Molecular Mechanistic Approach of Important Antileukemic Compounds Present in Honey

1

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Abstract

Homeostasis is a collective name for the self-regulating cellular processes that maintain cell stability and survival. Any variation from normal functioning in any biological process can lead to different diseases or syndromes such as cancer, diabetes, and metabolic syndromes. Cancer results by the uncontrolled division of cells in any organ or tissue and can metastasize to other organs as well. Derailment in the process of cell division is the main cause for caner development. Leukemia—a type of cancer in which the function and production of blood cells gets affected, is one of the leading cancers-related mortalities throughout the world. Scientific research has witnessed a great interest in the pharmacodynamics of naturally occurring food products or other plants or plant products of medicinal

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value in order to make them novel drug agents to target various diseases including cancer. This chapter vividly describes phenolic compounds that can be used as signature drugs to target leukemias. Gene expression and microarray studies have depicted the various signaling pathways regulated by these compounds and, hence, serve in inducing decreased cell growth and malignancy in leukemias. Honey is well-known for its nutritional and medicinal properties since ages and is continuously being explored for its wide pharmacological properties. Studies have attributed significant anti-cancerous action to honey, but very less literature is available on its antileukemic action. In this present chapter, we have summarized the anti-leukemic activities associated with bioactive components present in honey. Honey is a storehouse of biologically essential phenolic compounds such as phenolic acids, tannins, flavonoids, terpenoids, and coumarins. These compounds show tumor reduction or inhibitory action by arresting the cell cycle, up- or downregulating mRNA expression of proteins involved in apoptotic cascades like Bax, caspase-3, Bcl-2, NOXA, MCL-1, rTRAIL, FAS, SCF/c-Kit complex, p-ATM, p-ATR, 14-3-3 proteins sigma, MGMT, and HDACs; deactivating drug efflux ABC transporters; various cyclins and CDKs; and decreasing mitochondrial membrane potential. Till date no study elucidating the effect of raw honey-derived phenolic compounds has been undertaken and, therefore, a wide scope exists for studying the effective chemotherapeutic mechanisms of these compounds.

Keywords

Cancer · Leukemia · Blood cancer · Honey · Phytochemicals · Apoptosis · Chemoprevention · Chrysin · Quercetin · Apigenin · Kaempferol · Galangin · Hesperetin · Coumarin

1.1 Introduction

Several self-regulating biological processes take place in cells to maintain stability with survival conditions (homeostasis). To maintain cell homeostasis, many physiological processes take place inside the cell. Any derailment or fluctuation in the basic mechanisms maintaining cellular metabolism leads to different diseases (Biswal et al. 2017). Cancers occur due to the overgrowth of cells of any organ and can metastasize leading to various malignancies (Cooper 2000). Leukemia is one of the leading cancers affecting blood cell components and resulting in high risks to the life of patients, with a global prevalence of approximately 300,000 new cases every year. The cellular growth in these cancer cells can be kept under control by either apoptosis or autophagy. Hence, the escape of cells from apoptosis or autophagy stimulates proliferation of cells that can continue to grow rapidly leading to cancer, which can eventually metastasize to other organs. Scientific research has witnessed a great interest in the pharmacodynamics of naturally occurring food products or other plants or plant products of medicinal value in order to make them novel drug agents

to target various diseases. In this regard, phenolic compounds have been expressed notable potential. The antioxidant and antiinflammatory properties of phenolics make them potential candidates for therapeutic agents against remarkable diseases including cancers.

Honey is well known for its nutritional and medicinal importance and is continuously being explored for its wide pharmacological properties. Studies have revealed, potential anti-cancerous action to honey, but very less literature is available related to its anti-leukemic action. Honey is considered as a storehouse of biologically essential phenolic compounds such as phenolic acids, tannins, flavonoids, terpenoids, and coumarins. All these compounds have been studied to possess pronounced antitumorigenic action in various cancers including leukemias of different types. These compounds show tumor reduction or inhibitory action by arresting the cell cycle (Fig. 1.1), up- or downregulating mRNA expression of proteins involved in apoptotic cascades like Bax, caspase-3, Bcl-2, NOXA, MCL-1, rTRAIL, FAS, SCF/c-Kit complex, p-ATM, p-ATR, 14-3-3 proteins sigma, MGMT, and HDACs. Moreover, these compounds exhibit their anti-cancer action by deactivating drug efflux ABC transporters, various cyclins and CDKs, and decreasing mitochondrial membrane potential. But, in particular, further research is required to elucidate the antileukemic properties of these compounds after being isolated and characterized from honey. This chapter summarizes the molecular mechanisms associated with the antileukemic action of bioactive compounds present in natural honey.

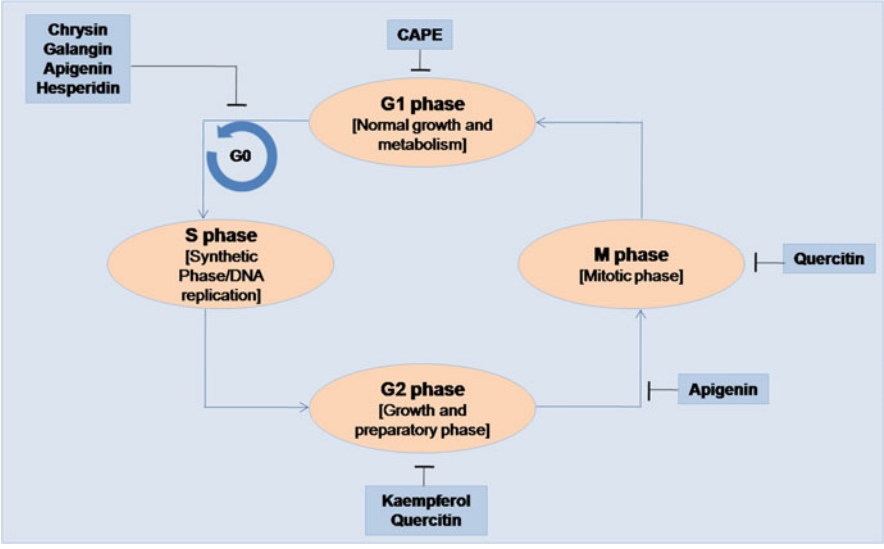

Fig. 1.1 Different chemical components present in honey causing cell cycle arrest at different phases of cell division during leukemia. Caffeic acid phenylethyl ester (CAPE) leads to cell cycle arrest at the G1 phase of cell division. Chrysin, galangin and hesperidin arrest cell cycle at the G1/G0 stages while apigenin does so at the G1/G0 and G2/M stages. Kaempferol induces arrest of the cell cycle at the G2 phase and quercitin at the G2/M phases of the cell cycle during leukemia

1.2 Leukemias

Hematologic cancers include myeloproliferative neoplasms, acute myeloid leukemia (AML), Hodgkin lymphoma, myelodysplastic syndromes, and chronic lymphocytic leukemia (CLL) (Genovese et al. 2014). AML is a major blood cell malignancy causing increase in the proliferation of clonal myeloid cell precursors and finally increased concentration of myeloid cells in the bone marrow (Zhu et al. 2019). It has been studied that most commonly CLL occurs due to an imbalance of lymphocyte apoptotic mechanisms leading to the abnormal proliferation of lymphocytes (Billard 2014).

Cancers occur due to any dysregulation in the normal physiological suicide programs. Programmed cell deaths can be widely of two types, viz., I and II, and are quite essential for the maintenance of steady cellular state (Nikoletopoulou et al. 2013). Hence, execution of these cellular death programs helps in the prevention of tumorigenesis. Apoptosis is an important suicidal program and is regarded as type I programmed cell death whereas cell death due to autophagy is regarded as type II programmed cell death (Towers et al. 2020). Interplay between apoptosis and autophagy is a vital phenomenon for cellular homeostasis (Li et al. 2020). Apoptosis in a cell is characterized by mitochondrial membrane potential loss, cytoplasmic blebbing, DNA fragmentation, and apoptotic body formation (Li et al. 2020). Another mechanism for cell death is autophagy characterized by lysosomal activation and the subsequent activation of degradation or phagocytotic pathways. A large set of proteins are associated with the activation of the apoptotic cascade or signaling in a cell. There may be proapoptotic and antiapoptotic classes of proteins which execute simultaneously to induce apoptosis. Cell death or apoptosis is notably induced by the upregulation of proapoptotic proteins and concomitant downregulation of antiapoptotic proteins. Bcl-2 family of proteins like Bcl-2, Bcl-XL, and Mcl-1 are regarded as antiapoptotic and are present in mitochondria (Bae et al. 2020). On the other hand, proapoptotic molecules like Bax, Bad, Bck, and BH3 domain (Puma, Noxa, Bid, Bim) are upregulated in cancer cells and cause decrease in the survival of cells (Bae et al. 2020; Yan et al. 2020).

Furthermore, any derangement in the cell cycle progression has a pivotal role in cancer development (Pirtoli et al. 2020). Cellular growth involves a series of molecular events in which a parent cell converts into new daughter cells, enabling cells to grow. The cell cycle has four important phases, namely gap 1 or the G1 phase, synthesis or the S phase, gap 2 or the G2 phase, and mitosis or the M phase. At the molecular level, two enzyme complexes, viz., cyclin A–cyclin T and the cyclin-dependent protein kinases (CDK 1–CDK 9) (Abubakar et al. 2012) are involved in the course of cell cycle.

1.3 Anticancer Compounds in Honey

Honey is a miraculous food and is a depot of almost 200 substances such as sugars including fructose and glucose, amino acids, proteins, trace amount of vitamins and enzymes, water, etc. (Wang and Li 2011) Phenolic acids and flavonoids are the two

Table 1.1 Phenolic Compounds Found in Honey

S. no.	Main bioactive compound	Type
1.	Phenolic acids	Caffeic acid
		Ellagic acid
		Ferulic acid
		p-Coumaric acids
2.	Flavonoids	Chrysin
		Apigenin
		Kaempferol
		Galangin
		Quercetin
		Pinocembrin
		Hesperetin
3.	Coumarins	Coumarin

major pharmacopotent classes of compounds present in honey (Stephens et al. 2010) (Table 1.1). Catalase, superoxide dismutase, reduced glutathione, tocopherols, and ascorbic acid are the major compounds present in honey with antioxidative properties. Honey and its many bioactive compounds possess antioxidative, antiinflammatory, chemopreventive, immunoregulatory, antiatherogenic, and wound healing properties (Fig. 1.2).

Phenolic acids are bioactive molecules present in many valuable foods including honey. They possess many essential biological activities like antiinflammatory, anticancerous, antioxidative, and antiatherogenic. Protocatechuic, p-coumaric, caffeic, and vanillic acids are the various constituents of honey derived from hydroxybenzoic acid and have potential antitumorigenic activity (Rocha et al. 2012; Tanaka et al. 2011).

1.4 Honey in Other Cancers

Honey has been used in its raw form to treat a number of cancers and has shown significant activity in combating cancerous growth. Several studies have reported potent anticancerous activities against many cancers like liver (Baig and Attique 2014), cervical (Fauzi et al. 2011), oral, bladder (Swellam et al. 2003), bone, and breast (Fauzi et al. 2011) cancers (Fig. 1.2). Vascular cell adhesion molecules (VCAM-1) and intercellular adhesion molecule or cluster differentiation 54 (ICAM-1 or CD54) are the important endothelial cell–associated adhesion molecules which are downregulated in prostrate (PC-3) and breast (MCF-7) cancer cell lines when exposed to different types of Greek honey (Spilioti et al. 2014). Furthermore, honey is a treasure of many compounds with properties of mitigating oxidative stress in cells. Spilioti and colleagues have reported that anticancer properties of honey were associated with its oxygen radical absorbance capacity (ORAC).

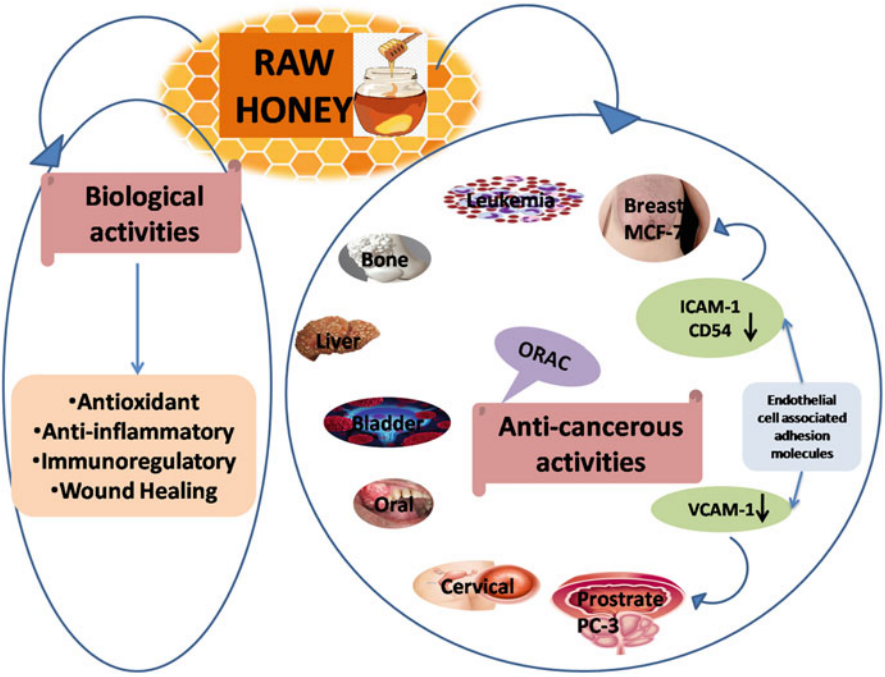

Fig. 1.2 Pharmacological activities of raw honey. Natural honey possesses numerous biological activities such as scavenging of toxic free radicals in the body by its antioxidant activity. Honey is a potent antiinflammatory agent resulting in the downregulation of key inflammatory markers (iNOS, Cox-2). Honey is a natural immunostimulant that protects cells from several pathogens. Honey enhances the wound healing process that may be interrelated with its antioxidant, antiinflammatory, and immunoregulatory activities. In addition, honey has been proved to be very beneficial against various cancers including leukemia, and bone, liver, oral, and prostate cancers

1.5 Honey in Leukemia

Till now very little literature is available reporting honey as an antileukemic substance in either in vitro or in vivo trials. Tualang honey (TH) is a widely studied variety of honey obtained from wild honey bees. Tualang trees present in the rain forests of Malaysia are home to these wild honey bees. It is the source of a number of biologically active compounds. It shows significant antileukemic effects by inducing apoptosis in leukemic cell lines. Microscopically apoptotic changes like membrane blebbing, apoptotic bodies, cell roundness, and fragmentations were seen in the TH-treated cell lines, which clearly depict the apoptosis-inducing ability of TH (Man et al. 2015). However, TH showed more pronounced antileukemic effects in acute leukemia as compared to chronic leukemic models.

An in vivo study revealed that raw honey and one of its phenolic compounds, eugenol, could not produce significant antileukemic activity against the rat leukemia

model. The median survival time (MST) showed nonsignificant increase using all the honey samples in comparison to the positive control (Jaganathan et al. 2014). However, additional research designed to validate the effective molecules in honey possessing antileukemic effects needs to be conducted in future.

1.6 Kaempferol

Bestwick et al. (2007) deduced the antiproliferative action of kaempferol in promyelocytic leukemia cells (HL-60), leading to various changes in cell cycle. A significant increase in the S-phase of cell cycle showed apoptotic changes like increased caspase-3 activity and decreased antiapoptotic Bcl-2 expression. In acute promyelocytic leukemia (APL), kaempferol treatment led to increase in apoptotic gene expression and concomitantly inhibited multidrug resistance. In HL-60 and NB4 leukemia cell models, kaempferol induced apoptosis by Akt and BCL2 downregulation while causing CASP3 and BAX/BCL 2 ratio upregulation (Moradzadeh et al. 2018). Cancer cells show prominent multidrug resistance that makes the anticarcinogens ineffective in these cells. ABC (ATP-binding cassette) transporters are upregulated in these cells causing efflux of the anticancerous drugs from the cancer cell, thus impeding the action of drug in the cancer cell (Chang et al. 2020). Kaempferol lead to a concentration-dependent decrease in the expression of ABCB1and ABCC1, which indicated inhibition of multiple drug resistance in leukemic cell lines (Moradzadeh et al. 2018). This suggests that kaempferol can be used as a potential anticancer drug substitute in cells that show resistance to chemotherapeutic drugs. Kim et al. (2016) found that G2 cell cycle arrest and mitochondrial system apoptosis led to cytotoxic effects due to kaempferol in leukemia. It was proposed that the antitumor activity of kaempferol might be due to hyperactivation of the ATM/ATR-Chk1/Chk2 pathway, which is important for inducing DNA damages in the cell. Furthermore, increase in phosphorylation at Ser-15 of the tumor suppressor p53 gene; upregulation of proapoptotic genes like Bak, PUMA (p53 upregulated modulator of apoptosis), and caspase enzyme (caspase 3, 8, and 9); and significant loss in mitochondrial membrane potential ($\Delta\psi m$) were responsible for antitumorigenic activity in leukemia cells (Fig. 1.3).

Comet tail formation and fragmentation of cellular DNA depicting cell apoptosis occurred in human promyelocytic leukemia cells (HL-60) when treated with kaempferol. Inhibition of the expression of a set of DNA damage repair associated protein like 14-3-3 proteins sigma (14-3-3σ), p-ATM, p-ATR, O6-methylguanine-DNA methyltransferase (MGMT), DNA-dependent protein kinases, p53 and MDC1, have been depicted in kaempferol-treated leukemic cell lines (Wu et al. 2015).

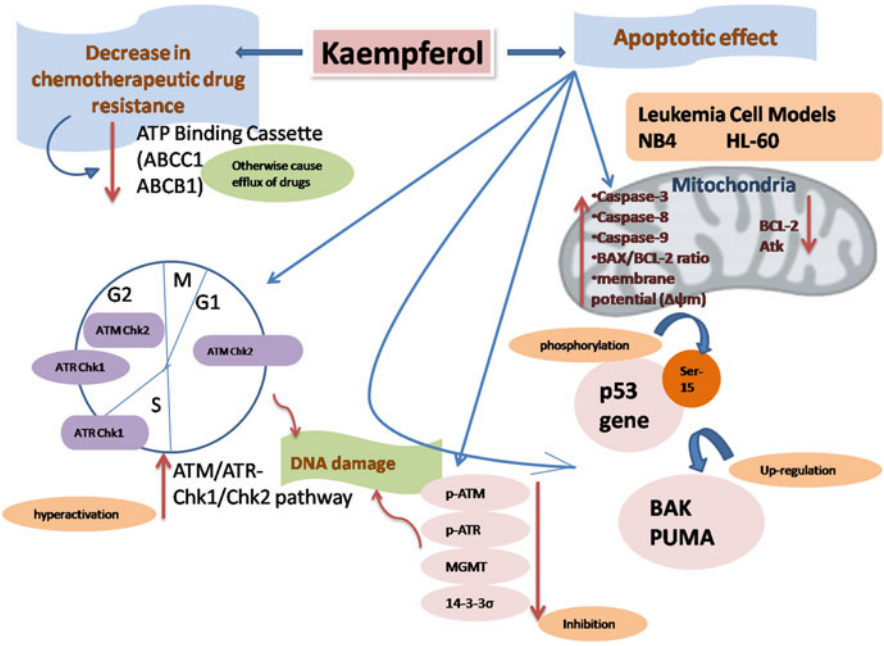

Fig. 1.3 Role of kaempferol in cell signaling pathways in leukemia cells. In leukemia cell models, kaempferol induces apoptosis by downregulating Akt and BCL2 and upregulating caspases and the BAX/BCL 2 ratio. Furthermore, kaempferol enhances the effect of other chemotherapeutic drugs (when given in combination) by inhibiting the ATP binding cassette (such as P-gp efflux pump), thus leading to increasing bioavailability of chemotherapeutic drugs during the treatment of leukemias. Kaempferol halts cell division by arresting cell cycle at the G1 stage resulting in cytotoxicity in leukemia. The antitumor activity of kaempferol is due to hyperactivation of the ATM/ATR-Chk1/Chk2 pathway, which is important for inducing DNA damages in the cell. Furthermore, increase in phosphorylation at Ser-15 of the tumor suppressor p53 gene; upregulation of proapoptotic genes like Bak, PUMA (p53 up-regulated modulator of apoptosis), and caspase enzyme (caspase 3, 8 and 9); and significant loss in mitochondrial membrane potential (Δψm) were responsible for the antitumorigenic activity in leukemia cells

1.7 Quercitin

Chemically, quercetin (3,3′,4′,5,7-penta-hydroxyflavone) is an important bioactive compound found in honey. Quercitin shows proapoptotic synergistic effect with cisplatin, when given in combined treatment in murine leukemia cell lines (L1210) (Čipák et al. 2003). Quercetin resulted in time- and dose-dependent decreases in the proliferation of HL-60 cells by trapping of cells in G(2) and M phases of cell cycle and over expression of apoptotic genes (Ren et al. 2010). Potentiating the effect of antileukemic drugs has been found with quercetin treatment in human leukemic cell lines like U937, HL-60, and THP-1. It has been reported that several anti-cancer drugs exert their anti-cancer action by inducing apoptosis in cancer cells due to loss

of mitochondrial membrane potential and decrease in reduced glutathione (GSH) content (Ramos and Aller 2008). Apoptotic cascade-like activation of Bax, caspase-8, cytochrome c, and Omi/Htra2 with Bcl-XL downregulation might be the driving mechanisms for the antileukemic effect in these cells. Kang and Liang demonstrated inhibition of proliferation of HL-60 cells by quercetin in a dose- and time-dependent manner via inhibition in the activities of TPK (tyrosine protein kinase) in the membrane and PKC (protein kinase C) in the cytosol (Kang and Liang 1997).

Quercitin led to enhanced Bax and caspase-3 expression activity with concomitant downregulated expression of Bcl-2 and NF-κB p65 mRNA levels, leading to cell cycle arrest at S phase in leukemic rat models (Han et al. 2015). Quercitin is a highly advantageous pharmacologically active compound showing prominent antileukemic effects but suffers due to its slow solubility and, therefore, decreased bioavailability. Therefore, to potentiate the pharmacokinetics and pharmacodynamics of quercetin, pharmaceutical nanotechnology comes to the rescue. Recently, quercetin-loaded polymer-lipid hybrid nanoparticles were used to potentiate the antileukemic effect of quercetin (Yin et al. 2019). Epigenetic modification, including posttranslational modifications, and DNA methylation of various proapoptotic genes also led to the antileukemic effects of quercitin. STAT-3-dependent increase in the expression of DNA methyltransferase (DNMT1 and DNMT3a) downregulated class I histone deacetylases (HDACs) and increased demethylation of apoptosis inducers, BCL2L11; DAPK1 genes were also seen in AML models in both in vivo and in vitro studies (Alvarez et al. 2018).

A crosstalk between apoptosis and autophagy has been reported by many studies for the pronounced antileukemic effect of quercetin. Quercetin in combination with green tea was able to cause significant reduction in BCL-2, BCL-XL, and MCL-1 proteins, overexpression of BAX, and caspase-3 stopping tumor growth in HL-60 (Calgarotto et al. 2018). Cells were trapped in the G1 phase of the cell cycle, and the activity of autophagy-inducing proteins was also enhanced by quercitin treatment. Chang et al. (2017) further demonstrated inhibition of CDK2/4, hence halting the cell cycle progression. Activation of proapoptotic signaling like the caspase pathway and poly (ADP ribose) polymerase (PARP)-1 cleavage are regarded as key mechanisms for tumor regression. Activation of the autophagic cascade marked by upregulated expression of LC3-II (light chain 3), downregulation of p62, and formation of acidic vesicular organelles were found to be associated with antileukemic effects in HL-60. Recently, a study on the CML cell line K-562 inferred the antiproliferative effect of quercetin as there was a reduction in the expression of some of the vital prosurvival proteins, especially heat shock proteins (HSP70), Bcl-X(L), and Forkhead box protein M1 (FOXM1), and simultaneous upregulation in proapoptotic genes like caspases (3 and 8) and Bax (Hassanzadeh et al. 2019). This suggests quercetin can be a candidate flavonoid for attenuating the proliferative mechanism by increasing apoptosis and antisurvival mechanism in leukemic cells.

Quercitin results in a dose-dependent reduction in levels of inositol 1,4,5-triphosphate (IP3) and expression of oncogenes, viz., c-myc and ki-ras, leading to a fragmentation of nucleosomal DNA in K562 human leukemia cells (Csokay et al. 1997). G2/M arrest associated with significant decline of cyclin D, cyclin E, and

elongation factors (E2F1and E2F2), and cyclin B overexpression occurred in quercetin-treated HL60 cells (Lee et al. 2006). Caspase-3 activation indicated by proteolytic cleavages of its target, i.e., PLC-γ1, led to DNA lysis and death in these leukemic cells. Spagnuolo et al. (2012) demonstrated that transcriptional activity of cell death-inducing proteins like recombinant tumor necrosis factor-alpha-related apoptosis-inducing ligand (rTRAIL) and CD95/FAS/apoptosis antigen 1 (APO-1) was upregulated in ALL when exposed to quercetin. Also, both mRNA and protein levels of Mcl-1 were decreased depicting the proapoptotic activity of quercetin.

Multiple myeloma (MM), a hematological cancer of plasma cells of bone marrow, incidence has increased recently all over the world. Quercitin led to the activation of a proapoptotic pathway in MM by upregulating p21, caspases (3,9), and poly(ADP-ribose)polymerase expression; c-myc downregulation; and G2/M cell cycle arrest (He et al. 2016). Furthermore, in vivo studies in xenograft models also revealed tumor growth inhibition using quercetin.

1.8 Chrysin

Chrysin or 5,7-dihydroxyflavone is a bioactive flavonoid found in honey and possesses a wide range of pharmacological activities including anticancer effects. Chrysin treatment in leukemic BALB/c mice enhanced the T and B cell populations and increased macrophage-induced phagocytosis and natural killer cell cytotoxicity, ascertaining the probable mechanism for antileukemia (Lin et al. 2012). These immunological effects were seen by increased number of cell surface markers of CD3 which is a T-cell maker, CD19, a B-cell marker, and Mac-3, indicating initiation of phagocytosis in the cells. Methylated chrysin, viz., 5,7-dimethoxyflavone (DMF), could be a potential candidate to treat ALL. DMF produces antileukemic effects by arresting the cell cycle (G0/G1), downregulates phosphorylated retinoblastoma-associated protein 1, and induces apoptotic changes in ALL (Goto et al. 2012). The apoptotic effect of chrysin has been established in ALL cell lines such as U937, MO7e, THP-1, and HL-60. However, Zaric et al. (2015) found that chrysin can induce proapoptotic and decrease antiapoptotic mechanisms in chronic leukemic cell lines like MOLT-4 and JVM-13 and lymphocytes isolated from B-CLL patients. A decrease in cell viability, lowered expression of Bcl-2, and activation of Bax, caspases, and mitochondrial cytochromes led to apoptosis in leukemic cell lines (Fig. 1.4). Stem cell factor (SCF) in combination with c-Kit executes its function in the proliferation and differentiation of hematopoietic stem cells. Chrysin shuts down the SCF/c-Kit-complex mediated pathways of cell differentiation via downregulation of the PI3K pathway and a concomitant upregulation of ERK5, CREB, and STAT3 (Lee et al. 2007). Woo et al. (2004) found an overexpression of caspase 3 and myr-Akt signaling pathways to be associated with apoptosis induction in U937 leukemic cell lines.

Decreased expressional activity of myeloid cell leukemia-1 (MCL-1) is associated with antileukemic properties of chrysin (Polier et al. 2011). MCL-1 belongs to the BCL-2 family and is, thus, a key regulator in the maintenance of

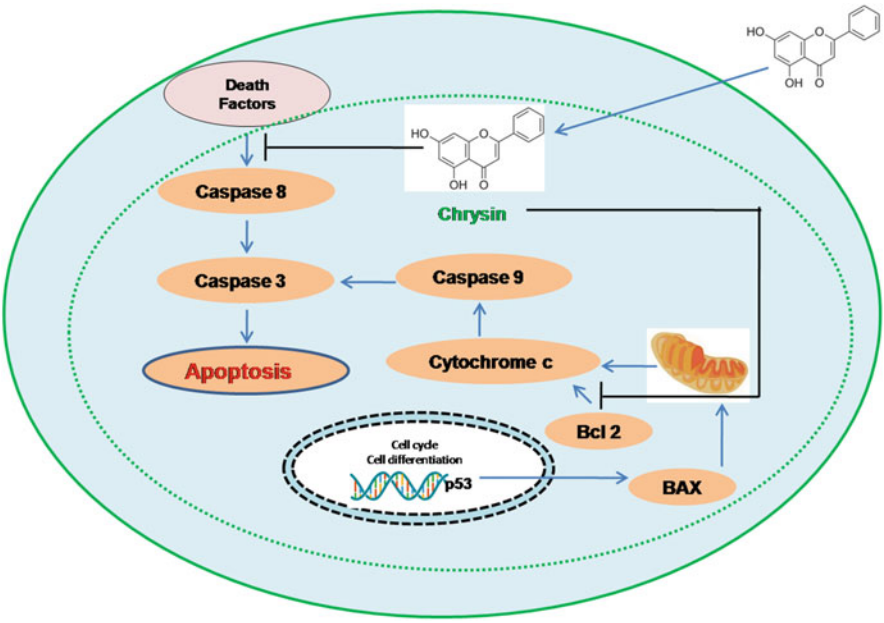

Fig. 1.4 Anticancer activity of chrysin, endorsing apoptosis. Chrysin influences death factors present on cell membranes (such as TNF-related apoptosis inducing ligands—TRAIL), downregulating antiapoptotic protein Bcl 2, and activation of Bax, caspases and mitochondrial cytochromes leading to apoptosis in leukemic cell lines

homeostasis and growth (Xiang et al. 2018). Hence, inhibition of MCL-1 is a target for designing drugs effective for cancer prevention. Polier et al. (2011) found the association of Mcl-1 gene downregulation with the inhibition of cyclin-dependent kinase 9 (CDK9) and Ser2 phosphorylation at the COO^{-} (carboxy) end of RNA polymerase II (RNA pol II). This decreased transcriptional activation of Ser2 finally stops the mRNA synthesis and protein formation. Chrysin-mediated apoptosis occurs due to the induction of mitochondrial membrane collapse, increased reactive oxygen species, caspase 3 activation, and selective inhibition of complex II and complex V (ATPases) in CLL (Salimi et al. 2017). Activation of caspase 3 and 8 pathways in human leukemia cell lines (U937) has also been linked with the chrysin-dependent antileukemic effect (Monasterio et al. 2004).

1.9 Galangin

Galangin's antileukemic effect in human leukemia cell lines (U937) was found to be associated with overactivation of caspases 3 and 8 inducing apoptosis and DNA fragmentation of cancerous cells (Monasterio et al. 2004). Tolomeo et al. (2008) found galangin potentiated imatinib's apoptotic and antiproliferative activity in sensitive and resistant leukemic cell lines. This was linked to an arrest of the cell

cycle at the G0–G1phase with a concomitant decrease in the level of cyclins (cdk1, cdk4, and cycline B), retinoblastoma (pRb) and BCL-2. Galangin (1–100 μM) exerted a dose- and time-responsive antiproliferative effect in HL-60, a human leukemia cell line (Bestwick and Milne 2006). Furthermore, DNA abnormalities like increased hyopodiplody, membrane disruptions, and caspase-3 activation in cancer cells were attributed with the antileukemic efficacy of galangin. Galangin has significantly shown antiproliferative potential in other cell lines like human hepatocellular carcinoma (HepG2) as well, by inhibiting protein kinase C/extracellular signal-regulated kinase (PKC/ERK)-mediated signaling pathway (Chien et al. 2015). It was also found that G2/M or G1 phase of the cell cycle are inhibited in colorectal cell lines (HCT116) on exposure to galangin (Sulaiman 2016).

1.10 Apigenin

Apigenin is a vital dietary flavonoid and has a chemical formula of 4′,5,7-trihydroxyflavone. Budhraja et al. (2012) found that apigenin treatment led to a pronounced apoptotic pathway through Akt, JNK, and caspase hyperactivation with concomitant cytochrome c release from mitochondria in human leukemic cells in dose- and time-responsive ways. In vivo administration resulted in decreased proliferation and subsequent apoptosis of tumors in U937 xenografts. Activation of caspase (9 and 3) and PKCδ, MAPKs, p38, and ERK as a result of their phosphorylation and induction of oxidative stress (ROS) inside the leukemic cells led to apoptosis and cellular death (Vargo et al. 2006). This study signifies the essential and pronounced role of PKCδ for the antileukemic nature of apigenin as was also observed after PKCδ silencing in leukemia cells.

Another mechanism for apigenin's apoptotic activity might be its potent CDK inhibition which, in turn, led to the downregulation of the prosurvival or antiapoptotic gene, Mcl-1 (Polier et al. 2011). Both MCL-1 and PI3K/AKT inhibition is known to cause apoptosis and, hence, significant antileukemic effects in CLL [Shehata et al. 2010]. Along with CDK, apigenin is believed to produce pronounced proteasomal inhibition which is linked in attenuating tumor growth, therefore, producing antileukemic actions in CLL cells (Chen et al. 2005).

NOXA or phorbol-12-myristate-13-acetate-induced protein 1, one of the major proteosome inhibitors, led to proapoptotic action in CLL when exposed to apigenin (Fennell et al. 2008). NOXA is responsible for stimulating various processes like caspase activation, changes in mitochondrial membrane constitution and potential, and proapoptotic cascade leading to apoptosis in cells. The Noxa/Mcl-1 axis serves as an effective target for generating apoptotic signals in CLL as NOXA is linked to proteosomal degeneration of MCL1 (Billard 2014) leading to decrease in survivability in cells.

Ruela-de-Sousa and colleagues have reported apigenin as a potent chemopreventive agent in erythroid and myeloid leukemic cell lines. Apigenin induced a G2/M and G0/G1 phase arrest of the cell cycle in myeloid myeloid (HL60) and erythroid (TF1) cells, respectively, which might be through

downregulation of the JAK/STAT pathway. Antiproliferation through the PI3K/PKB pathway inhibition which is, in turn, due to the mechanistic activation of PTEN and apoptotic caspase pathway activation was observed in myeloid cells only. On the other side, only increased autophagic activity was seen in erythroleukemic cells, indicating a dominant effect of apigenin in leukemia. Apigenin also induced autophagy through mTOR and P70S6K downregulation, in turn, reducing S6 protein phosphorylation. TF1 cells also showed reduction of autophagy-related genes, viz., Atg5, 7, and 12 with apigenin treatment.

1.11 Hesperidin

Hesperidin, a flavanone, is a bioactive component in many citrus fruits and honey that activates proapoptotic mechanisms in tumor cells leading to decrease in cell proliferation. However, very less research has been conducted until now regarding the antileukemic potential of hesperidin. One study by Ghorbani et al. (2012) showed the antileukemic effects of hesperidin in human pre-B cell lines (NALM-6). Further, significant growth inhibition was seen in these cell lines particularly through the overexpression of PPARγ under a hesperidin dosage of 10–50 μM. Apoptosis was induced by upregulation of antisurvival or proapoptotic protein, Bax, and a concomitant reduction in Bcl-2 and XIAP expression under a dose of 10–100 μM. Furthermore, involvement of p53 in tumor suppression was suggested as there was upregulated expression of p53 when NALM-6 cells were pretreated with hesperidin. Another study available suggests that hesperidin causes reduced cell proliferation in human CML cells (K562) by arresting cell cycle at G0/G1 phase and triggering programmed cell death (Adan and Baran 2016). Microarray analysis revealed involvement of downstream signaling pathways like JAK/STAT, KIT receptor, and growth hormone receptor for the antiproliferative tools of hesperidin. Interestingly, mitochondrial membrane depolarization and activation of caspase-3 led to apoptosis and cellular death in CML cells. This suggests hesperidin as an effective target for chemotherapeutic strategy in CML treatment protocols.

1.12 Caffeic Acid Phenylethyl Ester (CAPE)

Chemically CAPE is 2-phenylethyl, 3-(3,4-dihydroxyphenyl) acrylate and is also known as phenethyl caffeate or phenylethyl caffeate (Kumazawa et al. 2010). CAPE shows distinct pharmacological actions due to its varied properties, viz., antiinflammatory, antimicrobial, and cytotoxic (Murtaza et al. 2014). Probably the first study conducted by Chen and coworkers demonstrated decrease in cellular progression in HL-60 with exposure to CAPE in a dose-dependent manner. Almost about 74% growth inhibition was observed with 10 μM of CAPE exposure within 48 h. CAPE treatment was found to induce cytoplasmic blebbing, membrane disruptions, and chromatin condensation in HL-60 cell lines with about 25% and 67% growth reduction following 6 and 72 h treatment, respectively (Chen et al.

2001). Furthermore, apoptotic mechanisms like the activation of caspase-3 and Bax simultaneously with Bcl-2 expression caused inhibition to occur in CAPE-treated HL-60 cells. Mitochondria-mediated apoptosis involved increase in cytosolic cytochrome c, downregulation of Bcl-2 expression, and activation/cleavage of caspase-3 and PARP with overexpression of Bax in U937 cells when treated with CAPE (Jin et al. 2008).

Differentiation is a leading mechanism in cancerous cells that decreases malignancies, generating benign tumors and decreases self-renewal properties in cancer cells (Deng et al. 2018). CAPE enhances the granulocytic differentiation property of ATRA (all-trans retinoic acid) in HL-60 by causing arrest at the G1 phase via inhibition of cdk2-cyclin E complex formation (Kuo et al. 2006). Also, RARα, p21, and C/EBPε proteins showed enhanced activity leading to differentiation in ALL cells.

Recently CAPE exposure in lymphoblastoid cell line, PL104, lead to the activation of apoptotic mechanisms like loss of mitochondrial potential (Δψm), nuclear fragmentation, and G1 stage arrest (Cavaliere et al. 2014). Transcriptional analysis showed that survivin and Bcl-2 expressions were downregulated with subsequent increase in Bax, and caspases 3, 7, and 9.

1.13 Conclusion

Dietary phenolic acids and flavonoids, in general, and, specifically, in honey can be widely exploited as chemotherapeutic agents that can help in alleviating diseases and fight critical health conditions including cancer. Most of these antiproliferative compounds cause decrease in cell growth by putting the cell into an apoptotic environment, halting cell cycle, and activating tumor suppressor pathways. However, the mechanism of action of these compounds is still very ambiguous. This chapter vividly describes phenolic compounds that can be used as signature drugs to target leukemias. Gene expression and microarray studies have depicted the various signaling pathways regulated by these compounds and, hence, serve in inducing decrease cell growth and malignancy in leukemias. Till date no study elucidating the effect of raw honey–derived phenolic compounds has been undertaken and, therefore, a wide scope exists for studying the effective chemotherapeutic mechanisms of these compounds.

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