Research

Indole-3-carbinol: A glucosinolate derivative from cruciferous vegetables for prevention and complementary treatment of breast cancer

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Summary

Breast cancer is the most common malignancy in women today. Despite improved therapies, only every second woman with breast cancer can expect cure. If cancer is metastatic at diagnosis, or recurs with metastases, then treatment is limited to palliative measures only, and cure is usually not expected. Under these circumstances, quality of life as well as overall survival of the patient is significantly reduced. It is therefore advisable for patients, their physicians, and the entire society at large, to search for more effective and less toxic treatment methods and develop better prevention strategies that can reduce the burden of this cancer on the individual patient and society as a whole. Indole-3-carbinol, a glucosinolate derivative from cruciferous vegetables, seems to be a strong candidate to achieve these goals. It is abundantly available, well tolerated and non-toxic. Sufficient amounts for prevention of breast cancer can be taken up by daily consumption of cruciferous vegetables. Higher, therapeutic concentrations can be achieved with certain food supplements or functional foods. Indole-3-carbinol is known to have cancer preventive properties, reduces development and propagation of metastases, and enhances the therapeutic effects of various standard chemotherapy as well as other drugs used in conventional treatment regimens.

Key words: Breast cancer, prevention, complementary therapy, glucosin-

Introduction

Apart from cardiovascular diseases, cancer is the second leading cause of death in the Western world. Based on data from Europe and the United States, breast cancer is the most common malignant tumour in women. Today the majority of affected patients can be cured by adherence to modern therapy guidelines provided the disease is detected in its early stages. However, our standard therapies have proven unsuccessful in almost half of the patients and the tumours have relapsed. The resultant metastasization of the tumours limits the treatment strategy to palliative measures.

This notwithstanding, there have been significant improvements in recent years in the standard treatment of cytotoxic and anti-hormonal therapies, bisphosphonate treatment of bone infection as well as antibody therapies for human epidermal growth factor receptor 2 (HER2) positive tumours. Over the years, these improvements can lead to disease and symptom control depending on the individual case of the patient. However, the median overall survival of patients with remote metastatic breast cancer is still significantly low.

Against this background, it is easy to understand only too well why the health systems need to put more emphasis on effective prevention, and why doctors and patients are looking for effective additional therapies, with less toxic side effects that could lead to improved tumour control, survival, longevity as well as good quality of life.

Some of the best candidates in this respect seem to be secondary plant substances from cruciferous vegetables, such as indole 3-carbinol (I3C). This is a glucosinolate derivative containing sulphur whose metabolic products are widely known for their anti-cancer effects [34-36, 44, 46]. Detailed studies have been carried out regarding their preventive and therapeutic effectiveness in treating breast cancer and other types of cancer [7, 12, 18, 30, 62, 79]. Laboratory tests on cell cultures and animal experiments showed that I3C prevents the development of cancer in various organs in rodents, slows down the growth of existing tumours and reduces metastases [13, 14, 45, 66, 74, 82]. Besides, several partially synergistic mechanisms of action have been suggested based on experiments of I3C and its derivatives in cancer prevention and therapy. These include: inactivation of carcinogenic substances, protection against DNA damage, antiviral and antibacterial effects, anti-inflammatory effects, apoptosis induction, angiogenesis and cell migration inhibition. However, there is less clarity of epidemiological studies regarding the reduction of the risk of breast cancer in humans by I3C. For example, a meta-analysis of 8 prospective studies from the United States, Canada, the Netherlands and Sweden, could not establish any significant correlation [73]. Nonetheless, a recent meta-analysis of 13 epidemiological studies found a significant reduction in the risk of breast cancer with increased ingestion of cruciferous vegetables [53]. Different case and cohort studies also showed that women with higher consumption of cruciferous vegetables have a significantly lower risk of suffering from breast cancer [7, 34, 84]. Moreover, the study entitled "Women's Healthy

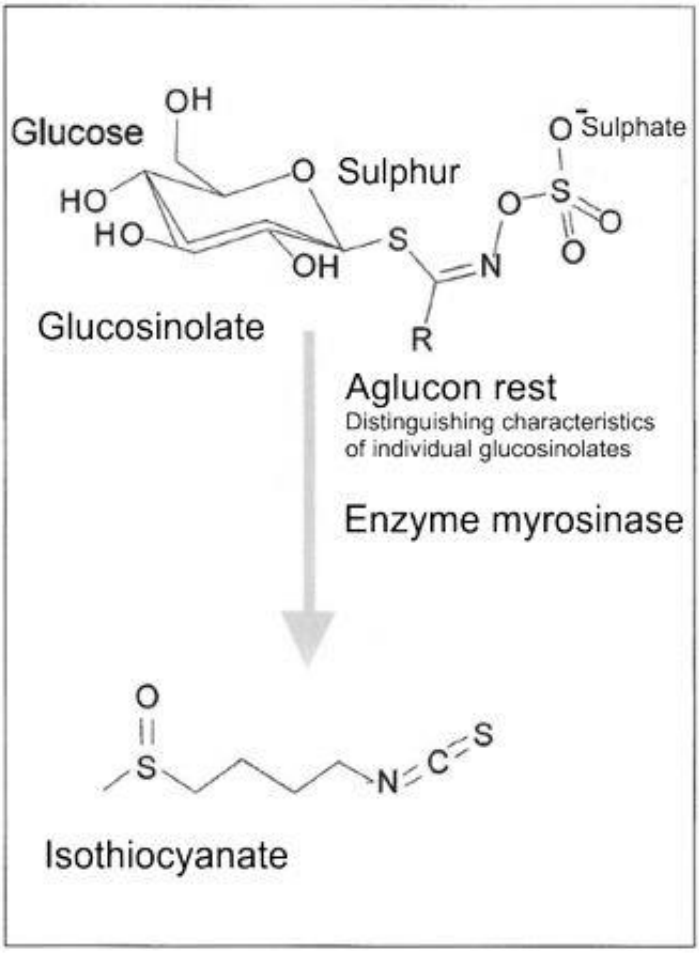

Fig. 1: Glucosinolate structure

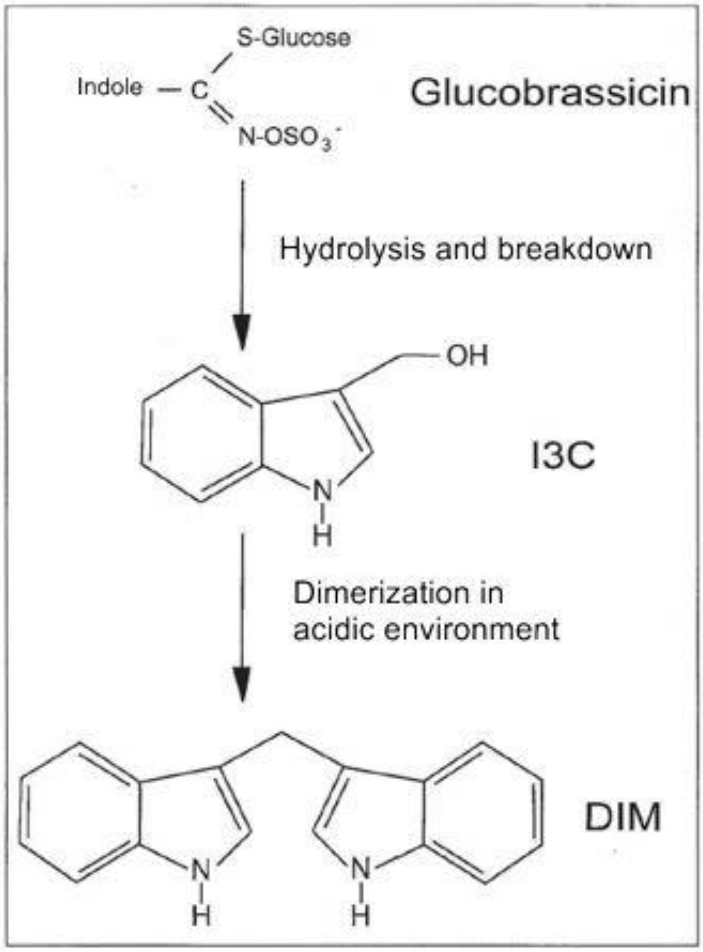

Fig. 1: Glucobrassicin catabolism to I3C and DIM

Eating and Living (WHEL)", in which more than 3,000 breast cancer patients participated, offered proof of additional reduction in the risk of recurrence when tamoxifen therapy was supplemented by the concomitant intake of cruciferous vegetables [78].

It may appear premature, based on this data, to give credence to I3C in complementary therapy of breast cancer and prevention. This notwithstanding, it is apparent that an increasing number of anxious and concerned patients and their doctors trust this substance in achieving their goal of effective prevention, improved tumour control, longer survival and a good quality of life. It is therefore the objective of this article to point out and discuss the new scientific findings regarding I3C.

Chemistry and Biology of I3C

I3C is produced by the metabolism of glucosinolates (glucobrassicin), which occur mainly in cruciferous plants. All glucosinolates have a typical basic structure, consisting of a glucose unit, a sulphur group, a group of aglucon products and a sulphate group (fig. 1). Over 150 different glucosinolates have hitherto been identified and described. These differ only in the aglucon products, which can have an alkyl, alkenyl, aryl or indolyl structure and are ultimately crucial for the physiological effects of the substances in the group.

The development of I3C occurs in successive stages. After splitting glucose (glycolysis) from the glucosinolate structure, in the initial stage, an unstable intermediate form is developed from which sulphur cyanate is separated in the second stage and thus the indole-ring of I3C is created. This differs from the aliphatic or aromatic isothiocyanates (such as sulforaphane from broccoli or PEITC from cress), due to the discarded cyanate.

The source of I3C for humans is mainly through the consumption of cabbage plants such as broccoli, Brussels sprouts or kale. Highest levels of I3C can be found in the seeds or young sprouts of the plants. During the preparation and consumption of cruciferous vegetables, plant cells are broken up and the glucosinolates metabolised by myrosinase (ß-thioglucosidase) into mustard oil, also referred to as isothiocyanates. In an acidic environment (e.g. gastric juice) I3C dimerizes to 3, 3'-diindolylmethan (DIM), which is the most important condensation product and pharmacologically active substance of I3Cs (fig. 2).

For more than 25 years, I3C/DIM and other glucosinolate derivatives have been the focal point of medical research due to their varied positive influence on the occurrence of cancer. Indeed, I3C and DIM serve as blueprints for the development of novel cancer therapeutics. They offer the link between orthodox medicine and evidence-based naturopathy where mustard plasters or cabbage poultices have been applied for generations for inflammatory processes, and also for cancer treatment.

Principles of I3C action	Mode of action	Molecular level	Synergisms
Epigenetic protection	Deactivation of oncogenes, activation of tumour suppressors by acetalization	HDAC, DNMT COX-2 ▼	
Protection from free radicals, Detoxification, DNA repair mechanisms	Breakdown in conversion of procarcinogenes to carcinogenes, detoxification systems are activated, DNA damages are compensated	GST, BRCA1 ▲	
Inhibition of hormone-dependent tumour growth	Hormone intake in the cell and transport into the cell nucleus is suppressed, no activation of hormone-dependent genes	ERb ▲ ERa, AhR ▼	Tamoxifen Herceptin
Inhibition of cell division (cell cycle arrest)	Inhibition of protein kinase, induction of inhibitors of kinases, stopping of regular organisation of tubulin, stopping of cell division	P21, P38 ▲ CDKs ▼	Paclitaxel Vinblastin
Inhibition of Neoangiongenesis, Reducing metastasis	Reduced organization of epithelial cells and formation of capillaries, inactivation of growth factors	Catenine ▲ VEGF ▼	Erlotinib Bevacizumab
Induction of apoptosis	Mitochondrial membrane depolarisation, cytochome C release, invalidation of cellular mechanisms and protection	Survivin, Cyclin D1, NF KappaB ▼ Caspase 9 ▼ P38, Bax, P21 ▲	
Increase of chemosensitivity	Overcoming resistance to therapeutics	NF KappaB ▼	Taxotere, Taxol Gemcitabin

HDAC	Histone deacetylases
DNMT	DNA methyltransferase
COX-2	Cyclooxygenase-2
ERb	Estrogen receptor beta
ERa	Estrogen receptor alfa
AhR	Aryl hydrocarbon receptor
P21	Inhibiting protein 21
P38	Inhibiting protein 38
CDKs	Cyclin dependent kinases
VEGF	Vascular endothelial growth factor
NF-kappaB	Nuclear factor kappa B
Bax	Cofactor suppressor protein P51

▲ activated, induced or highly regulated
▼ inactivated, repressed or degraded

Fig. 3: Pleiotropic mechanisms of action of I3C and DIM as well as their synergistic effects with established pharmaceutical drugs.

Bioavailability of I3C/DIM

Isothiocyanates and thiocyanates have exhibited a good bioavailability in animal experiments (CD-1 mice). With oral administration, I3C and DIM get quickly absorbed and distributed in tissues with good blood circulation such as heart, kidney, lung, liver and brain [8]. In humans, however, only the derived condensation product, DIM, was traced in serum after oral administration of 600 mg to 1,000 mg of I3C [69]. This suggests that DIM is responsible for the physiological effects of I3C.

An intake of 600 mg of I3C every day through the consumption of cruciferous vegetables is hardly possible. One would have to devour circa 0.5 kg of sprout material as well as 12 g of other glucosinolates. This would have either a toxic or at least, a higher laxative effect. The inclusion of I3C in cancer therapy therefore requires the use of isolated, enriched or chemically synthesized I3C.

Therapeutic effects - Pleiotropic mechanisms against cancer

I3C and DIM have detoxifying, anti-inflammatory and antioxidant effects, which play an important role in the prevention of breast cancer [33, 40, 41]. Furthermore, they induce programmed cell death (apoptosis) of cancer cells, which commonly represents the indispensable prerequisite for effective cancer therapy. Extensive studies show the interaction of I3C/DIM with multiple signalling cascades [1, 15, 19, 24, 29, 37, 38, 42], which ultimately lead to the apoptosis of cancer cells of different origin. This concerted action of different metabolic pathways (pleiotropism) elevates glucosinolates to one of the highly valuable natural components in the prevention and treatment of cancer (fig. 3).

These positive effects are complemented by the capability of I3C and DIM to increase the effect of different cytotoxic substances (synergism with standard chemotherapy) as well as make cancer cells more receptive to these substances, i.e. chemosensitization [2]. In previous studies, it was possible to show the significance of I3C and DIM in improving the effectiveness of taxol, taxotere, doxorubicin and cisplatin [68]. The apoptosis rate of breast cancer was increased significantly by inhibition of the Bcl-2 gene and of the NF-kB pathway. A chemosensitizing effect of I3C and DIM was demonstrated for breast cancer with regard to the medication taxotere and taxol [2, 4, 56] as well as for other anti-tumour drugs such as cisplatin, gemcitabine, oxaliplatin, doxorubicin and vinblastine [11, 17, 80].

I3C and DIM strengthen not only the effect of cytotoxic chemotherapy drugs, but also those of herceptin, tamoxifen and bortezomib [3, 54, 76]. Evidently, the combination of these medications with I3C/DIM was more effective than the single remedy. Thus, I3C and DIM are ideal candidates for combination therapy.

I3C/DIM detoxify, improve the redox status and have an anti-inflammatory effect

Humans are daily exposed to carcinogens and it is through the consumption of cruciferous vegetables that we are able to neutralize these cancer causing agents. Sourced from cruciferous vegetables, I3C and DIM are capable of inhibiting the conversion of procarcinogens into carcinogens. In the drug metabolism context, in phase 1, carcinogen activating enzymes (CYP 450) are probably competitively inhibited by I3C and DIM [51, 84], while in phase 2, the enzymes in the detoxification centres of the liver and intestinal epithelium are activated [72]. The result is an accelerated elimination of carcinogenic substances where I3C and DIM contribute to the increased formation of glutathione in liver cells, rendering a number of toxic substances, such as aflatoxin, dioxin, and heterocyclic aromatic amines, harmless [32].

In the same vein, I3C and DIM reduce reactive oxygen species (ROS) (fig. 4). According to animal experiments, the ingestion of I3C and other glucosinolate derivatives significantly contributes to the increase of glutathione-S-transferase and superoxide dismutase [23, 52, 72], as well as other enzymes that control the oxidative status of the cell. Therefore, I3C and DIM as secondary antioxidants are effective in this context.

Ultimately, I3C and DIM have an anti-inflammatory effect. This was proven in experiments with lipopolysaccharide - induced inflammation [16, 43, 81]. In the process, DIM inhibits the NF-kB activity as well as pro-inflammatory cytokines such as the TNF-alpha, IL-1-beta and IL-6. This detoxifying, antioxidant and anti-inflammatory properties of I3C and DIM are the mechanisms responsible for the reduction of cancer risk.

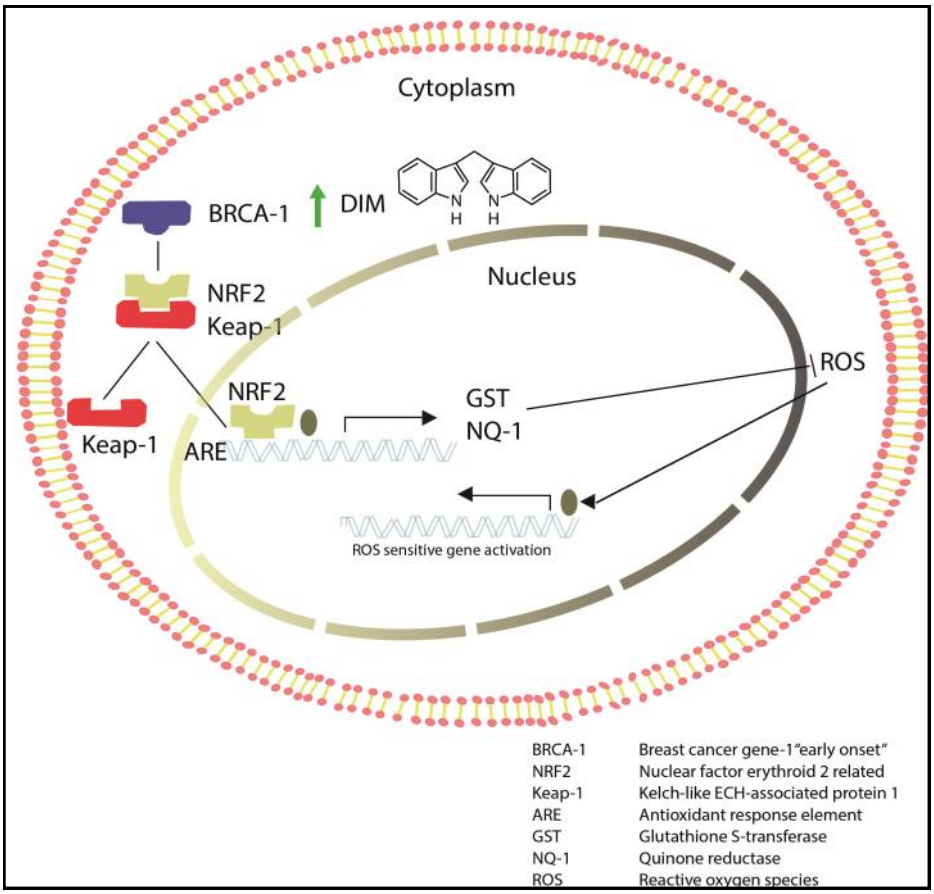

Fig. 4: Antioxidant effect of DIM is carried out by activation of phase II enzymes such as GST or NQ-1. The NRF2-Keap-1 complex is resolved by the induction of BRCA-1. NRF2 enters the nucleus and binds to the ARE promotor region. This leads to the induction of the transcription of phase II detoxifying enzymes such as quinon reductase or glutathione-S-transferase, which make reactive oxygen molecules harmless.

I3C and DIM inhibit cell division and promote apoptosis of cancer cells

Cell division is essential in the growth of tumours. Like all cells, tumour cells also go through the 4-phase cycle; G1, S, G2 and M. The phase sequence is controlled by a number of different protein kinases and their inhibitors. I3C and DIM intervene at different points in this circuit [55, 61, 65, 71, 75] and thereby often affect several signal cascades. Thus, I3C and DIM inhibit different protein kinases, such as CDK2, cyclin E and cyclin D1 or stimulate the production of specific inhibitors for cell division, for example p21 WAF1, and p27 KIP1 (fig. 5).

These effects lead to the termination of cell division and an interruption in the G1 phase and therefore to a halt in the duplication of chromatids and subsequent mitosis. Finally I3C and DIM inhibit "nuclear factor kappa B" (NF-κB) and different-dependent genes. They also highly regulate the p53 gene and reduce the mitochondrial membrane potential, thus finally leading to the apoptosis of cancer cells [15, 31, 67, 78].

I3C and DIM inhibit the formation of new blood vessels in the tumour

The overexpression of angiogenic factors such as vascular endothelial growth factor (VEGF), interleukin-6 and matrix metalloproteinases (MMP-9), is closely correlated with carcinogenesis and metastasis [5, 25, 26, 47, 50]. Tumour cells stimulate the formation of new blood vessels (neoangiogenesis) that provide various growth factors in the cell environment. In normal cells, the protein kinase G (PKG) regulates the "angiogenic switch" (beta catenin), which stimulates the formation of new vessels. In cancer cells, however, the production of the PKG is either regulated downwards or comes

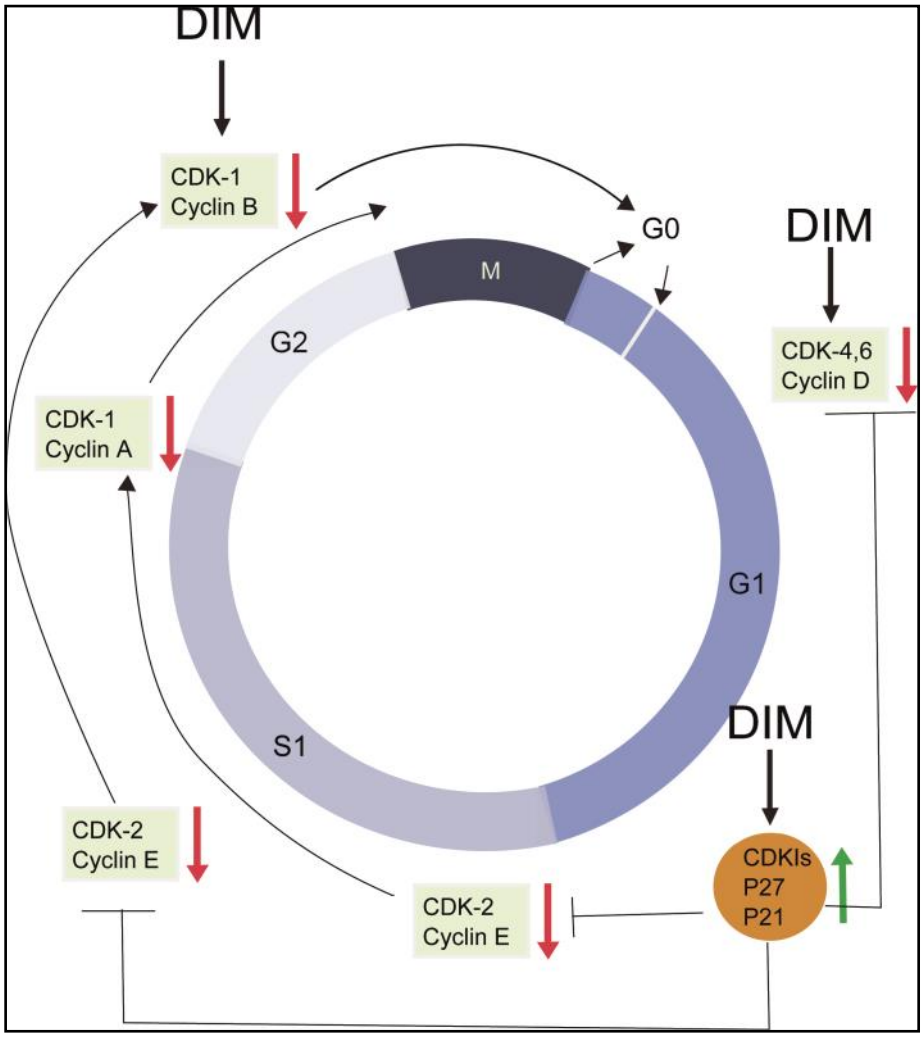

Fig. 5: DIM inhibits several protein kinases and breaks the cell cycle.

to a still-stand. The regulatory impact of this enzyme is thus eliminated and leads to unstoppable vascular regeneration. I3C, DIM and sulphoraphan compensate for the failure of PKG by stimulating the degradation of beta-catenin, reducing the secretion of VEGF, IL-6 and MMP-9 as well as inhibiting the growth of endothelial cells [9, 14, 22, 48, 49, 57]. At this point I3C and DIM intervene at the protein level of the cell without affecting the transcription of the genes involved.

Anti-angiogenic pharmaceuticals such as cetuximab, bevacizumab and erlotinib have been successfully used for several years in cancer medicine. They are aimed directly at fighting the VEGF or epidermal growth factor (EGF) receptors. So far, a synergistic effect has been proven for erlotinib and I3C/DIM [6].

I3C/DIM creates a healthy estrogen balance

Estradiol is the dominant and most effective estrogen in the human body. It is broken down into two metabolic pathways: on the one hand to the less potent 2-hydroxyestrone (estriol) and on the other to the potent and toxic metabolites 16-alpha-hydroxyestrone. The weaker estriol is considered the better estrogen, because it can block the strong signs of growth of the more active estradiol by competitive inhibition of the estrogen receptor.

I3C and DIM reduce the conversion of estradiol into the dangerous 16-alpha-hydroxyestrone, which is regarded as a tumour promoter [27], and thereby inhibit the development of breast cancer and other types of cancer. Women with higher levels of 2-hydroxyestrone and low 16-alpha-hydroxyestrone develop much less breast cancer than women with an inverse ratio of these metabolites. A favourable ratio of the two estrogen metabolites is now regarded as good cancer protection [28, 59, 60]. I3C and DIM work thus, in a positive way on the estrogen metabolism, by creating a favourable ratio of the two estrogen metabolites [61] (fig. 6). This positive hormone modulation is associated with a reduced risk of breast and cervix as well as prostate carcinomata.

For a long time the synthetic antiestrogen tamoxifen has been used in breast cancer therapy to reduce metastases and contribute to lifespan extension. I3C and DIM have similar effects as tamoxifen. However, I3C positively inhibits the growth of estrogen receptors in breast cancer cells more than tamoxifen (90% vs. 60%) [20].The combination of I3C and tamoxifen positively inhibits estrogen receptors in breast cancer cells (MCF-7) more effectively than either of the two substances alone. The growth of estrogen receptor negative cells is blocked by I3C by about 50%, while tamoxifen shows no effect [21]. Finally, whereas I3C activates specific signal proteins in the cancer cell (e.g. p21), which control cell division; tamoxifen shows no effect on p21 [21]. It is apparent that tamoxifen and I3C develop different mechanisms for the control of breast cancer. It is therefore recommended that also here a combination therapy be provided for.

Summary and closing remarks

The importance of cruciferous phytochemicals, particularly I3C and DIM is inherent in their potential for prevention and therapy of breast cancer and other types of tumour. Besides the detoxifying, anti-inflammatory and antioxidant effects of I3C and DIM, which play a role in the prevention of cancer, it is particularly the pleiotropic effects on different pathways of tumour cells that have brought these substances in the focus of research. I3C and DIM exert their effects on various corridors during cell division (for example: NF-κB, Akt, FoxM1, uPA-uPAR, survivin, BCL2, GSK-3beta / beta-catenin) and thus lead to cell cycle arrest and apoptosis. In this regard, NF-κB seems to play a special role (fig. 7).

In contrast, classical chemotherapeutic drugs are aligned only to a single target (molecule or signalling pathway). Classical chemotherapy typically leads to an efficient inhibition of specific targets. Initially, it slows the progression of the cancer. However, the cancer cells quickly switch to alternative pathways and can therefore survive. Thus resistance to chemotherapy develops and tumour growth continues. Indoles, such as DIM and I3C, modulate multiple targets and affect also the alternative signalling pathways required by tumour cells to survive. This capability to facilitate the switching off of "cross-talk" between the signalling pathways makes I3C and DIM ideal candidates for effective combination therapy against breast cancer.

For example, DIM renders various types of cancer in humans sensitive to taxol, taxotere, oxaliplatin, and gemcitabine. Thus, a reduction of these toxic chemotherapy doses could be undertaken to allow an improvement of the quality of life for the patient. However, prospective studies that support this finding are still lacking. Moreover, a synergistic effect between I3C/DIM and erlotinib, herceptin as well as tamoxifen could be established and make a combination therapy of these medicaments meaningful. Unfortunately, scientific evidence for the benefits of such a combination is still missing as well. Finally, I3C and DIM as well as some synthetic derivatives can also increase the sensitivity of cancer cells to radiation. This is a pointer to the possibility of a dose reduction of this type of cytotoxicity treatment.

Due to the location of most effects of I3C and DIM in the signalling pathways, independent of the cell type and the type of cancer, a vast clinical potential is available for successful and less toxic current therapies through the combination of suitable and optimized dosages. In this regard, I3C and DIM meet important prerequisites for appropriate applications in complementary oncological treatment (fig. 8).

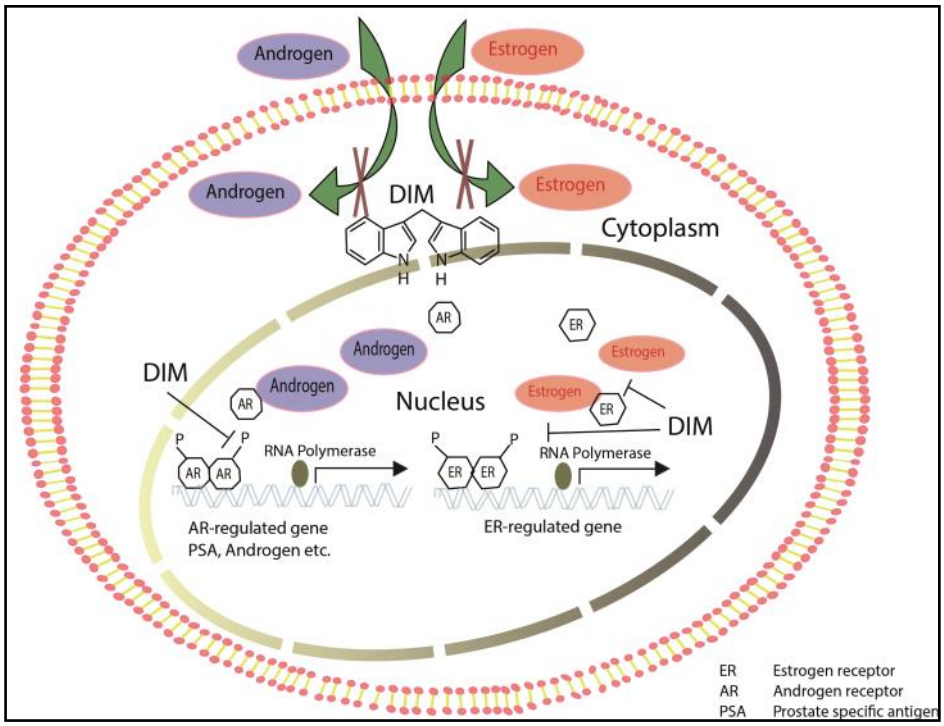

Fig. 6: DIM modulates estrogen and androgen effects. It modulates the androgen and estrogen receptors, and thus inhibits the absorption of these hormones in the cell. DIM causes the interruption of signal transduction and prevents the activation of hormone-dependent genes in the cell nucleus, which then can no longer be transcribed.

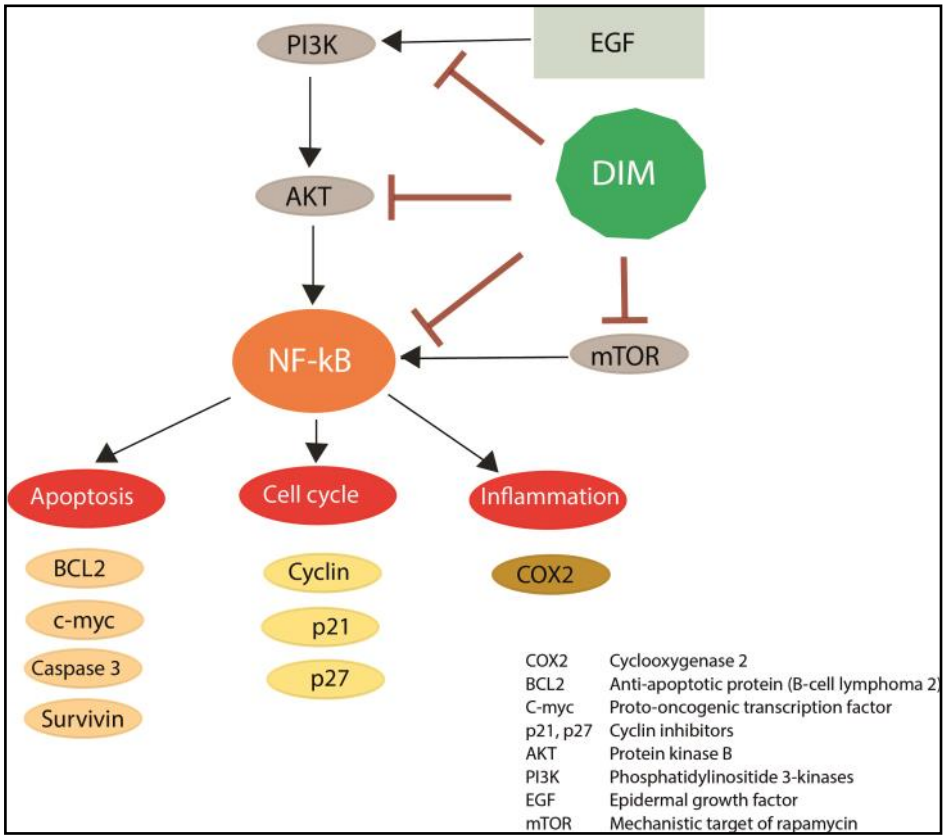

Fig. 7: DIM's effect on apoptosis, cell cycle and inflammation is carried out largely through the direct inhibition of NF-κB signalling cascade. DIM inhibits not only the activating kinases (PI3K, AKT and mTOR), but also the transcription factor itself. Consecutively the activity of BCL2, c-myc and survivin are reduced whereas caspase-3 is induced, the cyclin and COX2 inhibited and cyclin inhibitors activated, leading to cell cycle arrest and a reduced inflammatory response.

13C fulfills the basic prerequisite for complementary breast cancer therapy
Efficacy in estrogen-dependent tumors (breast, uterus, cervix, ovaries)
Efficacy in estrogen-independent tumors (prostate, colon, liver, lung)
Oral bio-availability, optimal efficacy after gastric passage
Good tolerability with long-term use
Pleiotropic effects
Positive synergies with complementary biological and synthetic therapeutics
Side effect free long term safety

Fig. 8: I3C and DIM - Candidates for prophylaxis and complementary oncological therapy in breast cancer.

Outlook

Both I3C and its phytonutrient DIM offer an excellent framework for the development of synthetic derivatives with pharmacological activity against cancer cells. Certain tetramers or alkylated indole derivatives show a similar efficacy spectrum as I3C/DIM but with 100-fold higher efficiency than the natural substances [70]. These synthetic analogues, similar to the natural "parents", affect multiple signalling pathways in tumour cells. Synthetic analogues do not affect healthy cells and thus provide the good chance for the development of new medicament with improved efficacy and without toxic side-effects.

It should be mentioned that some older reports about I3C have found no protection of indole substances against chemically induced carcinogenesis in animal experiments nor even described any activation of tumour genesis [10, 39, 63, 85]. In our opinion, the research results of the last 10 years have particularly exhibited the clear preponderance of positive effects of I3C and DIM in relation to the prevention and complementary treatment of breast cancer.

Caution: Pregnant patients should refrain from taking I3C or DIM, because these substances can modulate oestrogen balance and content which are needed for the normal development of the foetus. In this context, I3C seems to be an effective anti-oestrogen on both, the ovarian and the hypothalamic level, reducing the concentrations of the luteinizing (LH)- as well as the follicle stimulating (FSH) hormone [28].

Declaration by the authors: The authors hereby declare that there was no financial motivation leading to the writing of this manuscript, nor any financial support from companies selling phytotherapeutics and dietary supplements from cruciferous plants. The graphics were inspired by and adapted according to: Banerjee, S. et al. Attenuation of multi-targeted proliferation-linked signalling by 3, 3'-diindolylmethane (DIM): from bench to clinic. Mutat Res 2011; 728: 47-66.

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