

## Chemopreventive Activities of Shikonin in Breast Cancer

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Shikon (a Chinese herbal plant) has been used as an antitumor agent in complementary medicine for thousands of years [1,2]. Shikonin (SK), a key bioactive component of shikon with molecular weight of 288, has potent antitumor activity in a variety of human cancer cells [2-10] and is a novel natural compound in the database of the Developmental Therapeutics Program of the National Cancer Institute (NCI). SK inhibits tumor cell growth via various signaling cascades and different mechanisms including cell cycle arrest and induction of apoptosis [2-10]. SK administration produces no serious adverse events in clinical trials and animal studies [1,9,10]. Following ingestion, SK is metabolized in the gastrointestinal tract where it subsequently forms cycloshikonin and desoxyshikonin, which have no reported toxicity *in vivo* [11]. Finally, SK prevents chemical-induced gene mutations, and inhibits intestinal and skin tumorigenesis *in vivo* [12-14]. These results prove that SK has potential as a chemopreventive agent.

Breast cancer is the second most diagnosed cancer in women after skin cancer. Advancements in molecular biology techniques in the recent past have led to the identification of the heterogeneous nature of the disease consisting of various subtypes based on the underlying biological mechanisms. Technological advances in diagnostics have directed us in the identification of the condition at a very early stage that helps in the reduction in risk associated with progression to other acute stages. Estrogen receptor alpha (ER) signaling is considered to be a key contributing factor in the progression of mammary tumorigenesis. Although many anti-estrogen therapies are available for treatment of the condition, currently available treatments have major side effects (drug resistance and endometrial cancer) that may limit the survival rate. There is a pressing requirement for finding better alternatives and promising treatment strategies that will further reduce the post therapeutic complications and improve the survival rate and lifestyle of the survivors. In the process of identifying an ideal agent that will satisfy optimal treatment requirements, we identified that SK is a potent chemopreventive agent. We found that SK inhibits estrogen stimulated cell growth and have identified a key molecular mechanism through which it promotes ER ubiquitination and subsequently induces ER degradation in ER positive breast cells [15]. Our studies also show that the estrogen recruits ER at the estrogen responsive gene promoters (pS2 and c-myc), which can be inhibited by SK. We also found that treatment with SK can activate the Nrf2 pathway that is involved in the protection against estrogen induced DNA damage [15,16]. As studies have demonstrated the low toxicity of SK in normal human mammary epithelial cells and inhibitory effects on estrogen dependent cell growth, this compound has potential to be considered further for the drug studies against breast cancer.

SK is able to induce cell death in ER positive breast cancer cells distinct from apoptosis, with the characteristics of necroptosis [17]. Studies also demonstrate that SK down-regulates the expression of steroid sulfatase genes, which are the key regulators for the estrogen biosynthesis in breast tumor [18]. The cellular mechanistic actions of SK are intensively studied and treatment with SK can target multiple molecules or signaling pathways including activation of caspase-3, modification of the apoptosis-related genes Bcl-2 and Bax, and suppression of the NF- $\kappa$ B pathway through the down-regulation of p65 and inhibition of I $\kappa$ B- $\alpha$  phosphorylation [19]. A recent study

reports that treatment with SK can significantly suppress the HIF-1 $\alpha$  expression and inhibit ER negative human breast cancer cell growth [20]. Taxol is a widely used anti-cancer drug for several types of cancers and exerts its effect in ER negative breast cells by inducing cell cycle arrest at the G2/M phase. However, the drug resistance challenges its success as a chemotherapeutic drug. Combination treatment of SK and taxol sensitizes ER negative breast cancer cells (e.g., MDA-MB-231) to chemotherapy likely by inhibiting the activation of ERK, Akt, and p70S6 kinases, which are known to contribute to breast cancer drug resistance. *In vivo* studies show that mice treated with SK-taxol combination would survive longer than the mice treated with taxol only and their tumor size would be reduced [21]. Furthermore, treatment with SK analogues (e.g., 2-Methoxy-1,4-Naphthoquinone) suppressed matrix metalloproteinase-9 (MMP-9) activity and inhibited the migration and invasion of MDA-MB-231 cells. These studies suggest that SK-like compounds exert their anti-metastatic properties via the down-regulation of MMPs [22,23]. The ability of SK to inhibit the metastasis and invasion properties has been observed in different tumor types including osteosarcoma and lung metastasis [24], thyroid cancer [25], adenoid cystic carcinoma [23] and hepatocellular carcinoma [26].

In conclusion, according to the findings of our lab as well as other groups, SK appears as a strong candidate for being a novel therapeutic for breast cancer and its mechanism of action should be further studied for the identification of novel targets specific to breast cancer subtype. These findings clearly show that a) SK exerts its anti-tumor effects in breast cancer through targeting multiple signaling pathways, b) SK is able to sensitize breast cancer cells to several chemotherapeutic drugs, which might help solving the problem of drug resistance, which is one of the major challenges in breast cancer treatment, and c) SK has significant anti-migration and anti-invasion characteristics in several cancers. Collectively, SK has a great clinical potential for effective treatment of breast cancer.

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