cell lines. *Breast Cancer Res*. 2011;13(2):R33. doi: 10.1186/bcr2855.
121. Elsayed HE, Ebrahim HY, Mohyeldin MM, Siddique AB, Kamal AM, Haggag EG, et al. Rutin as a novel c-Met inhibitory lead for the control of triple negative breast malignancies. *Nutr Cancer*. 2016;69(8):1256-71. doi: 10.1080/01635581.2017.1367936.
122. Messeha SS, Zarmouh NO, Asiri A, Soliman KFAA. Rosmarinic acid-induced apoptosis and cell cycle arrest in triple-negative breast cancer cells. *Eur J Pharmacol*. 2020;885:173419. doi: 10.1016/j.ejphar.2020.173419.
123. Li YW, Xu J, Zhu GY, Huang ZJ, Lu Y, Li XQ, et al. Apigenin suppresses the stem cell-like properties of triple-negative breast cancer cells by inhibiting YAP/TAZ activity. *Cell*

Death Discov. 2018;4(1):105. doi: 10.1038/s41420-018-0124-8.
124. Bauer D, Mazzio E, Hilliard A, Oriaku ET, Soliman KFA. Effect of apigenin on whole transcriptome profile of TNFα-activated MDA-MB-468 triple negative breast cancer cells. *Oncol Lett*. 2020;19(3):2123-32. doi: 10.3892/ol.2020.11327.
125. Elkhalifa D, Alali F, Al Moustafa AE, Khalil A. Targeting triple negative breast cancer heterogeneity with chalcones: a molecular insight. *J Drug Target*. 2019;27(8):830-8. doi: 10.1080/1061186X.2018.1561889.
126. Oh YJ, Seo YH. A novel chalcone-based molecule, BDP inhibits MDA‐MB‐231 triple-negative breast cancer cell growth by suppressing Hsp90 function. *Oncol Rep*. 2017;38(4):2343-50. doi: 10.3892/or.2017.5925.
127. Greenshields AL, Doucette CD, Sutton KM, Madera L, Annan H, Yaffe PB, et al. Piperine inhibits the growth and motility of triple-negative breast cancer cells. *Cancer Lett*. 2015;357(1):129-40. doi: 10.1016/j.canlet.2014.11.017.
128. Burande AS, Viswanadh MK, Jha A, Mehata AK, Shaik A, Agrawal N, et al. EGFR targeted paclitaxel and piperine co-loaded liposomes for the treatment of triple negative breast cancer. *AAPS PharmSciTech*. 2020;21(5):151. doi: 10.1208/s12249-020-01671-7.
129. Mehta R, Katta H, Alimirah F, Patel R, Murillo G, Peng X, et al. Deguelin action involves c-Met and EGFR signaling pathways in triple negative breast cancer cells. *PLoS One*. 2013;8(6):e65113. doi: 10.1371/journal.pone.0065113.
130. Robles AJ, Du L, Cichewicz RH, Mooberry SL. Maximiscin induces DNA damage, activates DNA damage response pathways, and has selective cytotoxic activity against a subtype of triple-negative breast cancer. *J Nat Prod*. 2016;79(7):1822-7. doi: 10.1021/acs.jnatprod.6b00290.
131. Shao F, Sun H, Deng CX. Potential therapeutic targets of triple-negative breast cancer based on its intrinsic subtype. *Oncotarget*. 2017;8(42):73329-44. doi: 10.18632/oncotarget.20274.
132. Anand David AV, Arulmoli R, Parasuraman S. Overviews of biological importance of quercetin: a bioactive flavonoid. *Pharmacogn Rev*. 2016;10(20):84-9. doi: 10.4103/0973-7847.194044.
133. Vafadar A, Shabaninejad Z, Movahedpour A, Fallahi F, Taghavipour M, Ghasemi Y, et al. Quercetin and cancer: new insights into its therapeutic effects on ovarian cancer cells. *Cell Biosci*. 2020;10(1):32. doi: 10.1186/s13578-020-00397-0.
134. Lin Y, Shi R, Wang X, Shen HM. Luteolin, a flavonoid with potential for cancer prevention and therapy. *Curr Cancer Drug Targets*. 2008;8(7):634-46. doi: 10.2174/156800908786241050.
135. Abotaleb M, Samuel SM, Varghese E, Varghese S, Kubatka P, Liskova A, et al. Flavonoids in cancer and apoptosis. *Cancers (Basel)*. 2018;11(1):28. doi: 10.3390/cancers11010028.

Table 1. Mechanism and mutation of gene in TNBC

Gene	Location	Function	Type of Mutation	Reference
BRCA1	17q21	DNA damage response and repair, Tumour suppression	Inactivation	[31,46]
BRCA2	13q12	Tumour suppression, Homologous recombination	Inactivation	[31,47]
CHEK2	22q12.1	cell cycle checkpoint regulation, DNA repair	Inactivation	[36]
ATM	11q22.3	Cell cycle regulation, DNA repair mechanism	Inactivation	[39]
PALB2	16p12.2	DNA recombination repair and checkpoint functions by stabilization and localization within nuclear matrix and chromatin	Inactivation	[41]
RAD51	15q15.1	Homologous recombination	Inactivation	[44]
TP53	17p13.1	Apoptosis & DNA repair	Inactivation	[48,49,50,51]
PTEN	10q23.31	Tumour suppression, Cell cycle regulation	Deletion, Inactivation	[52,53]
PIK3CA	3q26.32	Proliferation, Differentiation	Activation	[54,55]
BCL2	18q21.33	Anti-apoptosis	Overexpression	[56,57]
EGFR	7p11.2	Cell proliferation, Metastasis	Overexpression	[58,59]
VEGFR2	6p21.1	Angiogenesis, Invasion	Overexpression	[60]
FGFR1	8p11.23	Cell proliferation, survival	Overexpression	[61,62]

ATM: Ataxia telangiectasia mutated; Bcl2: B cell lymphoma 2; BRCA: Breast cancer gene; CHEK2: Checkpoint kinase 2; EGFR: Epidermal growth factor receptor; FGFR - Fibroblast growth factor; PALB2: Partner and localizer of BRCA2; PIK3CA: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN - Phosphatase and tensin homolog; TNBC: Triple-negative breast cancer; TP53: Tumour protein 53; VEGFR: Vascular endothelial growth factor receptor;

Table 2. Role of various phytochemicals in TNBC

Sr. No	Phytochemical	Role	Mode of action	Reference
1.	Gossypol	Antimetastatic, Antiproliferative, Apoptosis,	The upregulation of proapoptotic genes TNFRSF9, BNIP3, and GADD45A genes. downregulation of Bcl2.	94,110
2.	Lycopene	Apoptosis, cell cycle arrest	Inhibits phosphorylation of Akt kinase and mTOR, Upregulation of pro-apoptotic Bax protein	98
3.	Curcumin	Apoptosis, Antiangiogenic, Immunomodulatory	Downregulate EGFR expression, Inhibition of Hedgehog, Notch signalling and Wnt/B-catenin signalling	100,101,103,104
4.	Genistein	Antiproliferative, Apoptosis, cell cycle arrest	Inhibits protein tyrosine kinases, Upregulation of BRCA-1 expression,	108,109
5.	Quercetin	Apoptosis, Antimetastatic	The upregulation of caspase-3 protein activity and downregulation of Bcl-2 protein and β-catenin.	111,112
6.	Luteolin	Apoptosis, Antimetastsic	The upregulation of Bax protein and downregulation of Bcl-2 protein alters the β-catenin pathway	113,114
7.	Fisetin	Cell cycle arrest, apoptosis	Suppress phosphoinositol 3-kinase (PI3K)-Akt-GSK-3β signalling pathway, Reverses epithelial to mesenchymal transition, Decrease histone H3	115,116

			phosphorylation	
8.	Resveratrol	Apoptosis, Antiangiogenetic, Antimetastsic	Fas/Fas ligand-mediated apoptosis Inhibits the matrix metalloproteinases enzymes	[117,118]
9.	Capsaicin	Apoptosis, cell cycle arrest	Increases in cytochrome C release, caspase 3/7 activity. Down- regulation of cyclin D1	[119]
10.	Indole 3 carbinol	Induction of autophagy	p21/CDKN1A and GADD45A overexpression	[120]
11.	Rutin	Antitumour activity	The modulation of diverse macromolecular targets	[121]
12.	Rosmarinic acid	Cell cycle arrest and apoptosis	The upregulation of GADD45A BNIP3 upregulation	[122]
13.	Apigenin	Inhibit Proliferation and migration of tumour	The downregulation of CXCL10, IL22RA2, ROS1, SLITRK6 and MMP13. Decrease YAP/TAZ activity and expression of CTGF and CYR61	[123,124]
14.	Chalcones	Apoptosis, antimetastatic, regulates proliferation	The upregulation of PARP, caspase 3, caspase 8. Inhibits the activity of MMP-9. Potent inhibitors of HDACs.	[125,126]
15.	Piperine	Apoptosis, Antimetastatic	The inhibition of Akt activation, Inhibit expression of metalloproteinase-2 and 9	[127,128]
16.	Deguelin	Antiproliferative, Apoptosis, Antimetastatic	Decrease PCNA level Downregulation of EGFR, p-AKT, p-	[129]

			ERK, c-met, NF-κB	
17.	Maximiscin	DNA damage response, Cell cycle arrest	Phosphorylation of p53, Chk1, and Chk2	[130]
18.	Cyclopamine	Antiproliferative	Inhibit Hedgehog pathway	[131]

Bax: BCL2 Associated X; Bcl-2: B-cell lymphoma 2; BRCA: Breast cancer gene; CDKN1A: Cyclin-dependent kinase inhibitor 1A; Chk: Checkpoint kinases; CTGF: Connective tissue growth factor; CXCL10: C-X-C motif chemokine ligand 10; CYR61: Cysteine-rich angiogenic inducer 61; EGFR: Epidermal growth factor receptor; ERK: Extracellular Signal-Regulated Kinase; Fas: Fatty acid synthase; GADD45A: Growth arrest and DNA damage-inducible alpha; GSK: Glycogen synthase kinase; HDACs: Histone deacetylase; IL22RA2: Interleukin 22 receptor subunit alpha 2; MMP: Matrix metalloproteinase; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor-kappa B; PARP: Poly (ADP-ribose) polymerase; PCNA: Proliferating cell nuclear antigen; SLITRK6: SLIT and NTRK like family member 6; TAZ: Transcriptional coactivator with PDZ-binding motif; TNFRSF9: TNF receptor superfamily member 9; YAP: Yes-associated protein;

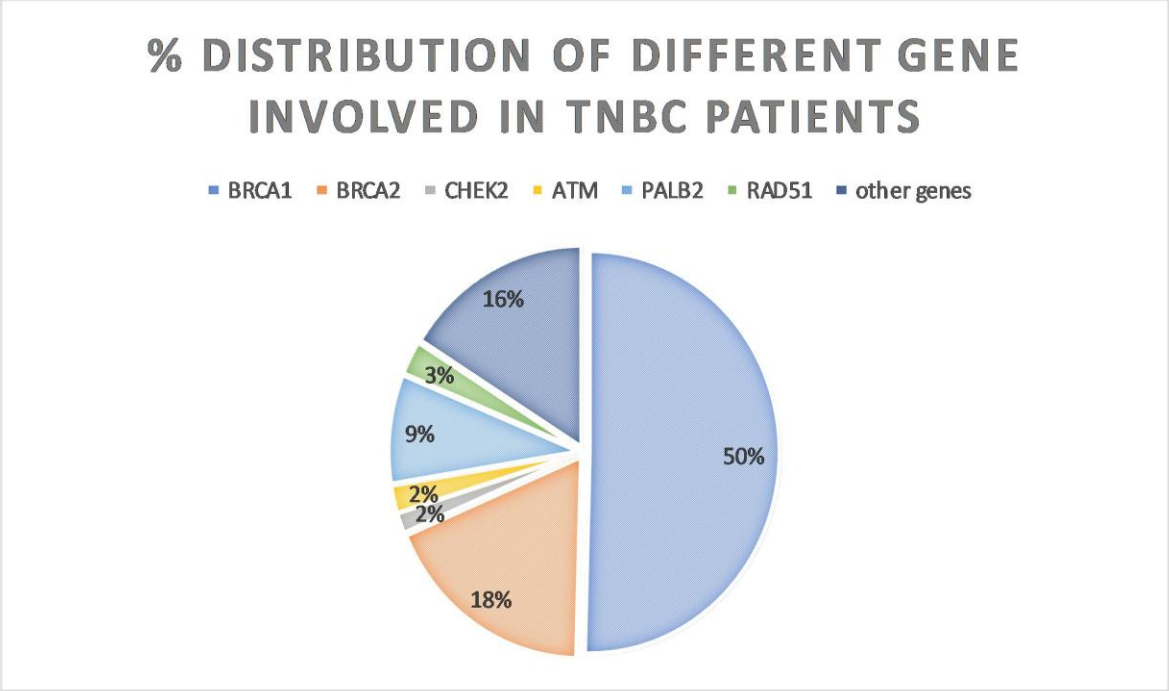

Figure 1. This figure shows the distribution of germline mutation in various genes among the women (n=692) with TNBC.[28]

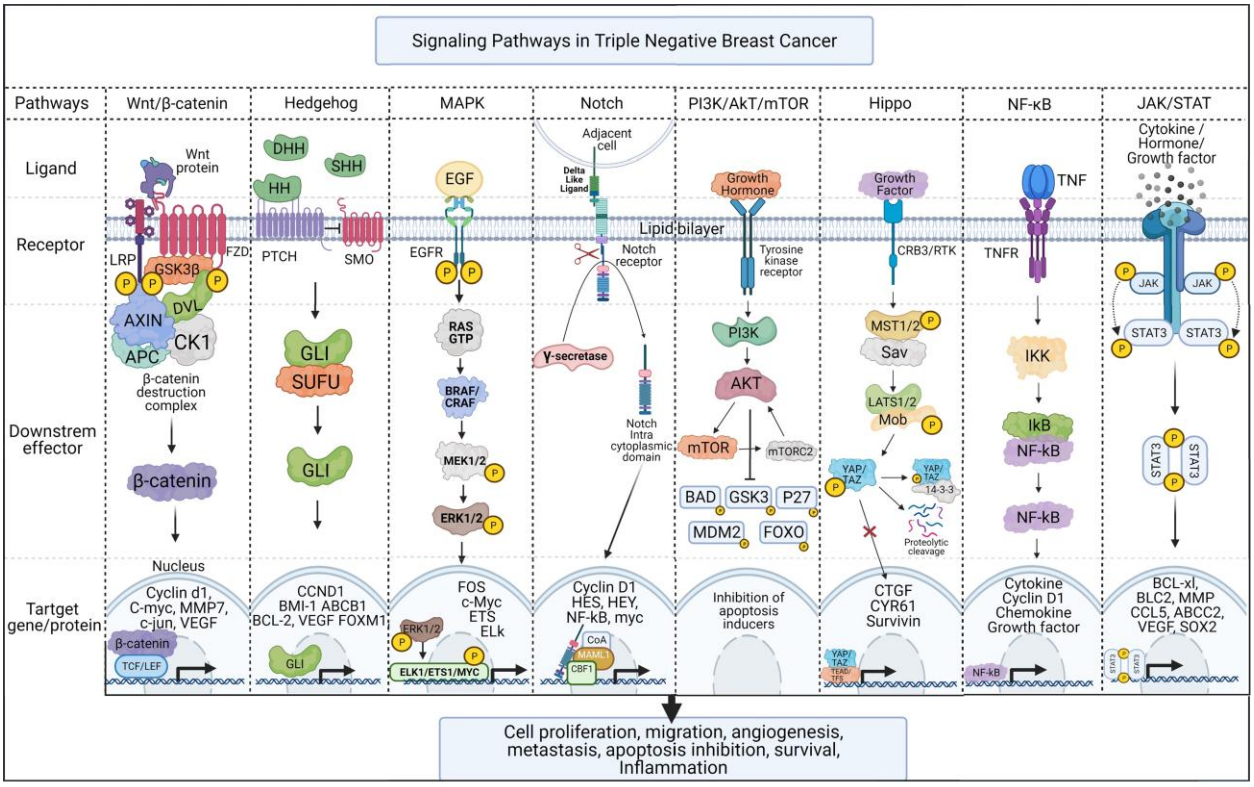

Figure 2. Different signalling pathways are involved in TNBC. Any aberration in signalling pathways leads to the transformation of cell. On binding of ligand to receptor cause a confirmational change in receptor and recruits downstream effector protein that inhibits or permits the function of transcription factors and regulatory proteins by multiple events of a signalling cascade to promote cell proliferation, metastasis, angiogenesis, apoptosis, survival and inflammation.

ABCB1: ATP-binding cassette sub-family B member 1; ABCC2: ATP-binding cassette subfamily C member 2; APC: Adenomatous polyposis coli; BAD: BCL2 associated agonist of cell death; BMI1: B lymphoma Mo-MLV insertion region 1; CBF1: Centromere binding protein 1; CCND1: Cyclin D1; CK1: Casein kinase 1; CTGF: Connective tissue growth factor; CYR61: Cysteine rich angiogenic inducer 61; DHH: Desert hedgehog; DVL: Dishevelled; EGF: Epidermal growth factor; EGFR: Epidermal growth factor receptor; ERK: Extracellular signal regulated kinase; ETS: E26 transformation specific; FOXM1: Forkhead box protein M1; FZD: Frizzled; GLI: Glioma associated oncogene homolog; GSK-3β: Glycogen synthase kinase 3 beta; Hes: Hairy/enhancer of split; Hey: Hairy/Enhancer of split related with YRPW motif; HH: Hedgehog; IKB: Inhibitor of nuclear factor kappa B; IKK: Inhibitor of nuclear factor kappa B kinase; JAK: Janus kinase; LATS1/2: Large tumour suppressor kinase 1/2; LEF: Lymphoid enhancer factor; LRP: Lipoprotein receptor related Protein; MAML1: Mastermind like transcriptional coactivator 1; MEK: Mitogen-activated protein kinase; Mob: Mps1 binder related; MST1/2: Mammalian sterile 20-like protein 1/2; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor kappa B; PI3K: Phosphatidylinositol-3-kinase; PTCH: Patched; RAF: Rapidly accelerated fibrosarcoma; RTK: Receptor tyrosine kinase; SAV: Scaffold protein salvador; SHH: Sonic hedgehog; Smo: Smoothened; SOX2: SRY-box transcription factor 2; STAT: Signal transducer and activator of transcription; SUFU: Suppressor of fused homolog; TCF: T-cell factor; TEAD: TEA domain family member; TNF: Tumour necrosis factor; TNFR: Tumour necrosis factor receptor; Wnt: Wingless related integration site.