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Mini-review

Bromelain's activity and potential as an anti-cancer agent: Current evidence and perspectives

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ABSTRACT

The medicinal qualities of pineapple are recognized in many traditions in South America, China and Southeast Asia. These qualities are attributed to bromelain, a 95%-mixture of proteases. Medicinal qualities of bromelain include anti-inflammatory, anti-thrombotic, fibrinolytic and anti-cancer functions. Existing evidence derived from clinical observations as well as from mouse- and cell-based models suggests that bromelain acts systemically, affecting multiple cellular and molecular targets. In recent years, studies have shown that bromelain has the capacity to modulate key pathways that support malignancy. It is now possible to suggest that the anti-cancer activity of bromelain consists in the direct impact on cancer cells and their micro-environment, as well as in the modulation of immune, inflammatory and haemostatic systems. This review will summarize existing data relevant to bromelain's anti-cancer activity and will suggest mechanisms which account for bromelain's effect, in the light of research involving non-cancer models. The review will also identify specific new research questions that will need to be addressed in order for a full assessment of bromelain-based anti-cancer therapy.

1. Introduction

Bromelain is an aqueous extract of pineapple that contains a complex mixture of thiol proteases and non-protease components. Proteases constitute the major components of bromelain and include stem bromelain (80%), fruit bromelain (10%), and ananain (5%). Among non-protease components are phosphatases, glucosidases, peroxidases, cellulases, glycoproteins and carbohydrates (reviewed in [1]). Assays for the individual protease components of bromelain have recently been established thus raising the possibility of standardizing bromelain preparations [2].

Bromelain can be absorbed in human intestines without degradation and without losing its biological activity [3]. Experiments in mice showed that antacids such as sodium bicarbonate preserve the proteolytic activity of bromelain in the gastrointestinal tract [4]. Taken orally, bromelain is well tolerated in high doses (up to 3 g/day) for prolonged periods of therapy, even up to several years (citations in [3,5–7]).

The evidence for the anti-cancer activity of bromelain comes from traditional observations (in Southeast Asia), studies of animal- and cell-based models and anecdotal clinical studies. The anti-cancer activity of bromelain is attributed predominantly to its protease components [1].

So far, bromelain as a cancer treatment has not been the subject of randomized controlled clinical studies. Anecdotal clinical studies of bromelain carried out in the 1970s offer early evidence suggesting the effectiveness of high dosages of bromelain (1–2.4 g/day) for treating some

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cancers, including breast and ovarian (citations in [1]). In 1995, Zavadova et al. [8] suggested that bromelain (as part of the multienzyme preparation Wobenzym) increases neutrophil activity, based on a study using healthy volunteers taking bromelain orally. Eckert et al. [9], performed clinical studies involving breast cancer patients and healthy volunteers and observed the stimulation of immunocytotoxicity of cancer-patient-derived immune cells following oral administration of bromelain. Most of the other evidence for bromelain anti-cancer activity comes from *in vivo* studies involving mouse cancer models as well as from *in vitro* observations of human and mouse cells (cancer and normal) treated with bromelain preparations. These studies are discussed below.

More numerous than clinical studies of bromelain in cancer are clinical trials of bromelain showing its effectiveness for treating various inflammation-based conditions. These include breast engorgement during lactation [10], osteoarthritis of the knee and hip [11,12], rhinosinusitis [13], sepsis in children [14] and urogenital inflammation [15]. Interestingly, no effect of bromelain was observed in the treatment of inflammation associated with relapsing multiple sclerosis, indicating the specificity of bromelain's targets [16]. Other studies demonstrated bromelain's antithrombotic, fibrinolytic, antiedematous properties and burn debridement properties (reviewed in [1,17]). Though these are not cancerous conditions, these studies are nonetheless very important for an understanding of bromelain's anti-cancer activity. Recent research has established that chronic inflammation, immune suppression as well as deregulation of the haemostatic system are implicated in carcinogenesis. These studies, therefore, allow one to speculate that bromelain targets the pathways that are directly involved in cancer initiation, growth and progression (the mechanisms of bromelain activity and its molecular and cellular targets are summarized in Tables 1 and 2).

Existing evidence indicates that bromelain can be a promising candidate for the development of future oral enzyme therapies for oncology patients. Previously, adjuvant therapy with external proteases has produced positive results in treating cancer, alleviating therapy side effects and prolonging survival [18]. Successful bromelain-based therapy development will be advanced by understanding the mechanisms of bromelain anti-cancer activity. In the following sections we will focus on evidence for the anti-cancer effects of bromelain that involve direct suppression of cancer cells as well as modulation of inflammatory, immune and haemostatic system function. In conclusion we will discuss directions for the further research and the prospects for the bromelain-based chemoprevention and adjuvant cancer therapy.

2. The effect of bromelain on cancer cells

2.1. Growth and invasive capacity

The *in vivo* evidence is consistent in demonstrating the tumor-inhibitory effects of bromelain. In chemically-induced mouse skin papillomas, topical application of bromelain reduced tumor formation, tumor volume and caused apoptotic cell death [19]. These findings are in agreement with other observations of bromelain's role in reducing metastasis [20] and of local tumor growth [20,21], resulting in increased survival rates. The *in vivo* activity of bromelain however involves not only direct inhibition of cancer cells, but also multiple systemic factors. These will be discussed later.

In vitro bromelain treatment of established mouse tumor cell lines resulted in inhibition of cell growth and matrigel invasion capacities [22,23]. Few experiments with human cancer cells were reported. One study involving bromelain treatment of gastric carcinoma Kato III cell lines demonstrated significant reduction of cell growth accompanied by 'significant DNA perturbation' (citation in [23]). Another study involving glioblastoma primary cells and cell lines demonstrated bromelain-induced inhibition of adhesion and migration [24]. The same study established that bromelain reduced the invasive capacity of glioblastoma cells and reduced *de novo* protein synthesis. However, in contrast to the growth-inhibitory effect observed in experiments with mouse cancer cell lines and human gastric carcinoma cells, bromelain did not affect glioma cell growth and the gene expression profile [24]. The apparent difference in bromelain's effect on human cancer cells might reflect cell and/or cancer type-specificity of bromelain action. This suggests that bromelain treatment might be more efficient for some types of cancer than the others. Further studies involving a variety of human cancer cell lines as well as clinical studies are needed to elucidate this possibility.

2.2. Apoptosis and cell survival

Bromelain was shown to increase expression of p53 as well as another activator of apoptosis, Bax, in mouse skin papillomas [19]. At the same time, bromelain decreased the activity of cell survival regulators such as Akt and Erk thus promoting apoptotic cell death in tumors.

In agreement with the previous observations, bromelain's effect on cell survival regulators was shown to be cell context-dependent. In mouse cardiomyocytes, bromelain *increased* cell survival and decreased apoptotic cell death leading to protection against ischemia–reperfusion injury. Here bromelain activated the cell survival mediator Akt and deactivated Akt-dependent pro-apoptotic regulator FOXO3A [25].

3. The effect of bromelain on regulators of inflammation

Chronic inflammation contributes to the development of cancer during cellular transformation, survival, proliferation, invasion, angiogenesis and metastasis. The effects of chronic inflammation depend on the tumor type and the micro-environment of the tumor. The leading viewpoint suggests that control of chronic inflammation could reduce the incidence of cancer as well as inhibit cancer progression [26].

3.1. NF-κB, Cox-2 and PGE2

There is accumulating evidence showing the role of NF-κB signaling and over-expression in many types of

Table 1
Cellular and molecular targets of bromelain related to its anti-cancer activity.

Target	Experimental approach	Effect
Neutrophils (human, healthy donors)	*In vitro* Wobenzym treatment	↑ROS, ↑ cytotoxicity towards tumor cell lines *in vitro* [8]
	In vitro bromelain	↓Chemotaxis towards IL-8 [59]
Neutrophils (mice)	*In vivo* bromelain + thioglycollate	↓Migration towards inflammatory stimulus [59]
CD4(+) T cells, activated (mice)	*In vitro* bromelain	↓CD25 [58]
Peritoneal lavage fluid (mice)	*In vivo* bromelain + thioglycollate (inflammatory signal)	↑KC(IL-8), =IFNγ, =TNFα, =IL-4,=IL-10, =IL-6, =MIP-1α, =MCP-1, =IL-12 [59]
Macrophages (mouse)	*In vitro* bromelain treatment + IFNγ	↑TNFα, ↑NO [42]
	In vitro bromelain treatment + LPS	=NO, =TNFα [42]
NK cells (mouse)	*In vitro* bromelain treatment + IL-2 + IL-12	↑IFNγ [42]
PBMC (human, healthy donors)	*In vitro* bromelain	↑TNFα, ↑IL-1β, ↑IL-6, ↑IFNγ, ↑GM-CSF, [41]
	In vivo bromelain followed by *in vitro* assay + IFNγ	↑TNFα, ↑IL-1β, ↑IL-6 [39]
PBMC (human, healthy donors)	*In vitro* bromelain + LPS	↓TNFα, ↓IL-1β, ↓IL-6 [32]
PBMC (human, healthy donors)	*In vitro* bromelain + CD2	↑Proliferation of lymphocytes [54,55]
PBMC (human, healthy donors)	*In vitro* bromelain	↓CD44 ↓CD128a/CXCR1, ↓CD128b/CXCR2 ↓CD7, ↓CD8a, ↓CD14, ↓CD16, ↓CD21, ↓CD41, ↓CD42a, ↓D45RA, ↓CD48, ↓CD57, ↓CD62L [55]
Blood samples from healthy donors (human)	Oral bromelain	↓ LAK cells activity =monocytic cytotoxicity, ↑IL-1β, ↑PTT, =PT, =plasminogen [9]
Blood samples from healthy donors (human)	Oral bromelain (Wobenzym)	↑ROS production in polymorphonuclear neutrophils [8]
Blood samples from breast cancer patients	Oral bromelain	↓CD44 on lymphocytes ↑monocytic cytotoxicity (MAK and bMAK cell activity), =IL-1β, =NK cell activity, =LAK activity [9]
IBD biopsies (human)	*In vitro* bromelain	↓G-CSF, ↓ GM-CSF, ↓IFN-γ, ↓TNFα, ↓CCL4/MIP1β [43]
Serum of RA, OMF, HZ patients with elevated TGFβ	Pholygenzyme	↓TGF-β [62]
Serum of mice	Oral immunization with bromelain	↑Anti-bromelain antibodies [70]
	Intraperitoneal immunization with bromelain	↑Anti-bromelain antibodies [22]
Tumors (mouse; chemically-induced skin papillomas; injected tumor cell lines: sarcoma L-1, P-388 leukemia, sarcoma (S-37), Ehrlich ascitic tumor, Lewis lung carcinoma, mammary adenocarcinoma)	*In vivo* topical or intraperitoneal bromelain application	↑Apoptosis, ↓NF-κB, ↓Cox-2 [19], ↓growth, ↓metastasis [20,21]
Tumor cell lines (mouse melanoma)	*In vitro* bromelain treatment	↓Viability [22,23], ↑growth [85]
Tumor cell lines (human glioma)	*In vitro* bromelain treatment	↓CD44, ↓ integrin α3β1, ↓adhesion, ↓invasion, ↓migration, =viability [24]
Tumor cells (human monocytic leukemia)	*In vitro* + LPS	↓NF-κB, ↓Cox-2, ↓PGE2 [32]
Haemostatic system (human)	*In vivo*	↓Platelets aggregation [81]
	In vitro + thrombin/ TRAP-6/ADP	↓platelets count, ↓platelets aggregation, ↓platelets activation [83]
		↓Blood coagulation, ↑fibrinolysis, ↓thrombus formation [1]
Kidney cells (pig)	*In vitro* bromelain treatment	↓AGE product-induced genotoxicity [48]

The effects of bromelain is marked as follows: ↓ decreased, ↑ increased, = unchanged.

cancers [26,27]. Emerging evidence also suggests that depending on cell context, NF-κB can also promote tumor suppression [28]. Among multiple target genes of NF-κB is Cox-2, a key player in chronic and cancer-related inflammation [29,30]. Cox-2 is involved in the synthesis of prostaglandin E2 (PGE2), a pro-inflammatory lipid that also

Table 2
Established mechanisms of anti-cancer activity of bromelain and future research directions.

Established mechanisms	Research directions
Inhibition of tumor cell growth and metastasis	
Stimulation of apoptosis activators and inhibition of cell survival activators in tumor cells	Bromelain effects on cell survival and apoptosis regulators in human cancer cell lines and primary cells
Cleavage of CD44	Bromelain effect on tumor markers of adhesion and invasion
Regulation of inflammatory mediators	
Inhibition of NF-κB/Cox-2/PGE2 expression in tumor cells	
Regulation of inflammatory cytokines and growth factors (TNF-α, IL-1β, IL-6 and IFNγ)	Bromelain effect on TNF-α, IL-1β, IL-6 and IFNγ in cancer patients-derived immune cells
Regulation of AGE mediated pathways	Bromelain effect on RAGE expression in cancer cells; Bromelain-RAGE-mediated effect on NF-κB
Immuno-modulatory activity	
CD44-mediated activation of lymphocytes	Bromelain effect on CD44-mediated activation of cancer patient-derived lymphocytes
CD25-mediated modulation of T lymphocytes activity	Bromelain effect on CD25-dependent response of cancer patient-derived lymphocytes
Stimulation of neutrophils	Bromelain effect on ROS production in cancer patients-derived neutrophils
Stimulation of monocytic cytotoxicity	
Down-regulation of immune system inhibitor (TGFβ)	Bromelain effect on TGFβ and IL-10 expression in cancer cells
Induction of antibodies that cross-react with cancer-expressed targets	Analysis of human anti-cancer targets of anti-bromelain antibodies
Alteration of tumor micro-environment	
Reduction of immune cells infiltration	Bromelain effect on tumor infiltrate in human cancers
Changing profile of secreted mediators (chemokines)	Bromelain effect on chemokine and chemokine receptors expression in tumor cells
Regulation of haemostatic system	
Inhibition of platelets activation and aggregation	Bromelain effect on cancer patients-derived platelet activation and aggregation
Reduction of blood coagulation capacity	Bromelain effect on coagulation parameters of cancer patients-derived blood
Reduction of elevated levels of soluble fibrin	Fibrinolytic 'un-coating' tumor cells and exposing them to immuno-editing

acts as an immunosuppressant and promoter of cancer progression. By facilitating conversion of arachidonic acid into PGE2, Cox-2 was shown to promote tumor angiogenesis and progression [30]. It is considered that inhibiting NF-κB, Cox-2 and PGE2 activity has potential as a treatment of cancer and chronic inflammatory diseases.

Bromelain was shown to down-regulate NF-κB and Cox-2 expression in mouse papillomas [19] and in models of skin tumorigenesis [31]. Additionally, in human monocytic leukemia and murine microglial cell lines, bromelain was shown to inhibit bacterial endotoxin (LPS)-induced NF-κB activity as well as the expression of PGE2 and Cox-2 [32]; [33].

Molecular mechanisms mediating this effect of bromelain are still unknown. We hypothesize that bromelain-induced cleavage of cell surface markers such as CD14 [32] could initiate an intracellular cascade that negatively regulates inflammation-induced NF-κB activation and its target genes. One of the interesting possibilities to investigate is whether bromelain generates cell-permeable peptide fragments similar to the synthetic NF-κB essential modulator-binding domain peptides that possess NF-κB suppressing capacity [34].

3.2. IFNγ, TNF-α, IL-1β and IL-6

Among the secreted regulators of inflammation that are connected to NF-κB pathways and that respond to bromelain are IFNγ, TNF-α, IL-1β and IL-6. Depending on the context and micro-environment, these regulators can either stimulate tumor growth and invasion or activate immune responses and cause tumor regression [35–38].

Experimental evidence derived from analyzing peripheral blood mononuclear cells (PBMC) from healthy volunteers as well as mouse macrophages suggested that bromelain can activate TNF-α, IL-1β and IL-6 secretion in an IFNγ-dependent mechanism [39–42]. IFNγ production, in turn, was also increased in the presence of bromelain [42]. These data allow us to hypothesize that bromelain has the potential to activate healthy immune system to ensure rapid response to pathogens and cellular stress.

However, in situations when immune cells are already stimulated, bromelain reduces TNF-α, IL-1β and IL-6 secretion. This occurs in the conditions of inflammation-induced over-production of cytokines. For instance, in the presence of LPS, which has the capacity to stimulate an acute inflammatory reaction, bromelain reduced elevated TNF-α, IL-1β and IL-6 expression in human PBMC [32]. A similar effect was observed in LPS-stimulated human monocytic leukemia cell lines [32]. Reduction of TNF-α and IFNγ expression was also observed in bromelain-treated inflamed tissues obtained from patients with inflammatory bowel disease (IBD) [43].

The described data demonstrate that the effects of bromelain on cytokine expression depend on the presence of inflammation-inducing conditions. This underlines the potential of bromelain for treatment of inflammation-based pathologies. Further studies with cancer patient-derived immune cells and tumor samples are required for

further elucidation of bromelain effects on cancer-induced inflammation and immune suppression.

3.3. Receptor for advanced glycation end products (RAGE)

RAGE presents an important possible target for bromelain. RAGE is a multi-ligand receptor expressed by many cell types, including cancer. It was found to be implicated in many inflammatory disorders, regulating activation of NF-κB and its target genes [44]. Recent studies established a novel role for RAGE, linking chronic inflammation and cancer [45]. Tumors and cells in the tumor micro-environment were found to express RAGE ligands that stimulate proliferation, invasion, chemoresistance and metastasis of cancer cells [46].

Among the RAGE ligands are advanced glycation end products (AGE). AGE are generated in the conditions of oxidative stress as a result of a non-enzymatic reaction between sugar ketone or aldehyde group and free amino group of proteins, lipids and amino acids [47]. Oxidative stress is one of the causes of inflammation and generates AGE products. At the same time, the interaction of AGE products with RAGE induces intracellular ROS formation and genotoxicity that can lead to cancer [48]. AGE products are usually accumulated in the process of aging and were recently implicated in the development of cancer and diabetes [48,49]. Evidence has emerged suggesting that bromelain can diminish the cell damaging effect of AGE products [48]. This study opens an exciting opportunity for the further unraveling of bromelain-mediated control of inflammation. We speculate that proteolytic degradation of RAGE could be among the mediators of bromelain effects. RAGE degradation could potentially ensure cellular AGE protection as well as mediation of bromelain effects on NF-κB and its targets that were discussed earlier.

4. Immuno-modulatory effects of bromelain

4.1. CD44

Among bromelain-sensitive surface markers is CD44. It is expressed by cancer and immune cells and is directly implicated in cancer growth and metastasis as well as in the regulation of lymphocyte recruitment to the vascular endothelium at the sites of inflammation [50,51]. Accumulation of soluble CD44 in circulation was found to correlate with cancer aggressiveness and lymph node metastasis and serves as a diagnostic and prognostic marker [51–53].

Bromelain was shown to reduce CD44 on the surface of mouse and human tumor cells. This was accompanied by diminished cancer cell invasion and substrate attachment as well as by attenuation of *de novo* protein synthesis [22,24]. It is important to note that bromelain causes degradation of the cleaved CD44 into at least three fragments [54] thus not allowing cancer-promoting accumulation of CD44 in circulation [51].

Lymphocyte-expressed CD44 and other markers implicated in lymphocyte adhesion and activation were also found to be bromelain sensitive [9,54–57]. The bromelain-mediated CD44 cleavage was associated with increased lymphocyte activation in healthy [54] and breast cancer patient-derived cells [9]. In breast cancer patients-derived cells, reduced CD44 expression was noted alongside increased monocytic cytotoxicity [9].

Based on these studies it could be hypothesized that the bromelain-induced reduction of CD44 suppresses the adhesion of lymphocytes to blood vessels and restricts the migration of lymphocytes to tumor sites. It should, however, be stressed that the impact of bromelain on lymphocytes will be determined by multiple bromelain-cleavable targets, the full spectrum of which has yet to be characterized. For example, recent studies by Sekor et al. showed that bromelain could induce cleavage of CD25 from the surface of activated mouse CD4(+) T cells [58]. These results indicate the potential of bromelain to modulate the activity of one of the most important regulators of immune function, IL-2. The implications of this activity for human cancer progression will have to be established in further studies.

4.2. Tumor micro-environment

As well as affecting CD44 (described above), bromelain was shown to reduce surface expression of a wide range of markers regulating lymphocyte homing and migration to the sites of inflammation (Table 2, [55]). Among other classes of regulators affected by bromelain are chemokines and chemokine receptors, which direct immune cells to the tumor micro-environment and to the sites of inflammation. Bromelain was shown to reduce CCL4/MIP-1b chemokine secretion by the inflamed tissues derived from inflammatory bowel disease (IBD) patients [43], induce cleavage of chemokine receptors on neutrophils and to inhibit IL-8 induced neutrophil chemotaxis [55,59]. Based on these studies we speculate that bromelain could control tumor micro-environment through modulation of chemokine expression. Further studies involving analysis of bromelain's effect on chemokine and chemokine receptors in cancer and immune cells from cancer patients are needed to validate this mechanism.

4.3. TGF-β and IL-10

Bromelain was found to modulate the expression of one of the major regulators of cancer-induced immune suppression and inflammation, TGF-β [60,61]. Orally applied bromelain was shown to reduce TGF-β expression. Interestingly, the effect was observed in a group of patients affected by rheumatoid arthritis, osteomyelofibrosis and herpes zoster only when the blood levels of TGF-β were elevated [62]. At the same time the basal level of TGF-β expression in healthy volunteers was not affected by bromelain [62]. The mechanisms involved in the physiological regulation of TGF-β by bromelain remain to be established. We speculate that these mechanisms might include the direct proteolytic impact on TGF-β or its precursor, as well as the indirect modulation of the TGF-β binding activity of α2-macroglobulin. The latter possibility was demonstrated in the study on healthy volunteers orally treated with a cocktail of proteinases, which included bromelain, trypsin and rutoside (Phlogenzym) [63]. The study demonstrated that proteinases induced the generation of alpha2-

macroglobulin with increased TGF-β binding capacity, which in turn was suggested to contribute to TGF-β clearance [63]. We hypothesize that in cancer bromelain acts analogously. Reducing elevated TGF-β levels would decrease immunosuppressive effects of malignancies and enable re-activation of the immune system. Further research is required to test this possibility.

The *in vitro* production of another regulator of cancer-related immunosuppression, IL-10, was shown not to be affected by bromelain [64]. This evidence, however, derived from experiments with lymphocytes obtained from healthy donors and is not sufficient for a definite conclusion regarding the effect of bromelain on IL-10. Further research is necessary to clarify this issue.

4.4. Reactive oxygen species (ROS)

Bromelain can stimulate the innate immune system by activating neutrophils to produce ROS. As part of a polyenzyme preparation, bromelain was shown to stimulate ROS production and tumor cell killing properties in neutrophils *in vitro* as well as in neutrophils from healthy volunteers taking the polyenzyme preparation [8,65]. ROS are also known to act as intracellular regulators of functional activity in neutrophils and other cell types including cancer [66,67]. Bromelain's capacity to change intracellular levels of ROS would have a direct impact on the modulation of signaling both in immune and cancer cells.

These data however cannot be easily interpreted as beneficial for cancer control. There is ample evidence suggesting that activated neutrophils and excessive ROS production induce DNA damage and cancer pathogenesis. Thus increased production of ROS leads to oxidative stress conditions that are beneficial for cancer [68]. Additionally, recent studies suggest that cancer cells increase functional activity of neutrophils including ROS production [69]. However considering overall anti-cancer properties of bromelain and its environment- and cell status-determined activity it could be speculated that bromelain's activity on ROS production in cancer patient-derived cells may be directed towards cancer inhibition. Further studies are necessary to elucidate these mechanisms.

4.5. Anti-bromelain antibodies

Recent studies in mice demonstrated that oral and intraperitoneal application of bromelain could induce anti-bromelain antibodies [22,70]. Proteolytic activity of bromelain was required for an orally-induced antibody response, however it was not suppressed by it [70]. Polyclonal anti-bromelain antibodies were shown to cross-react with mammalian melanoma cells. Antibodies against bromelain analogue, fastuosain, inhibited cancer cell viability suggesting the same potential for bromelain-induced antibodies [22]. Among the possible mammalian targets of the antibodies are cathepsins B and L, cysteine proteases of the papain family that are implicated in cancer progression [22,71]. Inhibitors of cathepsins are considered as possible anti-cancer therapeutic agents [71].

We hypothesize that the induction of anti-bromelain antibodies is among the mechanisms of bromelain's anti-cancer activity. Further studies are required to appraise this mechanism of bromelain activity in humans.

5. Haemostatic system mediated anti-cancer effects of bromelain

5.1. Fibrinolytic effects

Elevated levels of soluble fibrin were found to be a prognostic marker for cancer progression. Fibrin is directly involved in inhibiting lymphocytes-tumor adhesion and decreasing cytotoxicity [72]. Additionally, tumor cells are believed to form a protective coat by polymerizing fibrin and human serum albumin. This coat is resistant to the proteolytic activity of internal proteases such as plasmin and provides tumor cells with protection against the immune system [73]. A recent study of the thrombin receptor expression in primary tumor cells [74] can provide an insight into the molecular mechanisms of such coat formation. The fibrinolytic and anti-thrombotic action of bromelain has been recognized for a long time [1,5,75]. Fibrinolytic activity of bromelain can decrease amounts of soluble fibrin in circulation. Additionally, it could be speculated that bromelain can also cause "un-coating" of tumor cells, making them visible to the immune system. More research however is needed to confirm and elucidate this mechanism further.

5.2. Anti-platelets effects

The haemostatic system is now recognized as playing a significant role in stimulating inflammation and triggering many pathological conditions, including tumor growth, metastasis and angiogenesis. A recent review provides an analysis of the current understanding of the role of platelets in pathological conditions and malignancies [76]. As well as initiating blood clotting, platelets act as mediators of chronic inflammatory responses in various pathologies such as atherosclerosis, inflammatory bowel disease and rheumatoid arthritis. In the case of malignancies, there is a reciprocal stimulatory relationship between platelets and tumor cells. Tumor cells initiate platelet activation as well as the platelet-based production of multiple factors facilitating angiogenesis. Additionally, tumor cells in various degrees possess the capacity to surround themselves with platelets, forming tumor-platelet aggregates that protect tumor cells from immune recognition. In metastasis, these aggregates also serve to facilitate endothelial adhesion and tissue invasion.

Anti-platelet drugs were shown to be beneficial in controlling malignancies and conditions caused by chronic inflammation. Thus aspirin in lower doses corresponding to its anti-platelet activity was shown to be protective for colorectal [77], breast and ovarian cancers [78]. The improvement of survival was also observed in cancer patients treated with another inhibitor of coagulation, low molecular weight heparin [79,80].

Oral administration of bromelain as well as *in vitro* assays resulted in a reduction of platelet aggregation and activation [81,82]. A recent study demonstrated that *in vitro* bromelain treatment of platelets from healthy

volunteers significantly reduced platelet count [83]. Bromelain's ability to inhibit platelet activation is associated with its proteolytic activity [84]. In addition, non-enzymatic components are also believed to be involved in bromelain's interactions with the haemostatic system [75].

We hypothesize that bromelain's anti-platelet activity can interfere with platelet-mediated cancer growth and progression and can also prevent the generation of tumor-platelet aggregates, thus "un-coating" cancer cells and exposing them to the immune system.

6. Conclusion

Traditional and anecdotal clinical evidence suggest that bromelain could be an effective anti-cancer therapeutic agent. Laboratory evidence suggests that bromelain's anti-cancer effect could be the result of a systemic response, possibly involving a variety of targets. A summary of bromelain-sensitive targets is presented in Table 1. Possible mechanisms mediating anti-cancer activity of bromelain together with the areas for future research are presented in Table 2.

The molecular mechanisms of bromelain's anti-cancer activity are not fully understood. Many are plausibly inferred from bromelain's known activity in non-cancer environments. Existing experimental evidence for bromelain suggests that its activity could be modified in relation to the environment. Therefore, analyzing bromelain in relevant physiological conditions will be critical for assessing its role in cancer. Future approaches will require more clinical studies in patients with cancer and chronic inflammatory diseases. Priority in clinical studies should be given to identifying the effects of bromelain on tumor growth and metastasis, on tumor infiltrates, on blood coagulability and most importantly on patient survival.

The studies presented in this review indicate that bromelain affects major pathways and regulators implicated in cancer. We hypothesize that its activity is directed towards normalization of physiological balance. Building on recent *in vivo* investigations, laboratory studies and anecdotal clinical evidence, further research in this area may lead to promising results for the development of bromelain-based chemoprevention and adjuvant cancer therapy.

Conflicts of interest

None declared.

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