GENISTEIN: A MULTIMECHANISTIC ANTI-CANCER AGENT FROM SOYA

V.J.Pomfrey

CONTENTS

1 Introduction 3
2 Soya, genistein and cancer: the early links 3
3 Genistein a likely candidate for anticarcinogenicity 4
4 Pharmacokinetics and bioavailability 5-6
5 *In vitro* findings 6-21
5.1 Activities via oestrogen receptors 6-10
5.2 Non-ER-related mechanisms 10-14
5.2.1 Tyrosine kinase inhibition 12-13
5.2.2 Other anti-metastatic mechanisms 14
5.3 Is genistein selective for cancer? 14-19
5.3.1 Proteasome inhibition 14-16
5.3.2 Effects via DNA damage 16-19
5.4 Topoisomerase II inhibition 19-21
6 Possible adverse effects of genistein or soya 21-22
7 Can physiological genistein concentrations reach anticarcinogenic levels? 22-24
8 Clinical evidence 24
9 Conclusion 24-25

REFERENCES 26-30

APPENDIX I

Glossary 32

1 Introduction

Current cancer drug development focuses largely on 'rational drug design' based on the predicted ability of a synthetic molecule to cross cell membranes and to bind to a specific enzyme or receptor.

Unfortunately, functional groups on molecules often do not behave as predicted, rendering the compounds ineffective and/or toxic, with some even *causing* cancer.

Alongside the wholesale screening of novel molecules, other cancer researchers continue to study the roles of individual endogenous molecules, enzymes, genes and signalling pathways in carcinogenesis.

Whilst approximately 40% of cancers can now be cured by surgery and radiotherapy, and a few others by chemotherapy, the remainder of sufferers die from metastatic disease (Verweij and de Jonge, 2000). Chemotherapy has increased survival times for such patients, but not dramatically.

Most cancers are lifestyle-related, and an alternative approach to the 'rational drug design' route is to study epidemiological data on cancer incidence and mortality, and try to identify correlations such as diet and other environmental factors. This approach has led to the discovery of natural compounds which show great promise as preventative, and potentially curative, agents for human cancers.

This review will examine research on such a compound: genistein. Population-derived data and preliminary reports from clinical studies will be summarised, but the bulk of the review will comprise *in vitro* and *ex vivo* findings including pharmacokinetics, effects via oestrogen receptors, modulation of DNA transcription, enzyme inhibition, inhibition of cell proliferation and metastasis, DNA damage and selectivity for cancer cells. Evidence for adverse effects will also be outlined.

2 Soya, genistein and cancer: the early links

Soya (botanical name *Glycine max.*) has been consumed by humans, largely in the Far East, for over 5 000 years. The incidences of several cancer types, notably breast, prostate and colon, are significantly lower in the East than in the West. Studies of migrants indicate that this differential is not genetically based (Adlercreutz, 2002). In the mid-1980s some researchers (*e.g.* Setchell and Cassidy and Adlercreutz *et al*.) hypothesised a protective role for hormonally-active dietary components called **isoflavonoids** (types of **phytoestrogen**). In 1990 a National Cancer Institute workshop in the US identified several anticarcinogens in soya beans, and recommended investigation of the relationship between soya consumption and cancer risk (Messina *et al*., 1994).

3 Genistein a likely candidate for anti-carcinogenicity

Approximately 0.1% of the content of soya beans is genistin, the glycosidic conjugate of genistein, and 0.07% is daidzin, the glycoside of daidzein. These are metabolised by bacteria and enzymes in the gut to the aglycones genistein and daidzein; thus genistin and daidzin are **prodrugs**. Figure 1 illustrates this process for genistin/genistein.

Genistein is a heterocyclic diphenol (alternative term 'bisphenol') with three hydroxyl groups, and has a relative molecular mass of 270.24. Its chemical name is 4,5,7-trihydroxy-isoflavone or 5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one.

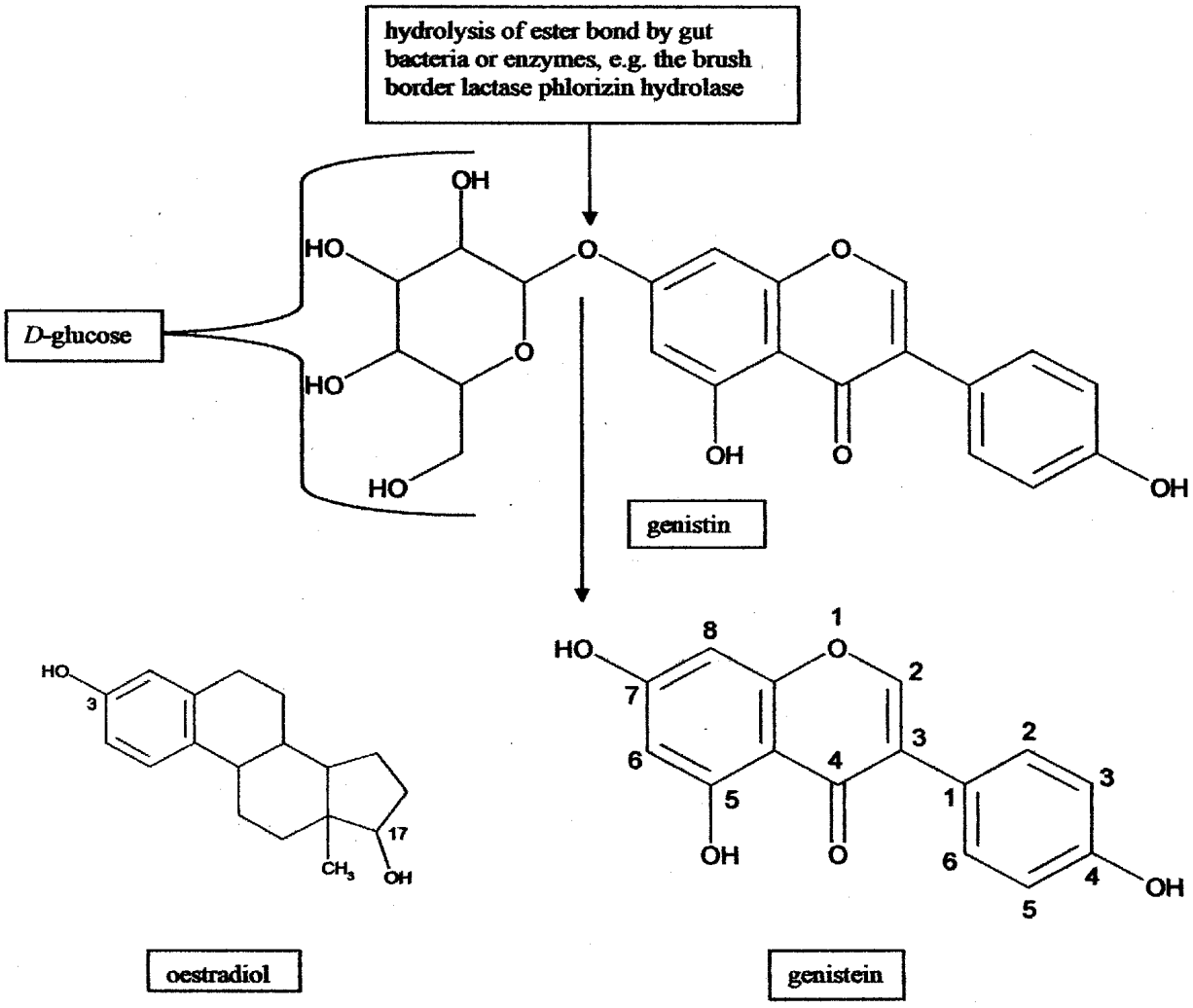


Figure 1 The hydrolysis of genistin to genistein in the gut. The endogenous oestrogen oestradiol is shown to illustrate similarities.

4 Pharmacokinetics and bioavailability

Figure 2 is an extreme simplification of the range of processes and metabolites which occur, but is useful to bear in mind when analysing the results of both *in vitro* and *in vivo* research: it cannot be assumed that only the studied compounds are implicated in the findings; their metabolites are also likely to have additive, synergistic or opposing activity.

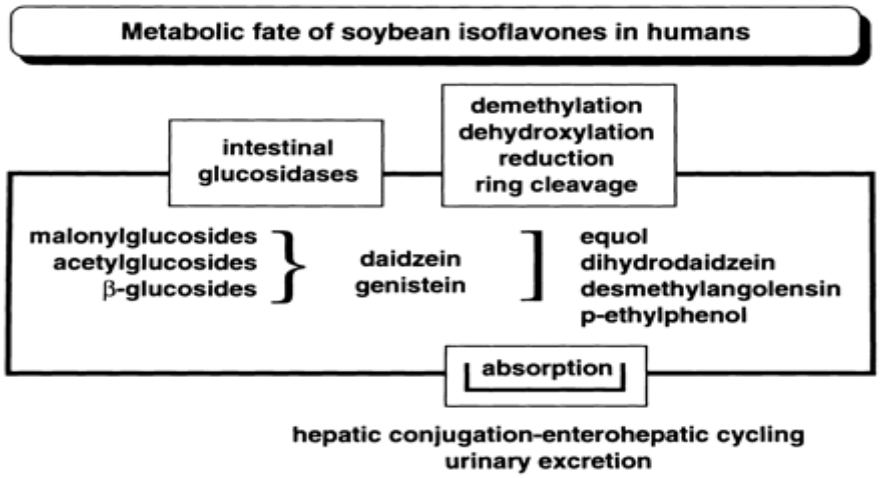


Figure 2 Schematic showing the major biotransformations in the metabolism of isoflavones in humans and animals

Source: Setchell and Cassidy (1999)

There are also significant inter- and intra-specific physiological differences. For example, only about 30% of people can metabolise daidzein to equol, the latter but not the former being strongly associated with a reduction in cancer risk. One reason for this metabolic differential appears to be variation in levels of fibre or carbohydrate in the gut (*see* Figure 3).

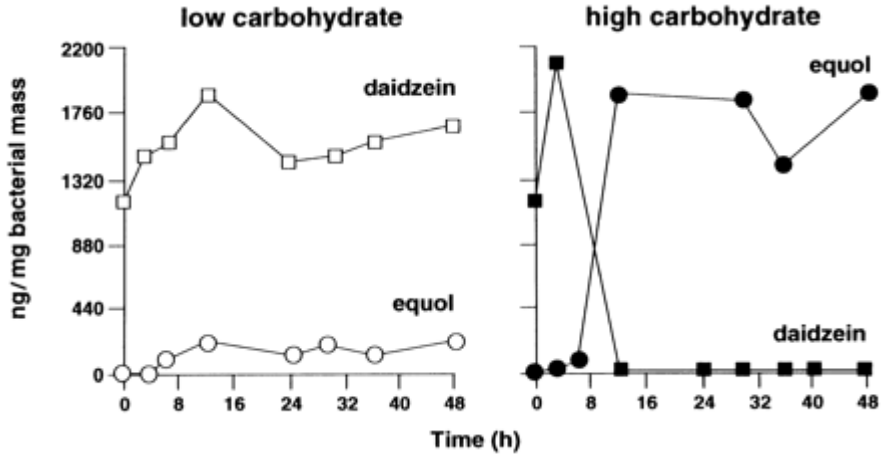


Figure 3 In vitro metabolism of daidzein in a colonic model of fermentation of human faecal flora showing the influence of a high carbohydrate milieu on the rate of conversion of daidzein to the intestinal metabolite equol.

Source: Setchell and Cassidy (1999)

Setchell and Cassidy's 1999 review states that soya formula-fed infants rarely produce equol, although they are able to hydrolyse glycosidic bonds to release aglycaglycones.

Aglycones are readily transported across human intestinal epithelial cell monolayers *in vitro* (Steensma *et al*., 1999) unlike their glycosidic conjugates, probably reflecting the smaller size and greater lipophilicity of the former. After entering the circulation, phytoestrogens may be metabolised in different ways in different tissues. Boersma *et al*. (2001) report their own studies finding that some human breast cancer cell lines, but not others, rapidly sulphate genistein. This conjugative process is generally much less prevalent than glucuronidation, both being general Phase II reactions which usually inactivate drugs.

Intakes of isoflavones vary greatly, the average Western intake being <1 mg per day per person compared with 20-50 mg/day in China and Japan (Setchell and Cassidy, 1999). A study of middle-aged Japanese women estimated a mean dietary genistein intake of 30.1 mg per day, producing mean plasma levels of 206.09 nM and urinary genistein excretion of 10.79 μM per day (Arai *et al*., 2000). Although estimated daidzein intake was much lower - 16.4 mg/day - plasma daidzein was a third, rather than half, the plasma genistein level, and the urinary excretion was twice that for genistein.

This suggests a significantly shorter plasma half-life for daidzein, but findings are inconsistent across studies and are confounded by different uses of the terms 'genistein' and 'daidzein', with some studies using them to mean both conjugated and unconjugated forms. Only about 14-16% of genistein is excreted unmodified (Boersma *et al*., 2001). Nevertheless, reported half-lives do not vary greatly, ranging from 3.2h for free (aglycone) genistein and 4.2h for free daidzein following a single intake of a formulation containing 90% genistein and 10% daidzein (Busby *et al*., 2002) to 10.1h for genistein and 8.0h for daidzein (Setchell *et al*., 2003). The latter team observed peak serum concentrations 4-8 h after ingestion of up to 39.2 mg of genistein and 26.4 mg daidzein. They found, additionally, that the mean fraction excreted in urine decreased with increasing intake in relation to the administered dose, indicating a trend toward nonlinear pharmacokinetics.

Plasma isoflavone levels in the West are, unsurprisingly, much lower than in the East. Griffiths *et al*. (1999) reported that mean total plasma genistein concentrations in Western men were less than 10 ng/ml (37nM). The highest plasma isoflavone levels have been found in infants fed soya formula, with genistein levels up to 4 μM (Setchell *et al*., 1997), leading to concern among some researchers.

There is a vast range of *in vitro* findings on likely mechanisms behind the epidemiological evidence. A representative selection will be analysed below.

5 *In vitro* findings

5.1 Activities via oestrogen receptors

Marked structural similarities to endogenous oestrogens suggested to researchers that phytoestrogens might exert anticarcinogenic effects via the oestrogen receptor (known as 'ER' due to the American spelling 'estrogen'). ERs occur extensively throughout the body (*see* Figure 4). They are nuclear receptors and transcription factors which, when activated by agonists, undergo conformational changes and bind to chromatin (DNA

plus protein) in various promoter regions on DNA, activating the transcription of the respective genes. ERα stimulates transcription and cellular proliferation, while ERβ inhibits the activation of ERα (Paech *et al*., 1997).

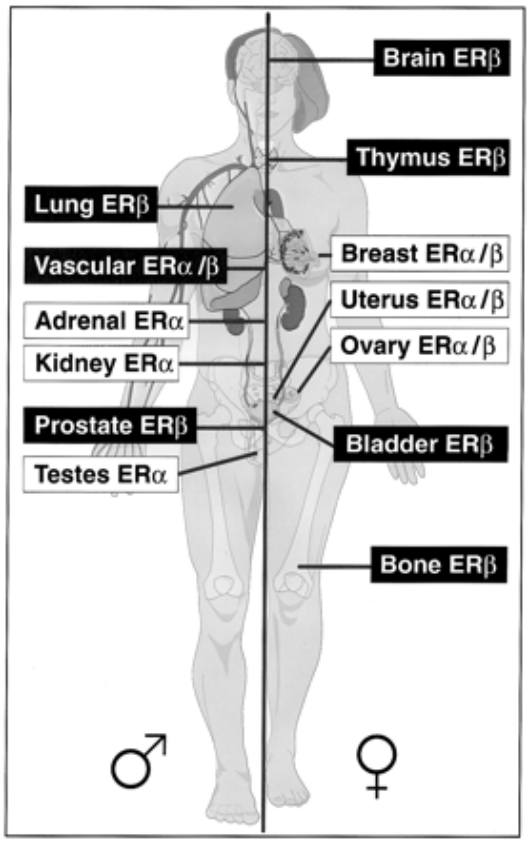


Figure 4

Simplified diagram illustrating the anatomical distribution of the more recently described oestrogen receptor, ERß, and the 'classical' ERα receptor.

Source: Setchell and Cassidy (1999)

Genistein has a 30-fold greater affinity for ERβ than for ERα (Kuiper *et al*., 1997; Barkhem *et al*., 1998) and is a partial agonist. Figure 5 shows how genistein and oestradiol bind to the ligand-binding domain (LBD) of human ERβ. The distance between the hydroxyl groups on opposite sides of the molecule (the 4' and 7' groups in genistein and the 3' and 17' groups in oestradiol) is important for hydrogen bonding.

Some promoters activated by ER binding to DNA contain a sequence called the oestrogen response element (ERE), but oestrogen receptors can also activate promoters without ERE. Hua and colleagues (2003) observed that genistein could stimulate expression, in colon and breast cancer cells, of an antioxidant and metal-binding protein - MTIIA - whose promoter does not contain ERE. The researchers found that the expression was instead effected via the GC (guanine-cytosine)-rich Sp1 binding sequence which is found in many genes. This includes the gene for the protein p21waf1, whose expression is upregulated by the anti-cancer drug tamoxifen and which inhibits cyclin-dependent kinases, thereby halting cell-cycle progression. Stimulation of MTIIA expression by genistein was increased in the presence of ER but also occurred, albeit to a lesser degree, in its absence. An ER- but not ERE-dependent upregulation by genistein was also observed by Wietzke and Welsh (2003) for the anti-mitotic and pro-differentiating Vitamin D receptor (VDR) in breast cancer cells.

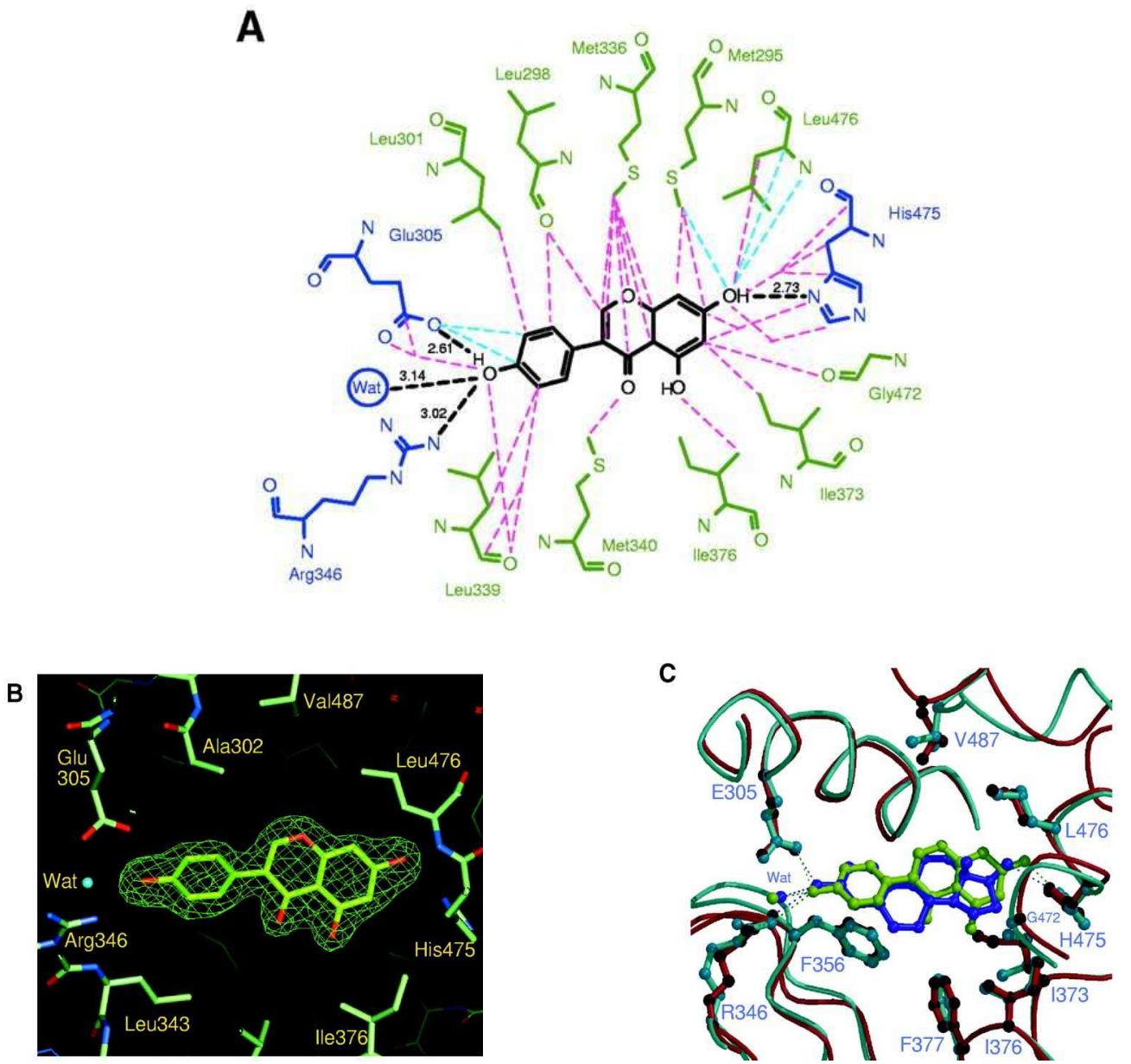


Figure 5

(A) Schematic representation showing genistein and its interactions with the hERβ-LBD. Residues within the binding cavity that make at least one contact within 3.8 Å of each ligand are shown. Residues making hydrogen bonds are coloured blue and those making van der Waals contact are coloured green. Interactions between the protein and ligands are depicted as broken lines (hydrogen bonds and distances in black; $d \leq 3.3$ Å in cyan; 3.3 Å$< d \leq 3.8$ Å in pink).

(B) Electron density map for the ligand-binding cavity in the hERβ-genistein complex. The map was phased using a model in which genistein had been excluded. Atoms are coloured according to type [carbon: pale green (protein), dark green (genistein); oxygen: red; nitrogen: blue].

(C) Comparison of ligand-binding mode of genistein (protein, turquoise; ligand, green) in hERβ-LBD and oestradiol (protein, brown; ligand, blue) in hERβ-LBD (PDB code: 1ERE) within the cavity. The ligands are viewed looking down from the β-face of the cavity and only those side chains that interact with the bound ligand or exhibit different orientations are shown. Hydrogen bonds are depicted as broken lines.

adapted from Pike et al. *(1999)*

Hua and colleagues concluded, from an array of assays in which they manipulated the sequences in the promoters, that genistein is not simply a general transcription promoter but might have its transcription-stimulating effects via interactions between the SP1 protein and the ER. In 2000 another team hypothesised a mechanism for such interactions, which could also explain differences between the actions of the two ER subtypes (Saville *et al*., 2000) (*see* Figure 6).

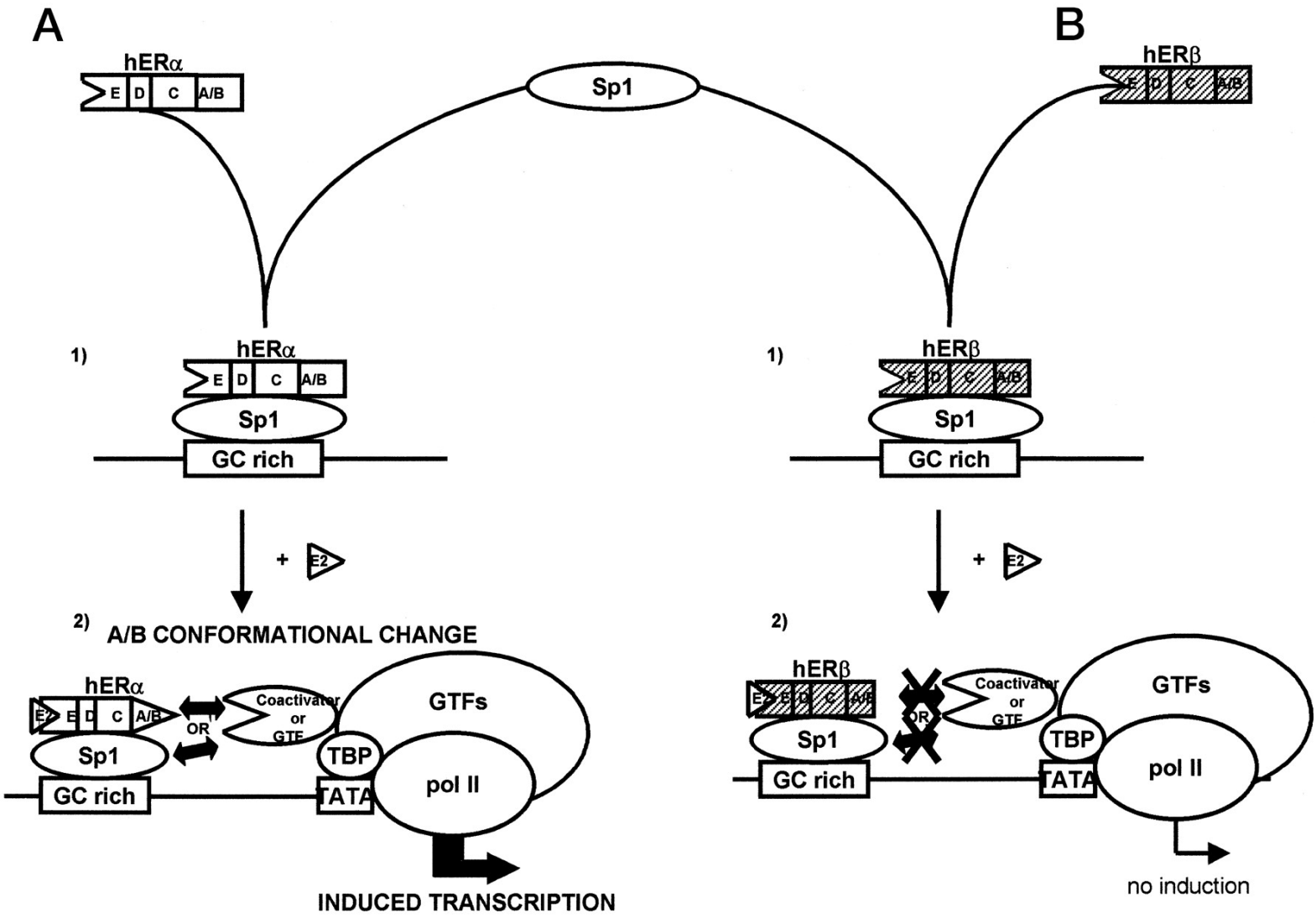


Figure 6

Hypothetical model to explain ERa/Sp1 and ERb/Sp1 differences. In step 1, Sp1 associates with both ERa and ERb and binds DNA at GC-rich elements. Upon binding of ligand (step 2), the AF-1 domain (A/B) of hERa undergoes a conformational change (denoted by transformation in shape from a rectangle to a triangle) required for interactions of ERa, Sp1, or both with coactivators or general transcription factors (GTFs) and for induction through the GC-rich element. In contrast, ligand binding to ERb does not result in formation of a transcriptionally active complex. *adapted from Saville* et al. *(2000)*

Some other ER-related ways in which genistein may be anticarcinogenic are:

(a) competition with endogenous oestrogens for ERs. Endogenous oestrogens promote the growth of many cancers. Binding to ER by genistein can produce weak oestrogenic activity - 1 000 to 100 000 times weaker than diethylstilbestrol (DES) or oestradiol (*see* review by Messina *et al*., 1994),

(b) inhibiting the phosphorylation by protein tyrosine kinase of receptors for oestrogen and progesterone, thus preventing their activation (ibid.), *and/or*

(c) downregulating the expression of ER-α (Chen *et al*., 2003).

5.2 Non-ER-related mechanisms

In addition to its effects via ER, genistein can decrease circulating oestrogen levels by

(a) stimulating the transcription of sex hormone binding globulin (SHBG), thereby decreasing levels of circulating oestrogens (and also androgens) (Adlercreutz, 2002), *and possibly*

(b) inhibiting the enzyme aromatase, which converts androgens to oestrogens, although le Bail *et al*. (1998b) did not find this in their cell-free study.

The versatility of genistein was illustrated by Hua and colleagues' (2003) findings of the upregulation of MTIIA being only partly mediated via ER. Chen and colleagues (2003) also found apparent partial dependency on ER of genistein's regulation of the transcription of serum response factor (SRF), a transcription factor which binds to serum response element (SRE) at the site on DNA where the transcription of many growth-related genes - such as *c-fos* - is initiated. SRF is implicated in cancer cell proliferation.

The Chen team also found that genistein upregulated the transcription of three genes associated with cellular stress response and apoptosis ('programmed cell death'): heat shock protein 105 (HSP 105), protein kinase, Y-linked (PRKY) and thymidine kinase 1, soluble (TK1). However, their finding relating to HSP 105, and their suggestion that upregulation of HSPs is pro-apoptotic, are at odds with the findings and conclusion of Zhou and Lee (1998). This latter team found that genistein *down*regulated the expression of another heat-shock protein - HSP 70 - and stated that HSP upregulation *protects* against programmed cell death. Induction of apoptosis is widely considered to be an anti-cancer mechanism, yet Zhou and Lee propose that *preventing* it may be anticarcinogenic. The methodologies were distinctly different between the two studies and, unlike Zhou and Lee, Chen and colleagues used exclusively human cancer cell lines. Zhou and Lee tested genistein's ability to counter a stress response induced by a synthetic compound, whereas Chen's team measured the effect of genistein without adding other active substances.

Measurement of gene expression was also conducted by notably different methods, with the Chen team verifying their findings from cDNA microarray analysis with the reverse transcriptase polymerase chain reaction (RT-PCR) technology. This study appears more rigorous and relevant, and the different findings and conclusions

illustrate the need to exercise caution when attempting to draw inferences from single studies.

Two of the *in vitro* studies cited above: Chen e*t al*. and Hua e*t al*., used concentrations of genistein up to 30 or 100 μM genistein, which have been found to decrease *in vitro* proliferation of a range of cancer cell lines. In contrast, Wietzke and Welsh (*see* Section 5.1) used concentrations from 5 nM to 5 μM, which *stimulated* proliferation, a low-concentration effect also observed by other researchers. Figure 7 illustrates the concentration-dependence of genistein's effects on *in vitro* proliferation in MCF-7 breast cancer cells.

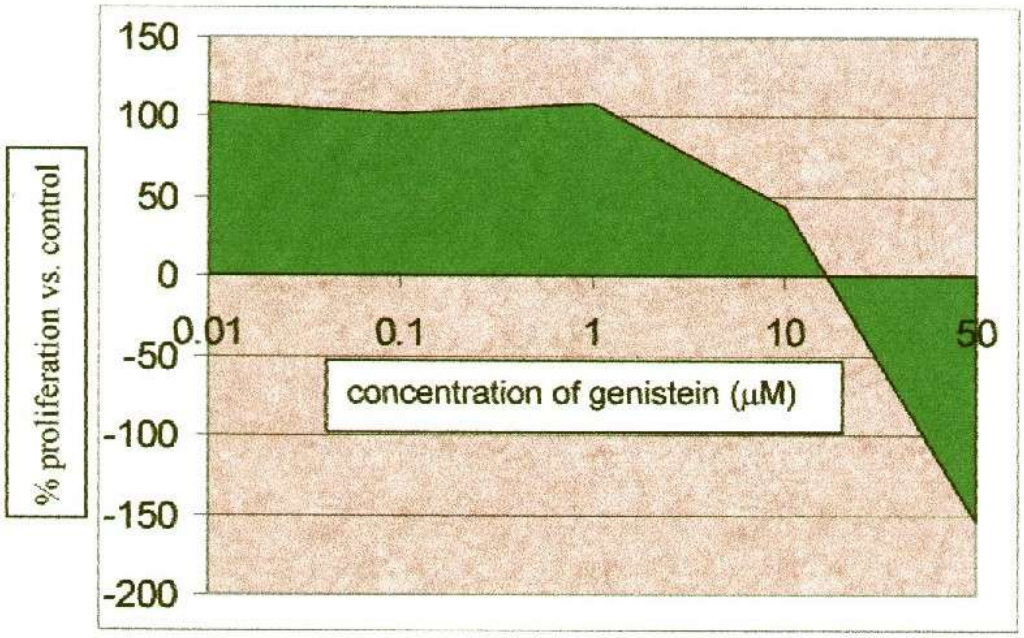


Figure 7 The effect of genistein on the proliferation of MCF-7 cells. The control medium, 10% charcoal-treated foetal calf serum (DCC-FCS), produced 0% proliferation, whilst addition to this of 0.01 μM oestradiol produced 100% proliferation.

Data source: le Bail et al. *(1998a)*

Chen and colleagues note that other studies have found genistein-stimulated proliferation to occur in ER-positive but not ER-negative cell lines, and that the properties of genistein also change with circulating oestrogen levels. Genistein's *anti*-proliferative effects at higher concentrations occur in both ER-positive and ER-negative breast cancer cell lines.

However, proliferation is not of itself malignant or lethal while the cancer remains *in situ* and does not invade other regions of the body (metastasis). Indeed, as noted in several papers (*e.g.* Adlercreutz, 2002; Morton *et al*., 1997), the incidence of small, latent prostate cancer foci (benign prostatic hyperplasia or BPH) is similar between men in Asia and in the West, whilst incidences of malignancy, metastasis and mortality are much higher in the West. Even the low genistein concentrations used by Wietzke and Welsh approximately doubled expression of the Vitamin D receptor and

also increased its levels, so any pro-proliferative effect might be offset by the anti-mitotic and pro-differentiation effects of the VDR in the presence of adequate levels of 1,25-$(OH)_2$Vitamin D_3, the anticarcinogenically-active form of Vitamin D which binds to the VDR. It is not stated whether such levels were present in the experiment.

5.2.1 Tyrosine kinase inhibition

About 50% of known **oncogenes** (mutated forms of proto-oncogenes which can over-stimulate cell division) code for proteins which catalyse or undergo phosphorylation by tyrosine kinases, and many cancers show increased tyrosine kinase activity. **Mitogens** (substances which enhance cell proliferation) activate proliferative cascades via transmembrane receptors bound to tyrosine kinases. Numerous studies have reported tyrosine kinase inhibition by genistein (but not its fellow-isoflavone daidzein, which lacks the 5' hydroxyl group) *in vitro*. Inhibition of tyrosine kinase phosphorylation of nuclear receptors, for example ER, may prevent the binding of their usual substrates (endogenous oestrogens) and is known to prevent receptors from binding to DNA, so that they cannot activate gene transcription.

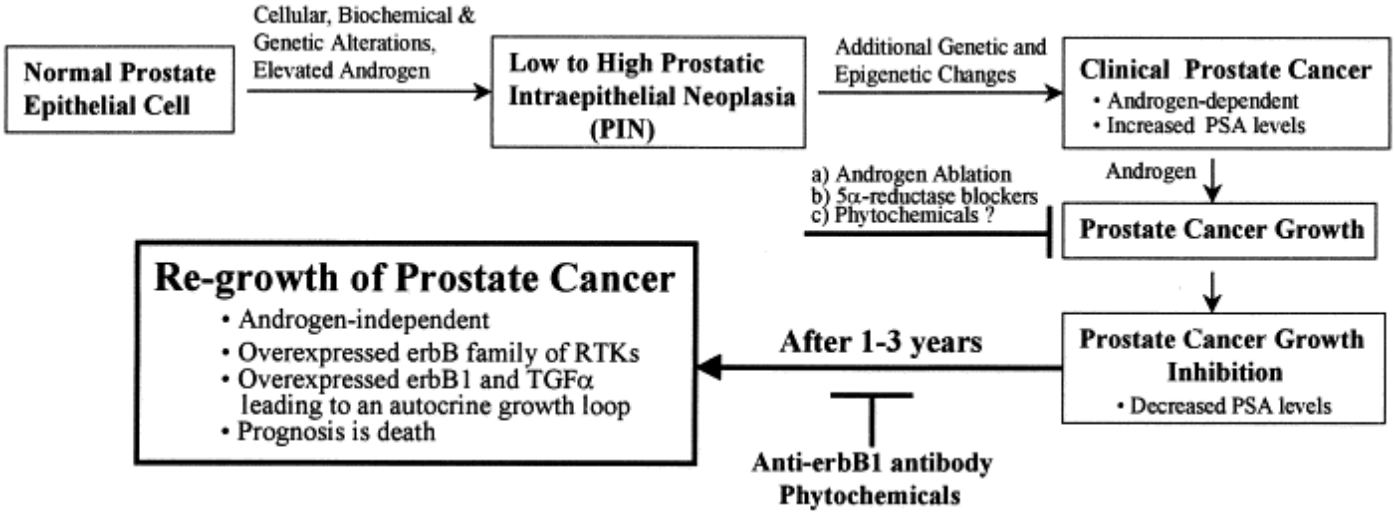
A type of receptor tyrosine kinase associated with metastasis is the epidermal growth factor receptor (EGFR) group, which is over-expressed in both primary and metastatic prostate cancers. EGFRs appear to participate in a pro-proliferative autocrine positive-feedback loop in advanced cancers which are no longer sensitive to growth stimulation by hormones (*see* review by Agarwal, 2000 and Figures 8 and 9 below).

Agarwal reports an experiment by his own team in which ≥100 μM of genistein (a concentration considered physiologically unattainable through diet) was tested for its ability to prevent such a loop from operating in DU145 prostate cancer cells.

Inhibition occurred (contradicting earlier findings reported in Messina *et al*'s 1994 review of such inhibition occurring in membrane preparations but not intact cells) although the mechanism was uncertain. One way in which genistein inhibits tyrosine kinase activity is by competing with ATP for the enzyme's catalytic domain (Akiyama *et al*., 1987).

Akiyama's team suggested that, the ATP-related inhibition they observed might not involve competition for the same site on the enzyme as ATP, referring to this as not 'true competition' but perhaps a consequence of genistein binding in 'multiple places in the reaction pathway'. Their study used more realistic genistein concentrations than Agarwal's team: 3.7-37 μM. It was performed on human epidermoid cancer cells but used murine EGF; without knowing the interspecific homologies for EGF it is impossible to know whether this species mixing might have confounded the outcome.

Another way in which genistein can decrease tyrosine kinase activity is by inhibiting the enzymes' transcription. The vascular endothelial growth receptor reported by Chen and colleagues (2003) (*see* below) to be 50% downregulated by genistein in breast cancer cells is a kinase-linked receptor also known as fms-related tyrosine kinase 1.



KEY

erbB1 = epidermal growth factor receptor (EGFR) RTK = receptor tyrosine kinase

TGF-α = transforming growth factor-α, a pro-proliferative ligand of EGFR

Figure 8 Genesis of human prostate cancer. Phytochemicals such as silymarin inhibit PSA levels regulated by both serum and androgen, causing strong inhibition of growth. Re-growth includes metastasis. *graphic from Agarwal (2000), caption abridged and modified*

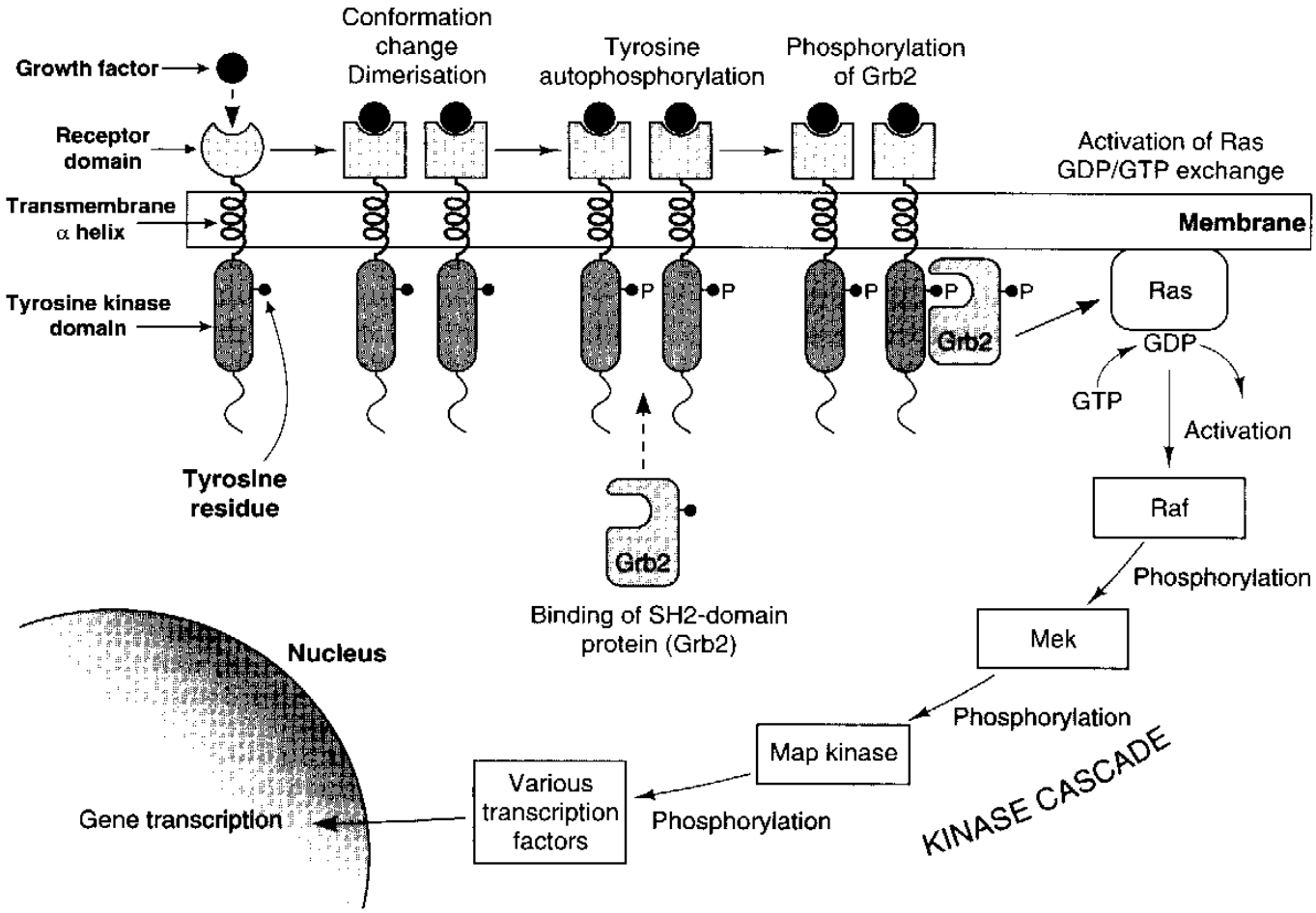


Figure 9 Transduction mechanism of EGFR receptors activated by growth factors. Growth-factors bind to the extracellular domains of the receptors, triggering dimerisation which in turn leads to autophosphorylation of the intracellular domains by tyrosine kinase. SH2 is a highly-conserved amino acid sequence present in many intracellular proteins including Grb2. Grb has high, selective affinity for EGFR, and is phosphorylated on binding to the phosphotyrosine residue of the receptor. *adapted from Rang, Dale and Ritter (2000)*

5.2.2 Other anti-metastatic mechanisms

The prognosis for metastatic cancer sufferers is poor. For metastasis to occur, the extracellular matrix must be broken down by extracellular proteases, enabling cells to escape from the tumour site, enter blood vessels and colonise new sites (Verweij and de Jonge, 2000). Three proteins involved in this process, and found at higher levels in invasive cancers, are matrix metalloproteinase 9 (MMP-9, also known as type IV collagenase), urokinase plasminogen activator (uPA) and its receptor uPAR. Li and Sarkar (2002) found the transcription of mRNA for MMP-9 to be downregulated 6.6-fold by 50 μM genistein after 72 hours, and 8.5-fold for uPAR mRNA. It is worth noting that synthetic MMP inhibitors have undesirable toxicity profiles (Gibbs, 2000).

Another process essential to metastasis is angiogenesis: the formation of new blood vessels providing access for *in situ* cancer cells to other parts of the body. Central to this are vascular endothelial growth factors and their receptors (VEGF and VEGFR).

Whilst Chen and colleagues reported a 50% downregulation of the transcription of VEGFR-1 by 50 μM genistein in breast cancer cells after 24 hours, the same genistein concentration used by Li and Sarkar produced tenfold decreases in another VEGF and VEGFR after only 6h in prostate cancer cells, a much lower decrease after 36h and an intermediate decrease after 72h, a paradoxical finding which they do not explain. The two teams used different culture media and treatments, and there were also differences in the cDNA microarray analysis equipment used, so the variations in findings may not merely reflect differences in the two cell types.

So far this review has looked at *in vitro* evidence for genistein's activity against the growth and spread of cancer cells. Most currently-used cancer drugs also damage healthy cells, producing often-severe side-effects. Thus it is necessary to try to ascertain genistein's ability to distinguish between cancerous and normal cells.

5.3 Is genistein selective for cancer?

5.3.1 Proteasome inhibition

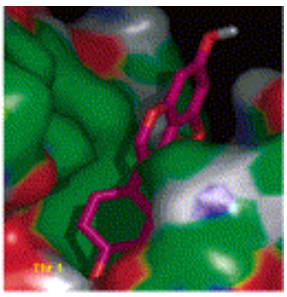
A 2003 study examined genistein's ability to inhibit proteasome activity in prostate and breast cancer cells, and also in normal human fibroblasts and fibroblasts immortalised by the oncogenic simian virus 40 (SV40) (Kazi *et al*., 2003). Protein degradation involving proteasomes is stated to be essential for tumour cell proliferation, and chymotrypsin-like proteasome activity is associated with survival in tumour cells.

Proteasome inhibition causes accumulation of ubiquitinated proteins: proteins 'labelled' for proteolysis. This accumulation is mirrored by an increase in anti-proliferative proteins such as p53 and p27, which would normally be degraded by proteasomes.

Kazi and colleagues carried out computational docking studies with genistein and yeast proteasome (*see* Figure 10). They state that the proteasomal 5 subunit is responsible for its chymotrypsin-like activity, which depends on the presence of the *N*-terminal Thr (Thr 1) residue, and that an S1 pocket of this subunit, defined by the

hydrophobic residues, is important for substrate specificity and proteasome inhibitor binding. For reasons given in the caption to Figure 10, plus the fact that green-tea polyphenol EGCG has twice as many potential hydrogen bonds as genistein, and the finding that genistein's docked free energy of binding was substantially higher than that for EGCG, the authors concluded that genistein was a weaker proteasome inhibitor than EGCG.

A



B

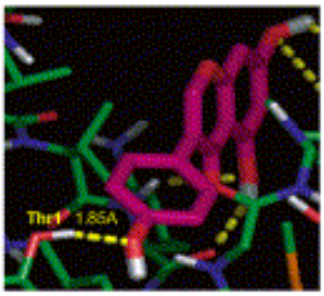


Figure 10

Docking solution of genistein to the proteasomal 5 subunit.

(A) The keto oxygen of genistein's C-ring was placed into the hydrophobic S1 pocket. This placement shifts the rings upwards from the pocket slightly. Furthermore, the presence of the oxygen in this hydrophobic environment may reduce the stability of genistein to remain in this position.

(B) The hydroxyl group of the B-ring of genistein lies in close proximity to Thr 1 (threonine 1) with a distance of 1.85 Å; this interaction may sterically block Thr 1.

adapted from Kazi et al. (2003)

Nevertheless, the team found that genistein concentration-dependently inhibited proteasomal activity in MCF-7 breast cancer cells and LNCaP prostate cancer cells (*see* Figure 11).

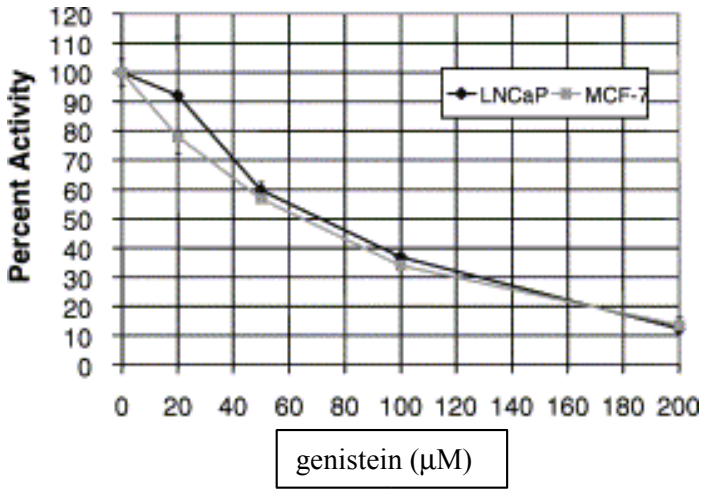


Figure 11

Inhibition of proteasomal chymotrypsin-like activity by genistein in solid tumour extracts. 10 µg each of LNCaP and MCF-7 cells extracts were incubated for 1h at 37° with 20 µM fluorogenic peptide substrate in presence or absence of genistein, followed by measurement of free 7-amido-4-methyl-coumarin (AMC) groups.

Source: Kazi et al. *(2003)*

This inhibition and concomitant increases in anti-proliferative proteins were closely followed by apoptosis, as measured by cleavage of poly(ADP-ribose) polymerase (PARP). The same experiment on normal and SV40-transformed human fibroblasts produced proteasome inhibition (as measured by levels of ubiquitinated proteins) and apoptosis only in the transformed, cancer-like cells (*see* Figure 12).

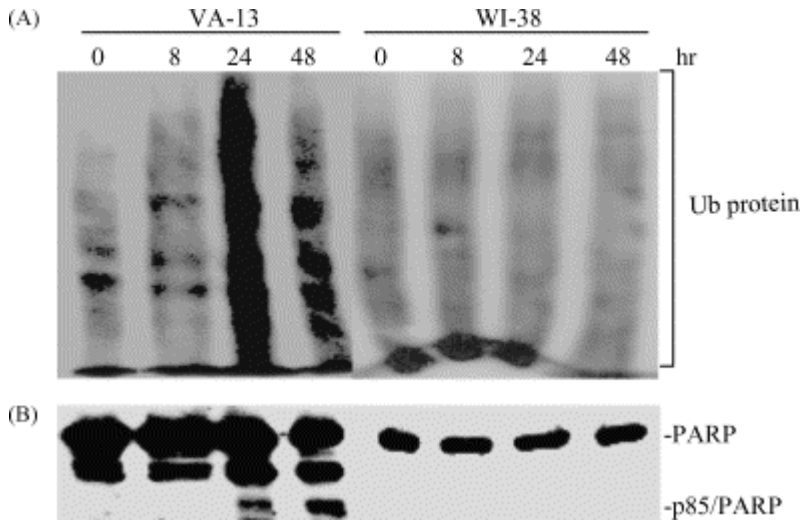


Figure 12

Preferable accumulation of ubiquitinated proteins and PARP cleavage by genistein in the transformed fibroblasts over the normal human fibroblasts. Exponentially growing normal (WI-38) and SV40-transformed (VA-13) human fibroblasts were treated with 100 μM of genistein for the indicated hours, followed by Western blot assay using specific antibodies to ubiquitin and PARP. Data are representative of at least three independent experiments.

adapted from Kazi et al. *(2003)*

In this context the high concentration of genistein used (100μM) serves to highlight the dramatic contrast between its effects on the two cell types, providing preliminary reassurance regarding its safety profile.

5.3.2 Effects via DNA damage

Further evidence for genistein's selective activity against cancer cells was provided by Pool-Zobel *et al*. (2000). These researchers found that 12.5-100 μM genistein potently induced DNA strand breaks in terminally-differentiated human colon cancer cells (HT29 clone 19A) but that up to 50 μM caused no strand breaks or base oxidation in normal colon cells. Although the cancer cells had been artificially differentiated, they might still have possessed characteristics enabling genistein to 'recognise' them as abnormal or cancerous. The lower genistein concentration tested on the normal cells was stated to be due to the cells' 'delicacy' and to 'exclude interference by cytotoxicity'. However, 50 μM is perhaps unlikely to be exceeded *in vivo*, even locally, at least through diet.

Although DNA strand-breaking is a common mechanism for many anti-cancer drugs, it does have disadvantages. Apart from the indiscriminate nature of most such drugs, there is a risk of strands rejoining in aberrant ways, producing mutant DNA and hence secondary cancers. This issue in relation to genistein will be addressed in Sections 5.4 and 6.

Another study on base oxidation was conducted by Davis *et al*. (2001). This team found that oxidative DNA damage, as measured by the presence of 5-hydroxymethyl-2'-deoxyuridine (5-OHmdU) - an indicator of oxygen free radical-induced DNA damage and thus a marker for oxidative stress - was decreased in the lymphocytes of healthy men given 100 mg of isoflavones daily for three weeks. The supplement concomitantly decreased the activation of nuclear factor-kappa B (NF-κB) by tumour necrosis factor-alpha (TNF-α), which the researchers deduced would render the cells more prone to apoptosis. The supplement used contained approximately 24% genistein and 19% daidzein, which constituted the bulk of the bio-active components. The plasma concentrations of the isoflavones are unfortunately not reported, but the intake would be likely to produce concentrations in the high nanomolar or low micromolar range.

A similar study by Mitchell and Collins in 1999 found that endogenous and hydrogen peroxide (H_2O_2)-induced DNA strand breakage in lymphocytes from ten healthy men was not decreased by consuming a litre of soya milk daily for four weeks. Soya milk consumers did, however, show progressively less oxidative damage to lymphocyte pyrimidine bases, in contrast to those assigned to cows' milk or a rice drink, as illustrated in Figure 13.

The Pool-Zobel study cited above also recorded a lack of antioxidant activity, in this case for high-concentration genistein added to cells *in vitro*. The latter finding appears to relate to tumour cells, but the paper often lacks clarity in distinguishing between normal and tumour cells when reporting outcomes.

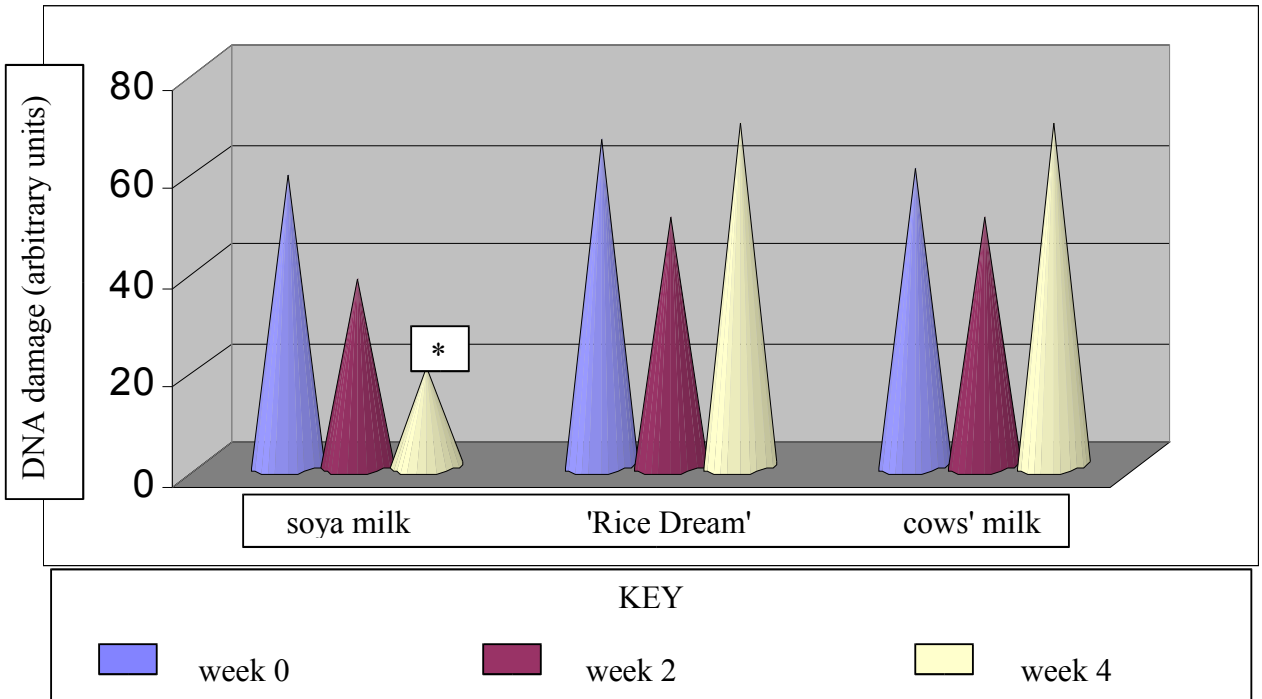


Figure 13

Effect of soya milk, 'Rice Dream' and semi-skimmed cows' milk on oxidative base damage to DNA as determined using the comet assay.

Soya milk *n*=4, 'Rice Dream' *n*=3, cow's milk *n*=3. * indicates P <0.05

source data: Mitchell and Collins (1999)

Interestingly, dihydrogenistein (*see* Figure 15 for 2 isomers), a gut bacterial metabolite of genistein, did decrease endogenous oxidative base damage in tumour cells in the Pool-Zobel study. This highlights the fact that genistein, and indeed other biologically-active food derivatives, are not present in physiological isolation and have a range of sometimes-opposing properties, making reductionist *in vitro* research of limited relevance to the human *in vivo* situation.

The Mitchell and Collins study recorded mean plasma genistein levels of 1.3 μM in the men assigned to the soya milk group; such levels are typical of those found in regions with high soya consumption, such as Japan and Hong Kong.

Kulling and colleagues (1999) also tested genistein's ability to damage DNA in healthy cells, and obtained results at odds with the Pool-Zobel and Davis teams, perhaps reflecting different methodology. Kulling's team tested lymphocytes from three healthy humans for the effects on DNA of 25 μM genistein. The isoflavone produced various chromosomal abnormalities including breaks in chromatids (*see* Figure 14).

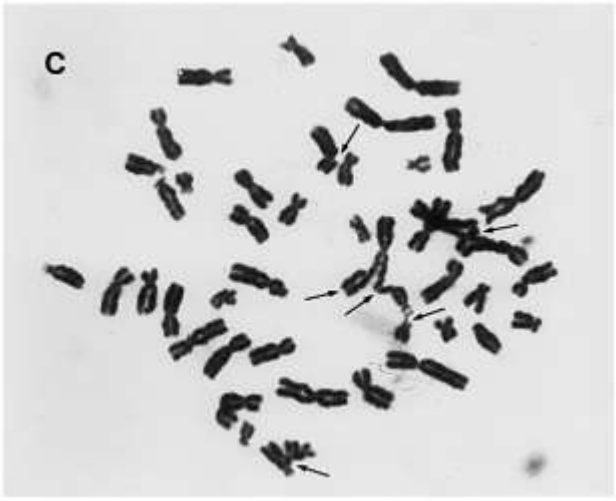


Figure 14

Metaphase spread of human peripheral blood lymphocyte exposed to 25 μM genistein for 6h and stained with Giemsa. Arrows indicate chromosomal damage.

Source: Kulling et al. *(1999)*

It may be significant that the cells were treated with phytohaemagglutinin (PHA) - a mitogen - prior to addition of phytoestrogens. As genistein has anti-mitotic properties, this treatment may lead to genistein 'recognising' cancerous or pre-cancerous qualities in the cells and consequently attacking them.

A later study headed by Kulling reported the production of catechols (hydroxylated metabolites) from both genistein and daidzein in human and rat microsomes, and also found such products in the urine of volunteers following ingestion of soya. The metabolites are shown in Figure 15.

The team proposed that, as catechol metabolites of isoflavones reportedly appeared to be poor substrates of catechol-O-methyltransferase (COMT), high levels might accumulate, giving rise to ortho-quinones and reactive oxygen species and consequent oxidative damage.

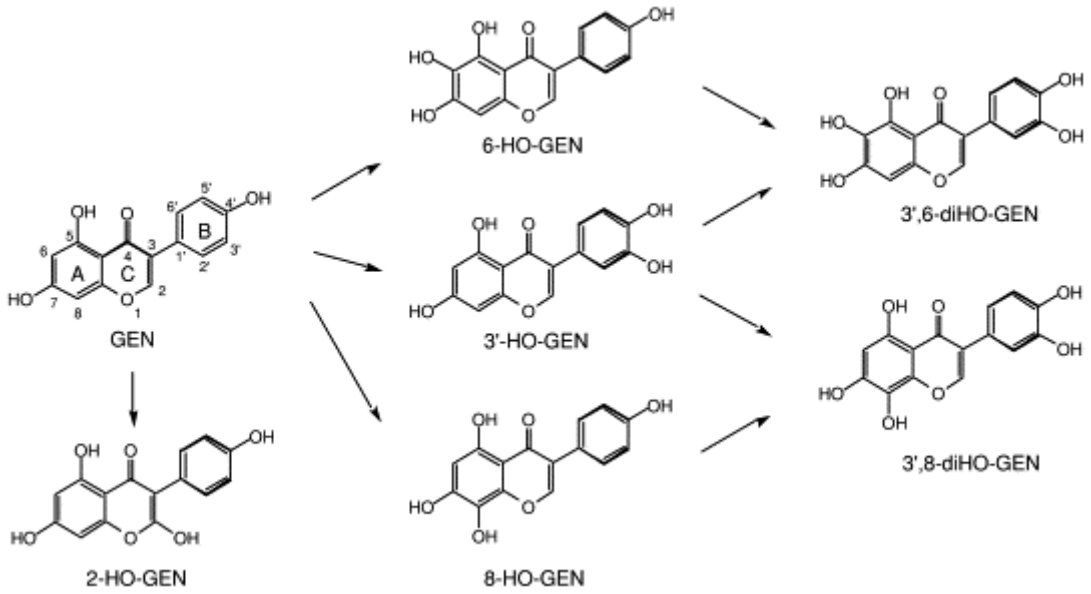


Figure 15 Oxidative pathways in the metabolism of genistein. 'GEN' = genistein.
Source: Kulling et al. *(2002)*

Thus the jury is still out over whether genistein is pro- or anti-oxidant in healthy cells.

Genistein's cytotoxic capacity, observed at high concentrations in other studies, is at least partly via inhibition of the enzyme topoisomerase II, and is characterised by the induction of double-strand breaks in DNA, similarly to many currently-used synthetic anti-cancer drugs. This review will now focus on genistein's effects on and via this enzyme.

5.4 Topoisomerase II inhibition

The enzyme topoisomerase II ('topo II') corrects topological problems arising in DNA during replication and other processes requiring the disruption of its helical structure. The enzyme is upregulated in proliferating cells such as cancer cells. Despite its highly planar structure {*see* structure (**1**)}, genistein is a *non-intercalative* topisomerase inhibitor - i.e. it does not inhibit the enzyme by inserting itself between base pairs - but is classified instead as a topoisomerase 'poison'.

(**1**) Edge-on ball-and-stick WebLab structure of genistein

This term describes compounds which stabilise the covalent topo II-DNA complex, preventing completion of the catalytic cycle, leaving the DNA with a double-stranded break and thereby converting the enzyme into a potent cellular toxin (*see* Figure 16).

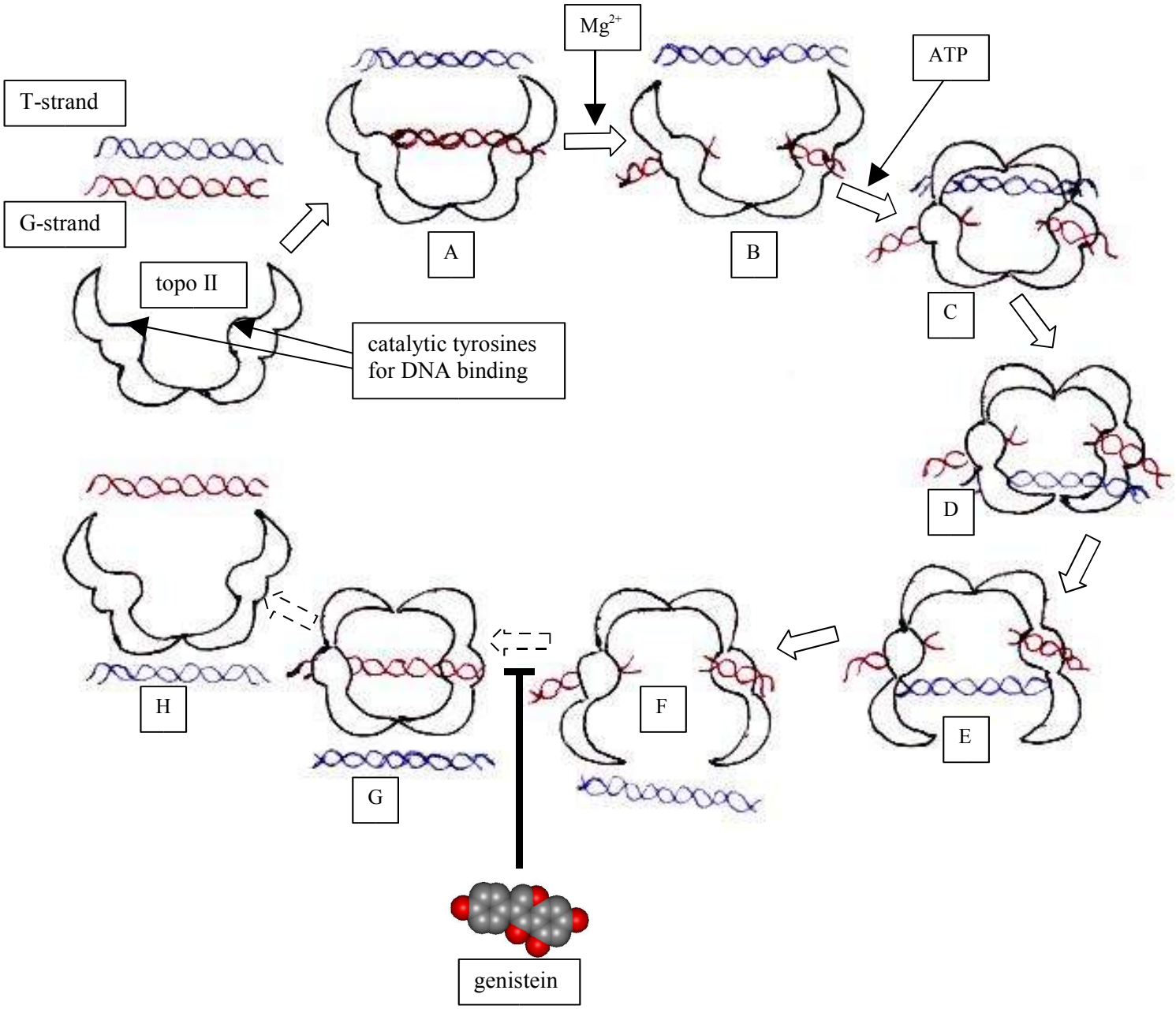


Figure 16 Enzymatic action of topoisomerase II. Genistein acts by trapping the G-strand-enzyme intermediate (step F in this figure), thus blocking religation and enzyme release, leaving the DNA with a permanent double-strand break.
adapted from Hengstler et al. (2002)

Accumulation of such breaks leads to apoptosis, but surviving cells may contain DNA which has been incorrectly rejoined following the topoisomerase inhibition, a potentially carcinogenic situation. This review will now look at possible adverse effects from soya or genistein.

6 Possible adverse effects from genistein use and/or soya consumption

Reports of adverse actual or potential consequences of the ingestion of soya or genistein are fewer and mostly weaker than those of actual or potential benefits.

However, translocations in the *MLL* gene often occur in cancer patients treated with synthetic topo II 'poisons' (*see* previous section), and are strongly suspected to be a causal factor in therapy-related leukaemia. Similar mutations are seen in infant leukaemia, which is considered to develop before birth (Strick *et al*., 2000). Findings of infant leukaemia levels almost twice as high in some Asian cities as in the West have fuelled concerns about the possible involvement of dietary topo II inhibitors.

Ross *et al*. (1996) found an approximately tenfold increased risk of infant acute myeloid leukaemia (AML) from medium-to-high maternal consumption of some topo II inhibitor-containing foods. However, Ross herself interpreted these data cautiously in a review (Ross, 2000) noting that, despite widespread consumption of the foods concerned, infant leukaemia is still very rare: 37 cases per million in the USA.

Another finding based on data from living humans relates to hypothyroidism. A review by Doerge and Chang cites a study which found that teenagers with autoimmune thyroid disease had received nearly three times as much soya-based formula as infants as those without the condition (Doerge and Chang, 2002). The review states that adding iodine to soya infant formula in the 1960s dramatically reduced reports of associated goitre. Findings in adults have varied, sometimes according to menstrual-cycle stage or pre- or post-menopausal status in women.

The authors also report *in vitro* and animal studies on the genistein/soya-hypothyroidism connection, highlighting the mechanism of inactivation of thyroid peroxidase (TPO) by inhibiting TPO-catalyzed iodination of thyroxine. Soya-induced hypothyroidism only occurred in iodine-deficient animals, and a general finding across study-types is of soya/isoflavone-associated goitres and biochemical signs of hypothyroidism (some in the absence of symptoms) being reversible on supplementation with iodine or cessation of isoflavone ingestion.

Despite the numerous human studies cited by Doerge and Chang, two other papers conclude, respectively:

"Data are lacking in infants for biological effects from exposure to phytoestrogens, with the exception of a study of cholesterol homeostasis..." (review by Setchell and Cassidy, 1999), *and*

" ...nor have deleterious effects of soy-based infant formulas become apparent despite having been in use for more than 30 years" (Setchell *et al*., 1997).

Doerge and Chang propose that preferential accumulation of genistein in animals' thyroids and reproductive organs is due to its lipophilicity, the clinical importance of which will be readdressed in Section 7.

Positive epidemiological correlations have also been observed between the consumption of fermented or fried soya products and some cancers (Messina *et al*., 1994).

Another potential adverse effect of genistein was examined by Ohno and colleagues (2002), using an adrenocortical tumour cell line as a model for human steroidogenic cells on which to assess the ability of flavonoids to inhibit cortisol synthesis. 12.5 μM of several of the compounds, including genistein and daidzein, inhibited cortisol synthesis, and structural comparisons suggested that this was partly dependent on a hydroxyl group at the 4' position of the benzene ring (*see* Figure 1). The researchers concede that *in vivo* evidence is lacking for this effect, and it should be questioned whether a tumour cell line, albeit apparently pluripotent, can usefully represent a complex normal system - the hypothalamic-pituitary-adrenocortical axis - particularly in the light of the cell line's admitted non-responsiveness to adrenocorticotrophic hormone (ACTH).

The other consistent finding which may be a cause for concern is genistein's pro-proliferative properties at low concentrations *in vitro* (*see* Section 5.2), but this is not reflected in epidemiological data on cancer risk.

Before extrapolating from *in vitro* experiments to preventative or therapeutic potential, it is necessary to ascertain whether levels of genistein found to be anticarcinogenic *in vitro* are achievable in living humans, especially through oral consumption. This would also support a causal basis for the mainly-negative epidemiological associations between genistein/soya and cancer.

7 Can physiological genistein concentrations reach anticarcinogenic levels?

Genistein levels found to have anti-proliferative activity *in vitro* are usually in the micromolar range.

IC_{50}s for cancer cell growth suppression *in vitro* by genistein are cited in Messina and colleagues' 1994 review as ranging from 5-40 μM. Agarwal's proteasome study found that IC_{50}s were higher in cell extracts than in purified proteasomes (65-70 μM for the former and 26 μM for the latter). It should be noted that the latter used rabbit proteasome, whereas the cell extracts were from human cancers. The finding is, however, consistent with genistein's tendency to bind to serum proteins, reducing its effective concentration (Kulling *et al*., 1999).

The highest plasma genistein levels recorded in adult humans are in the low micromolar range. However, genistein has been found at much higher proportions in its smaller, more lipophilic aglycone, and more *in vitro*-carcinogenic, form (genistein rather than genistin) in rat thyroid and reproductive organs (Doerge and Chang, 2002). Consistent with this, Farhan *et al*. (2002) found a tenfold higher genistein concentration in mouse prostates than serum following administration of genistein.

Caution is necessary when extrapolating animal data to humans, but Morton and colleagues (1997) measured mean levels of *daidzein* up to 3.5 times higher in human expressed prostatic secretions (EPS) than in plasma. The authors state that EPS 'may be partly representative of the extent of phytoestrogen penetration' of the prostate. Many cancer-vulnerable sites in the body are lipid-rich, and Strick *et al*. (2000) report studies finding high 'bioflavonoid' concentrations persisting in human milk 2-4 days after soya consumption. Such lipophilic partitioning suggests that diet-derived genistein may reach effective levels where needed, bearing in mind:

1. higher plasma concentrations of genistein than of daidzein (*see* Section 4),

2. genistein's greater lipophilicity than daidzein due to hydrogen bonding between its 5' hydroxyl group and its 4' ketonic oxygen (*see* Figure 17), *and*

3. EPS being only *partly* representative of prostatic fluid, and prostate *tissue* concentrations being possibly more relevant for anticarcinogenicity.

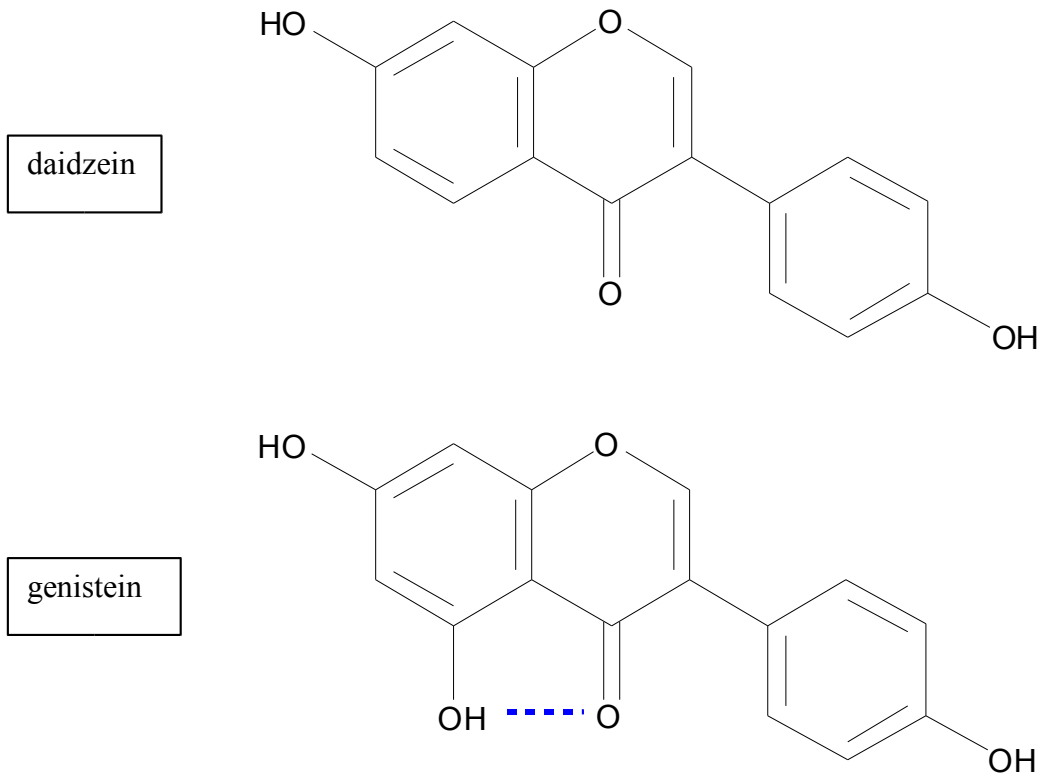


Figure 17 The isoflavones daidzein and genistein, showing intramolecular hydrogen-bonding only occurring in genistein (dotted blue line)

Evidence from a variety of studies strongly points to a potential therapeutic role for genistein and/or other soya components in cancer, and there is a smaller body of evidence for adverse effects, but only clinical studies can provide any degree of certainty for efficacy and safety. A brief overview of some preliminary clinical findings follows.

8 Clinical evidence

Clinical trials are still at an early stage for genistein and related compounds, with very few results published, even of a preliminary nature.

A Phase I trial gave a form of genistein: 'B43-genistein' - stated to be a tyrosine kinase inhibitor - to 15 acute or chronic leukaemia patients who had 'failed standard therapy' (AACR, 1999). The report states cites the drug's 'acceptable toxicity profile' and improvement or remission in three patients. It is not a peer-reviewed paper but a conference presentation abstract, and does not reveal the nature of the compound, the quantities administered, the route of administration, the duration of the trial or which patients appeared to benefit.

Another paper - again not a scientific journal article - reports a trial which measured levels of prostate-specific antigen (PSA) in 30 clinically stable prostate cancer patients (Barkin *et al*., 2000). It states that patients using a fermented soya drink for six months had a slower PSA increase than controls and patients given concentrated isoflavones, but PSA responses did not correlate with plasma genistein levels. EPS genistein levels and cancer progression are not reported. PSA levels do not necessarily correlate with cancer proliferation (Adlercreutz, 2002).

An original research communication on prostate cancer states that twenty patients treated for 28-56 days with high doses of genistein (300-600 mg per day) showed no significant genotoxicity arising from the treatment (Miltyk *et al*., 2003). The researchers report that there were no rearrangements of the *MLL* gene (*see* Section 6) in the three patients examined for such mutations. Plasma genistein levels did not exceed 0.32 μM despite the very high intake, although a level of 27.1 μM was recorded for 'total genistein', apparently meaning conjugated and unconjugated forms combined. Again, EPS genistein levels are not reported.

Clearly we must await more clinical trial reports before any firm conclusions can be drawn for genistein and/or other phytoestrogens as cancer treatments, and many such studies are ongoing and recruiting.

9 Conclusion

Notwithstanding the caveat at the end of the last section, the epidemiological evidence for soya-related anti-carcinogenic properties is strong. Many mechanisms have been identified *in vitro* to support genistein's role, at least in part, in the epidemiological associations seen. These include its ability to suppress oestrogen activity, to modulate gene expression, to inhibit specific enzymes and to selectively damage DNA in cancer cells. These activities are, variously, associated with inhibition of cell growth and metastasis and promotion of differentiation, and genistein has also been shown - if present at sufficient concentrations - to inhibit cancer cell growth.

Credible evidence exists for lipophilic partitioning of diet-derived genistein in cancer-susceptible organs at sufficiently high concentrations to have such effects in living humans.

Evidence for genistein or soya causing harm is scarcer and weaker than evidence for anticarcinogenicity.

It remains to be seen whether genistein and/or phytoestrogens, singly or in combination, can be therapeutic as well as prophylactic for cancer. If clinical findings are positive, the benefits to patients will be matched by savings in healthcare costs. Compared to the enormous sums spent developing synthetic anti-cancer drugs, the source of genistein - soya - is extremely cheap.

This work is a reformatted version of an assessable component of the author's Masters degree from the Open University and was produced in 2003. The author is now a freelance researcher and writer and has a website at http://www.vivienpomfrey.co.uk/.

REFERENCES

AACR (1999) NEW TYROSINE KINASE INHIBITOR SHOWS PROMISE IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA, Research results presented at International Conference sponsored jointly by AACR (American Association for Cancer Research), NCI and EORTC, accessed online 19.7.2003 at http://www.aacr.org/1000/1100/1120aa.html.

Adlercreutz, H. (2002) Phyto-oestrogens and cancer, *The Lancet Oncology*, **vol. 3**, Issue 6, 1 June 2002, pp. 364-373

Agarwal, R. (2000) Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents, *Biochemical Pharmacology*, v**ol. 60**, Issue 8, 15 October 2000, pp. 1051-1059

Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M. and Fukami, Y. (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases, *The Journal Of Biological Chemistry*, **vol. 262**, Issue 12, April 25, 1987, pp. 5592-5595

Arai, Y., Uehara, M., Sato, Y., Kimira, M., Eboshida, A., Adlercreutz, H. and Watanabe, S. (2000) Comparison of isoflavones among dietary intake, plasma concentration and urinary excretion for accurate estimation of phytoestrogen intake, *Journal Of Epidemiology/Japan Epidemiological Association*, **vol. 10**, Issue 2, March 2000, pp. 127-135

Barkhem *et al*., 1998, as cited in Pike *et al*. (1999)

Barkin, I., MD, Baranov, E., MD, Geller, J., MD, MACP and Hoffman, R., MD (2000) Comparison of the Effects of Two Different Soy Preparations with Placebo on Serum PSA and Plasma Genistein Levels in Patients with Early-Stage Prostate Cancer, EcoNugenics webpage, accessed online 2.10.2003 at http://www.econugenics.com/research_edu/trials.php3?trial=6

Boersma, B.J., Barnes, S., Kirk, M., Wang, C.-C., Smith, M., Kim, H., Xu, J., Patel, R. and Darley-Usmar, V.M. (2001) Soy isoflavonoids and cancer - metabolism at the target site, *Mutation Research* **vols. 480–481** (2001), pp. 121–127

Busby, M.G., Jeffcoat, A.R., Bloedon, L.T., Koch, M.A., Black, T., Dix, K.J., Heizer, W.D., Thomas, B.F., Hill, J.M., Crowell, J.A. and Zeisel, S.H. (2002) Clinical characteristics and pharmacokinetics of purified soy isoflavones: single-dose administration to healthy men, *The American Journal Of Clinical Nutrition*, **vol. 75**, Issue 1, January 2002, pp. 126-136

Chen, W-F., Huang, M-H., Tzang, C-H., Yang, M. and Wong, M-S. (2003), Inhibitory actions of genistein in human breast cancer (MCF-7) cells, *Biochimica et Biophysica Acta (BBA)*, Article in Press, Corrected Proof

Davis, J.N., Kucuk, O., Djuric, Z. and Sarkar, F.H. (2001) Soy isoflavone supplementation in healthy men prevents NF-κB activation by TNF-α in blood

lymphocytes, *Free Radical Biology and Medicine*, **vol. 30**, Issue 11, 1 June 2001, pp. 1293-1302

Dixon, R.A. and Ferreira, D. (2002) Genistein, *Phytochemistry*, **vol. 60**, Issue 3, June 2002, pp. 205-211

Doerge, D.R. and Chang, H.C. (2002) Inactivation of thyroid peroxidase by soy isoflavones, in vitro and in vivo, *Journal of Chromatography B*, **vol. 777**, Issues 1-2 , 25 September 2002, pp. 269-279

Farhan, H., Wähälä, K., Adlercreutz, H. and Cross, H. S. (2002) Isoflavonoids inhibit catabolism of vitamin D in prostate cancer cells, *Journal of Chromatography B*, **vol. 777**, Issues 1-2, 25 September 2002, pp. 261-268

Gibbs, J.B. (2000) Mechanism-based target identification and drug discovery in cancer research, *Science*, **vol. 287**, pp. 1969-1973

Griffiths, K., Morton, M.S. and Denis, L. (1999) Certain Aspects of Molecular Endocrinology That Relate to the Influence of Dietary Factors on the Pathogenesis of Prostate Cancer, *European Urology*, **vol. 35**, No. 5-6, 1999

Hengstler, J.G., Heimerdinger, C.K., Schiffer, I.B., Gebhard, S., Sagemüller, J., Tanner, B., Bolt, H.M. and Oesch, F. (2002) Dietary topoisomerase II-poisons: contribution of soy products to infant leukemia? *EXCLI Journal* 2002, **vol. 1**, pp. 8-14

Hua, P., Tsai, W-J., and Kuo, S-M. (2003), Estrogen response element-independent regulation of gene expression by genistein in intestinal cells, *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression*, **vol. 1627**, Issues 2-3, 19 June 2003, pp. 63-70

Jacobsen, B.K. (1998) Does high soy milk intake reduce prostate cancer incidence? The Adventist Health Study (United States), *Cancer Causes and Control*, **vol. 9**, Issue 6, December 1998, pp. 553-7

Kazi, A., Daniel, K.G., Smith, D.M., Kumar, N.B. and Ping Dou, Q. (2003) Inhibition of the proteasome activity, a novel mechanism associated with the tumor cell apoptosis-inducing ability of genistein, *Biochemical Pharmacology*, Article in Press, Corrected Proof

Kuiper *et al*., 1997, as cited in Pike *et al*. (1999)

Kulling, S.E., Rosenberg, B., Jacobs, E. and Metzler, M. (1999) The phytoestrogens coumoestrol and genistein induce structural chromosomal aberrations in cultured human peripheral blood lymphocytes, *Arch. Toxicol*. (1999) **vol. 73**, pp. 50-54

Kulling, S.E., Lehmann, L. and Metzler, M. (2002) Oxidative metabolism and genotoxic potential of major isoflavone phytoestrogens, *Journal of Chromatography B*, **vol. 777**, Issues 1-2, 25 September 2002, pp. 211-218

le Bail, J.C., Varnat, F., Nicolas, J.C. and Habrioux, G. (1998a) Estrogenic and antiproliferative activities on MCF-7 human breast cancer cells by flavonoids, *Cancer Letters*, **vol. 130**, Issues 1-2, 14 August 1998, pp. 209-216

le Bail, J.C., Laroche, T., Marre-Fournier, F. and Habrioux, G. (1998b) Aromatase and 17β-hydroxysteroid dehydrogenase inhibition by flavonoids, *Cancer Letters*, **vol. 133**, Issue 1, 13 November 1998, pp. 101-106

Li, Y. and Sarkar, F.H. (2002) Down-regulation of invasion and angiogenesis-related genes identified by cDNA microarray analysis of PC3 prostate cancer cells treated with genistein, *Cancer Letters*, **vol. 186**, Issue 2, 5 December 2002, pp. 157-164

Messina, M.J., Persky, V., Setchell, K.D.R. and Barnes, S. (1994) Soy intake and cancer risk: a review of the *in vitro* and i*n vivo* data, *Nutrition And Cancer* **vol. 21**, Issue 2, 1994, pp. 113-131

Miltyk, W., Craciunescu, C.N., Fischer, L., Jeffcoat, R.A., Koch, M.A., Lopaczynski, W., Mahoney, C., Crowell, C., Paglieri, J. and Zeisel, S.H. (2003) Lack of significant genotoxicity of purified soy isoflavones (genistein, daidzein, and glycitein) in 20 patients with prostate cancer, *American Journal of Clinical Nutrition*, **vol. 77**, No. 4, April 2003, pp. 875-882

Mitchell, J.H. and Collins, A.R. (1999) Effects of a soy milk supplement on plasma cholesterol levels and oxidative DNA damage in men in a pilot study, *European Journal of Nutrition*, **vol. 38**, Number 3 (1999) pp. 143-8

Morton, M.S., Chan, P.S.F., Cheng, C., Blacklock, N., Matos-Ferreira, A., Abranches-Monteiro, L., Correia, R., Lloyd, S. and Griffiths, K. (1997) Lignans and isoflavonoids in plasma and prostatic fluid in men: Samples from Portugal, Hong Kong, and the United Kingdom, *The Prostate*, **vol. 32**, Issue 2, pp. 122-128

Ohno, S., Shinoda, S., Toyoshima, S., Nakazawa, H., Makino, T. and Nakajin, S. (2002) Effects of flavonoid phytochemicals on cortisol production and on activities of steroidogenic enzymes in human adrenocortical H295R cells, *The Journal of Steroid Biochemistry and Molecular Biology*, **vol. 80**, Issue 3, March 2002, pp. 355-363

Paech, K., Webb, P., Kuiper, G.G., *et al*. (1997), as cited in Steiner *et al*. (2001)

Pike, A.C.W., Brzozowski, A.M., Hubbard, R.E., Bonn, T., Thorsell, A-G., Engström, O., Ljunggren, J., Gustafsson, J-Å. and Carlquist, M. (1999) Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist, *The EMBO Journal*, **vol. 18**, pp. 4608-4618

Pool-Zobel, B.L., Adlercreutz, H., Glei, M., Liegibel, U.M., Sittlington, J., Rowland, I., Wähälä, K. and Rechkemmer, G. (2000), Isoflavonoids and lignans have different potentials to modulate oxidative genetic damage in human colon cells, *Carcinogenesis*, **vol. 21**, No. 6, pp. 1247-1252, June 2000

Rang, H.P., Dale, M.M. and Ritter, J.M. (2000) *Pharmacology*, Churchill Livingstone, Edinburgh, UK, p.42, Fig. 2.17(A)

Ross, J.A., Potter, J.D., Reaman, G.H., Pendergrass, T.W. and Robison, L.L. (1996) Maternal exposure to potential inhibitors of DNA topoisomerase II and infant leukemia (United States): A report from the children's cancer group, *Cancer Causes & Control*, **vol. 7**, issue 6, pp. 581-590, Nov. 1996

Ross, J.A. (2000) Dietary flavonoids and the *MLL* gene: A pathway to infant leukemia? *Proc. Natl. Acad. Sci.*, **vol. 97**, April 25, 2000, no. 9, pp. 4411-4413

Saville, B., Wormke, M., Wang, F., Nguyen, T., Enmark, E., Kuiper, G., Gustafsson., J-A. and Safe, S. (2000) Ligand-, Cell-, and Estrogen Receptor Subtype (alpha/beta)-dependent Activation at GC-rich (Sp1) Promoter Elements, *J Biol Chem*, **vol. 275**, Issue 8, pp. 5379-5387, February 25, 2000

Setchell, K.D.R., Zimmer-Nechemias, L., Cai, J. and Heubi, J.E. (1997) Exposure of infants to phyto-oestrogens from soy-based infant formula, *The Lancet*, **vol. 349**, Issue 9070, 5 July 1997, pp. 23-27

Setchell, K.D. and Cassidy, A. (1999) Dietary Isoflavones: Biological Effects and Relevance to Human Health, *Journal of Nutrition* **vol. 129**, pp. 758-767

Setchell, K.D.R., Brown, N.M., Desai, P.B., Zimmer-Nechimias, L., Wolfe, B., Jakate, A.S., Creutzinger, V. and Heubi, J.E. (2003) Bioavailability, disposition, and dose-response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes, *The Journal Of Nutrition*, **vol. 133**, Issue 4, April 2003, pp. 1027-1035

Steensma *et al*., 1999, as cited in Dixon and Ferreira (2002)

Steiner, M.S., Raghow, S. and Neubauer, B.L. (2001) Selective Estrogen Receptor modulators for the chemoprevention of prostate cancer, *Urology* **vol. 57** (Suppl 4A), pp. 68–72

Strick, R., Strissel, P.L., Borgers, S., Smith, S.L. and Rowley, J.D. (2000) Dietary bioflavonoids induce cleavage in the *MLL* gene and may contribute to infant leukemia, *Proc. Natl. Acad. Sci.*, **vol. 97**, April 25, 2000, no. 9, pp. 4790-4795

The Lancet Oncology (2002), Webtable 3, *The Lancet Oncology* **vol**. **3**, June 2002, URL http://image.thelancet.com/extras/01oncl903webtable3.pdf, associated with Adlercreutz (2002)

Verweij, J. and de Jonge, M.J.A. (2000) Achievements and future of chemotherapy, *European Journal of Cancer*, **vol. 36**, pp. 1479-87

Wietzke, J.A. and Welsh, J. (2003) Phytoestrogen regulation of a Vitamin D3 receptor promoter and 1,25-dihydroxyvitamin D3 actions in human breast cancer cells, *The Journal of Steroid Biochemistry and Molecular Biology*, **vol. 84**, Issues 2-3, February 2003, pp. 149-157

Zhou, Y. and Lee, A.S. (1998) Mechanism for the suppression of the mammalian stress response by genistein, an anticancer phytoestrogen from soy*, Journal Of The National Cancer Institute*, **vol. 90**, pp. 381-388

APPENDIX I

Glossary

Glossary

Isoflavones/isoflavonoids	types of phytoestrogens; 3-phenyl-chromones; isomeric form of flavones with benzene group attached to the 3' rather than 2' position of benzopyran ring
Mitogen	substance able to induce mitosis of certain eukaryotic cells
Oncogene	mutated form of proto-oncogene which can over-stimulate cell division
Phytoestrogen	plant-derived compound or its metabolite able to mimic the action, or modulate binding, metabolism or production of endogenous estrogens in the body.
Prodrug	substance metabolised *in vivo* to liberate active drug agent
Proto-oncogene	gene coding for protein which stimulates cell division