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Inhibition of survival signalling by dietary polyphenols and indole-3-carbinol

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Abstract

Epidemiological studies have long hinted at the possibility that what we eat greatly influences our state of health, in particular our relative risk of developing cancer. In recent years there has been an exponential increase in the number of studies investigating how individual components of the diet interact at the molecular level to determine the fate of a cell. It is now apparent that many such molecules can preferentially inhibit the growth of tumour cells, by inducing cell cycle arrest or apoptosis. The number of signalling pathways and molecular targets involved is continually expanding. Consequently, the picture is becoming ever more complicated, not least because results often appear to be cell-type specific, dose–response relationships are critical, and any one agent appears to have multiple mechanisms of action. In addition most studies have been conducted in cell culture, often with physiologically unachievable concentrations of single agents, making extrapolation to the clinical situation difficult. In this review the mechanisms of action of a few well-studied dietary polyphenols (curcumin, epigallocatechin gallate and resveratrol) and indole-3 carbinol are considered in the light of these issues.

Keywords: Chemoprevention; Survival signalling; Curcumin; Epigallocatechin gallate; Indole-3-carbinol; Resveratrol; MAPK; PI3K; NF-κB; STAT

1. Introduction

A healthy cell is continuously receiving signals from its environment through many different receptors, both membrane bound and intracellular, which eventually feed through to the nucleus to influence the activity of various transcription factors. These in turn drive gene transcription, which determines the outcome for the cell – whether to arrest, proliferate, differentiate, senesce or die.

Signals in a normal, non-dividing cell are in balance between pathways which elicit proliferation and those dictating arrest. But when the first carcinogenic changes occur in preneoplasia, the balance begins to shift in favour of deregulated proliferation, until in tumours, the proliferative signals come to dominate. Thus the signalling pathways in tumour cells are very different from those in normal cells from which they are derived, affording opportunities for selective targeting in cancer treatment or prevention. Many cancer chemopreventive agents derived from dietary sources are now considered to exert their activity through disturbance of oncogenic signalling. In this review cellular signalling events thought to be affected by such agents are reviewed, in particular those germane to cellular survival. Four well-studied dietary agents are compared in terms of their effects on signalling pathways in order to illustrate the complexity of such interactions. The agents chosen are the polyphenols curcumin derived from turmeric, epigallocatechin gallate (EGCG) from green tea and

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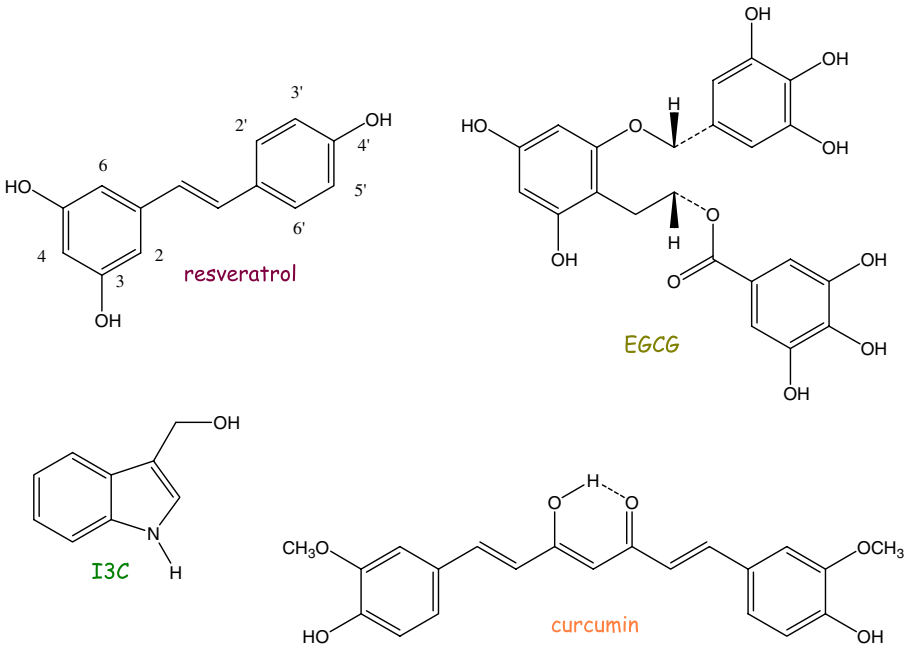

Fig. 1. Structures of curcumin, EGCG, resveratrol and I3C.

resveratrol from grapes and red wine, as well as indole-3-carbinol (I3C) from cruciferous vegetables (for structures see Fig. 1).

2. Oncogenic signalling and chemopreventive phytochemicals

The derangements in signalling for a fully malignant, invasive tumour, as summarised by Hanahan and Weinberg, [1] include:

1. Becoming self-sufficient in growth signals, such that the tumour cells produce their own, or the relevant pathways become constitutively active without the need for exogenous signals.
2. Becoming insensitive to signals which would normally inhibit proliferation.
3. Invoking survival pathways in order to avoid apoptosis, which would normally occur in irreversibly damaged cells.
4. Being able to replicate indefinitely, thus avoiding terminal differentiation or senescence.
5. Initiating angiogenesis to ensure sufficient oxygen and nutrient supply to sustain tumour growth.
6. Undergoing invasion and metastasis.

Numerous combinations of phenotypic or genotypic alterations provide limitless possibilities to achieve the same end result – a cancer cell.

Fortunately, however, there are dietary molecules with the potential to modulate each of these characteristics. So far, more than 1000 different phytochemicals have been credited with putative chemopreventive activity [2], and in recent years much effort has gone into elucidating the molecular mechanisms of a few of them. All the major signalling pathways, which are deregulated in cancer, and which have been examined as targets for chemoprevention, have responded to one or more agents. However, encouraging as this may be, there are many problems and from the studies carried out to date, several important caveats emerge.

Firstly, the effects of chemopreventive agents (as with any xenobiotic) are cell type- and dose-dependent. While some results from *in vitro* studies suggest similar mechanisms of action in cells of different origin, there are also data indicating distinct differences even in cell lines derived from the same target tissue. There are also examples of agents eliciting opposite effects at high and low doses.

Secondly, since the duration of a signal is important in determining the biological outcome, the effects of agents will differ depending on whether they target a signalling mechanism which is transient or sustained. The effect of signal duration on biological outcome was exemplified in a study by Murphy and colleagues [3]. They showed that the immediate early gene product and AP-1 transcription factor, c-fos, functioned as a sensor for signalling through the growth factor receptor (GFR)/mitogen activated protein kinase (MAPK) pathway. With transient extracellular signal regulated kinase (ERK) activation, there was no time for c-fos to accumulate and what little protein existed was unstable. However, in conditions where ERK signalling was

sustained, c-fos was phosphorylated by ERK and RSK (90K-ribosomal S6 kinase) causing stabilisation and ensuing transcriptional activity. One could imagine that for a normal cell with transient signalling, a chemopreventive agent would have little or no effect. But for a tumour cell heavily reliant on this pathway for proliferation or survival, an inhibitory effect by a chemopreventive agent could result in growth arrest or apoptosis. Outcome will also depend on the stability of the chemopreventive agent and whether its effects are sustained over time.

Thirdly, it is important when considering the chemopreventive activity of a particular compound, to differentiate between its effects on constitutive or inducible levels of aberrant signalling. In some cases, the effects can be quite different in these two scenarios. For example, an agent may inhibit the further activation of a signalling pathway when applied before, or along with, a suitable stimulus. But it may have no effect on the same pathway already constitutively upregulated in the absence of external stimuli. This might well affect its usefulness in some circumstances.

Finally, it is important to realise that most of the detailed mechanistic data have been obtained *in vitro* and therefore may not necessarily be physiologically relevant. However, encouragingly there are many examples of *in vitro* observations that can be reproduced *in vivo*. Also, as will be discussed later, different agents can exert similar effects, so that while *in vivo* the dose of any one compound may be very small, the combined effect of several agents (as could be achieved with dietary intake) may be more akin to the higher doses of single agents used *in vitro*.

3. Survival signalling pathways as targets for chemoprevention

Many *in vitro* studies have considered the effect of dietary molecules on cell proliferation, using growth inhibition, induction of cell cycle arrest or apoptosis as an end point. A large number have gone further, investigating the effects on components of survival signalling pathways that regulate the cell cycle or apoptosis. Some of the best understood survival pathways are those involving GFR/Ras/MAPKs, phosphotidylinositol-3-kinase (PI3K)/protein kinase B (Akt), signal transducers and activators of transcription (STATs) or nuclear factor κ B (NF-κB).

3.1. GFR/Ras/MAPK signalling

Due to its central role in regulating cell growth and survival, the pathway signalling through GFRs to MAPKs has been extensively investigated as a possible target for cancer treatments [4]. Signals from a variety of growth factors, cytokines and proto-oncogenes are transduced through Ras, leading to activation of a serine/threonine kinase Raf. This leads to consecutive activation of a MAPK kinase and MAPK(ERK). ERK is responsible for activation of various transcription factors, including c-fos. Other MAPK pathways signal through c-jun N-terminal kinase (JNK) or p38 to regulate c-jun or other transcription factors. Deregulation of these pathways in cancer involves amplified or overexpressed GRFs and oncogenic Ras leading to continuous activation of the pathway. In the case of JNK, both activation and de-activation have been shown to contribute to induction of apoptosis, depending on context [5,6].

3.2. PI3K/Akt signalling

Signalling through PI3K/Akt represents one of the most studied survival pathways in cells and a role in cancer has been well documented [7]. Signals received via GFR and non-receptor tyrosine kinases, such as the epidermal growth factor receptor (EGFR) or src, are transmitted through PI3K to Akt. Activation of this key component can block a number of mechanisms that would normally induce apoptosis or cell cycle arrest. Various components of this pathway are overexpressed or overactive in human tumours, for example the GFR, PI3K or Akt, while negative regulators of the pathway such as the tumour suppressor, PTEN, can be mutated or absent.

3.3. NF-κB signalling

Signalling through the transcription factor NF-κB is another well studied pathway that can aid survival. This pathway is central to the inflammatory response, but is also intimately involved in many cancers [8] and has been considered as a suitable target for cancer therapy [9]. A recent report defines NF-κB as a tumour promoter in inflammation-associated cancer [10]. Under normal conditions, NF-κB is sequestered in the cytoplasm by IκB. Signalling through receptors and a variety of adapter molecules activates a complex known as IκB kinase (IKK). IκB is phosphorylated by IKK and targeted for degradation, allowing NF-κB to translocate to the nucleus and activate transcription of many genes which induce proliferation or block apoptosis. Again there is plenty of evidence for deregulation in this pathway in cancer – overexpression of receptors or their ligands, constitutive activation of IKK, nuclear localisation of NF-κB or reduced levels of IκB. The consequences of activation of this pathway are, however, complex, with NF-κB exhibiting both anti-apoptotic and pro-apoptotic properties depending on context [8].

Furthermore, these signalling pathways do not necessarily act independently. In the case of PI3K/Akt and NF-κB for example, there is evidence of crosstalk, with

Akt activating NF-κB or *vice versa* [11–14]. Signalling through both pathways can therefore strengthen and reinforce the survival signals.

3.4. Signalling through JAK/STATs

Another pathway which has been less well studied with respect to modulation by chemopreventive agents, but which contributes to a tumour phenotype, is that involving Janus kinases and signal transducers and activators of transcription (JAK/STAT pathway) [15,16]. Normally STATs act to transmit cytoplasmic signals and regulate gene transcription in response to cytokine or growth factor stimulation of various receptors. However, in a wide variety of cancer cells STATs (particularly STAT 3 and 5) become overactive, due to the persistent activation of upstream tyrosine kinases. Such cells can become more dependent on STAT signalling

Table 1
Modulation by dietary agents of cell signalling components involved in proliferation and apoptosis

	Inhibition of proliferation	Induction of cell cycle arrest	Induction of apoptosis
curc	↓EGFR; ↓HER2; ↓ERK; ↓JNK; ↓AP-1; ↓Egr1; ↓c-fos; ↓c-jun; ↓COX2; ↓LOX; ↓PKC; ↓ODC; ↓src; ↓myc; ↓STAT 1/3; ↓JAK1/2; ↓β-catenin; ↓IL-8; ↓PGE;	↓cyclin D1; ↑↓p21; ↑p27; ↓pRB; ↑p53; ↓↑cdc2; ↓cyclin B1/E;	↓NF-κB; ↓IKK; ↓I κBα; ↓pAkt; ↓Bcl-2; ↓Bcl-xL; ↑Bax; ↑caspase3, 8, 9; ↓BID; ↑cyt c; ↑JNK; ↑GADD153/45; ↑smac; ↑AIF; ↓XIAP; ↓IAP; ↑PPARγ;
EGCG	↓EGFR; ↓HER2; ↓FGFR; ↓PDGFR; ↓↑ERK; ↓↑JNK; ↓↑AP-1; ↓Egr1; ↓c-fos; ↓c-jun; ↓p38; ↓COX2; ↓LOX; ↓PKC; ↓ODC; ↓STAT3; ↓telomerase;	↓↑cyclin D1; ↑p21; ↑p27; ↓pRB; ↑p16; ↑p53; ↓cdc2; ↓CDK2/4/6; ↑RARβ; ↓MDM2; ↓DNMT;	↓NF-κB; ↓IKK; ↓I κBα; ↓PI3K; ↓pAkt; ↓Bcl-2; ↓Bcl-xL; ↑Bax; ↑Bad; ↑caspase 3, 8, 9; ↑cyt c; ↑JNK; ↑APAF1; ↓p70S6K; ↓XIAP; ↓Mcl-1; ↑FasL; ↓FKHR;
resver	↓HER2; ↓ERBB3; ↓ERK; ↓JNK; ↓AP-1; ↑c-fos; ↑c-jun; ↓p38; ↓MEK; ↑Fra1/2; ↓COX2; ↓LOX; ↓PKC; ↓COX1; ↓src; ↓PR; ↑ER; ↓ODC; ↓PKD;	↓cyclin D1; ↑↓p21; ↑↓p27; ↑p53; ↓↑cdc2; ↓CDK2/4/6/7; ↓pRB; ↑CDK2; ↓cyclin D2/E; ↑PIG7; ↑cyclin A/E; ↑p300/CBP;	↓NF-κB; ↓IKK; ↑SIRT1; ↓↑PI3K; ↓↑pAkt; ↓↑GSK3; ↓Bcl-2; ↓Bcl-xL; ↑Bax; ↑caspase 3,9; ↑cyt c; ↑JNK; ↑APAF1; ↓p70S6K; ↑cdc42; ↑ASK1; ↓survivin; ↑FasL; ↑TRAIL receptor;
I3C	↓EGFR; ↑2OH:16 αOH; ↓ER; ↓STAT 3; ↓ODC;	↓cyclin D1; ↑p21; ↑p27; ↓pRB; ↑p16; ↑p15; ↓CDK2/6;	↓NF-κB; ↓PI3K; ↓pAkt; ↓Bcl-2; ↓Bcl-xL; ↑Bax; ↓BAD; ↑caspase 3,8,9; ↑cyt c; ↑TRAIL receptor;

Abbreviations: AP-1 – activator protein-1; AIF – apoptosis inducing factor; APAF1 – apoptotic protease activating factor 1; ASK1 – apoptosis signal regulating kinase; BAD – BCL2-antagonist of cell death; BAX – apoptosis regulator; Bcl – B-cell lymphoma, anti-apoptotic proteins; BID – BH3-interacting domain death agonist; CBP – CREB binding protein; cdc2– cell cycle controller 2; CDK – cyclin dependent kinase; COX-1/2 – cyclo-oxygenase 1/2; cyt c – cytochrome c; DNMT – DNA methyl transferase; EGFR – epidermal growth factor receptor; ERBB3 – EGFR family member; ER – estrogen receptor; ERK – extracellular regulated kinase; FasL – Tumor necrosis factor ligand superfamily; FKHR – forkhead transcription factor; HER2 – EGFR family member ERBB2; IL-8 – interleukin 8; erg-1 – v-ets avian erythroblastosis virus E26 oncogene related, transcription factor; FGFR – fibroblast growth factor receptor; Fra1/2 – AP-1 family transcription factors; GADD – growth arrest and DNA-damage-inducible; GSK3 – glycogen synthase kinase; IAP – inhibitor of apoptosis; IκB – inhibitor of κB; IKK – IκB kinase; JAK – janus kinase; JNK – c-jun N-terminal kinase; LOX 5′-lipoxygenase; MDM2 – transformed 3T3 cell double minute 2, p53 binding protein; MEK – mitogen activated protein kinase kinase; NF-κB – nuclear factor kappa B; ODC – ornithine decarboxylase; 2OH: 16αOH – ratio of 2:16α-hydroxyestrone; p70S6K – ribosomal protein S6 kinase; PDGFR – platelet-derived growth factor receptor; PGE – prostaglandin E; PIG7 – p53 inducible protein 7; PI3K – phosphotidylinositol-3-kinase; PKC – protein kinase C; PR – progesterone receptor; PKD – protein kinase D; PPARγ – peroxisome proliferative activated receptor, gamma; RARβ- retinoic acid receptor beta; RB – retinoblastoma protein; smac – second mitochondria-derived activator of caspase; SIRT1 – NAD-dependent deacetylase, which regulates processes such as apoptosis; STAT – signal transducer and activator of transcription; TRAIL – TNF-related apoptosis inducing ligand; XIAP – X-linked inhibitor of apoptosis.

Curcumin ("curc"), EGCG, resveratrol ("resver") and I3C have each been shown to affect expression or activity of a wide range of molecules involved in cell proliferation and apoptosis. These data are derived mainly from studies of epithelial cancer cell lines. The arrows indicate an increase or decrease in the levels, phosphorylation status or activity of the various components. The indicated changes do not imply that these molecules are direct targets of the agents. For some molecules studies on different cell types give opposing results such that it is quite possible to obtain contradictory data when comparing endothelial, neuronal, non-tumorigenic or even other epithelial cancer lines. In addition the effect is often determined by the dose of agent employed. Red denotes changes that have been observed for all agents. Green denotes changes common to the polyphenols, while blue shows changes observed for I3C and at least two polyphenols. References for curcumin [2,5,6,19,21–25,27–30,32–46,88,128,131]; EGCG [2,48–52,54,56–62,64,65,68,70,72–78,80,84,85,125,127,128]; resveratrol [2,86–98,100–107,109–112,116–118].

than normal cells, making them more sensitive to inhibitors of STAT activity. Src family kinases are involved in STAT 3 activation in many breast cancers, while IL6 autocrine or paracrine loops cause constitutive activation in myeloma and prostate lines. STAT 3 and 5 signalling can contribute to malignancy by preventing apoptosis through increased expression of anti-apoptotic proteins Bcl2 and Bcl-xl. Other anti-apoptotic or cell cycle regulatory proteins such as Mcl-1, survivin, c-myc, cyclins D1 and D2 and p53 are also targets. The JAK/STAT pathway contributes to a number of biological responses, including survival, proliferation, angiogenesis and immune evasion. Aberrant signalling through this pathway can occur as a result of altered GFR, STAT and src activities, as well as downregulation of tumour suppressors, suppressor of cytokine signalling (SOCS1) [17] and protein inhibitor of activated STAT 1 (PIAS) [18].

4. Inhibition of survival signalling by dietary agents

4.1. Inhibition of signalling through GFR/Ras/MAPK

The three polyphenols, curcumin, EGCG, and resveratrol have all been shown to downregulate ligand binding to, or phosphorylation of, GFRs, including the EGFR family, FGFR and PDGFR. This leads to downregulation of the MAPK cascade and ERK, resulting in decreased transcriptional activity via AP-1. There is also evidence of downregulation of signalling through JNK and p38 (see Table 1 for references).

4.2. Inhibition of signalling through PI3K/Akt and NF-κB

Curcumin has an impact on signalling through PI3K/Akt and NF-κB in a variety of tumour cell types (Fig. 2). It has been shown by us and others, to inhibit IKK activity. This leads to a decrease phosphorylation of IκB and nuclear translocation of NF-κB [29,38]. This can result in downregulation of cyclin D1, matrix metalloproteinases (MMPs), Bcl2, Bcl-xL, cyclo-oxygenase 2 (COX2) and inhibitors of apoptosis (IAPs (see references in Table 1). There is one report that suggests that curcumin may also directly target the p50 subunit of NF-κB [21]. The net result in many cell types is decreased proliferation and increased apoptosis.

With respect to the PI3K pathway, curcumin has been shown to inhibit phosphorylation of GFR and Akt, to enhance the growth inhibitory, anti-apoptotic effects, and in some cases reinforcing NF-κB inhibition (see references in Table 1).

Similarly we and others, have shown that I3C has the potential to modulate PI3K activity and inhibit phosphorylation of Akt [110,112,117]. There are also reports of it upregulating the cell cycle inhibitors p21 and p27 as well as the tumour suppressors PTEN and breast cancer gene BRCA1 [111,118] . It can inhibit NF-κB-DNA binding and downregulate Bcl2 and Bcl-xL (Fig. 3 and references in Table 1). Once again the net effect is inhibition of growth and induction of apoptosis. Two recent papers have suggested that the condensation product of I3C, diindolylmethane, which can be formed in the acid conditions of the stomach, may also have similar activities in prostate and breast cells [119,120].

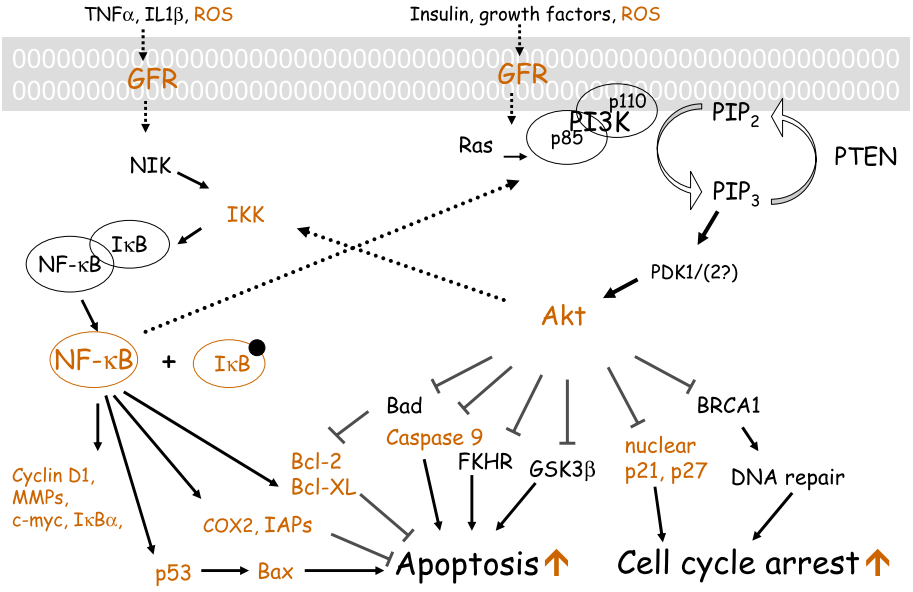

Fig. 2. Curcumin interacts with the PI3K/Akt and NF-κB pathways. The diagram illustrates the components of these two pathways that have been reported to be influenced by curcumin (coloured), but this does not imply that they are direct targets for this agent. For references see Table 1.

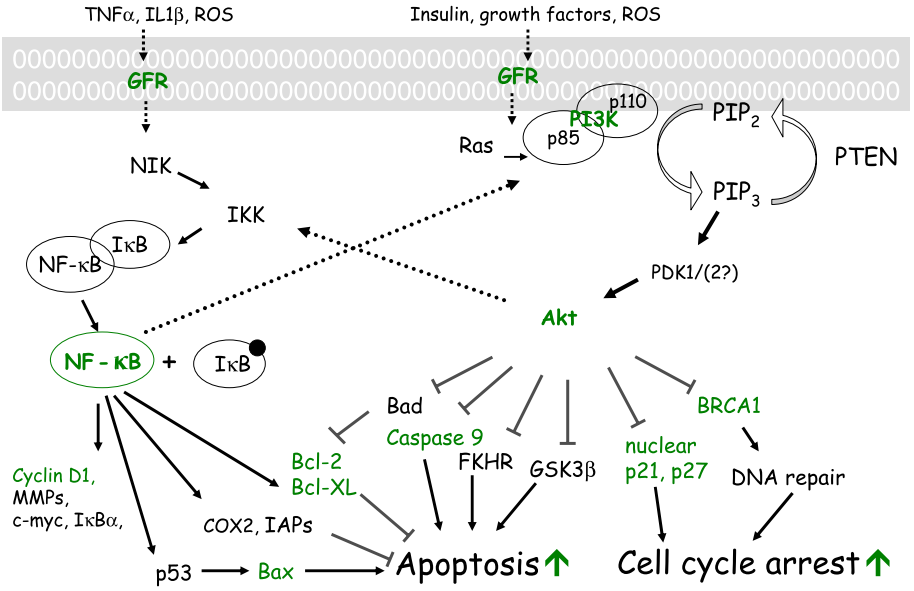

Fig. 3. I3C and signalling through PI3K/Akt and NF-κB. The diagram illustrates the components of these two pathways that have been reported to be influenced by I3C (coloured), but this does not imply that they are direct targets for this agent. For references see Table 1.

4.3. Inhibition of STAT signalling

Three of the dietary agents discussed here have been shown to downregulate STAT signalling.

Curcumin suppresses the JAK/STAT inflammatory cascade in brain microglial cells, while activating SHP2, a negative regulator of JAK1/2 [31], with suppression of COX-2 and iNOS. In multiple myeloma cells, curcumin suppresses constitutive and IL-6-inducible STAT 3 phosphorylation [20]. In T lymphocytes treated with curcumin, IL12-induced Tyr phosphorylation of JAK2 kinase, tyrosine kinase 2, and STAT 3 and 4 is inhibited [121]. In chondrocytes, curcumin was found to suppress oncostatin M-stimulated STAT1 phosphorylation and DNA binding activity, and JNK activation without affecting JAK1, 2 or 3, ERK1/2 or p38 phosphorylation [122].

EGCG inhibits EGFR signalling, STAT 3 activation, c-fos and cyclin D1 promoter activity in HNSCC and breast cells (Fig. 4), while sensitising the latter to taxol [72,73]. EGCG inhibited the growth of HNSCC cells, inducing cell cycle arrest and apoptosis. The latter, which was associated with a decrease in Bcl-2 and Bcl-xL (both of which are upregulated by STAT 3), showed an increase in the pro-apoptotic protein Bax and caspase 9 activity, and occurred via the mitochondrial pathway. To account for cell cycle arrest, EGCG decreased levels of cyclin D1 and phosphorylated Rb, while increasing p21 and p27. STAT 3 lies downstream of TNFα/EGFR signalling and can also regulate cyclin D1 and c-fos. Liang and colleagues [69] reported that EGCG could directly inhibit the binding of EGF to the EGFR and also directly inhibit the EGFR kinase activity. EGCG treatment also inhibited phosphorylation of Her2/neu receptor, which is associated with decreased STAT 3 activity, inhibition of c-fos and cyclin D1 promoter activity and a decrease in cyclin D1 and Bcl-xL (Fig. 4).

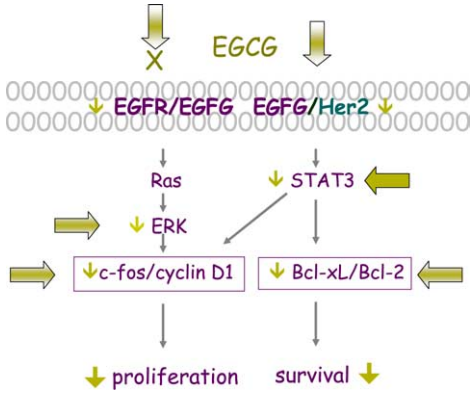

Fig. 4. EGCG disrupts EGFR signalling and downregulates STAT3. EGCG inhibits ligand binding to the EGFR and phosphorylation of EGFR and Her2. This leads to decreased signalling through ERK and STAT3, downregulation of c-fos, cyclin D1, Bcl-xL and Bcl2. The net result is decreased proliferation and increased apoptosis. Data taken from references [69,72,73].

I3C has been shown to downregulate constitutively active STAT 3 in human pancreatic tumor cell lines, followed by apoptosis [125].

In angiogenesis STAT 3 is a direct transcriptional activator of vascular endothelial growth factor (VEGF) signalling. Curcumin and EGCG can each inhibit STAT 3 activity, VEGF production and angiogenesis (see references in Table 2), and although so far a direct connection between these events has not been demonstrated, it is possible that they are linked.

Table 2
Modulation by dietary agents of cell signalling components involved in invasion and angiogenesis

	Inhibition of angiogenesis	Inhibition of invasion and metastasis
curcumin	↓VEGF; ↓STAT 3; ↑p53;	↓E-cadherin; ↓catenins; ↓MMP 2,3,9,13; ↓iNOS;
EGCG	↓VEGF; ↓VEGFR; ↓bFGF; ↓FGFR; ↓STAT 3; ↑p53; ↓67LR;	↓VE-cadherin; ↓β-catenin; ↓MMP 1,2, 7, 9,13; ↑TIMP1; ↓MT1-MMP; ↓iNOS; ↓67LR;
resver	↓VEGF; ↑p53, ↓survivin;	↓VE-cadherin; ↓β-catenin; ↓MMP 2,9; ↑GJIC; ↓iNOS;
I3C	↓STAT3;	↑E-cadherin/catenins; ↓Muc1;

Abbreviations: bFGF – basic fibroblast growth factor; FGFR FGF receptor; GJIC – gap junction intercellular communication; iNOS – inducible nitric oxide synthase; 67LR – 67kD laminin receptor; MMP – matrix metallopreoteinase; MT1-MMP-membrane-type-1 matrix metalloproteinase; Muc1 – mucin 1, transmembrane; STAT – signal transducer and activator of transcription; TIMP – tissue inhibitor of metalloproteinases; VEGF – vascular endothelial growth factor; VEGFR – VEGF receptor.
Data assembled as for Table 1. References for curcumin [20,26,31,45,47,121]; EGCG [53,55,63,66,67,71–73,79,81–83]; resveratrol [86,89,94,99,108]; I3C [113–115,118].

From available data, it appears that the polyphenols have greater inhibitory potential on angiogenesis, invasion and metastasis than I3C, although this may just reflect a lack of data for the indole. Targets common to all three polyphenols include downregulation of VEGF, matrix metalloproteinases (MMPs), β-catenin and inducible nitric oxide synthase (iNOS). It is therefore likely that resveratrol will also downregulate STAT 3, but this has not so far been examined.

5. Combination chemopreventive treatments

Evidence is fast emerging to suggest that combination therapy is often much more powerful that treatment with a single agent. While this strategy has been adopted for some years in the chemotherapeutic arena, it appears to be equally applicable in cancer prevention.

The combination could be designed to target multiple pathways, or to reinforce the effects on a particular pathway, at the same time allowing a reduction in dose of each component. For example, combination therapies that target both the EGFR and COX-2 prevent adenomas in APC^{min} mice, an effect thought to be due to the convergence of these two signalling pathways. Mice were almost completely protected from adenoma formation by a combination of the non-steroidal anti-inflammatory drug, sulindac and an EGFR inhibitor, EKB-569, allowing the effective dose of sulindac to be reduced by 75% [123].

Another approach has been to use a chemopreventive agent to lower the unwanted toxicity of an otherwise useful drug as has been shown in the case of I3C and ET743 (Trabectidin) [124]. A further use of combinations is to enhance the bioavailability of one or more components. Epicatechin increased the uptake of EGCG, inhibiting TNFα release and increasing the amount of apoptosis in human lung cancer cells. This led the authors to conclude that whole green tea was more efficacious that individual components [125].

Another study using the APC^{min} mouse model, but targeting a different pathway, showed that mice treated with white tea, green tea or sulindac had significantly fewer tumours than controls, but a combination of white tea and sulindac was even more effective. β-Catenin and two of the proteins it regulates, cyclin D1 and c-jun, were readily detected in polyps, but markedly reduced in normal-looking tissue [126]. The synergistic effects of EGCG with sulindac, against colon carcinogenesis in rats treated with azoxymethane, was demonstrated by Ohishi and colleagues [127].

Several studies have looked at the benefit of combining tea or EGCG with curcumin [128,129] and in human prostate cancer cells, a combination of low doses of curcumin and TRAIL was shown to cause increased cytochrome *c* release, activation of caspases 3, 8 and 9 and BID cleavage, where neither agent on its own was very effective [130].

Masuda and colleagues [73] found that EGCG markedly enhanced the growth inhibitory effects of 5-fluorouracil in HNSCC cells at concentrations that had been found in serum after oral administration. However, a study by Somasundaram [131] found that curcumin inhibited apoptosis induced by several chemotherapeutic drugs in human breast cancer cell lines. Using a tumour xenograft model they also showed that dietary supplementation with curcumin decreased the amount of apoptosis induced by cyclophosphamide. They hypothesised that curcumin inhibited apoptosis by inhibiting formation of reactive oxygen species and JNK activation and that exposure to curcumin might in some cases be detrimental to chemotherapy.

6. Conclusions

From the data currently available, it is obvious that dietary constituents can modulate cell growth and that in many cases tumour cells appear to be more sensitive that normal tissue. However, in interpreting the results from *in vitro* studies, care must be taken to take account of dose, cell type, culture conditions and treatment time, as each of these can affect the biological outcome. This may explain some of the apparently contradictory results that have been obtained in different laboratories.

Each of the four agents examined in detail here has a number of effects in common namely: inhibition of signalling through the EGFR family, NF-κB, and pAkt; induction of cell cycle arrest involving a decrease in cyclin D1 and phosphorylation of Rb, accompanied by upregulation of p21 and p27; and induction of apoptosis involving release of cytochrome c from mitochondria, activation of caspases 3 and 9 and downregulation of Bcl family members. Several other effects are common to the polyphenols, or to I3C and two of the polyphenols. However, depending on cell type and experimental conditions, there is evidence that the polyphenols can both up- and downregulate key molecules, including JNK, AP-1, p21, p27, cdc2, cyclin D1, p53, and PI3K. It is therefore important to determine the result of these opposing effects on biological outcome to predict unwanted effects.

As further data accumulate, it may be that other generic effects will emerge, or conversely distinct differences in activity will be identified. Because of the complexity and inter-relationships of signalling pathways, more information on the primary targets within cells for these dietary molecules is required in order to determine whether they are acting through common mechanisms to achieve the same end result.

The problem of bioavailability is of concern in trying to predict *in vivo* efficacy from *in vitro* studies. In this regard, combination treatments with low doses can be effective where individual agents are without effect. This therefore suggests that a diet providing multiple active constituents may indeed be effective, where it has been difficult to attribute any biological effect to a single agent.

Conflict of interest statement

None declared.

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