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# Molecular mechanism of the effects of quercetin on human breast cancer cells

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*Review*

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**The formation of breast cancer is a multi-step process which involves initiation, promotion and progression. Genetic mutations that control cell growth and division or the repair of damaged deoxyribonucleic acid (DNA) result in uncontrolled growth and division of the cells to form a tumor. This process is termed proliferation and it is regulated by cyclin dependent kinase (CDK 2), cyclin A and B, p53 and p57. Thereafter, angiogenesis enhances progression of the cancer cell. The process of angiogenesis is regulated by the vascular endothelium growth factor (VEGF), hypoxia-inducible factor (HIF-1) and activation protein (AP-1). The major treatment methods for breast cancer are chemotherapy, radiation therapy, gene therapy and biologic therapy; however, success rates are low. This has led to further investigations into other agents with a potential of acting on novel targets in breast cancer. Dietary polyphenols have been correlated with a reduced risk of developing cancer. Quercetin is a potential anti-cancer flavonoid molecule and is ubiquitous in nature. The suggested mechanisms for the chemoprevention action of quercetin include the capability to suppress cell proliferation and angiogenesis in breast cancer cells. In this review, we summarize the recent uncovered molecular mechanisms of quercetin on MCF-7 breast cancer cell lines biomarkers and molecular mechanism by which quercetin exhibits chemo preventive and chemotherapeutic effects have been discussed.**

**Key words:** Quercetin, breast cancer cell lines, angiogenesis, cell cycle.

## INTRODUCTION

Breast cancer originates from the breast tissue, especially from the inner lining of milk ducts or lobules. It results from a multistep process, which involves initiation, promotion and progression. (Kim et al., 2009). The development of breast cancer involves several proteins and genes, namely; CDK 2 (Cyclin dependent kinase), VEGF (Vascular endothelial growth factor), HIF 1 (Hypoxia-inducible factors), AP 1 (Activator protein), p53, p57, cyclin A and B (Sariego, 2010). Environmental and internal factors; for example smoking and oestrogen have been claimed to be the risk factors for breast cancer] (Anuso et al., 2010). Due to drug resistance and poor prognosis, low success rates have been observed in the management of breast cancer that necessitated the use of complementary and alternative medicine (CAM) and natural health products like dietary polyphenols (Jo et al., 2011). One such novel compound is; Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone), which is found in a variety of foods including apples, berries, red onions, tea, broccoli and as well as other green leafy vegetables. The chemo-preventive effect of quercetin has been hypothesized to result from inhibition of the cell proliferation (cell cycle arrest, apoptosis, etc.,) and angiogenesis in breast cancer cells (Kim et al., 2009; Jo et al., 2011).

### Quercetin and cell cycle to inhibit cell proliferation

Cell cycle comprises of a series of controlled events that drive the replication of DNA and cell division. It is divided

into several phases: G1 phase, S phase, G2 phase and mitosis Kim et al., 2009). In a study by Chou et al., (2010) quercetin was found to inhibit progression of human breast MCF-7 cancer cells through down regulation of proteins CDK2, cyclin A, D, E, p53 and p57 involved in cell cycle, which resulted in the arrest of cell cycle. Quercetin has also been found to block cell cycle at G2/M through up-regulation of p21 and cyclin B to regulate cell-cycle arrest at the G1 phase and G2/M phase in breast cancer cell lines (Moon et al., 2008). Likewise, *in vivo*, quercetin inhibited breast cancer cell line proliferation and led to chemoprevention (Miyamoto et al., 2009). It was also reported that quercetin doses ranging from 50 to 200 µM significantly inhibited proliferation when applied to cultured MCF-7 human breast cancer cells for a definite period of time (Duo et al., 2012).

## Quercetin and apoptosis

Apoptosis is a process of programmed cell death, where a cell is compelled to be obliterated through activation of proteins called *caspases*. The proteins break down the cellular components needed for the survival of the cell through a cascade of biochemical events that lead to change the cells morphologically changes and eventual disappearance of cell (Green, 2011).

## Apoptosis with regards to breast cancer

Normal breast development is controlled by a balance between cell proliferation and apoptosis. Breast cancer, which is formed due to tumor growth often results from uncontrolled proliferation. The balance between proliferation and apoptosis is important in determining the overall growth and or regression of the tumors in response to chemotherapy, radiotherapy and, more recently, hormonal interventions (Vakkala et al., 1999).

## Mitochondrial pathway

The mitochondrial apoptotic pathway is initiated via Bcl-2 and Bcl-2associated X (Bax) proteins which increase the mitochondrial membrane potential pore-size allowing cytochrome *c*, among other pro-apoptotic proteins, to leak out into the cytosol. Cytochrome c then activates apoptotic protease activating-factor 1 (APAF-1) and undergoes a conformational change forming the apoptosome. This enlists *caspase*-9 in order to activate executioner proteins, *caspase*-7 and *caspase*-3 to subsequently drive the cell to death (Tan *et al*., 2009). These workers have further reported that quercetin doses of 40–50 mM induced apoptosis in multiple cancer cell lines Tan et al., (2009). At 200 – 250 mM doses of quercetin, p53, *caspase*-9 activation, *caspase*-3, cytochrome *c* proteins were up-regulated *in vitro*, which induced apoptosis in human breast cancer cells MDA-MB-231 (Chien et al., 2009).

## Protein chaperone inhibition pathway

Quercetin promotes apoptosis by modulating the proliferation and cell maintenance pathways (Bcl-2 and XIAP inhibition) (Kim et al., 2013). However, it is emerging that quercetin directs protein chaperone inhibition and may play an important role in the stimulation to cell death (Aalinkeel et al., 2008). When protein chaperones are unable to perform their duties, cell functionality is decreased and cell death occurs. It was also revealed that quercetin initiates apoptosis via the mitochondrial pathway involves the activation of *caspase*-3 downstream from *caspase*-9 (Aalinkeel et al., 2008). Quercetin also inactivates these protein chaperones, possibly by its ability to inhibit the kinases that aid in HSP induction (Zhang et al., 2009).

The mechanism involves the expression of HSP70 after stimulation by radiation-induced heat in tumor cells. The heat induces the phosphorylation of heat shock transcription factor 1 (HSF1) by either casein kinase 2 (CK2) or calcium/calmodulin kinase II (CamKII). Once phosphorylated, these kinases activate HSF1, which catalyzes the transcription of HSP70. Quercetin can inhibit HSP70 expression but with no effect on HSP27 (Zhang et al., 2009).

## Endoplasmic reticulum (ER) stress pathway

ER is the cellular organelle responsible for the packaging and synthesis of many nutrients. Endoplasmic reticulum stress is also known as the unfolded protein response, as an accumulation of misshapen proteins increases endoplasmic reticulum stress in cells. It was found to initiate mitochondria-mediated apoptosis by increasing inter-mitochondrial calcium concentration (Lee et al., 2006) that leads to increased recruitment of Bax and thus decreasing mitochondrial membrane potential (Li et al., 2008).

This release triggers the activation of *caspase*-9 and -3, and then cell death. Inhibition of HSP70 in MCF-7, cell lines by quercetin (100 mM) was followed by initiation of the unfolded protein response initiating increased expression of glucose regulated protein 78 (GRP78) (Li et al., 2008). This provides a clue for cells to resist quercetin-induced apoptosis. However, it has been established that when GRP78 is inhibited, there is increased quercetin-mediated apoptosis (Lee et al., 2006). This provides evidence that quercetin may be able to work in collaboration with other compounds in order to mediate cell death via the endoplasmic reticulum stress pathway.

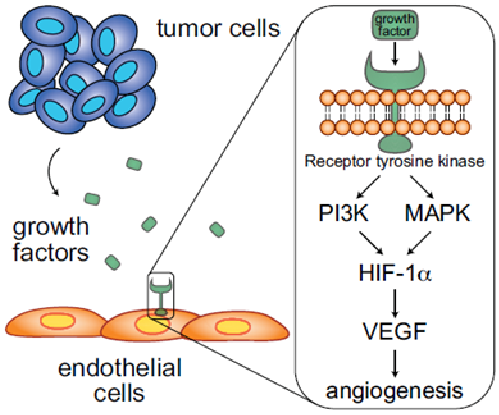


**Figure 1.**Pathways involved in angiogenesis (Cook et al., 2010).

## Angiogenesis

Many tumors outgrow their blood supply and are poorly perfused, giving way to areas of hypoxia (Wachsberger et al., 2005). This process involves the migration, growth, and differentiation of endothelial cells, which line the inside wall of the blood vessels. There are 6 essential hallmarks or processes, that are required for the transformation of a normal cell to a cancer cell proposed by Hao et al., (2005); (i) self-sufficiency in growth signals; (ii) insensitivity to antigrowth signals; (iii) evasion of programmed death (apoptosis); (iv) endless replication potential; (v) tissue invasion and metastasis; and(vi) sustained angiogenesis. There are several pathways involved in angiogenesis as shown in (Figure 1). Tumor cells secrete pro-angiogenic growth factor(s) that bind to receptors on dormant endothelial cells (ECs), leading to vasodilatation and an increase in vessel permeability. The ECs migrate and proliferate to form new branches from the pre-existing vasculature by detaching from the extracellular matrix and basement membrane. PI3K indicates phosphoinositide 3-kinase; MAPK, mitogen-activated protein kinase; HIF-1, hypoxia inducible factor-1; VEGF, vascular endothelial growth factor. Quercetin directly and indirectly inhibited these pathways thereby inhibited angiogenesis (adapted from Oha et al., 2010).

## Conclusion

We have reviewed the molecular mechanisms of quercetin on MCF-7 human breast cancer cell lines indicate that quercetin can suppress cell proliferation and angiogenesis in breast cancer cell lines. Therefore, quercetin is a dietary polyphenol that can be utilized as an adjunct treatment in the management of breast cancer. Therefore, it is suggested that quercetin may be one of the important molecules for the development of anti-breast cancer agent in clinical approaches. Nonetheless, in both prevention and treatment of this frightening health problem supplementation or another form of treatment(s) should be necessary for therapeutic responses.

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