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Sulforaphane and prostate cancer interception

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Whereas much attention is focused on distinguishing newly diagnosed prostate cancers that will progress to become aggressive forms of the disease from those that will remain indolent, it is also appropriate to explore therapeutic and lifestyle interventions to reduce the risk of progression. Diets rich in broccoli have been associated with a reduction in risk of progression, which has been attributed to the compound sulforaphane. Although the mode of action of sulforaphane has been extensively studied in cell and animal models and a multiple of mechanisms that could underpin its protective effects have been proposed, recent evidence from human intervention studies suggests that sulforaphane is involved in a complex interplay between redox status and metabolism to result in a tissue environment that does not favour prostate cancer progression.

Introduction

The incidence of prostate cancer has risen substantially over the past few decades. This is likely to be caused by an ageing population and a greater degree of diagnosis, largely owing to an increase in testing for prostate-specific antigen (PSA) in plasma and the introduction of routine screening programmes, with considerable implication for healthcare costs [1,2]. The dilemma that physicians and patients face is that only a minority of these newly diagnosed prostate cancers will become aggressive in nature with serious consequences for health but, currently, it is not possible to identify the 'pussy cats' from the 'tigers' [3]. Clinical and therapeutic interventions for all diagnosed prostate cancers are neither feasible nor advisable owing to the potential adverse nature of the treatments. One option that has been widely advocated and adopted is 'watchful waiting' or 'active surveillance', in which men with a diagnosis of low-grade cancer have regular PSA testing and annual prostate biopsies, with further therapeutic or clinical intervention only taking place upon evidence of cancer progression [4]. Lifestyle interventions, such as diet and exercise, might however be effective in reducing the probability of prostate cancer progression and could be readily integrated with an active surveillance or watchful waiting programme [5].

Diets that are rich in cruciferous vegetables such as broccoli have been associated with a reduction in progression from localised to more aggressive forms of prostate cancer [6], a phenomenon referred to as 'cancer interception' [7]. These vegetables uniquely contain a group of sulfur-containing glycosides known as glucosinolates that are hydrolysed upon consumption, either by the endogenous plant myrosinase or, if myrosinase has been denatured by cooking, by putative thioglucosidases within the gut microbiota to isothiocyanates and indoles [8]. These glucosinolate hydrolysis products have been shown in animal models to prevent or delay cancer development, and are thought to underpin the health-promoting properties of cruciferous vegetables [8]. The most studied of the glucosinolate hydrolytic products is sulforaphane (1-isothiocyanato-4-methylsulfinylbutane, SF), derived from glucoraphanin (4-methylsulfinylbutyl glucosinolate) (Fig. 1) which specifically accumulates in broccoli florets [9]. Following consumption of broccoli, sulforaphane is metabolised via the mercapturic acid pathway and excreted in urine predominantly as a conjugate with *N*-acetyl cysteine. In plasma, it has been found that approximately 50% of sulforaphane is found unconjugated with other thiols [10]. If a standard portion of broccoli is consumed in which the plant myrosinase enzyme is active, plasma levels of sulforaphane and its thiol metabolites peak at about 2 μM after one hour [10]; whereas if plant myrosinase has been denatured through cooking the peak sulforaphane concentration occurs after three to four hours and is less than 100 nM [11]. High-glucoraphanin broccoli has been developed through the introgression of a Myb28 allele from a wild brassica species that delivers 3–4-times

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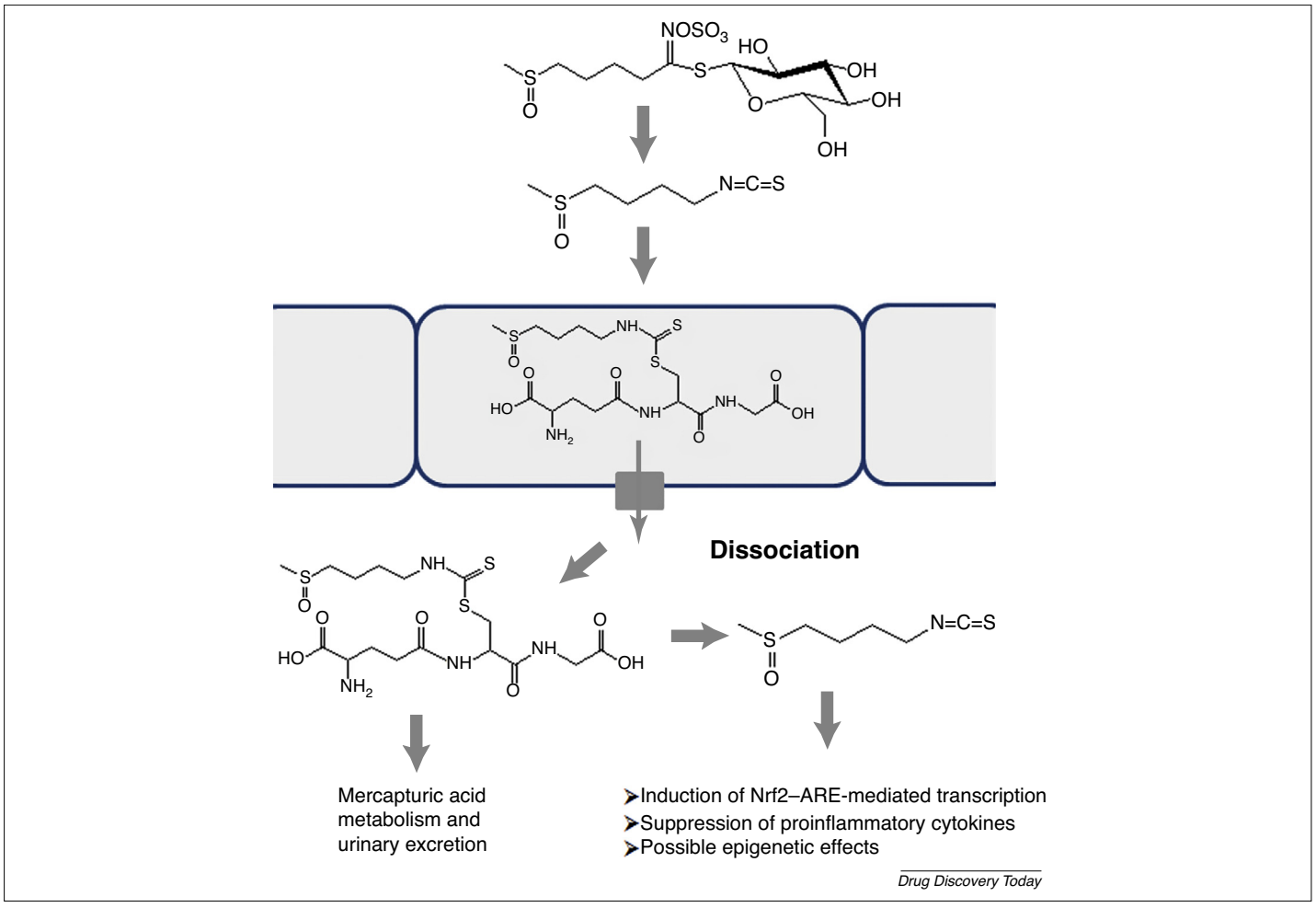

FIGURE 1

The hydrolysis of glucoraphanin that accumulates in broccoli to generate sulforaphane, and its subsequent metabolism and biological activity. Abbreviation: ARE, antioxidant response element.

more sulforaphane than standard broccoli [10,12]. Sprouted broccoli seeds have also been widely used as a means to deliver sulforaphane [13,14].

Consistent with epidemiological studies that have correlated diets rich in broccoli with a reduction in the risk of aggressive prostate cancer, sulforaphane has been shown to prevent or delay tumour development in a variety of animal models of prostate cancer, through a multitude of mechanisms [9,15–18]. However, the majority of these studies has exposed cells and animal models to levels of sulforaphane far greater than that which human tissues would be exposed to following broccoli consumption. In this review, we discuss two modes of action that we consider most likely to underpin the chemopreventive effects of sulforaphane obtained from diets rich in cruciferous vegetables, namely modification of redox status and its effects on cell signalling pathways and the suppression of proinflammatory cytokines.

The Warburg effect, prostate metabolism and the 'anabolic phenotype'

In contrast to normal differentiated cells that primarily generate ATP through oxidative phosphorylation, most cancer cells rely on aerobic glycolysis to generate their energy needs, a phenomenon known as the Warburg effect [19]. Energetic considerations suggest that this is not driven by a need to generate more ATP but by a need to provide the metabolic building blocks to make new cell membranes and associated structures, nucleotides and proteins to underpin cellular proliferation [19]. Thus, enhanced glycolysis is associated with an enhanced pentose phosphate cycle to generate NADPH required for lipid and steroid synthesis and nucleotides for DNA synthesis. Citrate is shunted out of the tricarboxylic acid (TCA) cycle, compensated for by enhanced glutamine and other amino acid anaplerosis [20]. This 'anabolic phenotype' is likely to be maintained through enhanced AKT/phosphoinositide-3-kinase (PI3K) activity that drives metabolic processes required for cellular proliferation [20]. Although mutation and inactivation of the tumour suppressor phosphatase and tensin homologue (PTEN) is a frequent occurrence within prostate tumours, PTEN can also be reversibly inactivated owing to the well characterised oxidative formation of a disulfide bridge between Cys-71 and Cys-124 in the active site of the enzyme following an increase in the oxidative status of the cells and tissues [21] (Fig. 2). Thus, lifestyle factors that increase oxidative stress such as diets rich in branch-chain fatty acids and a sedentary lifestyle combined with ageing can enhance AKT/PI3K signalling and drive the anabolic phenotype that contributes to cell proliferation. Important other proteins that could contribute to this anabolic phenotype are also redox sensitive, of

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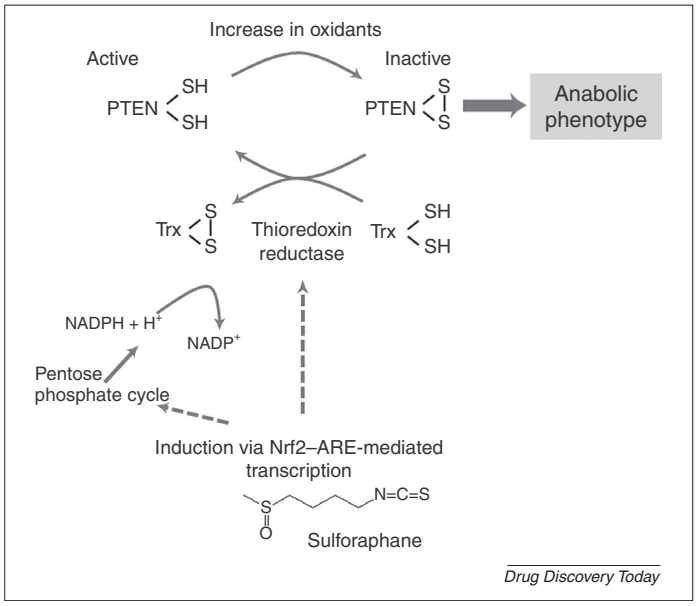

FIGURE 2

The inactivation of phosphatase and tensin homologue (PTEN) through enhanced oxidative stress, and its reversal through induction of thioredoxin reductase and NADPH synthesis as a result of sulforaphane-mediated Nrf2–antioxidant response element (ARE) gene induction.

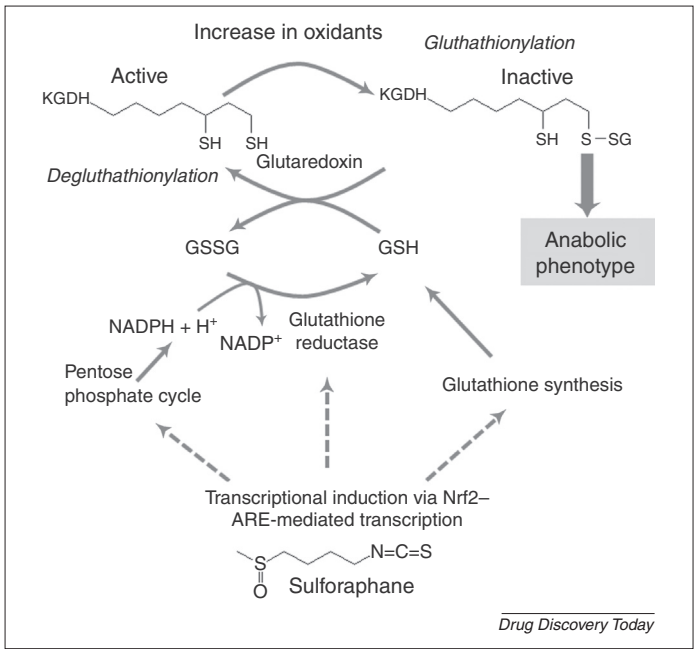

FIGURE 3

The inactivation of α-ketoglutarate dehydrogenase (αKGDH) through glutathionylation of the lipoic acid subunit, and its reversal through the coordinated induction of glutathione synthesis, NADPH synthesis and glutathione reductase, all of which are mediated by Nrf2–antioxidant response element (ARE) gene induction.

which α-ketoglutarate dehydrogenase (αKGDH), the key regulator enzyme of the TCA cycle, might be of particular importance. Increase in oxidative status results in the reversible inactivation of αKGDH through glutathionylation [22] (Fig. 3), preventing the catabolic oxidation of citrate within the TCA cycle and thus further driving the anabolic phenotype.

Prostate cancer usually arises in the peripheral zone of the prostate, which is dominated by epithelial secretory cells. These cells have three metabolic adaptations to facilitate the secretion of large amounts of citrate into the prostatic fluid. Firstly, these cells import Zn^{2+} which inhibits the activity of the enzyme m-aconitase preventing oxidative catabolism of citrate. Secondly, they import aspartate to replenish TCA intermediates and, thirdly, they increase glycolysis, presumably to compensate for the lack of oxidative phosphorylation [23–25]. Thus, there are similarities between the metabolism of a cancer cell and a normal prostate epithelial cell, which can be regarded as metabolically primed for carcinogenesis, and could partially explain the high incidence of prostate cancer in the ageing male.

Modification of redox status and cell signalling pathways

Sulforaphane is primarily a pro-oxidant. Once absorbed from the bloodstream into cells it spontaneously reacts with glutathione and other thiols enhancing the oxidative status of the cells leading to a brief increase in reactive oxygen species (ROS). The consequence of this change in oxidative status is the rapid activation of the Nrf2–antioxidant response element (ARE) antioxidant defence system that results in the increase in the transcription of many genes that are associated with restoring the redox status of the cells and preventing damage by ROS [26]. In the homeostatic state, the transcription factor Nrf2 is tethered to the cytoplasmic protein kelch-like ECH-associated protein 1 (KEAP1) and continually targeted for proteosomal degradation. SF induces conformational changes in KEAP1, either through direct interactions with sulfhydryl groups of KEAP1 or indirectly through the change in cell redox status, resulting in the separation of Nrf2 from KEAP1 and its translocation to the nucleus where it dimerises with Maf and binds to ARE resulting in the transcription of a large number of genes associated with antioxidant response [27]. Much of the diverse and apparent contradictory nature of the activity of SF can be explained by this biphasic response to SF – an initial increase in oxidative stress caused by glutathione depletion, followed by the Nrf2–ARE-mediated transcription of antioxidant genes [26,28] (Fig. 4).

The initial increase in ROS can activate cell-signalling pathways leading to mitochondrial dysfunction and apoptosis, as has been demonstrated by exposing many cell lines to sulforaphane [9]. Although certain cancer cells can indeed be more susceptible to oxidative bursts, owing to possibly a higher level of basal ROS or impaired antioxidant defences, induction of oxidative stress of such magnitude in non-cancerous healthy cells would risk lipid peroxidation, oxidation of amino acids and DNA damage. Moreover, although the level of sulforaphane required to induce levels of oxidative stress sufficient to induce apoptosis is variable between cell lines, it is substantially higher than that which could be achieved through dietary intake of cruciferous vegetables and is unlikely to underpin the ability of these vegetables to reduce cancer progression.

The immediate and short-lived burst of oxidative stress is followed by a sustained reduction in oxidative stress owing to Nrf2–ARE-mediated transcription of genes associated with functions such as antioxidant activity, free radical and xenobiotic detoxification, glutathione and NADPH syntheses, DNA damage recognition and inhibition of inflammation [27]. Chromatin

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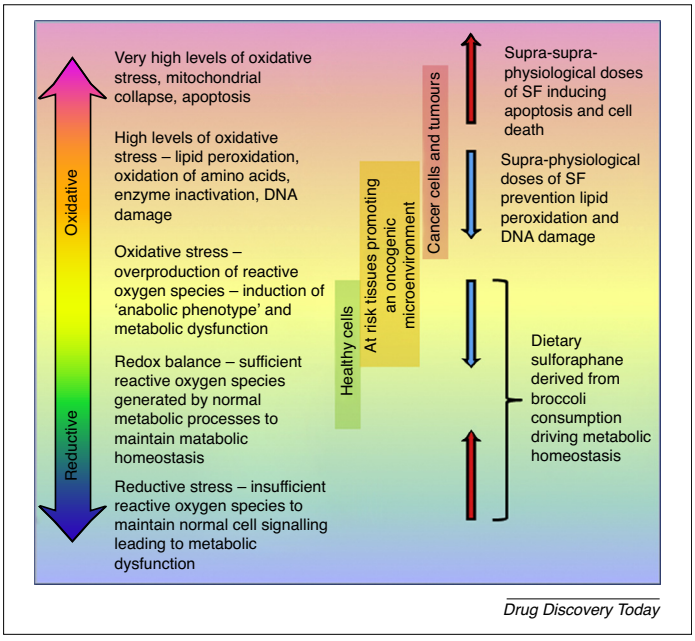

FIGURE 4

Modulation of cell redox status by sulforaphane. High levels of sulforaphane (SF) in cell and animal models can generate sufficient oxidative stress to induce apoptotic pathways and can synergise with cancer drugs to induce cell death. At lower levels the initial burst of oxidative stress is followed by induction of 'antioxidant' genes via Nrf2–antioxidant response element (ARE)-mediated transcription. This could be sufficient to prevent the damaging action of reactive oxygen species on DNA and other cell metabolites. However, in a normal dietary context the major consequence might be to 're-tune' metabolism through modulation of redox-sensitive proteins such as phosphatise and tensin homologue (PTEN) and α-ketoglutarate dehydrogenase (αKGDH).

Immunoprecipitation Sequencing (ChIP-Seq) analyses of human cell lines have suggested that there could be between 100 and 200 Nrf2-regulated genes [29]. Induction of these genes could have a direct effect because of the detoxification and excretion of potentially carcinogenic xenobiotics, such as heterocyclic amines within charred meat, or the reduction in endogenous ROS that can damage DNA, or an indirect effect through the changes in activity of redox-sensitive proteins. This would include enhancing the activity of PTEN (Fig. 2) and αKGDH (Fig. 3) and a resultant shift to a less anabolic phenotype reducing the rate of cell proliferation. In this manner, the mode of action of sulforaphane might be thought to be similar to that of exercise, in which an initial burst of oxidative stress is followed by Nrf2–ARE-mediated gene transcription [30]. Supporting evidence for the effect of a broccoli- and/or sulforaphane-rich diet on metabolism mediated by enhanced PTEN and αKGDH activity comes from a human study with high-glucoraphanin broccoli in which perturbations to the plasma metabolome were consistent in the majority of volunteers with enhanced TCA cycle activity and reduced lipid and steroid synthesis [31].

Sulforaphane and modulation of TLR4-mediated production of proinflammatory cytokines

Toll-like receptors (TLRs) are mediators of innate and adaptive immune responses to invading microbes. In particular, TLR4 senses lipopolysaccharide (LPS), an endotoxin present in Gram-negative bacteria, but is also highly expressed in a variety of cancers including prostate [32] and there is indication that it could also contribute to chemoresistance, in part through the activation of the PI3K/AKT pathway [33]. Whereas in chronic diseases, including cancer, activation of the TLR4 downstream signalling pathway is LPS-independent, stimulation of macrophages with LPS has been widely used as a model of inflammation. TLR4 exists as a complex with a co-receptor myeloid differentiation 2 (MD2). LPS interacts with both members of the TLR4–MD2 complex, triggers oligomerisation and the formation of homodimers, which switches on a signalling cascade that leads to the activation of nuclear factor kappa B (NFκB) and production of proinflammatory cytokines.

Sulforaphane has been shown to have anti-inflammatory activity in a variety of cell and animal models and suppresses the expression of inflammatory cytokines such as interleukin (IL)-6, IL-1β and tumour necrosis factor (TNF)α [34,35]. The mechanism of the anti-inflammatory action of SF has been tightly linked with suppression of TLR4 response through direct and indirect targeting of the TLR4–MD2 receptor complex. SF has been shown to suppress oligomerisation of TLR4, triggered by LPS, by forming adducts with cysteine residues in the extracellular domain of TLR4, thus preventing downstream inflammatory signalling [34]. Recently, SF was also found to form adducts with cysteine residues present on MD2, which blocked the recruitment of LPS in the TLR4–MD2 complex [36].

In addition to the direct interaction of SF with the TLR4–MD2 complex, SF can also indirectly affect TLR4 signalling by targeting the upstream PI3K/AKT. Increased TLR4 expression in response to hypoxic stress was found to be caused by phosphorylation of AKT and subsequent nuclear accumulation of hypoxia-inducible factor-1 (HIF-1), both of which were suppressed by pre-treatment with SF [37]. In prostate, specifically, SF also prevented the nuclear translocation of NFκB and induction of proinflammatory gene expression [38]. Interestingly, the anti-inflammatory activity of SF was abrogated in Nrf2 knockout macrophages [39], suggesting that the antioxidant activity of SF discussed above could have an indirect effect in preventing inflammation.

Concluding remarks

This brief review has focused on two potential modes of action that could reduce the risk of prostate cancer progression: the modulation of cell signalling pathways through changes in redox status and the suppression of TLR4-mediated transcription. Other modes of action have been described in model systems, including the inhibition of histone deacetylase activity with consequences for epigenetically mediated gene expression [40] and stability of the androgen receptor [41]. Obtaining evidence in humans as to the underlying mechanisms of how sulforaphane might be able to intercept prostate cancer progression is challenging and will require the move away from a focus on cell and animal models to short- and long-term human intervention studies.

Conflict of interest

The authors have no conflicts of interest to declare.

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