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Commentary

Occurrence, function and potential medicinal applications of the phytohormone abscisic acid in animals and humans

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ABSTRACT

Abscisic acid (ABA) is an important phytohormone that regulates plant growth, development, dormancy and stress responses. Recently, it was discovered that ABA is produced by a wide range of animals including sponges (*Axinella polypoides*), hydroids (*Eudendrium racemosum*), human parasites (*Toxoplasma gondii*), and by various mammalian tissues and cells (leukocytes, pancreatic cells, and mesenchymal stem cells). ABA is a universal signaling molecule that stimulates diverse functions in animals through a signaling pathway that is remarkably similar to that used by plants; this pathway involves the sequential binding of ABA to a membrane receptor and the activation of ADP-ribose cyclase, which results in the overproduction of the intracellular cyclic ADP-ribose and an increase in intracellular Ca^{2+} concentrations. ABA stimulates the stress response (heat and light) in animal cells, immune responses in leukocytes, insulin release from pancreatic β cells, and the expansion of mesenchymal and colon stem cells. ABA also inhibits the growth and induces the differentiation of cancer cells. Unlike some drugs that act as cell killers, ABA, when functioning as a growth regulator, does not have significant toxic side effects on animal cells. Research indicated that ABA is an endogenous immune regulator in animals and has potential medicinal applications for several human diseases. This article summarizes recent advances involving the discovery, signaling pathways and functions of ABA in animals.

1. Introduction

Abscisic acid (ABA) is one of five classes of hormones that play important roles in plants, including regulating plant growth and development, seed and bud dormancy, apical dominance, senescence and abscission as well as plant responses to stresses (low temperature, drought, salt and pathogens) [1,2]. The chemical structure and biosynthetic pathway of ABA in plants are shown in Fig. 1. Recently, it was discovered that ABA not only exists and functions in plants, algae [3,4], cyanobacteria [5] and fungi [6,7], but is also present in a wide range of lower animals, such as sponges [8–10], as well as higher mammals [11], including various human tissues and cells [12,13]. ABA can be produced and released from many animal and human cells (immune cells, cardiovascular cells, stem cells and pancreatic cells) under physiological or pathological conditions. ABA regulates cell growth, development and immune responses to various stimuli through a signaling pathway that is remarkably similar to that found in plants. This pathway involves a membrane G protein complex receptor, the phosphorylation and activation of ADP-ribosyl cyclase (ADPRC), the overproduction of the universal calcium mobilizer cyclic ADP-ribose (cADPR), and an increase in intracellular calcium concentration ($[Ca^{2+}]_i$). ABA was demonstrated to be a universal signaling molecule and has potential medicinal applications in several human diseases. This article summarizes the research behind the discovery, signaling pathways, functions and potential applications of ABA in animals and humans.

2. Production of ABA in animal and human cells

2.1. Pig and rat brains and other organs

In 1986, Le Page-Degivry et al. [11] reported the presence of ABA in the brains and other tissues of pigs and rats. They identified (+)-*cis*-ABA with radioimmunoassay and GC/MS methods in pig hearts, lungs, kidneys, livers, brains and in rat brains at concentrations of 13, 27, 37, 57, 180 and 248 ng/100 g fresh tissue, respectively. ABA content was significantly higher in the brain than in other tissues. They confirmed that the ABA from rat brains was not due to a diet containing ABA; animals were fed a synthetic diet containing very low levels of ABA for two generations. In fact, the brains of animals that were fed an ABA-

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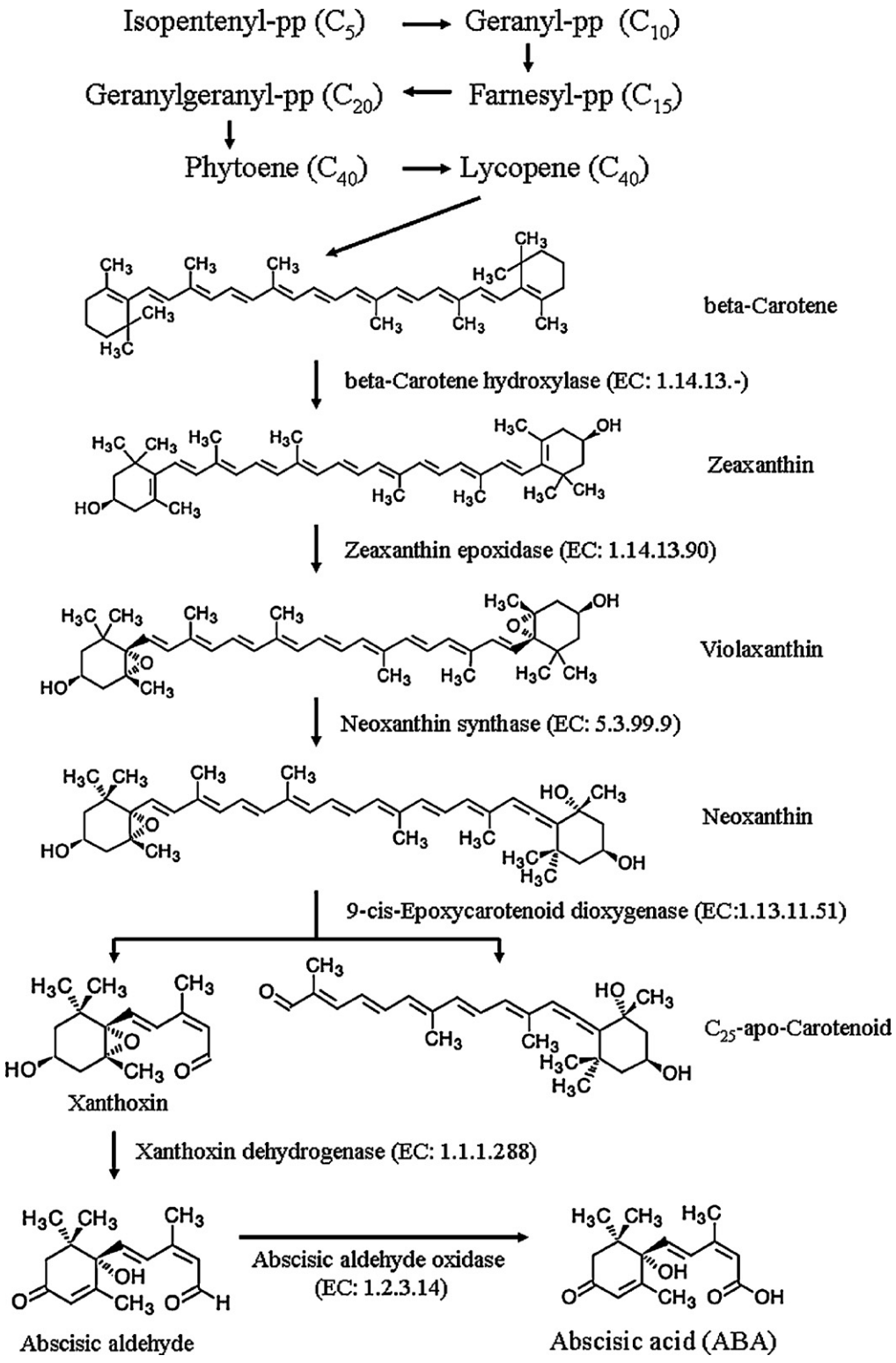

Fig. 1. The biosynthetic pathway of abscisic acid in plants (data from the GenBank).

poor diet contained more ABA than the brains of control animals [11]. ABA conjugates (ethers and glucosides) present in plants were also identified in the brains of these animals. ABA isolated from animal tissues showed the same biological activity as ABA isolated from plants in a bioassay that involved the inhibition of the stomatal opening of abaxial epidermis strips of well-watered plant leaves from *Setcreasea pururea* Boom (Commelinaceae) [11]. To the best of our knowledge, this was the first identification of ABA in the tissues of animals, although the authors did not speculate as to the possible function of ABA in brain tissue. ABA was also produced and released by rat insulinoma cell lines RIN-m and INS-1 [13], and murine microglial cell line N9 [14] under physiological and pathological conditions (Table 1).

2.2. Sponges – the oldest animal

Sponges (Phylum, Porifera) are the most phylogenetically ancient animals. Zocchi et al. [8] reported the discovery of ABA

Table 1
ABA (free) contents determined in animals tissues and cells.

Tissues or cells	[ABA]*i*	[ABA]*m*
Pig, ABA content in ng/100 g fresh weight, Le Page-Degivry et al. [11]		
Heart	13 ± 5	–
Lung	27 ± 10	–
Kidney	37 ± 7	–
Liver	57 ± 16	–
Brain	180 ± 37	–
Rat (Wistar) brain, ABA content in ng/100 g fresh weight, Le Page-Degivry et al. [11]		
With cereal diet	248 ± 54	–
With synthetic diet	429 ± 78	–
Rat insulinoma RIN-m cells, ABA content in pmol/mg cell proteins, Bruzzone et al. [13]		
Low glucose	10	15
High glucose	20	240
Rat insulinoma INS-1 cells, ABA content in pmol/mg cell proteins, Bruzzone et al. [13]		
Low (5.5 mM) glucose	8	12
High (16.7 mM) glucose	25	125
Murine microglial cell line N9, ABA content in pmol/3 × 10^7 cells, Bodrato et al. [14]		
Unstimulated control	4.1 ± 0.6	36 ± 6
100 ng/ml LPS, 72 h	14.3 ± 2.0	108 ± 18
LPS + 10 μM βA	15.2 ± 1.6	185 ± 29
PMA (0.1 μg/ml for 1 h)	4.9 ± 0.5	125 ± 18
f-MLP (1 μM for 1 h)	4.2 ± 0.4	206 ± 36
Sponges (*Axinella polypoides*), ABA content in pmol/g wet tissue, Zocchi et al. [8]		
Normal temperature 14 °C	4.1 ± 1.5	–
Heat stress at 26 °C	18.1, 80.0 or 104.2 For 1, 5 or 60 min, resp.	–
Hydroid (*Eudendrium racemosum*), ABA content in pmol/g wet tissue, Puce et al. [15]		
(1) 0 time (after sampling)	22	–
(2) Dark, 24 h	18	–
(2) +Fluridone (50 μM)	16	–
(3) Light, 15 min, 1 and 4 h	90, 105, 30, resp.	–
(3) +Fluridone (50 μM)	30, 15, 13, resp.	–
Parasite (*Toxoplasma gondii*), Nagamune et al. [10]		
Control	230 nM	–
Fluridone (50 μM)	40 nM	–
First 36 h after invaded	Between 0 and 1.3 μM	–
40th h (shortly before egress)	3.5 μM	–

[ABA]*i*, ABA in tissues or cells; [ABA]*m*, ABA in medium.

in the sponge *Axinella polypoides*. *A. polypoides* cells produced and responded to cADPR, a potent universal intracellular Ca^{2+} mobilizer, which is converted from its natural substrate NAD^+ by ADPRC. ADPRC activity was rapidly increased 3-fold after a short heat shock (2 min) at 26 °C (normally grown at 14 °C in the aquarium), which was paralleled by a 7-fold increase of intracellular cADPR. This finding prompted the researchers to investigate the effect of ABA on sponge cyclase activity. An increase in cyclase activity was also observed upon incubation of *A. polypoides* cells in the presence of ABA at nanomolar concentrations. Maximum activation (a 4-fold increase over the control) was observed in the presence of 50 nM ABA. Stimulation of cyclase activity by ABA (at concentrations ranging from 5 to 50 nM) was paralleled by an increase in [cADPR]*i*, a response that was similar in both extent and kinetics to that observed in the temperature stress experiment. Additionally, they found that heat shock stimulated ABA production in *A. polypoides*. ABA was detected in extracts from *A. polypoides* fragments at 4.1 pmol/g of wet tissue. The amount of ABA in sponge tissue increased rapidly after exposure to the 26 °C heat-shock from a basal value of 4.8 pmol/g at 14 °C to 18.1, 80.0 and 104.2 pmol/g after 1, 5 and 60 min at 26 °C, respectively.

2.3. Hydroids

Puce et al. [15] demonstrated that ABA is present and rapidly synthesized *de novo* under light conditions in the hydroid *Eudendrium racemosum* (Hydrozoa, Cnidaria). ABA content was found to be 18 pmol/g of wet tissue in *E. racemosum*, and the concentration decreased slightly after 24 h in the dark, but dramatically increased after 15 min of light exposure. The ABA levels reached approximately 105 pmol/g after 1 h and then decreased to near basal levels (approximately 30 pmol/g) after 4 h of light exposure. The amount of ABA (on a fresh weight basis) found in hydroids (20–100 pmol/g) is similar to that reportedly found in the tissues of higher plants (40–400 pmol/g) and is 2–12 times higher than that found in mammalian tissues (1–8 pmol/g). Carotenoids, which are ABA precursors in plants [16], were detected in acetone extracts from freshly collected *E. racemosum* colonies (42 μg/g wet weight). Regeneration of *E. racemosum* is decidedly light dependent, and exogenous ABA (5 μM) dramatically stimulated regeneration (4.5-fold compared with controls) in the dark compared to levels observed under light conditions. Light-stimulated endogenous ABA synthesis was inhibited by fluridone, which is an inhibitor of ABA synthesis from carotenoids in plants and works by specifically blocking phytoene desaturase activity [16,17]. Similar growth of ABA-treated fragments under light and dark conditions indicated that the effects of ABA and light on growth were not additive.

2.4. Human parasites

Nagamune et al. [10] reported that the parasite *Toxoplasma gondii* (an important human pathogen) produces endogenous ABA. Exogenous ABA induced the production of cADPR in *T. gondii*, which then activated Ca^{2+}-dependent protein secretion and induced parasite egress from the infected host cells. Production of endogenous ABA within the parasite was confirmed with HPLC purification and GC–MS analysis. ABA content did not significantly change during the first 36 h, but a rapid increase of ABA was observed after 40 h, shortly before egress. Selective disruption of ABA synthesis by the inhibitor fluridone (15 μM) delayed or completely blocked (50 μM) egress and induced the development of the slow-growing, dormant cyst stage of the parasite. Exogenous ABA reversed the inhibitory effects on egress resulting from the fluridone treatment. ABA is a natural agonist for host cell egress, and ABA-mediated calcium signaling controls the transition between lytic and chronic stage growth, which is a developmental switch that is central to pathogenesis and transmission. Fluridone, a registered herbicide with low toxicity to mammalian cells, prevented lethal infections in laboratory mice and may be exploited in the development of improved anti-parasitic drugs. Several genes that are similar to the genes involved in ABA synthesis from higher plants (*ABA1*, *ABA2*, and *ABA3*) as well as genes that are similar to ABA-response genes were found in the *T. gondii* genome.

2.5. Human granulocytes

Bruzzone et al. [12] reported the presence of free and conjugated ABA in human granulocytes. Free ABA was detected in unstimulated human granulocytes at a concentration of 0.23 pmol/mg protein. Approximately 30% of the total ABA was present as alkali-hydrolysable conjugated ABA which may represent an intracellular storage form of the hormone. Free intracellular ABA increased by 2.1- and 3.0-fold in granulocytes 30 min after treatment with chemical stimuli (0.1 μg/ml phorbol myristate acetate) or exposure to fever-like temperatures (39 °C), respectively. ABA was not released into the medium after granulocytes were exposed to high temperature, whereas intracellular ABA decreased and was released into the medium when the granulocytes were stimulated with zymosan (0.37 mg/ml) or latex beads. The total intra- and extracellular ABA content increased in particle-

stimulated cells compared to the controls, which indicated that ABA release is sustained by its intracellular production.

2.6. Human monocytes

Magnone et al. [18] reported that ABA was detected in extracts of human monocytes at 4.11 pmol/mg protein, which is a concentration that is 10 times higher than that observed in granulocytes (0.23 pmol/mg) [12]. The release of ABA by monocytes can be stimulated by heat shock, platelets, or monocyte chemoattractant protein-1 (MCP-1). When human monocytes were incubated for 60 min at 39 °C, intracellular ABA was not significantly modified, whereas ABA released into the medium increased 3.6-fold over the controls that were incubated at 37 °C. Human platelets stimulated ABA release from monocytes (9.2-fold increase over unstimulated monocytes) and intracellular ABA increased (1.6-fold over the controls) when monocytes (2×10^6 in 2 ml) and thrombin-activated platelets (10^8/ml, a density similar to that found in blood) were co-incubated for 1 h; this co-incubation simulates the environment of injured vascular endothelium. Incubation of monocytes with MCP-1 (50 ng/ml) for 60 min increased both intracellular ABA (2.7-fold compared to untreated controls) and the release of ABA into the medium (2.8-fold compared to controls).

Human platelets also stimulated the release of ABA, which was measured in the supernatant of cultured thrombin-activated platelets, but to a lesser extent (1.5%) than that observed with monocytes. Human arterial plaques (atherosclerotic) contained a 10-fold higher concentration of ABA compared to concentrations observed in normal arterial tissue (non-atheromatous). Exogenous ABA can activate human monocytes and vascular smooth muscle cells. ABA also functions as a signaling molecule in the development of atherosclerosis [20].

2.7. Human and murine pancreatic cells

To investigate whether ABA is produced by the autocrine system via pancreatic β cells in response to glucose, human islet and rat insulinoma cell lines, RIN-m and INS-1, were incubated in a low-glucose (LG, 5.5 mM glucose) or high-glucose (HG, 16.7 mM glucose) medium, and the ABA content in the cellular extracts and supernatants was determined using ELISA. The intracellular ABA concentration doubled in human pancreatic islets as well as in the rat insulinoma cell lines, and ABA released to the medium increased 25-, 20- and 10-fold, respectively, after a 15 min incubation in HG medium compared to levels in the LG medium. Neither human-blood-derived mononuclear cells nor murine exocrine pancreatic cell preparations released ABA when incubated in HG medium, which demonstrated that glucose induced ABA release from human islets; this finding is attributed to pancreatic β cells and not to contaminating cell types present in the human islet preparation. Glucose stimulates pancreatic cells to release ABA, which in turn increases insulin secretion, suggesting a positive-feedback mechanism that prolongs and enhances insulin release in response to glucose [13].

2.8. Human mesenchymal stem cells

Scarfi et al. [19] reported that ABA was detectable in human bone marrow (BM) derived human mesenchymal stem cells (MSC) at an extracellular concentration of 1.34 ± 0.3 pmol/ml per 10^6 cells and intracellularly at a concentration of 2.17 ± 0.4 pmol/mg protein, as estimated using both LC–MS and ELISA analyses of the medium and cell extracts. ABA production and release by MSC can be stimulated by specific growth factors [e.g., bone morphogenetic protein-7 (BMP-7)] or by inflammatory cytokines and lymphocyte-conditioned medium. Treatment with the MSC-specific growth factor BMP-7 (0.2 μg/ml) increased intracellular ABA by 1.6-, 6.0-, and 2.3-fold relative to untreated controls after 6, 24, and 48 h, respectively. PBMNC (peripheral blood mononuclear cells)-conditioned medium stimulated ABA release from MSC in a time-dependent manner. ABA released into the medium increased 4.7-fold after 24 h compared to the level at time 0 and returned to the time 0 level after 48 h. However, intracellular ABA concentrations were not changed throughout the culture period. The PBMNC-conditioned medium contains cytokines: TNF-α (tumor necrosis factor), M-CSF, IL-8, and RANTES. The effect of each of these cytokines on ABA release by MSC was investigated. M-CSF did not stimulate intracellular ABA production or ABA release into the medium by MSC. TNF-α, RANTES and IL-8 enhanced ABA release from MSC into the medium by 2.8-, 2.7-, and 2.8-fold after 6 h, respectively, and by 4.1-, 3.7-, and 5.5-fold after 24 h, respectively, compared to untreated cells at the same time points. ABA concentrations in all treatments returned to control levels after 48 h.

2.9. Murine microglial cells

Bodrato et al. [14] reported that murine microglial cell line N9 produces and releases ABA; both processes were enhanced by bacterial lipopolysaccharide (LPS), phorbol myristate acetate (PMA), the chemoattractant peptide f-MLP (formyl-methionyl-leucyl-phenylalanine), and β-amyloid (βA, the primary plaque component in Alzheimer's disease). Intracellular ABA from the N9 cell line was 0.31 ± 0.07 pmol/mg protein by LC-MS and increased to 1.32 ± 0.29 pmol/mg protein after the cells were incubated for 72 h in the presence of 100 ng/ml LPS. ABA concentrations in the medium of LPS-treated cells increased 3-fold compared to control levels after a 72 h incubations in the presence of LPS. After treatment with LPS for 48 h, N9 cells were further treated with βA for 24 h, and the concentration of ABA in the medium increased 5-fold and intracellular ABA increased 3.7-fold compared to the controls. Treatment with f-MLP or PMA for 1 h also increased ABA in the medium by 5.7- and 3.4-fold, respectively, compared to untreated control cells. ABA stimulated several functional activities in N9 cells, including the production of NO and TNF-α, as well as cell migration. These results indicate that ABA is a pro-inflammatory hormone that induces autocrine microglial activation, potentially representing a new target for anti-inflammatory therapies aimed at limiting microglia-induced tissue damage in the central nervous system.

Bassaganya-Riera et al. [20] noted significant levels of ABA in the plasma of mice that were fed AIN-93G-based diets. For quick reference, the ABA concentrations found in all tissues and cells of animals and humans are summarized in Tables 1 and 2.

3. ABA signaling pathway in animal and human cells

3.1. ABA signaling pathway in lower animals

Zocchi et al. [8] demonstrated that, similar to the mammalian K^+ channel, *A. polypoides* cells express a heat stress- and mechanical stress-activated cation channel, which functions as a thermoreceptor. The cation channels can be opened by heat and mechanical stresses or by micromolar concentrations of arachidonic acid (AA, which mimics mechanical activation by modifying the membrane curvature). The cation channels can be blocked by PKA-dependent phosphorylation, micromolar concentrations of Gd^{3+} and by local anesthetics (e.g., bupivacaine). A concentration of 50 μM AA induced a greater than 4-fold increase of ADPRC activity in intact *A. polypoides* cells, which was completely inhibited in the presence of 50 μM Gd^{3+}. The temperature-induced activation of the ADPRC was also prevented by Gd^{3+} and 1 mM bupivacaine. A rapid increase in ABA was induced by both AA and heat shock and was completely abolished by the pretreatment of the cells with

Table 2
ABA (free) contents determined in human tissues and cells.

Tissues or cells	[ABA]*i*	[ABA]*m*
Mesenchymal stem cells (MSC), Scarfì et al. [19,46]		
[ABA]*i*: pmol/mg protein, [ABA]*m*: pmol/ml per 10^6 cells		
Control (untreated)	2.17 ± 0.4	1.34 ± 0.3
BMP-7 (0.2 μg/ml)	Up 1.6-, 6.0-, 2.3-fold for 6, 24, 48 h, resp.	–
PBMNC-conditioned medium	No change	Increase 4.7-fold after 24 h Return to normal after 48 h
M-CSF	No change	No change
TNF-α, RANTES or IL-8, 6 h	–	Up 2.8-, 2.7- or 2.8-fold
TNF-α, RANTES or IL-8, 24 h	–	Up 4.1-, 3.7- or 5.5-fold
Lymphocyte-stimulated MSC	–	1.5, 7.2, 3.1, 2.7 for 0, 24, 48, 72 h, resp.
Granulocytes, Bruzzone et al. [12]		
Temperature stress treatment, ABA content in pmol/mg protein		
Control	0.23 ± 0.09	–
0.1 μg/ml PMA, 20 °C	Up 2.1 ± 0.3-fold	No ABA release
39 °C, 30 min	3.0 ± 0.5-fold	No ABA release
Chemical or mechanical stimuli, ABA content in pmol/5×10^7 cells		
Control	1.7	0.2
Zymosan (0.37 mg/ml)	1.3 (down)	1.6 (up with total ABA up)
Latex bead	0.5 (down)	2.8 (up with tatal ABA up)
Monocytes, ABA content in pmol/mg protein, Magnone et al. [18]		
Control (37 °C)	4.11 ± 0.82	–
39 °C, 60 min	No change	3.6 ± 0.6-fold
Thrombin-activated platelet, 1 h	1.6 ± 0.5-fold	9.2 ± 0.7-fold
MCP-1 (50 ng/ml, 1 h	2.7 ± 0.3-fold	2.8 ± 0.3-fold
Pancreatic islets, ABA content in pmol/mg cell proteins, Bruzzone et al. [13]		
Low glucose, 15 min	5	2
High glucose, 15 min	10	48
Platelet, ABA content in pmol/mg wet weight, Magnone et al. [18]		
Non-atheromatous	0.003 ± 0.002	
Atherosclerotic plaque	0.023 ± 0.018	–

[ABA]*i*, ABA in tissues or cells; [ABA]*m*, ABA in medium.

Gd^{3+} or bupivacaine. These results indicate that opening of a heat-activated cation channels is the first event in the signal transduction pathway leading to ADPRC activation in *A. polypoides*, and ABA generation occurs downstream of cation channel activity.

Both ABA and temperature stress induced an increase in ADPRC activity, and intracellular cADPR synthesis was completely inhibited by the addition of the protein kinase inhibitor K252a (1 μM) during incubation, demonstrating that ABA induces ADPRC activation by means of its PKA-dependent phosphorylation. cADPR binds to a microsomal receptor/Ca^{2+} channel, which is known as the ryanodine receptor, and causes the immediate release of calcium from the same source as ryanodine [8].

Based on these results, Zocchi et al. [8] proposed that a temperature-signaling-based pathway exists in *A. polypoides*. The membrane cation channels sense the temperature stress and open (also opened by mechanical stress and AA, but inhibited by Gd^{3+} or bupivacaine), which causes an increase in intracellular ABA. ABA activated protein kinase A and stimulated the multiple-phosphorylation of ADPRC and the overproduction of cADPR. cADPR induced an increase in intracellular Ca^{2+}; the pathway (channel) is feedback-inhibited by PKA-mediated phosphorylation.

A similar ABA signaling pathway has also been reported in a hydroid, *E. racemosum*, by Puce et al. [15] and in human parasites by Nagamune et al. [10]. In the hydroid, exogenously added ABA stimulated ADPR cyclase activity via the activity of protein kinase A (PKA)-mediated phosphorylation, and increased the regeneration of *Eudendrium* fragments in the dark to levels observed under light conditions. The signaling pathway that stimulates light-induced regeneration in *E. racemosum* is as follows: light → ABA↑ → PKA activation → ADPR cyclase activation → [cADPR]*i* → $[Ca^{2+}]i$ → regeneration [15].

3.2. ABA signaling pathway in human leukocyte and pancreatic cells

Bruzzone et al. [12] proposed a mechanism in which ABA stimulates several functions of human granulocytes through a sequential signaling pathway. ABA first interacts with a transmembrane binding site and activates PTX-sensitive G protein(s), which in turn activates PLC (phospholipase C) and results in rapid increase in diacylglycerol and IP3. Diacylglycerol activates PKC and PKC stimulates adenylyl cyclase, which increases [cAMP]*i* levels. The increased [cAMP]*i* activates PKA, leading to ADPRC CD38 phosphorylation and the rapid up-regulation of its ADPRC activity. ADPRC stimulation in turn results in an increase in [cADPR]*i*. The increase in [cADPR]*i* induces an influx of extracellular Ca^{2+} through either or both of the following mechanisms: (1) Ca^{2+} release from ryanodine receptor-gated stores, or (2) direct cADPR-mediated opening of plasma membrane Ca^{2+} channels.

Paracrine signaling between pancreatic β cells and inflammatory cells is recognized as a pathogenetic mechanism in metabolic syndromes and type II diabetes [21–23]. Bruzzone et al. [13] reported that ABA increases glucose-stimulated insulin secretion from RIN-m and INS-1 cells and from murine and human pancreatic islets. ABA-induced insulin secretion was inhibited by pertussis toxin, by inhibitors of the ADPRC/cADPR system (nicotinamide, ryanodine, and 8-Br-cADPR) and by a PKA-specific inhibitor, which suggested the involvement of the second messengers, cADPR and cAMP, in ABA signaling. Similar to the ABA signaling pathway in human granulocytes, the signaling cascade triggered by ABA in insulin-releasing cells [13], as well as in human monocytes [18] sequentially involves a pertussis toxin-sensitive G protein, cAMP overproduction, protein kinase A-mediated activation of the ADPRC CD38, and cADPR overproduction.

However, Jiang et al. [24] determined the response to ABA and its analogues by murine primary microglia, immortalized murine microglial cell line BV-2 and N9 cells. Both the primary microglia and the microglial cell lines (BV-2 and N9 cells) showed significant increases in intracellular calcium in response to treatment with ATP and ionomycine. ABAs failed to evoke dose- and time-dependent $[Ca^{2+}]i$ changes in mouse primary microglia, BV-2 and N9 cells when treated with (−)-ABA, (+)-ABA, (−)-trans-ABA and (+)-trans-ABA. They proposed that ABA invokes $[Ca^{2+}]i$ in microglia, which is a conclusion that requires more evidence and further study.

In plants, it has been demonstrated that ABA stimulates the release of intracellular calcium in conjunction with the upregulation of cADPR [25]. ABA is produced and released by various lower animals and a variety of mammalian tissues and cells; ABA functions through a strikingly similar signaling pathway in both plants and animals, which involves cADPR and Ca^{2+} functioning as intermediates. These results are consistent with an early idea proposed by Huddart et al. [26] based on experiments with various smooth muscle preparations and a cyanobacterium, which suggested that ABA may serve as a universal Ca^{2+} agonist across taxonomic kingdoms.

4. ABA receptors

4.1. ABA receptors in plants

ABA receptors have both intracellular and extracellular evidences in plants. The evidence for the intracellular receptor is based on the studies on the ABA inhibition of stomatal openings via the passive uptake and microinjection of ABA into guard cells [27], which rely on an influx of Ca^{2+} as a second messenger [28] and cADPR as an intermediate [25]. Evidence for the extracellular receptor of ABA is based on the monitoring of ABA-regulated gene expression in a suspension of *Arabidopsis thaliana* cells, which were treated with an impermeate ABA-BSA conjugate [29], and the ABA-binding sites on the plasma membrane of stomatal guard cells were visualized using biotinylated ABA (bio-ABA) [30,31].

Several ABA receptors (or binding proteins) have been reported in plants, including the membrane-bound G protein-coupled receptor 2 (GCR2) [32,33], the chloroplast Mg-chelatase subunit H (CHLH) [34], the type 2C protein phosphatase (PP2C) [35] and the PYR/PYL protein family [36]. However, contradictory results regarding GCR2 [37,38] and CHLH [39] as ABA receptors were reported in subsequent studies. Additional studies are needed on the nature of ABA receptors in plant cells.

4.2. The presence of ABA receptors on the plasma membrane of human cells

Pertussis toxin (PTX) abrogated or inhibited all biochemical changes triggered by extracellular ABA, similar to the effect observed with ABA receptor sites on the plasma membrane of human granulocytes. The presence of ABA-binding sites on human granulocytes was revealed by incubating the cells with bio-ABA and the subsequent detection of surface fluorescence was accomplished using FITC-conjugated streptavidin and a Scatchard plot analysis of the specific binding of radioactive ^{3}H-ABA to granulocyte cells [12]. ABA is known to bind to the plasma membrane of human monocytes and aortic smooth muscle cells (AoSMC) [18], and to the surface of MSC, via the detection of fluorescence from bio-ABA [19]. The presence of high-affinity ABA binding sites in N9 cells have also been reported, at levels up to 5.4×10^3 high-affinity binding sites/cell, and a K_d of 4 nM. Both values are similar to those reported in human granulocytes [14].

4.3. LANCL2 is necessary for ABA-binding and functioning in granulocytes and insulinoma cells

The lanthionine synthetase C-like protein 2 (LANCL2, NP 061167, 450 amino acid residues) is a member of the LANCL protein family and is associated with the plasma membrane through N-terminal myristoylation. Based on the sequence homology between the putative *Arabidopsis* ABA-receptor protein GCR2 [32] and the human LANCL protein family and the reported association of LANCL2 with the plasma membrane, Sturla et al. [40] investigated the involvement of LANCL2 in ABA sensing in mammalian ABA-responsive cells. Human granulocytes were transfected with a plasmid containing full-length LANCL2 cDNA. In parallel, granulocytes were also transfected with the empty plasmid (control) or with the same plasmid in the presence of a siRNA for LANCL2. Real-time PCR confirmed that LANCL2 mRNA is present in freshly isolated human granulocytes and in siRNA-transformed cells. Western blot analysis demonstrated the efficient expression of recombinant LANCL2. The ABA-induced Ca^{2+} response in granulocytes expressing recombinant LANCL2 was significantly increased compared to that of cells transfected with the empty plasmid. LANCL2-silenced cells did not respond to ABA, confirming that the silencing of LANCL2 abrogates the ABA-induced Ca^{2+} response in human granulocytes. LANCL2 silencing abrogated the ABA-induced responses in granulocytes, including the intracellular increase in $[cAMP]i$ and $[Ca^{2+}]i$, chemotaxis (cell migration through the filter), and the stimulation of reactive oxygen species (ROS) production and particle phagocytosis.

Rat insulinoma cell lines, RIN-m and INS-1, as well as human and murine pancreatic β cells, were revealed to express LANCL2 based on real-time PCR analysis. RIN-m and INS-1 cells were transfected with a siRNA specific to rat LANCL2 and in parallel with a negative control siRNA. The silencing of LANCL2 (LANCL2 siRNA-transfected cells) inhibited the ABA-triggered increase in $[cAMP]i$ and $[Ca^{2+}]i$, as well as the ABA-triggered insulin release in the presence of both high and low glucose concentrations [13].

The granulocyte ABA-signaling pathway was reproduced by expression of LANCL2 in CD38-transfected HeLa cells. ABA induced a concentration-dependent, sustained $[Ca^{2+}]i$ increase, intracellular $[cAMP]i$ increases, and the activation of ADPRC activity in $CD38^+$ HeLa cells co-expressing LANCL2. The $CD38^+$ HeLa cells transfected with an empty vector did not respond to ABA, which is similar to the results in human granulocytes and human and rat insulin-releasing cells [13]. Pretreatment with PTX inhibited the increase in $[cAMP]i$ that was triggered by ABA in $LANCL2^+/CD38^+$ HeLa cells.

The ABA-induced $[cAMP]i$ increase was inhibited by PTX and a protein kinase C-specific inhibitor in human granulocytes. Transformation of a α-transducin (α_t), a scavenger of free βγ subunits released from activated Gi (a G-protein) into $LANCL2^+/CD38^+$ HeLa cells resulted in the inhibition or strong reduction of ABA-induced increases in intracellular $[cAMP]i$ and $[Ca^{2+}]i$. Transformation of a chimeric G-protein ($G\alpha_{q/i}$) into granulocytes and $CD38^+/LANCL2^+$ HeLa cells resulted in a significant increase in $[cAMP]i$, $[IP_3]i$ and $[Ca^{2+}]i$ in response to ABA. Further experiments with xestospongin (an inhibitor of IP_3-mediated Ca^{2+} release) and 8-Br-cADPR (an antagonist of cADPR) revealed a two-step mechanism involved in the ABA-triggered $[Ca^{2+}]i$ increase: a fast and transient $[Ca^{2+}]i$ increase immediately after the addition of ABA by $[IP_3]i$ (xestospongin-sensitive), followed by a sustained, cADPR-mediated Ca^{2+} increase [40].

Based on these results, Sturla et al. [40] suggested that LANCL2 is coupled to Gi in granulocytes and $LANCL2^+/CD38^+$ HeLa cells, which enables the ABA-induced activation of both PLC and AC. The analysis of [^{3}H]-ABA binding to intact cells and a surface fluorescence analysis of bio-ABA binding indicated that the C-terminal domain of LANCL2 is intracellular, whereas the ABA-

binding sites on LANCL2$^+$ HeLa cells are extracellular. If ABA binds directly to LANCL2, this protein should span the plasma membrane, or LANCL2 is necessary for the activity of an ABA-binding protein complex, where the ABA-binding domain is extracellular and LANCL2 lies on the intracellular side of the plasma membrane.

Bassaganya-Riera et al. [20] reported that ABA-mediated activation of macrophage PPARγ (nuclear receptor peroxisome proliferator-activated receptor γ) is dependent on LANCL2 expression through LANCL2 knockout experiments. An examination of the distribution of the binding sites on LANCL2 suggested that ABA preferentially binds to the loop regions of LANCL2. Homology modeling using the three-dimensional structure of LANCL2 also indicated that ABA has a binding site on the surface of LANCL2 [41].

4.4. PPAR γ

PPAR γ is a key metabolic regulator and transcription factor. It is a member of the nuclear receptor superfamily, which consists of 48 ligand-induced transcription factors that respond to nutrients, xenobiotics, various hormones and endogenously produced compounds. PPAR γ is found in high concentrations in white adipose tissue (WAT), immune cells, and the colonic epithelium. Following ligand binding, PPAR γ heterodimerizes with retinoid X receptor (RXR), another member of the nuclear receptor superfamily, and subsequently binds to a PPAR response element (PPRE) on DNA that initiates the transcription of responsive genes. Many of the genes induced by PPAR γ, such as aP2 and CD36, are important in fatty acid transport, adipogenesis, and lipid uptake. Studies have shown that PPAR γ is an essential participant in the differentiation of pre-adipocytes into adipocytes, particularly in the subcutaneous adipose tissue depots. Studies have also shown that PPAR γ activation directly inhibits inflammation through multiple mechanisms. Synthetic PPAR γ agonists, such as thiazolidinediones (TZDs), have been shown to be very effective in improving systemic insulin sensitivity. TZDs serve as synthetic ligands for PPAR γ. Because of the many side effects associated with TZDs, researchers are seeking more effective natural or synthetic chemicals that target PPAR γ [20].

Bassaganya-Riera et al. [20] identified 3404 ABA-related genes in *Arabidopsis* using the Arabidopsis Information Resource and Arabidopsis Hormone Databases. These ABA-related genes include those in the ABA biosynthetic, metabolic or signaling pathways that responded to ABA treatment, which, as a consequence, have been designated as ABA-related. In humans, 1677 genes were found with orthology to *Arabidopsis* ABA-related genes using *Arabidopsis*-human orthology information. By exploring the protein interaction network, 1048 genes among the putative ABA-related genes from humans were found to be of highly functional relevance, and could interact with 3780 genes in the network. PPAR γ and its related genes are within the putative ABA-related human genes.

As ABA is structurally related to TZDs, PPAR γ-responsive genes were found to be induced or activated by ABA in 3T3-L1 pre-adipocytes *in vitro*. Dietary ABA-increased mRNA expression was found to increase the expression of PPAR γ and its responsive genes (i.e., adiponectin, aP2, and CD36) in WAT [42].

The deficiency of PPARγ in immune cells, including macrophages, impaired the ability of ABA to suppress the infiltration of F4/80hi ATMs into WAT, to repress WAT MCP-1 expression and to improve glucose tolerance [43]. ABA's beneficial effects on disease activity were completely abolished in T cell-specific PPAR γ null mice (PPAR γ deleted from macrophages and other immune cells) [44]. ABA improved colon histopathology, reduced blood F4/80+CD11b+ monocytes, increased the percentage of CD4$^+$ T cells expressing the inhibitory molecule cytotoxic T lymphocyte antigen 4 in blood and increased the number of T_{reg} cells in the mesenteric lymph nodes and colons of PPAR γ-expressing mice, but not in T cell-specific PPAR γ null mice. ABA increased PPAR γ activity in RAW 264.7 macrophages and increases PPAR γ expression *in vivo* [45]. It has been proposed that ABA improves insulin sensitivity and obesity-related inflammatory diseases through a PPAR γ-dependent mechanism.

However, ABA was demonstrated not binding to the ligand-binding domain (LBD) of PPAR γ. LANCL2 knockout studies indicate that ABA-mediated activation of macrophage PPAR γ is dependent on LANCL2 expression. They concluded that ABA decreases LPS-mediated inflammation and regulates innate immune responses through a bifurcating pathway that involves LANCL2 and an alternative LBD-independent mechanism of PPAR γ activation [20]. More work is required to understand the function and mechanism of PPAR γ and its regulation of immune responses as well as its functional relationship to LANCL2.

5. ABA function and potential medicinal application in animals and humans

5.1. ABA function in stress responses of animals

Heat stress stimulates ABA production and ABA-mediated responses to temperature and mechanical stresses in sponges. Micromolar concentrations of ABA or heat stress caused a decrease in amino acid incorporation, protein synthesis, oxygen consumption and water filtration in sponges [8]. Light-stimulated endogenous ABA synthesis and ABA mediated the light-stimulated response in *E. racemosum,* which induces the regeneration of *Eudendrium* fragments. The regeneration of *Eudendrium* fractions is light sensitive. ABA, added at a concentration of 5 μM, dramatically stimulated regeneration (4.5-fold compared to controls) in the dark, similar to the effects observed under light conditions (4-fold compared to controls). Fluridone, an ABA biosynthetic inhibitor, reduced ABA-induced hydroid regeneration in a dose-dependent manner [15].

Stress signals, such as TNF-α, RANTES or IL-8, released by peripheral blood mononuclear cells stimulate MSC to release ABA, which in turn induces MSC functions, including the release of cytokines that stimulate hematopoiesis, the production of immunomodulatory chemokines and cytokines, and the stimulation of MSC mobilization from the bone marrow. These results indicate that ABA functions as a stress hormone in animals, similar to the drought stress response in plants [46].

5.2. ABA function in stem cells

Scarfi et al. [19] reported that ABA stimulates the *in vitro* expansion and proliferation of MSC through the cADPR/[Ca^{2+}]*i* signaling pathway, without affecting differentiation. ABA at concentrations of 0.1, 1.0, and 10 μM increased colony output by 1.3-, 1.7-, and 1.4-fold, and stimulated MSC proliferation by 1.25-, 1.6-, and 1.3-fold, respectively. ABA induced a significant increase in prostaglandin E2 (PGE2) production, induced chemokinesis or cell migration and stimulated the release of several cytokines known to mediate the trophic and immunomodulatory properties of MSC. In MSC, ABA production and release were stimulated by specific growth factors (e.g., bone morphogenetic protein-7), by inflammatory cytokines, and by lymphocyte-conditioned medium. ABA is an autocrine stimulator of MSC function; stress signals, such as TNF-α, RANTES, and IL-8, which are released by peripheral blood mononuclear cells, stimulate MSC to release ABA, which in turn induces ABA-related MSC functions.

Pre-treatment of cells with ABA also promoted cell expansion (but not increases in cell numbers) among the first generation of human uncommitted hemopoietic progenitors (HP) *in vitro*, and increased cell numbers among the second generation, through the cADPR/$[Ca^{2+}]i$-mediated signaling pathway. The cell expansion factors for the first generation of cells were 1.6-, 2.2-, and 1.35-fold, and the colony numbers from the second generation increased 3.4-, 3.7-, and 3.5-fold, for 0.5, 2, and 20 μM ABA treatments, respectively. Pre-treatment of $CD34^+$ cells with ABA caused significant changes in gene expression compared to control CD34 cells. Incubation of $CD34^+$ cells with micromolar concentrations of ABA also induced transcriptional effects, which included NF-κB nuclear translocation (which increased 2.8-fold with 10 μM ABA for 30 min) and the transcription of genes encoding several cytokines, such as vascular endothelial growth factor (VEGF), IL-8, IL-9, Oncostatin-M, GRO, RANTES, Fas-L, and IL-1b; the largest increases included a 4-fold increase in VEGF and a 3-fold increase in IL-8. ABA-treated $CD34^+$ cells release cytokines and growth factors (VEGF and IL-8) that stimulate hemopoietic, stromal, and endothelial cell growth and proliferation. Human MSC stimulated with a lymphocyte-conditioned medium produced and released ABA at concentrations sufficient to exert growth-stimulatory effects on co-cultured CD34 cells, as demonstrated by the inhibition of colony growth in the presence of an anti-ABA monoclonal antibodies [46]. ABA participated in paracrine signaling among MSC, inflammatory/immune cells, and HP. ABA is recognized as a new hemopoietic growth factor involved in the cross-talk between HP and MSC [46].

ABA may represent a new autocrine and paracrine-signaling molecule in the bone marrow environment, targeting both MSC and HS, and possibly regulating the response of the hemopoietic niche to alarm inflammatory signals originating from other parts of the organism. MSC-derived ABA induced a continuous supply of stimulatory cytokines at nanomolar concentrations, which reduced their effective concentration by 2–3 logs compared to pulse addition and stimulated HP growth. As ABA can be released by activated granulocytes, ABA may function as a paracrine alarm signal, stimulating granulocyte production in the bone marrow and activating mature cells under infection/inflammation conditions in the response to cellular and environmental stresses [46].

5.3. ABA function in immune cells

5.3.1. Granulocytes

Of the many mammalian cell types, granulocytes are the most exposed to environmental stimuli, they function as the first line of defense against pathogens. In response to pathogen attacks or chemotactic stimuli, granulocytes can be rapidly recruited to the infected site, where they kill the pathogens via phagocytosis and the production of cytotoxic ROS, including nitric oxide (NO). These granulocyte functions are dependent on the cADPR-induced $[Ca^{2+}]i$ increase [12].

ABA stimulates several functional activities, including phagocytosis, ROS and NO production, and chemotaxis in human granulocytes. ABA increased phagocytosis significantly when present at 50 nM and to a marked level when present at 20 μM. ABA at 20 μM induced a 6-fold increase in ROS, a 1.83- to 9.42-fold (median 3.55-fold) increase in NO production, and a slight, but consistent, increase in chemokinesis (untargeted cell movement) of granulocytes, compared to the controls. ABA also induced a concentration-dependent chemotactic response, resulting in a significant increase in cell migration at 50 nM and the maximal migration at 20 μM. However, ABA-methylamide and (±)-trans, trans ABA did not have an effect [12].

ABA release by granulocytes is sustained by its intracellular production. Removing the increased ABA from the supernatant of particle-stimulated granulocytes with an anti-ABAmAB reduced ROS production by 80%, which can be restored by the addition of ABA. These results demonstrate that ABA behaves as a pro-inflammatory endogenous autocrine cytokine that is capable of stimulating granulocyte functions and provide a new approach for the development of new anti-inflammatory drugs [12].

5.3.2. Monocytes

Monocytes play a key role in both inflammation and immunity by performing antigen presentation, phagocytosis, and immunomodulation through the production of various cytokines and growth factors. Incubation of monocytes with ABA led to NF-κB activation and subsequent increases in cyclooxygenase-2 (COX2) expression and prostaglandin E_2 (PGE2) production as well as enhanced release of MCP-1 and metalloprotease-9 (MMP-9). All of these events are reportedly involved in atherogenesis, which is a chronic immune inflammatory disease [18].

NF-κB regulates the expression of several inflammatory proteins in monocytes [18]. In response to inflammatory stimuli, cytosolic NF-κB translocates to the nucleus via Ca^{2+}- and PKC-dependent phosphorylation of the NF-κB inhibitor, IκB. Incubation of monocytes for 30 min with ABA showed a dose-dependent increase in NF-κB nuclear translocation, with the highest value being a 2.17-fold increase with the 10 μM ABA treatment compared to the controls. The expression of NF-κB-controlled genes, COX-2, MCP-1 and MMP-9 (which catalyzes the proteolytic cleavage of the extracellular matrix and promotes monocyte extravasation and VSMC migration through the blood vessel wall), were increased. COX-2 expression was increased 1.3-, 1.4- and 1.6-fold in the cells treated for 6 h with 0.1, 1.0, and 10 μM ABA, respectively. The release of MCP-1 and MMP-9 peaked with 2.3-fold and 1.8-fold increases, respectively, when monocytes were incubated with 10 μM ABA for 6 h. The synthesized prostaglandin E_2 (PGE2) was increased 1.8-fold (maximum), compared to the control, by concentrations of ABA as low as 0.1 μM. These data indicate that ABA induces over-expression of NF-κB-controlled atherosclerosis-related proteins in human monocytes [18].

AoSMC migration and proliferation are critical events in atherogenesis. ABA stimulates human AoSMC migration when cells were incubated or pretreated at concentrations of 0.1 or 10 μM. ABA treatment or pretreatment for a short time (2 h) increased AoSMC proliferation by 1.3- and 1.6-fold at 0.01 and 0.1 μM ABA, respectively. Short-term exposure to ABA (in both monocytes and AoSMCs) is sufficient to induce long-term functional responses, including the release of PGE2 and MCP-1 from monocytes, and AoSMC migration or proliferation. ABA released from monocytes can also stimulate AoSMC migration and proliferation [18].

Human atherosclerotic plaques produces much lower levels of ABA than monocytes, but concentrations of ABA were 10-fold higher than non-atheromatous vascular tissue, or 0.02 pmol/mg wet weight ABA, which is equivalent to 20 nM, a concentration within the range of concentrations (10–100 nM) that induce functional effects in both monocytes and AoSMCs. MCP-1 is believed to be the primary chemoattractant for monocytes to the atherosclerotic plaques, and stimulates VSMC proliferation. Nanomolar concentrations of ABA stimulates MCP-1 release from monocytes, and MCP-1, in turn, stimulates ABA release from monocytes. This autocrine and paracrine positive feedback mechanism cascade induces anti-atherosclerotic responses at the site of endothelial lesions. ABA plays a central role in the molecular interactions between platelets, monocytes and AoSMCs, leading to a series of cell responses to atherogenesis [18].

5.4. ABA stimulates insulin release from human pancreatic islets and ameliorates glucose tolerance

5.4.1. Insulin secretion from pancreatic cells

Bruzzone et al. [13] reported that insulin secretion by RIN-m in low (LG) or high glucose (HG)-modified Krebs–Ringer–Hepes-buffer (KRH) was 1.3 ± 0.1- or 2.2 ± 0.2-fold higher, relative to that in glucose-free KRH. The maximum insulin secretion was obtained in HG buffer with 30 mM KCl, with an insulin release that was 3.4 ± 0.4-fold higher than observed in glucose-free KRH. Insulin secretion from RIN-m cells in LG-buffer was 2.6 ± 1.3 ng insulin/10^6 cells/h and was increased by ABA by as much as 1.6-fold, with 10 nM ABA being the saturated concentration for insulin induction. No significant difference was found between insulin release at concentrations of 10, 10^2, 10^3, or 10^4 nM ABA in LG. ABA at 1 nM or lower did not significantly stimulate insulin release from RIN-m cells, in either LG medium or HG medium. ABA at a 1-μM concentration can also increase insulin secretion from RIN-m cells in the absence of glucose. Both ABA enantiomers, (+)- and (−)-*cis*, *trans*-ABA, were similarly effective in stimulating insulin release from RIN-m cells. Rat INS-1 cells had a similar insulin secretion in response to LG (1.5-fold), HG (2.3-fold), KCl-HG buffer (6.6-fold) and different concentrations of ABA (maximal 2-fold at 10 nM).

Incubation with ABA for 30 min stimulated insulin release from freshly isolated murine pancreatic islets in a concentration-dependent manner; concentrations of ABA as low as 1 nM significantly increased insulin release in LG buffer, and 10 nM was as effective as 1 μM, increasing the SI (stimulation index) 1.6-fold compared to the control in LG buffer. Maximal stimulation of insulin release, as induced by HG-KCl, resulted in a 3.8-fold increase in the SI, and glucose induced a faster response than ABA. SIs were 1.51 ± 0.15 *versus* 0.95 ± 0.17 at 7 min and 2.02 ± 0.18 *versus* 1.55 ± 0.012 at 30 min, for HG *versus* 10 nM ABA, respectively, and no significant difference was noted between them at 120 min. Similar to the murine pancreatic islets, ABA induced maximum stimulation of insulin secretion from human pancreatic β cells when present at a concentration of 10 nM, with similar SI values at 120 min for 10 nM ABA (2.7) or HG (2.9) treatment. Stimulation of insulin secretion by HG was faster compared to ABA, with SI values of 2.3 and 1.5, respectively, after 30 min of incubation [13].

Pretreatment with PTX, a PKA-specific inhibitor (myristoylated peptide, I-PKA), ADPR cyclase inhibitor (nicotinamide), cADPR antagonists (8-Br-cADPR and ryanodine) and intracellular calcium chelator EGTA-AM abolished or severely reduced ABA-induced insulin release, whereas 8-Br-cAMP (the PKA activator and membrane-permeable cAMP analog) stimulated insulin secretion [13].

The results suggest that a positive feedback mechanism exists to prolong and enhance insulin release in response to glucose. ABA is an endogenous stimulator of insulin secretion in human and murine pancreatic cells. Autocrine release of ABA by glucose-stimulated pancreatic cells and the paracrine production of the hormone by activated granulocytes and monocytes suggest that ABA is involved in the insulin release as well as in the dysregulation of insulin under conditions of inflammation [13].

5.4.2. Type II diabetes

Type II diabetes is an insulin resistance disease. TZDs are a class of the most effective insulin-sensitizing drugs that increase the sensitivity of peripheral tissues to endogenous insulin. TZDs serve as synthetic ligands, and activate the key metabolic regulators as well as the transcription factor PPAR γ. Therefore, PPAR γ agonists are considered to be a promising target for future drug design and treatment of diseases related to insulin resistance and the related conditions of hyperlipidemia, hyperglycemia, and hyperinsulinemia [42].

Guri et al. [42] studied the effect of ABA on high fat diets and the onset of type II diabetes in obese db/db (leptin receptor-deficient) mice. Mice were fed a range of ABA concentrations (0, 100, 200, 400, or 800 mg/kg as part of a high-fat diet) for 5 weeks. Dietary ABA-supplementation was associated with the following effects: significantly improved glucose tolerance, or the glucose-normalizing ability; decreased fasting blood glucose concentrations; reduced TNF-α mRNA and the number of macrophages; reduced average adipocyte size; increased adipocyte differentiation and adipogenesis; and increased the expression of PPAR γ and its responsive genes (i.e., adiponectin, aP2, and CD36), which are involved in lipid metabolism in white adipose tissue. The lowest effective dose of dietary ABA was 100 mg/kg. No side effects were observed in ABA-fed mice, such as excess weight gain and fluid retention, which are commonly observed with TZDs. They suggested that ABA could be used as a nutritional supplement to combat type II diabetes and obesity-related inflammation. ABA-supplementation was also associated with significant improvements in hepatic steatosis and plasma triglyceride levels [42,43].

ABA has structural similarities to TZDs and increased the expression of PPAR γ. ABA-induced glucose homeostasis was affected, macrophage infiltration, and the normalization of fasting blood glucose concentrations were abolished or significantly reduced in PPAR γ-deficient immune cells. These results indicated that PPAR γ expressed by immune cells is a key player in the mechanism of ABA's anti-diabetic actions [43]. PPAR γ activation directly inhibits inflammation through multiple mechanisms including the reduction of MCP-1 and the corresponding migration of monocytes, making it very probable that the anti-inflammatory actions of PPAR γ in cells other than adipocytes are involved in the insulin-sensing activities.

Although ABA activates PPAR γ, it does not bind directly to PPAR γ's ligand binding domain, as reported by Bassaganya-Riera et al. [20]. Magnone et al. [18] reported contradictory results, which challenge the anti-inflammatory effects observed in the white adipose tissue of mice. They did not observe any stimulation of PPAR γ expression by ABA in cultured human monocytes when ABA was present at concentrations ranging from 0.1 to 10 μM over 6–48 h. Moreover, incubation with ABA (0.01–100 μM) for 6 h consistently increased MCP-1 release from human monocytes. The stromal vascular cells extracted from murine white adipose tissue are a mixed cell population that is composed of macrophages, pre-adipocytes, T-lymphocytes, and endothelial cells; this mixed population may account for the discrepancy between the two groups' results, which need to be studied in further detail.

5.5. ABA function on other inflammation diseases

5.5.1. Microglial cells

Microglial cells are the monocyte/macrophage equivalent in the central nervous system and represent the first line of defense in the brain by removing infectious agents and damaged cells. Microglia can also release a variety of trophic factors and cytokines that are able to regulate the communication between neurons and other glial cells and can contribute to tissue repair and neuroprotection. ABA priming stimulates N9 cell migration towards β-amyloid. Their results indicate that ABA is a pro-inflammatory hormone that induces autocrine microglial activation, potentially representing a new target for anti-inflammatory therapies aimed at limiting microglia-induced tissue damage in the central nervous system [14].

5.5.2. Atherosclerosis

Magnone et al. [18] reported that ABA levels are increased 10-fold in human atherosclerotic plaques and functions as an endogenous regulator that minimizes inflammatory lesion devel-

opment during atherosclerosis. Guri et al. [47] reported that mice treated with ABA dietary supplements had significantly improved systolic blood pressure and decreased accumulation of F4/80+CD11b+ macrophages and $CD4^+$ T cells in aortic root walls. ABA significantly enhanced aortic endothelial nitric oxide synthases (eNOS) and tended to suppress aortic vascular cell adhesion molecule-1 (VCAM-1) and MCP-1 expression as well as plasma MCP-1 concentrations. ABA caused a dose-dependent increase in the intracellular concentrations of cAMP and NO and contributed to the up-regulation of eNOS mRNA in human aortic endothelial cells. Their results indicated that ABA has an anti-atherosclerotic and anti-hypertensive effect in ApoE−/− mice, which functions by suppressing macrophage and $CD4^+$ T cell infiltration into the aortic root walls. ABA supplementation has no effect on body weight, cholesterol, glucose or insulin concentrations [47].

5.5.3. Inflammatory bowel disease

Guri et al. [48] reported that ABA prevents experimental inflammatory bowel disease (IBD). ABA significantly ameliorated disease activity, colitis and reduced colonic leukocyte infiltration and inflammation. These improvements were associated with the decreased expression of VCAM-1, E-selectin, and mucosal addressin adhesion marker-1 (MAdCAM-1). ABA also increased the numbers of CD4 and CD8 T-lymphocytes in the blood as well as MLN and regulatory T cells in the blood.

5.6. Anticancer function of ABA

ABA was reported as an anti-cancer compound in a US patent issued to Dr. Virginia Livingston in 1976 [49]. The inventor reported that ABA "neutralized" the human chorionic gonadotropin (hCG), which is a negatively charged glycoprotein that reportedly coated cancer cells and prevented immune cells (the outer membranes are normally negatively charged) from getting close to attack the cancer cells, thereby facilitating anticancer immune responses. ABA treatment (1 or 10 mg/kg) substantially increased survival rates in C57BL/6J mice that were transplanted with tumor C1498 (a cancer that is lethal in mice within 10–15 days) at 14 days after transplantation, when compared to control mice. ABA did not have any toxic side effects in mice, even when administered at concentrations corresponding to approximately 10% mouse's body weight. However, ABA cannot be administered during pregnancy due to the presence of hCG in the placenta [50], indicating that ABA may interfere with mammal [49,51] and insect reproduction [52]. ABA was also demonstrated to induce a positive membrane potential which causes adhesion of plant root tips to a negatively charged glass surface [53,54].

Tan et al. [55] reported in a China patent that ABA effectively inhibits the proliferation of tumor cells; stagnates the cells in S-phase, stops cell division, and induces differentiation of tumor cells or reverts cancerous cells to normal cells. They also reported that ABA induces apoptosis and inhibits angiogenesis in a variety of cancer cells. ABA also showed synergistic inhibition of the cellular proliferation of mouse leukemia L178Y cells when it was simultaneously administered with trans-retinol [56]. ABA was reported to inhibit proliferation and induced differentiation in human SMMC- 7221 cells [57] and in nude mice transplanted with human hepatocarcinoma [58]. Cell proliferation was inhibited at ABA concentrations of 4×10^{-3} M or higher, and the inhibition increased with the length of treatment time and concentration of ABA. The cells were arrested in the G0/G1 phase. ABA induced cell normalization, and the SMMC-7221 reverted to normal cells after ABA treatment. ABA may inhibit growth and proliferation of cancer cells by regulating the cell cycle, by inhibiting the expression of mtP53, Ki67 Cyclin D1 and hTERT [59] and by increasing the expression of caspase-3 mRNA and inducing apoptosis [60].

Because calcium signaling is a key regulator of apoptosis, changes in calcium distribution in the cell activate cellular cascades, which lead to cell death [61]. The calcium signaling pathway activated by ABA is similar to the pathway activated by certain types of chemotherapeutic agents used in cancer treatment, such as staurosporine, doxorubicin, tamoxifen, and etoposide. These drugs function by increasing oxidative stress and apoptosis rates in cancer cells, both of which are mediated by increasing $[Ca^{2+}]i$ [62]. The ABA induced hyperpolarization across the membrane and inhibited K^+ and/or Na^+ uptake, which modulates cellular water content and metabolic activities leading to cellular process of destruction and fragmentation [63]. Rakic et al. [64] reported that a ABA analog origamicin inhibits HCV replication through the inhibition of host protein folding, indicating that ABA or its analog may target protein folding.

6. Conclusion and prospects

Previous research has demonstrated that ABA exists and functions in a wide range of animals, from lower animals to a variety of mammalian tissues and cells. ABA is involved in a very similar signaling pathway in both plants and animals. The universal signaling pathway is a sequential process that involves ABA perception at the plasma membrane, and cADPR and cytoplasmic Ca^{2+} functioning as intermediates. The perception and functions of ABA directly depends on the membrane protein LANCL2 and/or the nuclear receptor PPAR γ. ABA significantly stimulates immune cells, pancreatic cells and vascular cells under various inflammatory disease conditions and significantly inhibits cancer cells. Therefore, ABA is a good candidate for the development of therapeutic drugs for several human diseases.

Despite this work, research on ABA function in humans is in its early stages. Several important aspects of ABA function need to be studied, including its function in brains and the central nervous system [11,14], the biosynthetic and metabolic pathways that involve ABA in animal cells, its mechanisms of action and, finally, clinical tests in the form of disease treatments. In addition, current research on ABA in animals needs to be expanded to include more species, tissues and cell lines, and more experimental conditions need to be included.

Additionally, the jasmonates represent another group of plant growth regulators with some functions that are similar to ABA that can also suppress proliferation and induce apoptosis in certain mammalian cancer cells (lymphoblastic leukemia, prostate, breast and melanoma cancer cells). One of the most important characteristics of jasmonates, as with ABA, is that they do not affect normal human lymphocytes, in contrast to their strong effects on lymphoblastic leukemia cells, even within a mixed population of normal and lymphoblastic leukemic cells [65, for reviews 66, 67]. The plant signaling molecular salicylic acid inhibited the growth and proliferation, and induced apoptosis of cancer cells [65,68,69]. Cytokinins delay or prevent the onset of age-related changes in human skin fibroblasts [70] and protect DNA and proteins from oxidative damage [71–73].

Unlike some chemotherapeutic agents which act as cell killers, growth regulators such as ABA and the jasmonates regulate diseased cells by facilitating normal growth and differentiation, stimulating the immune system in response to environmental stresses or diseases, or inducing cancer cells to undergo apoptosis without significant toxic effects on normal cells. The function and potential application of plant growth regulators towards animal and human diseases are not limited to ABA. Broad interactions and cross functions of bioactive substances between plants and animals are anticipated.

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