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Review Article

Attenuation of ROS Mediated Regulation of Cell Signaling by Bee Venom and Melittin in Cancer Progression

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Abstract

Reactive oxygen species (ROS), a component of free radicals has emerged as a potential target that can elicit a cascade of cell cycle regulatory events to facilitate tumor progression. Recent reports suggest G-protein coupled receptors (GPCRs) mediated ROS activation promotes deregulated cellular signaling. Melittin, a bee venom product competently targets ROS-mediated signaling such as GPCR, mitogen-activated protein kinase (MAPK), and nuclear factor-κB (NF-κB) signaling pathways, which will eventually lead to regression in tumor development. Soaring interests toward natural anticancer drugs, Bee venom is on the bright side for future use in clinical practices when coupled with nanoparticles and other therapeutic agents. Melittin can further provide a potent system for better drug delivery to the targeted tumor. This review enlightens the efficacy of bee venom and melittin to hinder ROS-mediated tumor progression.

Keywords: Reactive Oxygen Species; G Protein Coupled Receptors; Cell Signaling; Bee Venom; Melittin; Cancer

Abbreviations

ROS: Reactive Oxygen Species; GPCRs: G-protein Coupled Receptors; MAPK: Mitogen-Activated Protein Kinase; NF-κB: Nuclear Factor-Kappa b; Nox: Nicotinamide Dinucleotide Phosphate (NADPH) Oxidase; AngII: Angiotensin II; LPA: Lysophosphatidic Acid; VSMCs: Vascular Smooth Muscle Cell; PI3K: Phosphoinositide 3-Kinase; GTP: Guanosine Triphosphate; GDP: Guanosine Diphosphate; cAMP: Cyclic Adenosine Monophosphate; PKC: Protein Kinase C; MMP: Matrix Metalloproteinase; MDA: Malondialdehyde; GSH: Glutathione; EGFR: Epidermal Growth Factor Receptor; NAC: N-acetyl-L-cysteine; JNK: c-Jun N-Terminal Kinase; ERK1/2: Extracellular Signal-Regulated Kinase1/2; TGF-β: Transforming Growth Factor-Beta; IκBα: Nuclear Factor of Kappa Light Polypeptide Gene Enhancer in B-Cells Inhibitor α; VEGF: Vascular Endothelial Growth Factor; AIF: Apoptosis-inducing Factor; Endo G: Endonuclease G; CypA: Cyclophilin A; PLA2: Phospholipase A2; sPLA2: Secretory Phospholipase A2; CaM: Calmodulin; TNF-α: Tumor Necrosis Factor α; PIP3: Phosphatidylinositol-3,4,5-triphosphate; HIF-1: Hypoxia-Inducible Factor

Introduction

Cellular signaling pathways form a close network between each other, in which cells receive signals from various growth factor receptors, from cell-matrix and cell-cell communication,

and with the amalgamation of all signals, cells synchronize diverse processes like protein synthesis, cell proliferation, differentiation, and apoptosis. Poor regulation of cellular signaling leads to cancer progression [1]. G-protein coupled receptors (GPCRs) are the largest and most diverse group of membrane receptors in eukaryotes that have attracted several interests due to their multifaceted physio-pathological roles elicited as transducers of extracellular signals into intracellular effector pathways and are involved in vital process development, hematopoiesis, migration, angiogenesis, inflammation, invasion, and cell viability [2]. GPCRs family are the diversified therapeutic targets encoding human genomics. GPCRs can also regulate the activity of key intracellular transducing molecules, including small GTP-binding proteins of the Ras and Rho families and serine/threonine protein kinases such as Akt and mitogen-activated protein kinases (MAPKs) [3]. It is well documented that Gq and Gs-coupled GPCR are involved in cancer which is regulated by PKC and cAMP/PKA, respectively and there are cites of regulation of nuclear factor-κB (NF-κB) by PKC in various intracellular mechanisms in the cancer cell [4].

Reactive oxygen species (ROS) is a phrase used to describe several chemically reactive molecules and free radicals derived from molecular oxygen having a single unpaired electron in their outermost shell of electrons and this makes ROS highly reactive. ROS is generated in the body from a wide range of complexes, including Nicotinamide dinucleotide phosphate (NADPH) oxidase (Nox), Xanthine oxidase, uncoupled endothelial nitric oxide, and assorted enzymes in the mitochondrial electron transport chain [5]. ROS and Nox go hand in hand in GPCR signaling, various GPCR agonists such as angiotensin II (Ang II), lysophosphatidic acid (LPA), endothelin 1, and thrombin are involved in ROS production in Vascular Smooth Muscle Cell (VSMCs) [6]. ROS evokes various downstream signaling cascades including the Ras/mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/protein kinase B(Akt) pathways thus leading to cellular proliferation, migration, differentiation, and cell survival [6]. Phosphorylation of ERK via the Src family and the Ras-dependent pathways is stimulated by hydrogen peroxide in a dosage-dependent manner and inhibition of MAPK phosphorylation plays a central role in averting apoptosis all leading to cell proliferation and cell survival [7].

Recently cancer treatment has made a move towards natural products and their extract that can be affordable for emerging drug industries. So, Apitherapy has emerged as a target that suffices traditional products which can be used as a treatment in cancer therapy. Apitherapy refers to the usage of bee products such as bee venom, melittin, and propolis that sustain health parallelly with disease treatment [8]. This paper highlights the evolved role of bee venom and melittin in modulating ROS-mediated cell signaling keeping in view G-protein coupled receptors (GPCRs) and its downstream targets regulation, which could be a therapeutic boon in the field of cancer research.

GPCR structural and molecular activation in carcinogenic response

G-protein coupled receptors (GPCRs) are ubiquitous and play a pivotal role in communicating extracellular cues into intracellular responses [9]. The conserved structure of GPCR is characterized by an extracellular N-terminus with seven transmembrane-spanning Alpha–helices (namely I, II, III, IV, V, VI, and VII) that are connected by three intracellular (ICL1, ICL2, and ICL3), three extracellular (ECL1, ECL2, and ECL3) loops and finally an intracellular C-terminus [10]. G proteins are specialized proteins with the ability to bind the nucleotide guanosine triphosphate (GTP) in their active form and guanosine diphosphate (GDP) in their inactive form. The G proteins which are involved with GPCRs are heterotrimeric, having three different subunits: an alpha subunit (α), a beta (β) subunit, and a gamma (γ) subunit. Out of these three, two subunits -(α-) and (γ) - are attached to the plasma membrane by lipid anchors [11]. A plethora of GPCR ligands including inorganic ions, amino acids, proteins, lipids, and nucleotides, as well as GPCR sensory stimuli such as light, tastes, and odorants, transduce a wide range of extracellular signals into intracellular information to direct various physiological processes [12]. Upon binding with ligand, GPCR undergoes a conformational change, replacing the bound GDP with GTP on the Gα subunit and activating the G-protein. The active form of the G-protein is then released from the surface of the receptor, dissociating into its α- and β/γ subunits which then activate the adenylyl cyclase, ion channels, and phospholipase c (PLC) leading to an increase in cyclic adenosine monophosphate (cAMP), Calcium and protein kinase C (PKC) activity [11,13].

A great deal of GPCRs has been involved in the growth and spread of tumor cells. As mentioned in various studies, GPCRs of potent mitogens and chemokines are constitutively overexpressed in diverse types of tumors and play a major role in directing migration, invasion, and metastasis of cancer cells [14,15]. Several GPCRs like

angiotensin, thrombin, prostaglandin, lysophosphatidic acid, and chemokine receptors are involved in angiogenic responses which lead to the progression of many tumors acting either directly on endothelial cells or indirectly through the release of proangiogenic factors from stromal, immune and cancer cells [16,17].

ROS: Origin and the crosstalk between ROS and GPCR signaling

ROS constitutes a wide range of molecules that are grouped into two classes: free oxygen radicals and non-radical ROS. Free oxygen radicals include superoxide ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), nitric oxide ($NO^{\bullet}$), organic radicals ($R^{\bullet}$), peroxyl radicals ($ROO^{\bullet}$), alkoxyl radicals ($RO^{\bullet}$), sulfonyl radicals ($ROS^{\bullet}$), thiyl peroxyl radicals ($RSOO^{\bullet}$), and disulfides (RSSR). Non-radical ROS include hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), ozone/trioxygen (O_3), organic hydroperoxides (ROOH), hypochlorite (HOCl), peroxynitrite (ONO^-), nitrosoperoxycarbonate anion ($O=NOOCO_2^-$), nitrocarbonate anion ($O_2NOCO_2^-$), dinitrogen dioxide (N_2O_2), nitronium (NO_2^+) out of which hydroxyl radicals, superoxide, and hydrogen peroxide are the hot topics in cancer biology and involved in controlling various cell signaling process [18]. ROS are naturally occurring molecules produced in the organism as by-products of the normal aerobic metabolism of oxygen [19], exposure to UV light [20] or X-rays [21] and have important roles in cell signaling acting as second messengers harmonizing various signal transduction, gene expression, normal functioning of the immune system, homeostasis [22] and apoptosis [23].

Heightened levels of the epidermal growth factor receptor (EGFR), a growth-factor-receptor tyrosine kinase, and/or its cognate ligand are responsible for multiple cancer types and appear to promote solid tumor growth. Acting as a strong prognostic indicator in head and neck, ovarian, cervical, bladder, and oesophageal cancers, increased EGFR expression resulted in reduced recurrence-free or overall survival rates in 70% of studies [24]. It has been reported that EGF-induced ROS generation favors cell survival, proliferation, and activation of Akt and MAPK signaling pathways, inhibition of EGF-induced ROS formation by N-acetyl-L-cysteine (NAC) which is an antioxidant inhibits the phosphorylation of Akt, extracellular signal-regulated kinase 1/2 (ERK1/2) and c-Jun N-terminal kinase (JNK) in human epithelial cells thereby affecting cell proliferation and cell survival [25].

ROS/Nox dependent systems are known to induce GPCR transactivation of EGFR in three ways, first through an increase in intracellular ROS which then activates matrix metalloproteinase (MMP) that cleaves heparin-binding EGF-like growth factor (pro-HB-EGF) and release of EGF ligand leading to EGFR activation and following phosphorylation of ERK1/2, second via Src-dependent pathway and third through inhibition of protein tyrosine phosphatase [6]. Transforming growth factor-beta (TGF-β) is a multipotent growth factor affecting cell differentiation, proliferation, apoptosis, and migration [26]. They carry out the signal transduction, binding with their cognate receptor through Smad-dependent and Smad – independent pathways. Some of the recent findings outline the specific involvement of ROS production in TGFBR1 activation and the underlying mechanism is its role in the TGF-β/ALK5/SMAD2/3, MAPK, ERK, p38, and JNK signaling pathways [6]. The incident of GPCR transactivation of TGFBR1 involves cytoskeletal rearrangement with leads to the activation of ROCK signaling leading to the activation of integrin which then binds to the large latent TGF - β complex (LLC) which then undergoes a conformational change, exposes TGF- β ligand. Binding with TGFBR1, TGF- β phosphorylates the downstream intermediate Smad2 in the carboxy-terminal [27]. In recent studies, the endogenous pharmacological stimulation of ROS in human VSMCs is known to activate ROCK and integrins. These findings lead to an assumption that ROCK signaling is a redox-sensitive pathway and that GPCR-mediated generation of ROS could play a major role in GPCR transactivation of TGFBR1 via rock signaling [27] (Figure 1). Activated G-protein coupled receptors by various agonists such as angiotensin, and thrombin triggers ROS production. ROS activation stimulates various signaling that leads to the proliferation, migration, and metastasis of cancer cells. Inhibitory Effect of Bee Venom and Melittin (-|).

Figure 1

According to the studies, ROS activates NF-κB through the alternative nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, α (IκBα) phosphorylation that may lead to the degradation of IκBα or may not. Along with that, ROS-dependent RelA phosphorylation drives NF-κB activation in an elevated manner. Activation of pro-inflammatory transcription factors like NF-κB steers to an enhancement in cancer progressive events like proliferation, and metastasis [28].

Alleviative action of bee venom and melittin in cancer

Bee venom is extracted from the venom gland present in the abdominal cavity. It comprises various biologically active peptides such as melittin, apamin, mast cell degranulating peptide, and enzymes (phospholipase A2, and hyaluronidase) as well as non-peptide components, such as histamine, dopamine, and norepinephrine [29]. Bee venom can be accessible from the markets in multiple modes such as ointments, balms, acupuncture, lotions, bee bites, injections, and raw liquid venom [30]. Furthermore, the fatal dose (LD50) for an allergic person ranges from 2.8 mg to 3.5 mg of bee venom per kg of the human body [30]. Experimental studies revealed that bee venom has anti-carcinogenic [31-34], anti-inflammatory [35], anti-mutagenic [36], radio-protective features, and radio-protective features [37]. Bee venom impedes lipid peroxidation activity leading to suppression of free radical generation to a certain extent [38]. *In vivo* data showed bee venom hinders cancer cell migration and proliferative events. This inhibition is correlated with the development of cellular immune responses in lymphatic nodes [32]. Bee venom as well as its components primarily aims for pathways like the ERK pathways, the PI3K-AKT pathways [31,34,39], JNK pathways, and NF-κB pathways [40].

Melittin, isolated from the honeybee, *Apis mellifera*, comprises 50-70% of bee venom [32]. It has a peptide length of 26 amino acids. Bee venom specifically melittin possesses antioxidative properties to rule out free radicals to a certain extent. This is consistent with the data that the bee venom gland tissue contains antioxidative enzymes such as superoxide dismutase 1 (SOD1), glutathione-S-transferase sigma 1 isoform A (GSTS1), peroxiredoxin 2540 (PXR2540), and thioredoxin peroxidase 1 isoform A (TPX1). *In vitro* data concluded that at a concentration of 0.1 mg/ml, the bee venom extract from *Apis dorsata* has the highest antioxidant characteristic to efficiently regress free radicals activity [41]. Reports suggest that they also show anti-tumor activity [42]. Melittin shows anti-proliferative [43] and anti-angiogenic activity against cancer *in vivo* [40,44]. Bee venom extract from *Apis dorsata* possessed the most enhanced scavenging property against 2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH). Melittin inhibits malignant cells at IC50 values of 3.125 and 2.5 g/mL in A549 (Lung adenocarcinoma cell line), 6.25 and 3.125 g/mL MDA-MB-231 (Breast carcinoma cell line), and 12.5 and 6.25 g/mL HeLa (Cervical carcinoma cell line) over the timeframe of 24, and 48 h, respectively [45].

Multimodal effects of bee venom and melittin in different cancer signaling cascades

The anti-cancerous or anti-tumor effects of bee venom as well as its component melittin have been studied in various types of cancer. Melittin is being focused on due to its abundance and its diversified nature for cancer treatment. Moreover, since the cancer cells exhibit increased membrane potential compared to normal cells, melittin could directly aim at tumor cells while exerting no impact on the development of normal cells at the same dosage concentration [30]. Heterogenous actions of melittin are reported in different types of cancer. In breast cancer, melittin attenuated the PI3K/Akt/mTOR axis, in ovarian cancer, it suppressed the JAK2/STAT3 pathway, MAPK pathway inhibition in melanoma, and in lung carcinoma, the NF-κB signaling pathways also inhibited [46]. Studies have reported bee venom-induced apoptosis leads to a decrease in cellular viability and lessens MMP-2 expression, thereby inhibiting metastasis of glioblastoma cells [47]. Bee venom is seen to mediate calcium concentration, a rapid increase in the concentration of calcium is observed with the application of bee venom that in turn may give rise to the generation of reactive oxygen species leading to a breach in mitochondrial membrane potential transition, due to which Apoptosis-inducing Factor (AIF) and Endonuclease G (Endo G) are released into the nucleus that gives rise to cell death by apoptosis in a caspase-independent manner in melanomas [48]. Declining Bcl-2 and surge of Bax protein levels have a positive relationship with the administration of bee venom that drove to the liberation of cytochrome c which caused the rise in caspase-9 leading to caspase-3 activation and then apoptosis [31]. Bee venom may also induce apoptosis through caspase-3 independent (AIF and Endo G) pathways [48]. Flow

cytometry data revealed the presence of phosphatidylserine on the outer surface of the cell membrane upon melittin treatment [49,50]. This data confirms the induction of apoptosis on cancer cells via melittin.

The study conducted has shown that bee venom and melittin-induced suppression of MMP-9 expression come up with the anti-tumor properties in Caki-1 cells and MCF-7 cells, whereas the cytolytic activity of melittin/avidin conjugate has been proven to work wonder against cancer cells with elevated MMP-2 activity as in DU 145 prostate cancer cells and SK-OV-3 ovarian cancer cells [51]. Various processes are involved in the regulation of tumors, for example, cholesterol homeostasis is greatly affected in tumorigenesis, in a study it is reported that melittin downregulates CLU, a gene involved in the cholesterol pathway by inducing NONHSAT105177 expression. Overexpression of NONHSAT105177 showed decreased expression of mesenchymal markers [52]. Bee venom restricts the angiogenesis process by reducing the Ca2+/CaM and Vascular Endothelial Growth Factor (VEGF) signaling [43] (Figure 2). Multiangle effects of bee venom and melittin in inhibiting cancer progression and various physiological processes.

Figure 1

It has been demonstrated that bee venom in both time-dependent and dose-dependent fashion repressed multiplication of MCF7 cells via S-phase arrest along the elevation of p53, p21, p27, and the manifestation of Cdk2 [53]. Cyclophilin A (CypA) is a cytosolic protein produced in a wide range of cells in response to oxidative stress [53]. CypA acts as a ligand for CD147 and which in turn can stimulate numerous signal transductions and perform chemotactic functioning [54]. Melittin in a dose-dependent fashion is known to suppress the invasion level of MCF-7 cells by counteracting CD147 mRNA expression stimulated by CypA. A transmembrane glycoprotein CD147, stimulate the expression of MMP family members exclusively, particularly in malignant breast tumor [54,55]. Bee venom and its constituent melittin intervene with the phosphorylation of EGFR and HER2 by its ligand in breast cancer, and specifically, melittin inhibits growth factor-dependent RTK dimerization by critically targeting HER2- and EGFR-overexpressing breast cancer cells [47]. Apart from the breast cancer cell, EGFR overexpression is also seen in glioblastoma, lung, and colorectal cancer [56], and HER2 overexpression is also found in the colon, endometrial and ovarian cancer [57]. Experimental studies suggested that a concentration of 0.7 μM of melittin regresses breast cancer cells' invasive and migratory capability [58].

Modulation of ROS mediated signaling by bee venom and melittin

ROS regulates multiple cellular signaling pathways and the ROS interlinked routes such as MAPK, JNK, EGFR signaling, and others help in tumor progression. Melittin suppresses tumor necrosis factor α (TNF-α) gene expression and also induces apoptosis via the inactivation of NF-κB signaling [59-61]. Degradation of the Extracellular matrix (ECM) is one of the contributing factors to the invasion and metastasis of tumors. Melittin inhibits MMP-2 and MMP-9 activity by suppressing NF-κB via p38 MAPK and JNK signaling pathways in MCF-7 cells thus checking metastasis of tumor [42]. Epidermal growth factor receptor (EGFR) aberrant expression contributes to breast carcinogenesis. Melittin/Bee venom components have targeted various signaling components such as Ras-MAPK, PI-3KT, and PLCY-CAM. They dephosphorylate or degrade their downstream signaling pathways halting proliferation, metastasis, angiogenesis, and apoptosis [61].

Melittin is known to primarily activate phospholipase A2 (PLA2) crotoxin, an anti-tumor protein that results in cell toxicity that corresponds with EGFR expression providing a promising strategy for the anti-cancer activity of bee venom [62]. Along with that, a secretory phospholipase A2 (sPLA2) present in bee venom is involved in the catalysis of the sn-2 fatty acyl ester bond of membrane glycero-3-phospholipids hydrolysis to produce free fatty acids and lysophospholipids leading to subsequent degradation of the constituents of cell membrane disturbing the integrity of the membrane that makes the cell prone to degradation [63-65]. Structurally resembling synthetic analogs of sPLA2 are

used for their antiproliferative effects and cytotoxicity in cancer therapies which occurs by the inhibiting ERKs and PKB/Akt pathways [66-68]. NF-κB signaling, an important mediator of oxidative stress and inflammation is also disrupted by bee venom/ melittin. Bee venom directly binds to NF-κB thereby preventing it from binding with the DNA along with the inhibitory effect of bee venom on phosphorylation of IκB and nuclear translocation of p50 and p65 in colon cancer cells [69]. A highly anticipated model of calcium/calmodulin-induced Akt activation and cell survival has been brought into light in which the alliance between Ca^{2+}-bound activated calmodulin (CaM) and Akt leads to the movement of Akt to the plasma membrane, where Akt binds to phosphatidylinositol-3,4,5-triphosphate (PIP3) a product of PI-3 kinase, leading to activation by phosphorylation [61].

Studies have also reported the role of Ca^{2+}/CaM in angiogenesis by triggering hypoxia-inducible factor 1 (HIF-1) which then induces the expression of VEGF a pro-angiogenic factor. Therefore, PI-3 kinase suppression, intracellular calcium chelation, and downregulation of calmodulin results in apoptosis and Akt inhibition acting as potential therapeutic targets for the treatment of angiogenesis-related diseases, including cancer. On that account antagonists such as melittin may attenuate Ca2+/CaM signaling thereby repressing angiogenesis [43].

Conclusion

Cancer mortality is increasing as the day passes by, and better treatment options are being developed to tackle its growth and prevention [43]. GPCRs have become well-known potential targets for drug treatment and act as markers in cancer therapeutics. ROS plays a prominent role in averting apoptosis leading to cell proliferation and cell survival [7]. ROS has been shown to play various roles such as it exerts a pro-apoptotic effect by accumulating in the cell leading to the cytotoxicity of the cancer cell, inducing apoptosis [70] and it also plays a part in tumor progression by working along with GPCR and its downstream targets [6]. So, attenuating the production of ROS may lead to checking cell proliferation, angiogenesis, and metastasis. And emerging awareness of natural anticancer drugs, bee venom is on the bright side for future use in clinical practices.

Bee venom is involved in ROS production leading to cytotoxicity but here comes the twist, it also shows high levels of antioxidants which can check the hazardous effect of ROS thereby showing positive results towards cancer regression. And it also has been established that with the detoxification of bee venom, its antioxidant property can be enhanced [71]. Combinational strategies of melittin with different therapeutic agents like cisplatin [72] and docetaxel in cervical and lung cancer cells respectively have been proven to work better [73]. Debatable properties of bee venom were confirmed in a dose-dependent experiment where doses of 0–0.05 μg mL^{-1} provoke the matrix MMP-2 and MMP-9 activation and the inhibitory effect of bee venom at a dose higher than >0.05 μg mL^{-1} [74]. Coupled with Nanoparticles, melittin, and other peptides can further provide a robust system for better drug delivery to the targeted tumor which has been validated in liver metastasis [75]. Melittin present in bee venom has become renowned for its chemotherapeutic effects, being a good cytotoxic agent and acting as a tumor suppressor by attenuating various growth factor receptors, blocking cell proliferation by promoting apoptosis and triggering death receptors.

It can be summarized that the action of bee venom is multifaceted, where a few of the components of the bee venom acts as a hindrance for the receptors at the surface by either destroying them or inhibiting their activity by dephosphorylating them, repressing processes like cell proliferation, programmed cell death like apoptosis, angiogenesis, and metastasis [43]. Since GPCRs are evolving as a pivotal target in the field of cancer research. A deep insight into the molecular mechanism of bee venom in restricting GPCR signaling through ROS impediment could provide a breakthrough in this field of research.

Conflict of Interest

The authors declare that they have no competing interests.

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