Abstract. Diet is believed to play an important role in cancer. It has been revealed by epidemiological studies that Asian populations, who consume phytoestrogens in large amounts, have a lower incidence of prostate cancer in comparison with the Western world, where consumption of soy is lower. Genistein and daidzein, the soy phytoestrogens most widely studied, are believed to be potent anticancer agents and have been shown to possess anticancer properties. It has been shown that these compounds inhibit the growth of cancer cells through the modulation of genes controlling cell-cycle progression. Genistein inhibits the activation of the kappa light polypeptide gene enhancer in B-cells (NF-κB), signaling pathway, which is implicated in the balance between cell survival and programmed cell death (apoptosis). Antioxidant and antiangiogenesis properties of genistein have been also described. Soy isoflavones are also implicated in reversion of epigenetic events observed in prostate cancer. Significant advances have been made for understanding how soy isoflavones are implicated in protection against prostate cancer. However, more studies are needed to better-understand and elucidate all pathways mobilized by genistein and daidzein, in order to fully exploit their anticancer properties.

Prostate cancer is the most frequently diagnosed cancer in men in the Western world (1). New prostate cancer cases numbered 66,000 in France in 2008; while in Europe in general, this number was 3.4 million (2). The incidence of prostate cancer is increasing in numerous Western countries, however, mortality remains stable in these countries due to early diagnosis (3). Prostate cancer is a disease caused by multiple interacting factors. A family history of prostate cancer, age and ethnic origin are well-established risk factors for this pathology (4-6). Others factors also play a role in prostatic carcinogenesis events (7). Human diet has been reported to have a strong impact on the genesis of prostate cancer. Indeed, several studies have shown nutrients such as phytoestrogens to possess anticancer properties. Epidemiological evidence and several studies suggested that the risk of prostate cancer is reduced due to the increased consumption of phytoestrogen (8-10). The two major phytoestrogens studied, genistein and daidzein, have been reported to induce several biological effects, such as inhibition of prostate cancer proliferation and cell-cycle arrest, both in vivo and in vitro. This review presents the different molecular effects of soy phytoestrogens on prostate cancer.

Biological Effects of Soy Isoflavones

Phytoestrogens are defined as non-steroidal compounds naturally occurring in foods of plant origin (especially soy-based foods) that structurally resemble natural estrogens and compete with them for binding estrogen receptors (ERs) (11). These compounds are found to have stronger affinity for binding ER-beta than ER-alpha. By interacting with ERs, soy isoflavones are able to exert estrogenic effects on human. However, according to the dose or the exposure duration, soy isoflavones can also act as antiestrogenic agents (12). These compounds are believed to possess anticancer properties. Soy isoflavones, especially genistein, have many other health benefits, such as a protective role in cardiovascular diseases, attenuation of post-menopausal problems and prevention of osteoporosis (13). In prostate cancer, soy isoflavones intervene in several biological processes such as cell-cycle control and angiogenesis, and may also be involved in the regulation of gene activity by modulating epigenetic events, such as DNA methylation and/or histone acetylation.
Effects on cell-cycle control and growth. One of the characteristics of tumor cells is their capacity for uncontrolled growth. This feature is caused by a defect in the regulation of cyclins and cyclin-dependent kinases (CDKs) which control several checkpoints of the cell cycle. The cyclin/CDK complexes are needed for cell-cycle progression. It has been shown that genistein and daidzein affect cell-cycle control by impacting expression of these genes. Hedlund et al., reported the inhibitory effects of soy phytoestrogens on the proliferation of prostatic epithelial cells (14). According to the authors, equol and daidzein caused a cycle arrest in phase G0/G1, while genistein caused cycle arrest in G2/M. These results suggested that soy phytoestrogens induced cycle arrest through different biological pathways. Other studies showed the capacity of genistein to induce cell-cycle arrest in the G2/M phase and in G0/G1 for daidzein (15-17). This report was confirmed by Sarkar and colleagues, who reported that genistein induced a G2/M cell-cycle arrest in prostate cancer cell lines (18). Interestingly, it has been shown that cell-cycle arrest by genistein is partially mediated by a down-regulation of cyclin B. Cyclin B is necessary for forming the cyclin B/CDK complex which controls the G2/M phase progression. Indeed, a dose-dependent decrease of cyclin B expression was observed after genistein treatment in prostate cancer cell lines, whereas other cyclins and their associated CDKs showed no alteration in their expressions. The authors reported the ability of genistein to act on cell-cycle through a CDK inhibitor (CDKI) which is another controller for cell-cycle progression. This family of proteins negatively regulates cyclin/CDK complexes in order to maintain a balance between cell proliferation and apoptosis in normal cells. Up-regulation of one of these CDKIs, p21, was described by the authors (19). Another study conducted by Shen et al., reported that genistein induced up-regulation of two CDKIs, p27 and p21, in prostate cancer cell lines (20). However, contrary to the other previous studies, Shen and colleagues reported a cell-cycle arrest by genistein in LNCaP cell lines in the G0/G1 phase.

Cell-cycle arrest may be intimately related to growth inhibition of prostate cancer cell lines by soy phytoestrogens. According to Choi et al., the inhibition of human prostate carcinoma cell growth is associated with cell-cycle arrest in the G2/M phase and inhibition of cyclin B1 and induction of p21 expression (21). Several in vitro studies have reported that genistein, daidzein and equol are potent inhibitors of prostate cancer cell proliferation (14, 17). A study conducted by Hedlund et al., showed that exposure of the prostate cancer cell line LNCaP to genistein reduced cell growth by 10%, compared to the control cells (22). Effects of soy phytoestrogens on prostate cancer cell growth inhibition are probably mediated via the inhibition of protein-tyrosine kinase (PTK) activities. PTKs are a family of proteins implicated in several signaling pathways which are responsible for cell survival and proliferation (23). Indeed, it has been reported that genistein has an inhibitory effect on PTK activities (24). Genistein inhibited the growth of prostate cancer cells by counteracting the ability of insulin-like growth factor I (IGF-I) to stimulate cell proliferation. Cell proliferation stimulated by IGF-I depends on activation of several PTK activities such as extracellular signal-regulated kinases (ERK1/2) and phosphatidylinositol 3-kinases (PI3K). Genistein has been demonstrated to inhibit the activation of these proteins by suppressing their phosphorylation. Indeed, phosphorylation of tyrosine residues of these proteins is an important mechanism which modulates their enzymatic activities (25). Genistein inhibited prostate cancer cell line proliferation stimulated by IGF-I by inhibiting components of IGF-I signaling cascade. A recent in vivo study, in contrast, demonstrated that genistein stimulated tumor cell proliferation by increasing phosphorylation of PTKs such as epidermal growth factor receptor (EGFR) and sarcoma (SRC) (26). These are members of the PTK superfamily. EGFR is the cell-surface receptor for members of the epidermal growth factor family (EGF) and it is implicated in the activation of numerous downstream molecules which affect cell proliferation, division and cell migration (27). SRC is a proto-oncogene playing pivotal roles in numerous cellular processes such as proliferation, migration, and transformation (28). This report (26) corroborated the results of a phase I clinical study that reported an increase in PTK phosphorylation after oral ingestion of genistein (29). According to two reports (24, 26), the differential effects of genistein on cell proliferation, as observed in in vivo and in vitro studies, may be attributed to cell interaction within the tumor microenvironment and may, thus, only be observable in in vivo models. Another hypothesis which may explain the differential effect and which is independent of the type of study, in vitro or in vivo, could be related to the concentration of genistein used. Indeed, it has been shown that at a lower concentration, genistein increases cell proliferation and the PTK phosphorylation in vitro, while at higher concentration (≥100 μM), genistein is considered as a PTK and cell growth inhibitor (30). Soy phytoestrogens especially genistein is able to have heterogeneous actions which promote prostate cancer growth or inhibition.

Effects on the induction of apoptosis. In addition to cell-cycle arrest and cell growth inhibition, apoptosis or programmed cell death is another mechanism controlling uncontrolled proliferation of cancer cells. Several studies reported that the soy phytoestrogens genistein, daidzein and equol induced prostate cancer cell apoptosis (31-34). Different hypotheses were proposed to explain the mechanisms by which soy phytoestrogens act on apoptosis of prostate cancer cells. One mechanism suggested is the inhibition of the activation of NF-κB which is a pleiotropic transcription factor involved in many biological processes such as the inflammatory response...
and apoptosis (35-37). Genistein modulated the regulation of proteins involved in cell-cycle progression by up-regulating cell-cycle inhibitors or down-regulating proteins which promote cell-cycle progression. This may be another mechanism by which soy phytoestrogens promote prostate cancer cell death. Induction of apoptosis by genistein, equol and daidzein can be attributed to their capacity to enhance tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptotic death of prostate cancer cells (38). TRAIL is a member of the tumor necrosis factor (TNF) superfamily which induces selective apoptosis of cancer cells, with no toxicity towards normal cells (39). Kazi et al., demonstrated that genistein induced apoptosis by the inhibition of proteasome activity and the induction of cyclin-dependent kinase inhibitor 1B (p27KIP1), NF-κB, and B-cell CLL/lymphoma-2 (BCL2)-associated X protein (BAX) (40). BAX is a member of BCL-2 family of proteins which regulate and contribute to programmed cell death or apoptosis. The down-regulation of proteins controlling cell-cycle progression, inhibition of proteasome, activation of NF-κB, can represent mechanisms by which genistein induces apoptosis in prostate cancer.

**Effects on inhibition of angiogenesis and metastasis.**

Angiogenesis is defined as the formation of new blood vessels from pre-existing vessels. It is essential for embryonic growth, development, tissue repair and regeneration (41). However, it is also a prerequisite event for promoting the proliferation, invasion and metastasis of prostate cancer cells (42). A study conducted by Guo and colleagues demonstrated that genistein may inhibit prostate tumor angiogenesis through the expression of vascular endothelial growth factor (VEGF) (43), a signaling protein involved in the regulation of angiogenesis and vasculogenesis (44). Several gene expression profiling studies have showed that genistein and daidzein down-regulated many genes such as matrix metallopeptidase-2 and -9 (MMP2, MMP9), EGF and angiopoietin-2 (ANGPT2), and up-regulated genes such as connective tissue growth factor (CTGF) and connective tissue activation peptide (CTAP) (17, 45). These genes are all related to angiogenesis or metastasis. Genistein was also found to inhibit the activation of p38 mitogen-activated protein kinase (p38 MAPK) and MMP2 (46). These two proteins are important regulators of cell invasion and metastasis, and their inhibition by soy isoflavones lead to the suppression of these two mechanisms necessary for cancer progression. According to Xu et al., inhibition of the activation of MMP2 by genistein is mediated through the inhibition of transforming growth factor beta-2 (TGFβ)-mediated phosphorylation of MAPK activated protein kinase 2 (MAPKAPK2) and the 27-kDa heat-shock protein (HSP27), which are downstream regulators of p38 MAPK (47). Indeed, activation of p38 MAPK by TGFβ leads to the phosphorylation and activation of MAPKAPK2 and HSP27, these two proteins are important regulators of MMP2 activity (48) in prostate cancer. The results of in vitro studies corroborated these in vivo studies. Down-regulation of genes implicated in metastasis has been reported in a severe combined immunodeficient human model (SCID-hu) (49-50). This SCID-hu was obtained by injecting prostate cancer cells into human bone fragments previously implanted in severe combined immunodeficient (SCID) mice. These findings clearly demonstrated that genistein is a potent inhibitor of angiogenesis in prostate cancer.

**Antioxidant effects.** In prostate cancer and prostatic intraepithelial neoplasia (PIN), a down-regulation of three antioxidant enzymes, namely copper-zinc superoxide dismutase (SOD1), manganese superoxide dismutase (SOD2), and catalase (51) has been reported. Genistein has been shown to stimulate expression of antioxidant proteins such as the enzymes, SOD2 and catalase (52). The ability of genistein to enhance the expression of the genes for these enzymes is mediated through AMP-activated protein kinase (AMPK) activation and increased expression of phosphatase and tensin homolog deleted from chromosome 10 (PTEN). An up-regulation of glutathione peroxidase-1 (GPX1) gene expression in prostate cancer cell lines after exposure to genistein has also been reported (53). A global study conducted by Raschke and colleagues on 192 genes involved in bio-transformation and cellular stress response, showed up-regulation of three genes, metallothionein 1X (MT1X); glutathione reductase (GSR) and microsomal glutathione s-transferase-1 (MGST-1), implicated in DNA protection from H2O2-induced strand breaks (54).

**Regulation of Gene Activity by Soy Isoflavones**

**Effects on epigenetic events.** Epigenetic events are defined as alterations in gene expression that are heritable through cell division, without changes in the DNA coding sequences (55). Two mechanisms are integral to epigenetic transcriptional control: DNA methylation and histone modification. These two epigenetic alterations play an important role in the progression of prostate cancer. DNA methylation refers to the covalent addition of a methyl group, catalyzed by DNA methyltransferase (DNMT), to the 5-carbon of cytosine in a CpG dinucleotide (56). Promoter hypermethylation is associated with the loss of expression of tumor-suppressor genes in prostate cancer (57-67). In addition to DNA methylation, post-translational histone modifications are the second type of epigenetic event. Histones are subject to a wide variety of posttranslational modifications including but not limited to, lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, and lysine ubiquitination and sumoylation (68). In prostate cancer, the two histone modifications essentially studied are
histone methylation and histone acetylation. Although histone modification and DNA methylation are both independent processes, they are closely related (69). A number of in vitro studies have demonstrated that DNA methylation and histone deacetylation may co-operate to repress gene transcription (70).

Several studies have reported the ability of soy isoflavones to induce inhibition of epigenetic events observed in prostate cancer cell lines. According to Majid and colleagues, genistein is able to induce up-regulation of two tumor suppressor genes p21\(^{WAF1/CIP1}\) and p16\(^{INK4A}\) implicated in cell-cycle control. The enhancement of the expression of these proteins is linked to the ability of genistein to induce an increase of acetylation of histones H3 and H4, and methylation of histone H3 lysine-4 in prostate cancer cells (71). Histone acetylation is generally associated with an active transcription. Another study conducted by the same group concluded that genistein reversed hypermethylation of B-cell translocation gene-3 (BTG3/ANA/APRO4) which is a candidate tumor suppressor in prostate cancer. The up-regulation of this gene expression by genistein is mediated through an inhibition of DNMT activity and the enhancement of histone acetyltransferase (HAT) activity (72). Fang et al. reported a reversal of hypermethylation of p16\(^{INK4A}\), retinoic acid receptor-beta (RARB), and O-6-methylguanine-DNA methyltransferase (MGMT) in prostate cancer cell lines after exposure to soy isoflavones. Hypermethylation reversion leads to the activation of gene expressions and is related to the decrease of DNMT activity (73). According to another study, genistein induced de-methylation of lysine 9 of histone H3 (H3K9) of PTEN and p53, two genes involved in apoptosis. The authors reported that methylation of H3K9 is correlated to the absence of expression of these two genes in cancer cells (74). Our laboratory (Department of Oncogenetics) has studied the effects of genistein and daidzein on DNA methylation of four genes: glutathione S-transferase P1 (GSTP1), ras association domain family-1 (RASSF1A), ephrin B2 (EPHB2) and breast cancer-1 (BRCA1) in prostate cell lines. A reversion of DNA hypermethylation was found in prostate cancer cell lines after soy isoflavone treatment. The demethylation of these genes was confirmed by immunohistochemistry which showed an increase of the expression of the corresponding proteins (75). These findings were confirmed by a recent quantitative study which demonstrated a decrease of the methylation percentage of the four genes after soy treatment (76).

**Conclusion**

In summary, genistein exerts its action against cell cycle, cell growth, angiogenesis, metastasis, apoptotic process and epigenetic modifications. These effects are mediated through different signaling pathways. All these findings taken together demonstrate clearly that genistein is a powerful agent with anticancer properties. However, most of these studies used a stronger dose of soy isoflavones than those found in the plasma of the population who regularly consume soy. For the practical application of genistein and daidzein in the prevention of prostate cancer, a study with doses of genistein and daidzein reached in tissues should be undertaken.

**References**


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