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Aloe emodin: from anti- to pro-tumor action

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Abstract

Poor response of highly invasive forms of cancer to the treatment can be explained not only by the cell death resistant phenotype of low/undifferentiated cell subpopulation but even more, by tumor repopulation as a reaction to damage triggered by the chemo- or radiotherapy. Regarding the serious limits of regular healing protocols, one of the pivotal challenges for biologists is to prove the relevance and discover the mechanisms behind traditional medicine-based tumor healing. One of the oldest and most powerful plants with 4000 years long tradition in folk medicine, Aloe vera is intensively studied during last century due to the treasure of active compounds with proven biological potential attractive not only for physicians and patients but also for scientists. Anthraquinones, emodin and aloe emodin (AE), derived from Aloe vera and different plants from Polygonaceae family are definitely the most researched constituents. Aloe emodin owns multiple anti-tumor properties realized through induction of cell cycle arrest, cell death, differentiation and suppression of the malignant cell motility. However, its interaction with tumor cells is not unidirectional and due to the complex network of signals in the tumor microenvironment can be easily transformed from tumor destructive to tumor-stimulating. Therefore, this review will summarize direct tumoricidal effects as well as the interaction of AE with cells and mediators of the immune system. In addition, the potential of AE as chemo- or photosensitizer will be elaborated. Finally, new designs including chemical interventions on this molecule and nanotechnology will be discussed.

Key words: *Aloe emodin, anticancer properties, interplay with microenvironment, cytostatic drugs, photodynamic therapy*

INTRODUCTION

Cancer is a disease developed from permanent loss of balance between death and life at the intercellular level. Abnormal proliferation, ruined tissue, and organ architecture lead to local and soon after, systemic dysfunction. Because of rowdy tissue organization, the main impression is that tumor grows randomly and without any rules. Today is well recognized that strategies employed by tumor at cellular as well as intercellular level are fascinating, and altogether demonstrates the superiority toward all therapeutic approaches which ignored their "higher intelligence" and treat them as randomly generated proliferative mass of the cells. Their plasticity and an enormous capacity to change phenotype and avoid host immune defense became more and more powerful through the process of dedifferentiation [1]. According to this parameter, tumors are classified into grades. So, high-grade tumors are invasive/metastatic and, in general, almost incurable. The difference in sensitivity to treatment between well differentiated and low or non-differentiated forms of cancer can be explained not only by the cell death resistant phenotype of poorly differentiated cell subpopulation but even more by specific intercellular communication inside of the heterogeneous tumor mass. Tumor repopulation frequently observed upon the chemo- or radiotherapies of metastatic tumors arose as a consequence of this specific and complex interplay between members of tumor commune [2,3]. Proliferation in response to death induction basically compromised healing protocols in high-grade tumor treatments [4]. As a consequence of this, traditional medicine and alternative approaches, which are not accepted by conventional medicine as legitimate, scientifically are very frequently explored during the last decades. Numerous data generated from investigation of antitumor potential of different herbs, fractions or isolated compounds give to biologist hard but important task – to select the most potent between them, to explain how they work at the molecular level, define their targets and then assess possibility to improve them and use as a drugs in treatment of cancer. For all, scientist and people out of the science, the main task remains to understand – how people, starting from 4000 years ago were able to recognize and treat diseases using certain herbs.

ALOE VERA- FROM ANCIENT TIME TO DATE

One of the oldest and most powerful plants with extraordinary potential to heal is, for sure, *Aloe vera*. Its potential to regenerate the wounds, inhibit inflammation, suppress infection, stimulate the clearness of the gut and in general bring the refreshment for the whole body, and be used for beauty care, as well as preservation, is described by old civilization such as Sumerian and Egyptians, starting from 4000 years BCE [5]. There are data indicating that the main motive of Alexander the Great for capturing Island of Socotra in the Indian Ocean was to come close to the famous *Aloe* plants growing there, required for healing the wounds of soldiers [5]. Today, at the market, the most popular product made from this herb is aloe gel, which from the middle of the last century, became popular nutritional drink with a long list of beneficial effects to the health maintenance but also in different pathologies. This potent influence on human physiology is pivotally ascribed to different carbohydrates but also numerous proteins, lipids, amino acids, vitamins, enzymes, inorganic compounds, and small organic molecules present in *Aloe vera* leaf pulp. Polysaccharide fraction is found to be very important in the stimulation of innate immunity and regarding this, *Aloe vera* gel was indicated as an immune booster [6]. Synergistic actions of numerous other ingredients of *Aloe vera* leaf extract protect healthy tissue from destruction and intoxication triggered by the chemotherapy. However, according to double edge sword principle, consumption of the gel concomitantly with chemotherapy can be questionable since compounds from *Aloe vera* gel can be cytoprotective even for malignant cells, decreasing the effects of chemotherapy.

In addition to numerous constituents listed above, many secondary metabolites anticipated as a product of III polyketides (PK) as well as lately discovered novel octaketide synthase, PKS4, and PKS5, were found to possess strong biological activities as an anti-inflammatory, lipid-lowering, antioxidant, microbicidal and laxative [7]. Recently, this kind of molecules became the most famous concerning their potent direct antineoplastic effects [8–12]. The main merit for the antitumor potential of *Aloe vera* belongs to one of them-Aloe emodin (AE). Even the name suggested that its source is exclusively *Aloe* species, this anthraquinone is found in many plants frequently used in folk medicine from Asia to Balkan. Among them are *A. barbadensis miller*, rhubarb (*Rheum palmatum*), buckthorn (*Rhamnus frangula*), *Senna* etc. In addition to the direct effect on the viability of malignant cells, long list of AE biological effects on mammalian cells, in general, should be taken into account when its anticancer features are assessed [8]. Additionally, potent influence of AE on different signaling pathways involved in essential cellular processes, multiply reflected on immune cell-mediated antitumor activities basically through changed gene and protein expression, as well as the activity of different mediators implicated in the transfer of information at the intracellular level. According to this, the effectiveness of the drugs applied concomitantly with AE can be affected. How extreme oscillation in effects on tumor cells AE exerts, depending on circumstances, such as intercellular contact, the presence of proinflammatory cytokines and the chemotherapeutic drug will be reviewed in this paper.

DIRECT ANTITUMOR EFFECTS OF AE- FROM CELL DEATH TO DIFFERENTIATION

A major criterion for characterization of any compound as antitumor is the ability to induce cell death, preferentially apoptosis in transformed cells. Following this, AE for sure belongs to this list. It is intensively studied on a wide range of tumor cell lines and in addition to numerous original papers its feature to promote cell death in different forms is also reviewed. Cell cycle arrest and apoptosis as a final outcome are noted in various *in vitro* studies on glioma, melanoma, bladder, breast, gastric, oral squamous cell carcinoma, colon, cervical, prostate, leukemia cell lines and even those that are resistant to conventional chemotherapeutic [8,12]. Cell cycle arrest is found to be connected with the AE interference with cyclin and cyclin-dependent kinases expression [11,13]. Apoptosis as a result of AE treatment, depending on the type of the tumor, has been realized through the receptor-mediated or mitochondrial pathway, and regarding high correlation with oxidative stress, can be the consequence of reactive oxygen species (ROS) production. AE triggered the activation of caspase-3, -6, -7, -8 and -9, regulates the expression of numerous transcriptional factors like nuclear factor kappa B (NF-κB), p53, pro/antiapoptotic members of B-cell lymphoma-2 (Bcl-2) family etc., affecting almost all intracellular pathways involved in the realization of death program [10–19]. Intensified production of ROS upon AE exposure in colon cancer cell lines induces endoplasmic reticulum stress characterized by glucose-related protein 78, phosphorylated protein kinase R-like ER kinase, phosphorylated eukaryotic initiation factor-2α expression, and increased cytosolic calcium level [17]. Additionally, AE elevated the permeability of lysosomal membranes in cervical adenocarcinoma HeLa cells, followed by the release of cathepsins, showing that the drug initiates lysosomal pathway-dependent apoptosis [20]. AE targeted various signaling pathways included in tumor cell proliferation, differentiation and death like the extracellular-signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38, mammalian target of rapamycin/protein kinase B (mTOR/AKT) etc., revealing its strong antitumor competence but also, its potential to affect different aspects of cell functioning [10,11,15,19]. During the last decades, more attention is devoted to AE ability to trigger autophagy. Its potential to promote this process strongly reflected on its antitumor

but, importantly, also protumor activities generated in interplay with other agents or molecules. It is documented that fundamentally autophagy has a bivalent role in homeostasis maintains and ballast between death and self-renewal. An addition to apoptosis, its involvement in drug cytotoxicity realization is recognized as highly important since can vary from "safe" mode to being the type of cell death. According to this, the definition of its contribution to any kind of cancer treatment became a very important task. For the first time, it was observed that the treatment of glioma cells with AE, apart from apoptosis, is tracked with the appearance of acidic vesicles in the cytoplasm, indicative for the autophagy. Regarding this, we speculated that both type of programmed cell death – type I (apoptosis) and II (autophagic cell death) were triggered by AE [10]. However, AE triggered autophagy as opposed to the apoptotic process in concomitant treatment with other molecule or agent with apoptosis-inducing potential [21]. So far, only one study showed that AE is able to lead to mitotic catastrophe. Treatment of HeLa cells with AE resulted in the appearance of multinucleate cells, giant and micronuclear cells. Mitotic index was diminished and the prevalence of cells in the metaphase was noted [22]. Serious facts about lack of success and even contraindications of killing based protocols in the treatment of highly invasive tumors, underline the advantage of differentiation based therapies, even with all difficulties in research settings and time frames needed for the evidence-based assessment of this kind of approach [23]. The most intriguing feature of AE, in this context, is its potential to act as a differentiation-inducing agent. Conversion of un/low differentiated malignant phenotype into a more mature stage might be of great benefit from multiple points of view. First, this phenotype conversion is accompanied with the decreased proliferation rate and the reversion of the higher grade to lower, less aggressive form. In parallel, the process of change into a more mature stage remarkably enhances their sensitivity to chemotherapy. This effect became profoundly important *in vivo* and in the context of high grade, heterogeneous tumor mass consisted of tumor cells with different phenotype and level of differentiation. Presence of "stem" cells is a critical point for undesirable tumor repopulation in reaction to aggressive treatment since these cells start to proliferate in response to damage in the surrounding [23]. Regarding this, it could be speculated that AE has the potential to promote differentiation and subsequently, minimize tumor tissue proliferation in response to apoptosis induced by itself or other agents. Tabalocci *et al*. found that AE endorsing macrophage differentiation from a human U937 monoblastic leukemia cell line, followed with amplified transglutaminase activity. Therefore, AE can serve as a differentiation-inducing agent in the treatment of leukemia [24]. Beside hematological malignancies, abundant evidence *in vitro* showed its potential to different cell lines representing solid cancers. We found that treatment of B16 cells, derived from solid melanoma tumor, with AE, resulted in inhibited cellular proliferation, acquisition of flattened enlarged morphology and at the biochemical level, intensified melanin production and tyrosinase activity. Such transformed the melanoma cells lost their potential to induce tumors in syngeneic C57/BL6 animals confirming once again that AE might be useful in differentiation based therapy of melanoma [11]. Alteration of B16 metastatic clone isolated from lung metastasis – B16-F10, by AE was followed with an elevation of the activity of the transamidating form of TG2 while the invasiveness and production of matrix metalloproteinase-9 were inhibited [25]. Similarly, intensification of transamidating activity of transglutaminase was found upon the treatment of human melanoma SK-MEL-28 and A375 cells. AE significantly diminished the proliferation, stemness, and invasive potential of melanospheres indicating its activity against cancer stem cells [26]. Treatment of rat astrocytoma C6 with AE beside apoptotic and autophagic cell death resulted in phenotype change of survived cells that display elongated morphology accompanied with elevated glial fibrillary acidic protein (GFAP) expression. While GFAP is well-known marker of astrocyte lineage, upregulated expression of this protein clearly indicated the process of glioma cell maturation upon AE. Having in mind that inhibitor of ERK1/2, PD98059, imitated the differentiation effects of AE on glioma cell without triggering tumor cell death, we concluded that differentiation of astrocytoma cells was connected with the inhibition of this signaling pathway [10]. There are also other types of tumors that underwent differentiation in the presence of this herbal anthraquinone. It was found that cervical carcinoma HeLa and oral KB tumor cells displayed a panel of molecules that can be connected with differentiation. For example, alkaline phosphatase activity was increased by AE treatment, while proliferating cell nuclear antigen (PCNA) expression, cyclin A and cyclin-dependent kinase 2 (CDK2) were diminished [27,28]. Interestingly but not so surprisingly, AE ability to induce differentiation and inhibit proliferation of gastric cancer cells was synchronized with repression of alkaline phosphatase, oppositely to oral cancer cells [29]. This cell-specific feature of AE to regulate the same protein in the opposite manner is also observed in the comparative analysis of its influence on ERK1/2 in two different melanoma cell lines. The same dose and time frame exposure of less invasive B16 clone and the inducible nitric oxide synthases^{+} (iNOS^{+}) highly invasive amelanotic melanoma cell line-A375 resulted in phosphorylation of p44/42 in mirror image mode-strong time-dependent inhibition in B16 cells opposite to remarkable, also time-dependent, potentiation in A375 cell line [11].

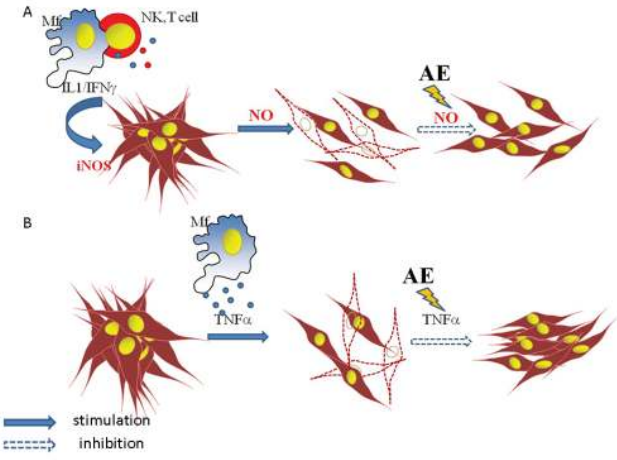
Finally, AE interfered with migration, invasion, and adhesion of tumor cells influencing their dissemination. AE repressed cancer metastasis through the inhibition of epithelial-mesenchymal transitions transition. Exposure of human epidermal growth factor recep-

tor-2 (HER-2) overexpressing breast cancer cells to AE blocked their motility *in vitro*, suppress YB-1 expression through the down-regulation of the intracellular integrin-linked kinase (ILK)/protein kinase B (Akt)/mTOR signaling pathway, diminishing further downstream HER-2 expression. Moreover, its activity was confirmed *in vivo*, in xenograft model induced in nude mice [30]. In a colon cancer cell, WiDr cells, AE inhibited migration and invasion induced by the phorbol-12-myristyl-13-acetate. AE targeted a lot of proteins responsible for the mentioned effects like matrix metalloproteinase (MMP)-2/9, Ras-homologous (Rho) B and vascular endothelial growth factor (VEGF) expression. The described effect might be due to suppressing the nuclear translocation and DNA binding of NF-κB [31]. AE also inhibits invasion of nasopharyngeal carcinoma cells (NPC) by suppressing the expression of MMP-2 via the p38, mitogen-activated protein kinase (MAPK)-NF-κB signaling pathway [32].

INTERPLAY WITH MICROENVIRONMENTAL FACTORS: FROM ANTI- TO PROTUMOR ACTION

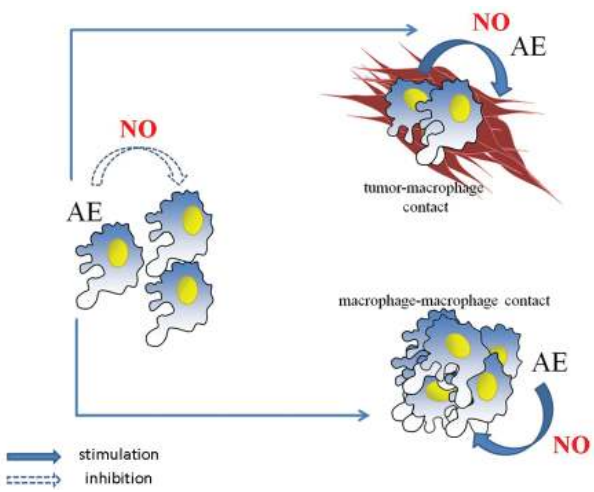
Today is well recognized that micro-environmental factors are able to transform signals triggered by the drug into unexpected and in an unpredictable direction. There are more and more data about the sensitive and very fragile relationship between signals leading to death and those leading to proliferation [2]. Only one additional molecule possesses a power to completely convert apoptotic into dividing stimulus, and subsequently, the outcome of the drug application turns into opposite than anticipated. Numerous molecules created with the purpose to induce apoptotic death of cancer cells, under certain conditions promoted their growth [23]. Plenty of data obtained from clinical trials showed that effectiveness of tested compounds was dramatically lower or even opposite than expected at least partly due to this fascinating biological phenomenon that extremes- death and life, pro- and anti- always go together [3]. Data obtained about AE anticancer activities greatly illustrated how context defines the outcome. Many studies describe its potential to reduce the number of tumor cells in culture and few of them confirmed this *in vivo*. Mechanistically, AE works as an intercalating agent classified as topoisomerase II inhibitor [33]. According to this feature and many other data about its influence on main signaling pathways involved in cell proliferation, death, and differentiation, AE is characterized as an agent with strong antitumor potential. However, it was clearly showed that even the simplest variation in cultivation conditions can radically change the outcome of the treatment with this compound. Cell density in cultures at the beginning of the treatment, for example, can be of crucial importance for AE effectiveness. While in low-density cultures AE was efficient in the range from 20 to 40 μM, applied on the subconfluent/confluent state of the same cell type, it became completely inefficient [9]. It means that intracellular features previously declared as essential for the sensitivity to the treatment with AE easily become irrelevant when cells reached the confluence. Close intercellular contact dramatically affected AE influence on the tumor cells *in vitro*, making questionable compound effectiveness *in vivo*. Since AE potential to reduce tumor volume *in vivo* was already documented [16,34], it is clear that the spectrum of its influence on tumor progression is more complex than it is possible to be simulated *in vitro*. Loss of AE potential to directly affect tumor cell viability in confluent cell cultures was discovered by the case in experiments designed in order to explore its effectiveness in the presence of proinflammatory cytokines in rat astrocytoma C6 and mouse fibrosarcoma L929 cell cultures [9]. Namely, the presence of proinflammatory cytokines in mentioned cell cultures triggered production of endogenous nitric oxide (NO) through enhanced expression of iNOS. To quantify NO and to determine the AE influence on its production, cells were exposed to the compound when they were in the high-density state. In addition to the fact that AE remarkably inhibited iNOS expression and NO production in the presence of cytokine stimulation, it becomes clear that even unstimulated, but AE treated cells were insensitive to the treatment when the drug is applied on subconfluent/confluent culture, oppositely to their low-density counterpart. Furthermore, generated NO negatively regulates viability of its own producers – C6/L929 cell, through induction of apoptosis. This mechanism is known as suicidal and is established by our immune cells and their products- proinflammatory cytokines, as a part of a defensive mechanism against the tumor. Under these circumstances, the iNOS inhibiting the potential of AE consequently abolished interleukin 1(IL1)/interferon-γ (IFNγ) antiglioma and antifibrosarcoma effects (Scheme 1A). Not too far, our group discovered that AE neutralizes tumoricidal potential of tumor necro-

Scheme 1. Interplay of AE with cytokines in tumor microenvironment. A) AE inhibited on IL1/IFNγ triggered NO production in tumor cells. B) AE diminished TNFα mediated tumoricidal action.



sis factor α (TNFα) through induction of autophagy, which opposes to TNFα mediated apoptosis (Scheme 1B) [21]. In addition, since ERK1/2 is very important for the propagation of TNFα triggered signal, diminished activation of this protein upon AE became, at least partly, responsible for the observed tumor protective activities of the agent. Here we arrived to one more paradoxical level of antitumor/protumor potential of AE, facing with the fact that same signal, like the inhibition of ERK1/2, mediates both- tumor destructive activities such as blockage of proliferation, induction of differentiation and even chemo-sensitization, and concomitantly, seriously opposes to some of the important cytokine-mediated aspects of antitumor immune response. A long list of contradictions is further extended by AE influence on macrophages (Mf) (Scheme 2). In concordance with its ability to down-regulate iNOS expression and NO production in C6 and L929 cells, as well as their healthy counterpart- astrocytes and fibroblasts, cultivation of Mf in the presence of the drug resulted in suppression of NO release [35]. This result indicated the diminished antitumor capacity of Mf when they are exposed to AE. Instead of this, co-cultivation of macrophages with glioma cells in the presence of AE revealed even enhanced the cytotoxic potential of Mf and, concordantly, enhanced nitrite accumulation in culture supernatants, as a consequence of the increased release of NO. The observed contradiction between AE influence on Mf NO production when cells were cultivated alone and in co-cultures with tumor cells can be connected with some additional signal generated from the contact between tumor cells and Mf, which in combination with AE resulted in hyper- instead of hypo-production of NO. However, the same effect was observed in high-density cultures of peritoneal Mf alone, indicated ones more, the pivotal importance of cell to cell contact and its influence on signals triggered with AE. Importantly and differently to the effect of AE on NO production determined in C6/L929 cells as well as primary astrocytes and fibroblasts, hyper-production of NO observed in the high-density culture of mouse Mf was iNOS independent and resistant to treatment with inhibitors of transcription or translation, actinomycin, and cycloheximide, respectively [35, unpublished data]. It is clear that AE is able to interfere with NO production at the level of iNOS gene as well as protein expression. In addition to NO, it was discovered that AE down-regulated production of interleukin-6 and interleukin-1β in macrophage cell line RAW264.7 cells upon stimulation with bacterial lipopolysaccharide and realized the suppressive effect on leukocytes isolated from Sprague-Dawley rats, decreasing the phagocytic potential of Mf and NK cells activity. Contradictory, measurement of cytokine production showed that AE augmented the interleukin-1 β and TNFα [36,37]. Altogether, it is clear that outcome of the treatment with AE presents the net effect of the complex network of hardly predictable interactions generated through collection of signals triggered at the level of DNA and genes, proteins and cell membrane. More than that, further integration of the stimuli modified by AE happens in communication between different cells in heterogeneous tumor mass- tumor cells in the different stage of differentiation and their non-transformed counterparts, stromal cells, and various immune cells.

Scheme 2. Influence of AE on macrophage NO production depending on cell-cell contact. AE inhibited NO production in low-density macrophage cultures while in high-density or in co-cultivation with tumor cells hypo production of NO is converted to hyper production.



INTERPLAY WITH CYTOSTATIC DRUGS: FROM SYNERGISM TO ANTAGONISM

Usage of aloe derived anthraquinones as chemosensitizing agents are well described in the literature. Mostly literature data evaluate the effect of emodin as an amplifier of chemotherapy, especially cisplatin. The interaction with the drug basically refers to ROS production and their sensitizing features, interference with multi-drug resistant transporters and induction of autophagy [38,39]. Even though the antitumor action of AE is well studied there are only a few papers describing its interaction with chemotherapeutic drugs. The co-treatment of Merkel cell carcinoma with cis-platinol (abiplastin), doxorubicin (adriablastin), and 5-fluorouracil (5-Fu), and AE resulted in potentiation of their cytotoxicity [40]. Similarly, AE amplified the cytotoxicity of tamoxifen against MCF-7 breast cancer cells through reduction of epidermal growth factor receptor (EGFR), rat sarcoma (Ras), ERK, c-Myc, and mTOR protein expression. In addition, the activity of Ras/ERK and phosphatidylinositol 4,5-bisphosphate 3-kinase (PI3K)/mTOR pathways was suppressed. Production of ROS and enhanced cell death in co-treatment was potentiated indicating that AE can act as a strong chemosensitizer [41] The potentiation of 5-fluorouracil was also achieved in epidermoid carcinoma A431 and head and neck squamous cell carcinoma SCC25A431 through regulation of caspase-8, -9, and -3 expression

[42]. Oppositely to the beneficial chemosensitizing effect of AE, there are few data indicating opposite effect. The possibility that the concomitant application of AE and certain cytostatic can lead to neutralization of tumoricidal effects of chemotherapy, underlines that special caution is needed in the eventual design of combined treatment with conventional drugs. We already described that AE as a single agent possesses strong antitumor potential on two different melanoma cell lines, human A375 and mouse B16. However, parallel treatment with doxorubicin or paclitaxel resulted in antagonistic action *in vitro* [11]. In line with this, AE neutralized cisplatin-induced apoptosis and necrosis on murine L929 fibrosarcoma and C6 glioma cell lines. The counteracted action of cisplatin was due to opposite regulation of ERK in tumor cells, but not c-Jun N-terminal kinase [43].

ALOE EMODIN AS A SENSITIZER IN PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) is a clinically approved, noninvasive therapeutic method that includes the application of a photosensitizing agent concomitantly with a particular type of light [44]. This procedure can be valuable particularly for early-stage tumors but also can extend survival and quality of life of patients with inoperable cancers. Its advantage is marginal toxicity for normal tissues, minor systemic effects, diminished morbidity and development of resistance mechanisms. Photosensitizing substances are activated by a specific wavelength that usually triggers the production of ROS that is harmful to cancer cells. Besides the direct killing of cancer cells, such treatment affects blood vessels in the tumor and triggered an immune response against the tumor. Also, repetition of PDT and concomitant treatment with surgery, radiation, and chemotherapy is possible. So far, PDT is approved by the Food and Drug Administration for the treatment of esophageal cancer and non-small cell lung cancer. Recent findings indicate that AE can be a useful drug as photosensitizer during PDT. Therefore, it was shown that AE, as the part of this therapeutic approach, inhibited proliferation of oral mucosa carcinoma KB cells in G1 phase and triggered apoptotic cell death due to the massive ROS production. Apoptotic cell death was followed with an up-regulated expression of caspase-3 and Bax together with strong suppression of Bcl-2 expression. Moreover, AE-PDT had marked inhibitory effect *in vivo* and extended the survival time of animals without notable side effects [45]. Similar findings were observed in human gastric cancer SGC−7901 cells where the AE-induced PDT resulted in the activation of mitochondrial-dependent apoptosis [46]. Apoptosis is also the main outcome of AE-PDT of osteosarcoma MG63 cells. Fast intracellular ROS production led to the disruption of mitochondrial membrane potential, the release of cytochrome *c*, enhancement of caspase-3, -9, and -12, CCAAT-enhancer-binding protein homologous protein (CHOP) and glucose-regulated protein 78 (GRP78) [47]. Apart from apoptosis, AE-PDT induced the autophagy of human osteosarcoma cell line MG-63 through the activation of the ROS-JNK signaling pathway [48]. In addition, AE as a sensitizer for PDT interferes with the metastatic spreading of MCF-7 breast cancer cells. Oxidative stress targeted the expression of MMP2, MMP9, VEGF, and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) as well as cytoskeleton organization after treatment with AE-PDT [49]. In lung cancer, H460 cells photo-activated AE provoked a disturbance of cytoskeleton and triggered anoikis, as the programmed cell death that occurs in adherent cells detached from the extracellular matrix. Anoikis is related to α-actinin and MAPK expression and led to the mitochondrial permeability transition pore opening and elevated expression of apoptosis-related proteins [50]. Aloe emodin and irradiation stimulated the expression of protein kinase Cδ (PKCδ) in H460 cells and its translocation of to the nucleus. Additionally, AE-PDT triggered numerous proteins important for cytoskeleton organization like RAS, ras homolog gene family member A (RHO), p38, heat shock protein 27 (HSP27), focal adhesion kinase (FAK), α-actinin and tubulin [51]. Finally, AE-PDT interfered with angiogenesis process that is crucial for both growth and metastatic spreading of cancers. Aloe emodin photodynamic therapy suppressed formation branching points, tubule number, and length. Also, the number of capillary structures was significantly reduced by AE-PDT treatment. Migration and invasion of human umbilical vein endothelial cells were also affected by the treatment. Mention processes are connected with the activation of p38, ERK, but not JNK, while the expression of vascular endothelial growth factor was diminished upon the treatment. In addition, AE-PDT altered the organization of F actin cytoskeleton [52].

NEW CHEMICAL DESIGNS FOR IMPROVEMENT OF AE ANTICANCER POTENTIAL

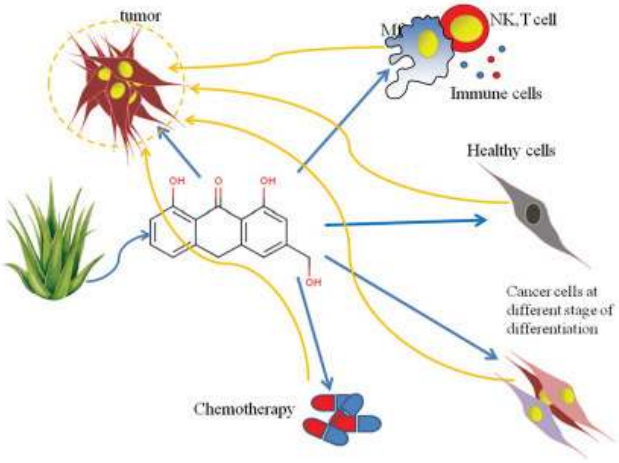
The antitumor activity of AE is highly compromised by its rapid degradation and low bioavailability. A lot of effort was made to make a different formulation of AE to improve its features. One of the approaches includes the application of nanotechnology. Wu *et al*. made a poly (lactic-co-glycolic acid) based AE nanoparticles. The application of AE in this form was more efficient than an original compound in inhibition of human lung squamous cell carcinoma proliferation, induction of cell cycle arrest and further apoptotic cell death. Apoptosis was followed with activation of Caspase-3, poly (ADP-ribose) polymerase (PARP), Caspase-8 and Caspase-9. In parallel, ROS production was elevated. Nano-AE stimulated MAPK activation and suppressed PI3K/AKT signaling pathway. More importantly, nano-AE suppressed the tumor growth *in vivo* with insignificant toxicity [53]. The other group prepared surface-functionalized polyethylene glycol liquid crystalline nanoparticles (PEG-LCNPs) of AE to enhance its water solubility and enable its anticancer use. Particle size was 190 nm and their stability in serum was ele-

vated. Further studies showed a good safety profile of PEG-LCNPs of AE [54]. One more strategy involves solid lipid nanoparticles with a stable particle size at approximately 90 nm with good drug entrapment efficiency and stability. AE loaded in solid lipid nanoparticles displayed amplified cytotoxicity against human breast cancer MCF-7 cells and human hepatoma HepG2 cells in comparison to the AE. The toxicity toward human mammary epithelial MCF-10A cells was diminished. AE loaded in solid lipid nanoparticles induced apoptosis in MCF-7 cells probably due to increased cellular uptake of AE [55]. Similar improvement was achieved by integrating AE into the liposomal formulation. That formulation augmented cell death of A431 and SCC25 cells and improved transdermal delivery of AE [42]. Other approaches aimed to make water-soluble formulation consider the chemical modification through coupling with various amino acid esters and substituted aromatic amines. Derivate significantly reduced the growth of human liver cancer cells HepG2, and lung cancer cells NCI-H460, human epithelial carcinoma cells HeLa and prostate cancer cells PC3 more potently than AE alone [56]. The attachment of an amino-sugar unit to AE formed a new class of AE glycosides (AEGs) with improved cytotoxic potential even in doxorubicin-resistant cell lines probably through interference with P-glycoprotein efflux pumps [57]. Finally, with an attempt to improve tumoricidal potential hybrid molecule from rhein and AE was synthesized. This chimeric molecule was more efficient against human hepatoma HepG2, human nasopharyngeal carcinoma CNE, human lung cancer NCI-H460, human ovarian cancer SK-OV-3, and human cervical cancer HeLa cells than separate compounds [58].

CONCLUSION

Summarizing all the mentioned effects of AE, it is clear that its direct tumoricidal potential and synergistic action with some of the conventional drugs is undoubting (Scheme 3). However, it is not possible to overview it separately and independent from the other actors in tumor microenvironment or applied therapy. It is more than clear that serious observation is necessary because the line between the cure and harm is so tiny and hard to predict. "Magic bullet" for cancer therapy still not exists but the definition of conditions and protocols when the phytotherapeutics can be beneficial is highly valuable. Thousands of years old ethnic- medicine can definitely mark the right way, but gathering an experimental knowledge and clinical experience is also necessary. The study of Lissoni et. on 240 patients with metastatic lung, colorectal and pancreatic tumors who have received chemotherapy in parallel with Aloe arborescens treatment showed significant tumor regressions, disease control and increased survival rate, claiming that this class of drugs might have a clinical potential [59]. We can have multiple benefits from phytotherapy if we understand and utilize it correctly.

Scheme 3. Influence of AE on tumor is a net effect of complex network involving different members of tumor commune. In addition to direct effect on tumor cell AE influence tumor growth through modulation of immune cells, normal cells, heterogeneous population of tumor cells as well as chemotherapy.



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ALOE EMODIN U TRETMANU TUMORA: SAVEZNIK ILI PROTIVNIK

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Kratak sažetak

Loš odgovor visoko invazivnih formi kancera na tretman se ne može objasniti samo fenotipom ćelija rezistentnih na indukciju smrti već i repopulacijom tumora u odgovoru na oštećenja nastala usled primene hemio- ili radioterapije. Ozbiljna ograničenja regularnih terapeutskih pristupa jedan su od glavnih izazova za biologe da utvrde relevantnost i istraže mehanizme u osnovi lečenja malignih bolesti zasnovanih na tradicionalnoj medicini. Jedna od najstarijih i najmoćnijih biljaka sa 4000 godina dugom tradicijom u narodnoj medicini, Aloe vera, je intenzivno izučavana u poslednjem veku zbog čitave riznice aktivnih komponenti privlačnih kako lekarima i pacijentima, tako i naučnicima. Antrahinoni, emodin i aloe emodin (AE), izolovani iz Aloe vera i drugih biljaka iz porodice Polygonaceae su definitivno najviše izučavani konstituenti. Aloe emodin poseduje višestruka antitumorska svojstva realizovana kroz indukciju zastoja u ćelijskom ciklusu, ćelijske smrti, diferencijacije i supresije motiliteta malignih ćelija. Međutim, njegova interakcija sa tumorskom ćelijom nije jednosmerna i zbog kompleksne mreže signala u mikrosredini tumora može lako biti konvertovana sa destruktivne u promovišuću za tumor. Ovaj pregledni članak će sumirati direktne tumoricidne efekte ali će razmatrati i interakcije AE sa imunskim ćelijama i njihovim medijatorima. Takođe, biće elaboriran i potencijal AE kao hemio- i fotosenzitizatora. Konačno, diskutovaće se i novi pristupi u modifikaciji ovog molekula koji uključuju hemijske intervencije i primenu nanotehnologije.

Ključne reči: *Aloe emodin, antikancerska svojstva, interakcija sa mikrosredinom, citostatici, fotodinamička terapija*