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Bromelain as a Potential Material in Future Chemotherapy: A Review

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Abstract

Cancer is an uncontrolled cell growth-type chronic illness. It is usually associated with oxidative stress and chronic inflammation. Pineapple and its components have been shown in several studies that significantly reduce cancer risk. One of these substances is the bromelain enzyme, which exhibits anti-inflammatory, reduces metastasis which can stimulate cell death and support the white blood cell activity of some cancer cells that may reduce inflammation and minimize oxidative stress. Herein, a comprehensive review of the potential of bromelain as a treatment of chemotherapy to be employed in the future medical sector is discussed in this paper. This review study focuses exclusively on the effect of bromelain on distinct cancer cell types that use experimental approaches *in vitro* and *in vivo*.

Disciplinary: Chemotherapy, Health and Science, Plant Science.

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1 Introduction

Bromelain is a complex mixture of proteases and non-protease components found in pineapple (Ananas comosus) extracts. Protease is a part of the major components of bromelain in pineapple which consists of stem bromelain (80%), fruit bromelain (10%), and ananain (5%) (Maurer, 2001). Investigations into the use of a variety of proteolytic enzymes in pineapple plants for therapeutic purposes have been conducted. Bromelain is stable without deterioration or loss of its biological properties and well absorbed, whereby it is exposed to the gastrointestinal tract upon oral administration (Hale et al., 2005). The therapeutic effects of bromelain are dose-dependent,

and it has been shown that bromelain is both safe and reliable as a food supplement (Tochi et al., 2008). Bromelain is popularly known for a wide spectrum of therapeutic benefits, including a nutritional supplement to promote digestion, improved drug delivery and absorption, fibrinolytic effects, and wound debridement (Bhattacharyya, 2008). Bromelain also has anti-cancer properties and is able to facilitate cell death through apoptosis (Pavan et al., 2012).

Cancer is an invasive cell that tends to proliferate with uncontrolled growth. The evolution of cancer is defined by hallmarks that are dependent on the tumor suppressor genes and proto-oncogenes (Mamo & Assefa, 2019). Replicative immortality and angiogenesis are hallmarks, as is the ability to evade growth suppressors and activate invasion and metastasis, as well as resistance to cell death and angiogenesis. (Hanahan & Weinberg, 2011). Cancer cells are self-regulating and self-sufficient which is in contrast with the behavior of normal healthy cells due to DNA mutation (Torgovnick & Schumacher, 2015). It is independent of growth signaling in proliferation cause interruption of the normal homeostatic mechanism of cells in tissue (Yousef Ahmad & Carmen, 2017). Mitigation against cancer cell growth requires concentrating on the lead factors in uncontrolled proliferation, angiogenesis, metastasis, and other development factors (Doughlas & Weinberg, 2000).

Adjuvants and monoclonal antibodies are commonly used to supplement chemotherapy in the treatment of cancer. The combination therapy of two or more medicines or herbal medicinal concoctions has been of interest in the fight against diverse illnesses, in particular cancer. Nutritional supplements or herbs have long been used to regulate the immune system and enhance the efficacy of the medications that are being implemented. Various fruits have been documented with anti-inflammatory and immunomodulatory properties, including pineapple.

Bromelain is a protease found in pineapples. It has been described as having immunostimulatory and anti-inflammatory properties, as well as being easily absorbed when taken orally. It has been reported bromelain isolated from pineapple stem attributed to immunomodulatory and anti-inflammatory effects in In-vivo research treatment of P-388 leukemia, sarcoma (S-37), ascitic tumor, Lewis lung carcinoma, MB-F10 melanoma, and mammalian adenocarcinoma (ADC-755) model (Dhaval et al., 2016).

2 Effect of Bromelain on Cancer Cell

The unique properties of bromelain increase interest in its anti-cancer activity studies in deeper purpose for advanced chemotherapy technology. Its capability affects the cancer cell with numerous studies on the effect of bromelain on cancer cells have been established as shown in Table 1. Bromelain is shown to affect cancer activities via interrupting the cancer cell growth and proliferation, metastasis, inflammation, and immunodulation. Several bromelain studies have been performed, and evidence indicates that bromelain was able to treat breast, ovarian cancer and other cancers (Mohamad et al., 2019). Some investigations of bromelain studies on different types of cancers are listed in Table 2.

Table 1: Established Anti-cancer effects of bromelain

Parameters	Cellular/Molecular effect	Reference
Cancer cells growth	Bromelain reduces tumor size and metastasis	(Mohamad et al., 2019)
	Bromelain inhibits tumor development	(Hale et al., 2005; Tochi et al., 2008)
	Bromelain reduces proliferation and cell viability	(Chermahini et al., 2020; Oliveira et al., 2017)
	Bromelain induces apoptosis via activation of survival cellular proteins	(Bhatnagar et al., 2014; Bhui et al., 2009; Pillai et al., 2014)
Metastasis	Bromelain reduces metastatic foci by inhibiting platelet aggregation by endothelial cell	(Béez et al., 2007)
Inflammation	Bromelain downregulated the inflammatory-related genes	(Mohamad et al., 2019)
Immunomodulation	Bromelain enhances IFN-γ-derived signals in primary macrophages as well as macrophage cell lines.	(Manosroi et al., 2017)

Table 2: Investigation of anti-cancer properties of bromelain

Target	Experimental Approach	Result	Impact	Reference
4TI triple-negative breast cancer cell (Mice)	In vivo bromelain treatment + Cisplatin	Low weight and size of tumor, downregulates GREM1, IL-1β, IL-4, NFκB1, PTGS2, NO, IL-1β, and IL-4	Bromelain induces apoptosis, causes reduce tumor size, reduces lung metastasis of 4TI	(Mohamad et al., 2019)
Skin tumorigenesis (Mice)	In vivo bromelain treatment	Reduces the volume of tumor up to 65%, upregulate sp53, Bax, caspase 3, caspase 9, downregulate sBcl-2, Cox-2, NF-κB. Cutdown activity of ERK1/2, MAPK and Akt	Bromelain inhibits tumor development	(Bhui et al., 2009)
AGS, PC3, MCF-7 (Human)	In vitro of bromelain treatment	Suppresses growth and colony formation	Bromelain inhibits cell proliferation and colony formation	(Raeisi et al., 2019)
Malignant Peritoneal Mesothelioma (MPM)	In vitro bromelain treatment + Cisplatin	Downregulates NF-κB, p-NF-κB and p-AKT. Activates caspase 7, 8, 9, cytochromes c, and PARP	Bromelain induces cell apoptosis and autophagy	(Pillai et al., 2014)
Human Breast Cancer (MCF-7)	In vitro bromelain treatment	Reduces cell viability and cell generations	Bromelain as an anti-proliferative effect	(Fouz et al., 2013; Oliveira et al., 2017)
HEK293, MCF-7, HeLa, A549, HaCaT, EAC Mice	In vivo and In vitro bromelain treatment	Upregulates (p53, p21, Bcl2, Bax), increases ROS, loss of MMP	Bromelain induces cell apoptosis	(Bhatnagar et al., 2014)
HepG2, HT-29, A549, KB, HeLa and HuTu-80 cell	In vitro bromelain treatment	Induces apoptosis	Bromelain as anti-proliferation and induce apoptosis.	(Manosroi et al., 2017)
Prostatic Carcinoma (PC3)	In vitro bromelain treatment + Cisplatin	Upregulates p53	Inhibits colony formation and reduces cell viability	(Amini Chermahini et al., 2020)
Colorectal cancer	In vivo and In vitro bromelain treatment	Induces high levels of ROS, ATG 5/12, beclin, p62, LC3, Endo G, and caspases-3, -8, and -9	Bromelain induces apoptosis	(Chang et al., 2019)
Lung cancer	In vivo	Downregulates tPA receptor	Bromelain reduces metastasis	(Béez et al., 2007)

3 Discussion

3.1 Cell Growth and Proliferation

Regulated cell growth and proliferation are the normal processes that undergo by each normal cell. The uncontrolled cell proliferation could cause an imbalance cell cycle and leads to the formation of cancer cells. Cell cycle is a series of events that include phases of cell growth and division. The cell cycle composes of four phases which are mitosis, synthesis and two phases of gaps that correspond between synthesis and mitosis (Kastan & Bartek, 2004). Cyclins is responsible at every checkpoint of the phases for determining the confirmation of each cell to proceed to the next phase (Lim & Kaldis, 2013). In a cancer cell, the cell cycle is interrupted and irregulated as a normal cell cycle whereby the function of the cell cycle checkpoint is limited (Evan & Vousden, 2001). It leads to uncontrolled cycling even in the presence of DNA damage as a cancer cell is independent of growth signal and it will initiate the cell cycle even though cell division is unrequired. Cancerous cells have their own tendency to resist receiving growth suppression signals in order to continue to grow and proliferate through different pathways over time (Elledge, 1996).

The mechanism by which cells grow uncontrollably is dependent on oncogenes and tumour suppressor genes. The most mutated oncogene in human cancers is the RAS oncogene (DeBerardinis et al., 2008). An increase in RAS oncogene motivates more cancer cell growth and proliferation. Oncogenes are a part of cell DNA and initiate the production of proteins that are important for cell growth, cell survival, and cell activity (Gariglio, 2012). RAS stimulates the activity of several transcription factors, which results in the activation of genes involved in cell cycle progression and division. RAS gene is activated through the exchange of protein that is bound to the RAS gene which is GDP substituted with GTP. After sufficient cell growth, the RAS gene needs to be deactivated. However, the mutated RAS gene is unable to be deactivated and keep on activated due to the failure of exchange of protein factor. Continuous activation could lead to active cancer cell growth (Evan & Vousden, 2001). Irregulated cell cycles cause loss of control of protein complex in cancer cells. The majority of chemotherapy aims to stop cancer cells from growing and spreading by reverting them to their normal cell cycle (Levine & Puzio-Kuter, 2010).

Commercial and recombinant bromelain has shown the reduction of cytokinetic activities of MCF-7 breast cancer cells by decreasing the cell viability with IC50 values of 5.125 µg/mL and 6.25 µg/mL, respectively. Bromelain, both commercial and recombinant, has a better anti-proliferative effect by reducing the number of cell generations of MCF-7 cells from 3.92 to 2.81 compared to taxol reducing the cell generations from 3.92 to 3.12 (Fouz et al., 2013). The highest anti-proliferative effects were revealed in a study of lung tissue cancer cells (A549) by freeze-dried and the spray-dried bromelain with the ic50 values of 18.31±5.11 and 26.36±9.76 μg/ml, respectively (Manosroi et al., 2017). Bromelain also proved to have anti-proliferative effects on human AGS, PC-3, and MCF-7 cancer cells with bromelain at concentrations >75 μg/ml (Raeisi et al., 2019).

Apoptosis, a programmed cell death, is one of the phases in a normal cell cycle that is vital in the homeostatic preservation of cells in a body (Evan & Vousden, 2001). Loss of apoptotic pathways could cause advanced development growth of cancer cells. The apoptotic mechanism could happen through several pathways including the intrinsic pathway, extrinsic pathway, or apoptosis-inducing factor (Vermeulen et al., 2005). Current chemotherapy is targeting apoptosis to increase cell death of cancer cells to reduce the availability of cancer cells to spread likely in the body (Kerr et al., 1994). The effect of bromelain on cell death does link with the reduction of tumor volume by up to 65% (Bhui et al., 2009). Intrinsic pathways, also known as mitochondrial pathways, involve the tumor suppressor gene p53 in the upregulation of Bcl-2-like protein 4 (Bax) expression in B-cell lymphoma 2 cells (Bcl-2) (Haupt et al., 2003). Bax functioning is a pro-apoptotic protein while Bcl-2 is an anti-apoptotic protein. The upregulation of Bax could donate a higher ratio of pro-apoptotic over anti-apoptotic protein whereby increases the chances of the apoptotic pathway occurring (Zhang et al., 2000). Bax disrupts the protective protein Bcl-2 on the mitochondrial membrane, allowing cytochrome to enter the cytosol, and interacts with the apoptotic protease activating factor-1 (Apaf-1) to create the apoptosome complex, a specialized structure. Apoptosomes cause the activation of a specific type of protease protein called caspase-9 (Martin, 2014). The active caspase 9 complexes is formed when caspase-9 is activated. The complex will move around the organelles of the cell and breakdown the organelles which leads to breakdown of DNA causing apoptosis (Ahn & Metallo, 2015).

Bromelain has been mainly killing the cancer cells by induction of apoptosis to reduce the number of malignant cells (Bhui et al., 2010). Furthermore, evidence shows bromelain inhibits of NF-κB and induces apoptosis by causing G2/M arrest of epidermoid carcinoma and melanoma cancer cells (Bhui et al., 2012). Studies have shown that exposure to bromelain in advanced colorectal cancer (CRC) causes induction of high levels of apoptosis with elevated amounts of pro-apoptotic proteins including Endo G, and caspases-3, -8, and -9 (Chang et al., 2019). Another study shows that stem bromelain induces apoptosis activity on A549, HeLa, DU145, HT-29, KB and HuTu-80 cell lines (Manosroi et al., 2017). Bromelain was found to induce apoptosis by triggering the mitochondrial pathway with inhibit the expression of COX-2 and nuclear factor κB (NF-κB). At the same time, downregulation of Bcl-2 and upregulation of p53, Bax and activation of caspase-3 and caspase 9 are also found in studies on skin tumors whereby it is a strong proof of the ability of bromelain on induction of apoptosis on malignant cells (Bhui et al., 2009; Kalra et al., 2008). Bromelain diminishes the activity of cell survival proteins such as NF-kB, Ikkb, p-Ikkb, p-AKT and p-NF-kB thus endorsing apoptotic cell death in tumors (Pillai et al., 2014).

3.2 Angiogenesis and Metastasis

Angiogenesis is the process by which endothelial cells migrate and proliferate to form new blood vessels from the pre-existing vasculature (Folkman, 1985). Angiogenesis is a vital biological event for tumor growth and metastasis to provide nutrients needed by rapidly proliferating cancer cells. As the tumor grows bigger, tumor cells become hypoxic and stabilize hypoxia-inducible factor

(HIF) proteins (Senger & Davis, 2011). HIF proteins will trigger pro-angiogenic proteins such as vascular endothelial growth factor (VEGF) to induce angiogenesis. The existence of ECM acts as a barrier for VEGF to reach its target receptor which is the endothelial cell membrane of the blood capillary. Hence, the recruitment of macrophage and mast cells from nearby stroma will aid the degradation of ECM using matrix metalloproteinases MMPs. The breakdown of ECM releases other pro-angiogenic factors such as platelet-derived growth factors (PDGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and transforming growth factor (TGF-β), angiogenin and angiopoetin-1 and 2 (Kerbel, 2008).

The increased expression of pro-angiogenic factors could contribute to the higher ratio of pro-angiogenic factor over anti-angiogenic factors leading to promote angiogenesis (Folkman, 1995). Bromelain is known to downregulate TGF-β which acts as pro-angiogenic growth factor to counteract the effects of anti-angiogenic factors (Béez et al., 2007). The spread of cancer from the primary tumor is one of the causes related to high mortality rates (Paroulek et al., 2010). Metastatic is a process of spread of tumor cells from the primary tumor to a secondary site in the body (Sagar et al., 2006). The spreading of cancer depends on the location of the primary tumor. Malignant tumors can undergo metastasis through lymphatic, hematogenous spread or body cavity passages. Tumor metastasis mechanism invasion of extracellular matrix (ECM), vascular dissemination, homing of tumor cells, and colonization (Gupta & Massagué, 2006). Invasion of ECM starts with cell detachment from the primary tumor where the loss of intercellular e-cadherin force. The migration of tumor cells could be through ameboid migration or local degradation of the extracellular matrix (ECM) (Chaffer & Weinberg, 2011). Ameboid migration occurs through spaces in the matrix instead of cutting its way through, perhaps explaining the low performance of metalloproteinases (MMP) inhibitors as chemotherapy in some clinical trials (Egeblad & Werb, 2002). Local degradation of ECM occurs when tumor cells interact with ECM proteins. The interaction causes loss of integrins, secretion of proteolytic enzymes and increase of metalloproteinases (MMPs), cathepsin D and urokinase plasminogen activator (Mohamad et al., 2019). Subsequently, tumor cells invade the lumen of blood vessels and are carried away via blood to a distant target organ. Tumor cells are able to survive from the interaction with host lymphoid cells due to autocrine motility factor (AMF) making the tumor more motile. Then, extravasation of tumor cells from the blood lumen to the secondary location. Tumor cells will be homing and colonizing the new location. Survival of tumor cells is also extended with growth factors provided by the host tissue and cleavage products of matrix ECM such as PDGF, FGF, TGFβ and VEGF (Sasaki et al., 2013).

Other than focusing on the impairment growth of malignant cells, bromelain also has a captivating feature of an anti-cancer agent which is the ability to inhibit of cancer metastasis process (Doughlas & Weinberg, 2000). The inhibition is by interfering with the pivotal biological events in the progression of cancer metastasis such as cell migration, inflammation, and cell adhesion on the target site. The ability of bromelain to inhibit platelet activation is due to its

proteolytic activity (Bhui et al., 2009). The reduction of platelet aggregation shows that the anti-coagulant activity of bromelain helps to enhance the blood flow circulation and prevention of formation of a thrombus (Béez et al., 2007). Bromelain's anti-platelet activity thus interferes with the growth and development of platelet-mediated cancer and prevents the development of tumor-platelet aggregates by uncoating and exposing cancer cells to the immune system (Kalra et al., 2008). Studies on human cholangiocarcinoma cell lines show bromelain downregulated the expression level of MMP-9 and other epithelial mesenchymal-transition markers. Downregulation of MMP-9 by inhibition of activator protein 1 (AP-1) and NF- κB signaling pathways remarks reduction of inflammation and deployment of tissue-bound growth factors and proteins (Müller et al., 2016).

3.3 Inflammation Regulator

Inflammation is known as one essential during the development of cancer in the cellular transformation phase, cell growth, metastasis, and angiogenesis (Coussens & Werb, 2002). It is shown that cancer progression could be suppressed and reduce the chance of cancer incidence with suppression of chronic inflammation (Paroulek et al., 2010). Chronic inflammation could trigger chronic diseases including cancer depending on the tumor type, and micro-surroundings of the tumor. Inflammations require pro-inflammation protein to promote progression such as prostaglandin E2 (PGE 2) synthesized by Cyclooxigenase-2 (COX-2) makes COX-2 as an important molecule in cancer-associated inflammation (Grivennikov et al., 2010). Some studies have shown that bromelain is able to reduce inflammation by inhibiting cyclo-oxygenase-2 activity by patterning a downregulation of COX-2 and PEG-2 levels (Sagar et al., 2006). It is also shown that bromelain is able to reduce the migration of neutrophils to inflammation sites hence reducing the expression of cytokines that aid amplifies inflammatory reactions (Fitzhugh et al., 2008). The majority of proinflammatory mediators are eliminated by bromelain, which indicates a significant function as an anti-inflammatory agent in a variety of conditions and circumstances (Mynott et al., 1999).

3.4 Immuno-modulatory

The ability to modulate immune response activity is one of the targets in chemotherapy and various clinical testing. The immunological response is a natural response of body defense and modulation of the immune system involves the use of therapy to modify the action of the immune response (Engwerda et al., 2001). Clinical trial on immunomodulating activity of bromelain has been proved capable of modulating and enhancing cellular responses of lymphocytes (S. Müller et al., 2013). The cluster of differentiation 44 (CD44) is a cell surface marker that is expressed by cancer and leukocyte cells. The level of CD44 expression is also used as a diagnostic and prognostic marker on a cancer patient. Expression of CD44 indicates inflammation, cancer growth and metastasis. The upregulation of CD44 indicates an increase in lymphocyte homing to the vascular

endothelium at inflammatory sites. CD44 expression at high levels in the blood was found to be associated with cancer progression aggressiveness and lymphatic metastasis.

Bromelain was shown to reduce CD44 expression in breast cancer cells which conclude that the reduction could impede the adhesion of lymphocyte on endothelial cells (Munzig et al., 1994). Moreover, in murine microglial cells and human monocytic leukaemia cell lines, bromelain has been shown to reduce PGE-2 and COX-2 expression (Engwerda et al., 2001). Bromelain increases the development of granulocyte-macrophage-colony stimulating factors, IL-2, and IL-6 and decreases the activation of T-helper cells by activating natural killer cells. The implications of bromelain in immune system response for progression of human cancer would have to be identified in further studies (Chobotova et al., 2010).

4 Conclusion

Bromelain has been shown to be a safe and effective therapeutic agent. Clinical evidence indicates that bromelain has a bright potential to become an important therapeutic agent against cancer. Laboratory evidence indicates that the anti-cancer effect of bromelain may be the result of a systemic reaction, likely involving a variety of target sites and pathways. Bromelain inhibits tumor growth, which has antitumor initiating and proliferative properties, as evidenced by induction of p53, shifts in the decreases in Cox-2 expression, induction of caspases, Bax/Bcl-2 ratio and inhibition of the NF-κB pathway through regulation of the MAPK and Akt/PKB signaling pathways. Studies of bromelain in relevant psychological and conditions are pivotal for evaluating its role as an anti-cancer agent. More clinical assessments in cancer patients are suggested to be done in the future with priority focusing on detecting the effects of bromelain on patient survival in terms of tumor growth and metastasis rate, chronic inflammation, tumor infiltrates, and blood coagulability. In this review, all studies are interpreted to show that bromelain's anti-cancer activity affects major pathways and regulators. Bromelain-based chemotherapy could benefit from more in-vivo studies, laboratory assessments, clinical trials, and further research in this area.

5 Availability of Data and Material

All information is included in this study.

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