

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Anticancer Properties of Cardiac Glycosides

Varisa Pongrakhananon

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55381>

1. Introduction

Cardiac glycosides comprise a large family of naturally derived compounds, the core structures of which contain a steroid nucleus with a five-membered lactone ring (cardenolides) or a six-membered lactone ring (bufadienolides) and sugar moieties [1]. A few widely recognized examples of cardiac glycosides are digoxin, digitoxin, ouabain, and oleandrin. The cardenolides digitoxin and digoxin, two well-known cardiac glycosides, are inhibitors of the plasma membrane Na^+/K^+ -ATPase that are clinically used for the treatment of heart failure. Their positive inotropic effects help suppress the active counter-transportation of Na^+ and K^+ across the cell membrane, leading to an increase in the intracellular Na^+ concentration, a decrease in the intracellular K^+ concentration, and a consequent increase in cardiac contraction [2]. Epidemiologic evidence suggests that breast cancer patients who were treated with digitalis have a significantly lower mortality rate, and their cancer cells had more benign characteristics than those from patients not treated with digitalis [3,4]. Interestingly, the concentrations of cardiac glycosides used for cancer treatment are extremely close to those found in the plasma of cardiac patients treated with the same drugs, suggesting that the anticancer effects of these drugs are exerted at non-toxic concentrations [5]. Furthermore, studies have suggested that cardiac glycosides target cancer cells selectively [6]. These encouraging findings have gained considerable attention in the field of anticancer research, and subsequent studies on the anticancer properties of these compounds have been conducted. These studies investigated not only digoxin and digitoxin but also other related cardiac glycosides, such as ouabain, oleandrin, proscillaridin A, and bufalin [7-10]. Several mechanisms of action, including the inhibition of cancer cell proliferation, the induction of apoptosis, and chemotherapy sensitization, have been reported in a large number of published articles that support the potential use of these compounds for cancer treatment [11-14]. However, further clinical studies are still ongoing to better characterize the pharmacological and safety issues associated with these compounds. This chapter provides an overview of the anticancer activities of cardiac glyco-

sides and describes the selectivity of these compounds, which could prove to be promising treatments in cancer therapy.

2. The chemistry of cardiac glycosides and their biological activities

Cardiac glycosides from both plants and animals have been known for over one hundred years [14]. Major plant-derived cardiac glycosides include digitoxin, digoxin, ouabain, oleandrin and proscillaridin, which are extracted from the plant families Scrophulariaceae, Apocynaceae, and Asparagaceae (*Digitalis purpurea*, *Digitalis lanata*, *Strophanthus gratus*, *Nerium oleander* and *Urginea maritima*). These compounds consist of a steroidal nucleus linked with a sugar at position 3 (C3) and a lactone ring at position 17 (C17) (Fig 1) [15]. The various types of sugar moieties and lactones provide a large number of cardiac glycosides that, based on their lactone moieties, can be divided into two sub-groups: cardenolides, which contain a five-membered unsaturated butyrolactone ring, and bufadienolides, which contain a six-membered unsaturated pyrone ring. The core steroidal portion of each molecule has an A/B and C/D cis-conformation, which has significant pharmacological relevance. The attached sugars, such as glucose, galactose, mannose, rhamnose, and digitalose, determine the pharmacodynamic and pharmacokinetic activities of each cardiac glycoside.

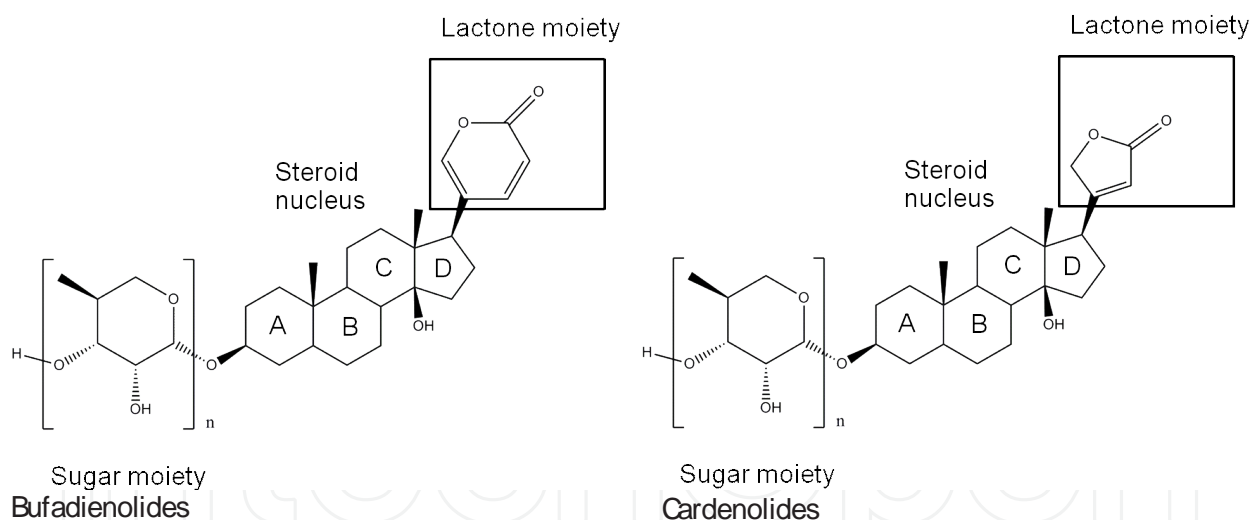
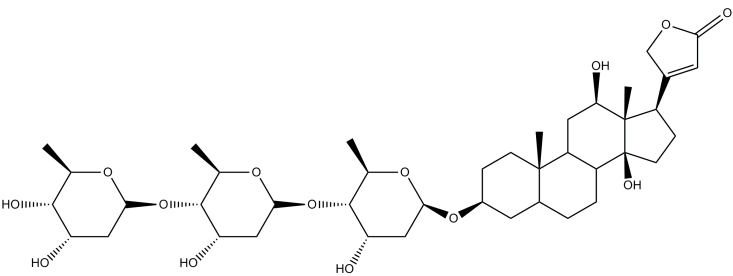
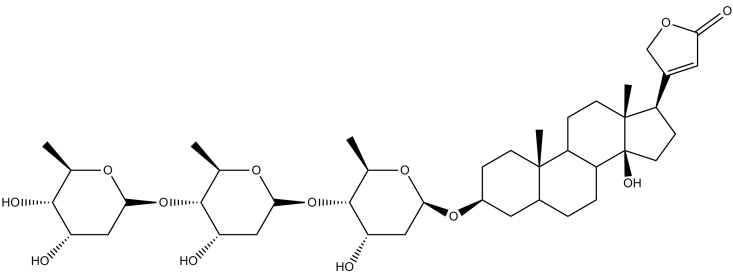


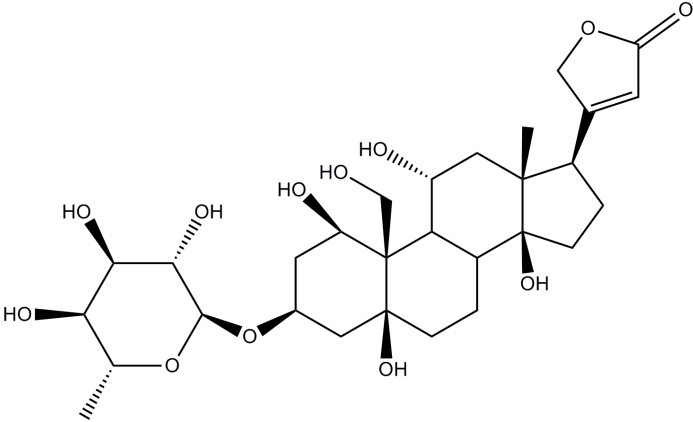
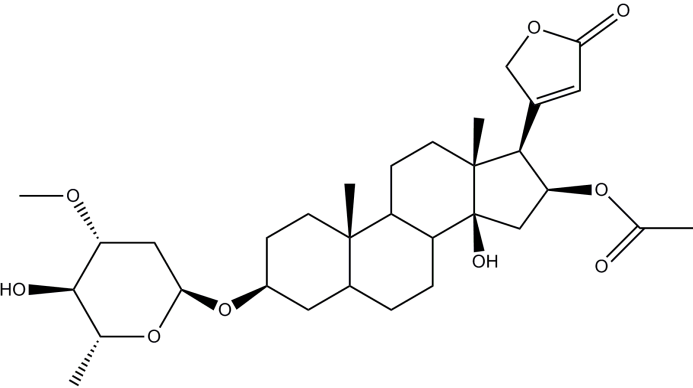
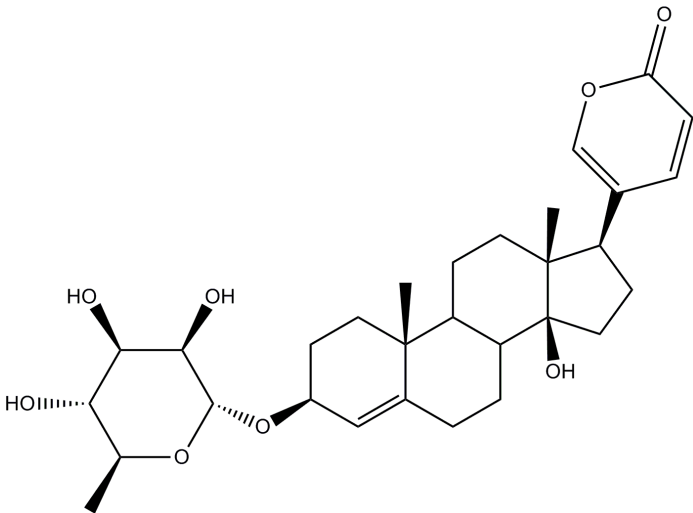
Figure 1. Structural characteristics of cardiac glycosides

Cardiac glycosides have been found in animals as well as plants; for example, bufadienolide was isolated from the venom of a toad species [16], and endogenous digitalis-like compounds have been found in mammalian tissues [17,18]. Several studies have reported that ouabain and proscillaridin A are found in human plasma, that digoxin and marinobufagenin are present in human urine, and that 19-norbufalin exists in cataractous human lenses [18-22]. Table 1 presents a list of the cardiac glycosides found in plants and animals along with their chemical structures.

A digitalis preparation from *Digitalis purpurea* was first used for the treatment of congestive heart failure by William Withering in 1785 [23]. Currently, digoxin is recognized as a primary treatment for patients with heart failure. Its mode of action has been identified as the potent inhibition of Na⁺/K⁺-ATPase. Na⁺/K⁺-ATPase, a ubiquitous transmembrane enzyme, is a p-type cation transporter that actively drives two K⁺ ions into the cell and drives three Na⁺ ions out of the cell using ATP as an energy source. This pump plays a vital role, acting as a secondary transporter of nutrients such as glucose and amino acids and helping to maintain the electrochemical gradient by keeping the intracellular Na⁺ concentration low [24]. The elevation of the intracellular Na⁺ level in response to cardiac glycosides stimulates the Na⁺/Ca²⁺ exchanger mechanism. As a result, the intracellular Ca²⁺ concentration is increased, consequently promoting cellular events such as myocardial contractibility, accounting for the positive inotropic effects of the cardiac glycosides.

Accumulating evidence has established that the Na⁺/K⁺-ATPase acts as a scaffold for signaling molecules or for the formation of a signalosome complex that activates various signaling cascades. Several signaling molecules, such as caveolin, SRC kinase, epidermal growth factor receptor (EGFR), and the inositol 1,4,5-triphosphate (IP3) receptor, have been investigated [25-27]. The inhibitory effects of cardiac glycosides on Na⁺/K⁺-ATPase activity might lead to alterations in these downstream transduction pathways, which could account for the biological properties of these compounds, including their anticancer activities.

Name	Structure
<ul style="list-style-type: none"> • Digoxin (Cardenolide) • From <i>Digitalis purpurea</i> • Family: Scrophulariaceae 	
<ul style="list-style-type: none"> • Digitoxin (Cardenolide) • From <i>Digitalis purpurea</i> • Family: Scrophulariaceae 	

Name	Structure
<p>Ouabain (Cardenolide)</p> <p>From <i>Nerium oleander</i></p> <p>Family: Apocynaceae</p>	
<ul style="list-style-type: none">• Oleandrin (Cardenolide)• From <i>Nerium oleander</i>• Family: Apocynaceae	
<ul style="list-style-type: none">• Proscillaridin (Bufadienolide)• From <i>Urginea maritima</i>• Family: Liliaceae	

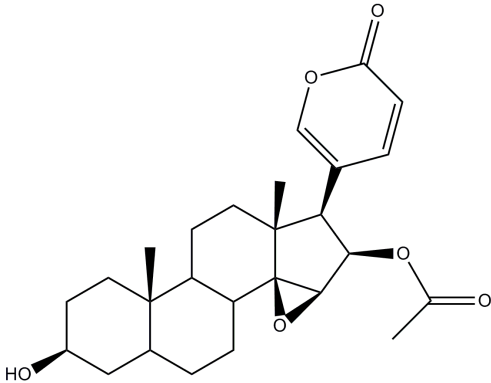
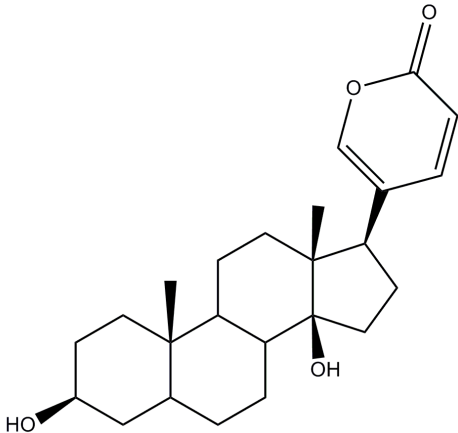
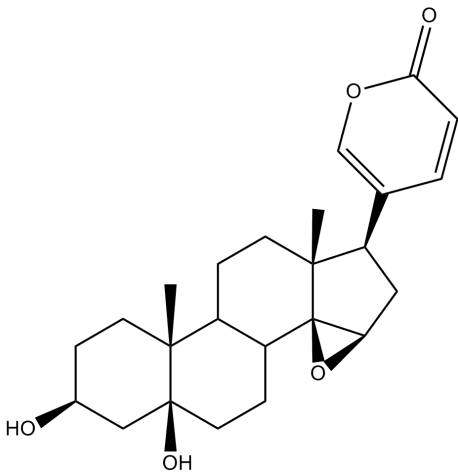
Name	Structure
<ul style="list-style-type: none"> • Cinobufagin (Bufadienolide) • From <i>Bufo bufo gargarizans</i> • Family: Bufonidae 	
<ul style="list-style-type: none"> • Bufalin (Bufadienolide) • From <i>Bufo gargarizans</i> • Family: Bufonidae 	
<ul style="list-style-type: none"> • Marinobufagenin (Bufadienolide) • From <i>Bufo marinus</i> • Family: Bufonidae 	

Table 1. The chemical structures of cardiac glycosides

3. Clinical analysis of the effects of cardiac glycosides on cancers

Epidemiologic evidence for the anticancer effects of digitalis was first reported in 1980 by Stenkvis and colleagues. Their study indicated that breast cancer tissue samples from congestive heart failure patients treated with cardiac glycoside therapy exhibited more benign characteristics than cancer tissue samples from control patients who were not treated with the cardiac glycoside regimen [28]. In addition, 5 years after undergoing mastectomy, the recurrence rate for the cardiac glycoside treated-group was 9.6 times lower than that for the control group [28-29]. Four years later, Glodin and colleagues investigated the mortality in 127 cancer patients who received digitalis therapy. These researchers reported that up to 21 patients in the control group died from cancer, whereas only one member of the digitalis-treated group died [30]. Interestingly, the long-term observations of Stenkvis and colleagues also supported the previous finding that digitalis therapy significantly reduces the mortality rate of breast cancer. Among 32 breast cancer patients treated with digitoxin, only two (6%) died, whereas the control group of 143 patients had 48 cancer-related deaths (34%) [4]. Several types of cancer other than breast cancer have also been examined. Recently, Haux and colleagues published an analytical descriptive study on the antineoplastic effects of cardiac glycosides on leukemia and cancers of the kidney/urinary tract [31]. This study indicated that the doses of cardiac glycosides that are active against cancers are similar to the therapeutic plasma concentrations found in cardiac patients treated with these drugs. These clinical observations have established the beneficial outcome of cardiac glycosides for cancer therapy. Although these agents seem to be safe at the doses used for the treatment of cardiac disorders, further supporting evidence is still needed before these compounds can be used clinically.

4. Anticancer properties and their mechanisms

At present, cancer is one of the major causes of death worldwide. Extensive research has been conducted over the last decade in an attempt to identify promising compounds that have anticancer effects. Cardiac glycosides are natural compounds that have been previously documented to be antiarrhythmic agents, and their potential anticancer properties were identified thereafter. Cardiac glycosides have been shown to have anticancer activities during various stages of carcinogenesis. These activities include antiproliferative, pro-apoptotic, and chemotherapy sensitization effects.

4.1. Antiproliferative effects

Aberrant cell growth is recognized as one hallmark of cancer [32]. Excessive cell replication is the basic characteristic of cancer progression that facilitates tumor formation and expansion. Defects in normal growth signals result in the inadequate regulation of cell division, which drives quiescent cells to proliferate [33]. Cardiac glycosides have been demonstrated to have antiproliferative activities via their regulation of the cell cycle. The extract from the skin glands of *Bufo bufo gargarizans*, which contains bufalin, is able to induce arrest in human malignant

melanoma cells in the G2/M phase of the cell cycle [34]. In lung cancer cells, bufalin upregulates p21 WAF1 and suppresses cyclin D expression in response to the activation of p53 [35]. Because the tumor suppressor p21 WAF1 acts as a potent inhibitor of cell cycle progression [36] and because cyclin D1 is a subunit of cyclin dependent kinase (Cdk)-4 and Cdk-6, which are responsible for cell cycle progression from G1 to S phase [37], these changes prevent cells from entering the next phase of the cycle.

Likewise, digitoxin causes cell cycle arrest in G2/M in a dose-dependent manner, resulting in a large increase in the number of cells in the sub-G0 phase [38]. A synthetic monosaccharide analog of digitoxin, D6-MA, has 5-fold greater potency than digitoxin. The mode of action of D6-MA has been reported to involve the downregulation of key elements required for cell replication, including cyclin B1, cdc2 and survivin. It has been suggested that these events might be downstream signaling events resulting from the modulation of second messengers, such as tyrosine kinase Src, PI3K, phospholipase C and Ras/MAPK pathway components, by cardiac glycoside-bound Na⁺/K⁺-ATPase [25-27].

An antiproliferative effect of ouabain against human breast and prostate cancer cells has also been reported [39]. Ouabain mediates the depletion of the Na⁺/K⁺-ATPase through endocytosis and a degradation-dependent pathway, which in turn elevates the level of the cell cycle inhibitor p21. It has been suggested that the cellular level of Na⁺/K⁺-ATPase plays an important role in determining the rate of cell growth. Additional mechanistic studies have demonstrated that an increase in the intracellular Ca²⁺ concentration following treatment with digoxin, digitoxin, or ouabain is associated with the antiproliferative effects of these compounds in androgen-dependent and androgen-independent prostate cancer cell lines [40]. Because Ca²⁺ serves as a mediator in several signaling pathways, the elevation of the Ca²⁺ concentration may stimulate cellular processes that switch the cells into a growth-retarded state. Several of the antiproliferative effects of cardiac glycosides are summarized in Table 2.

4.2. Induction of apoptosis

Resistance to apoptosis in response to stress conditions is a basic feature of cancer cells and results from the overactivation of survival pathways or the attenuation of cell death mechanisms. The primary readout for screens of anticancer agents is thus usually an apoptosis-inducing effect. Cardiac glycosides have been established as cytotoxic agents that are active against various types of cancers. As mentioned above, the inhibition of Na⁺/K⁺-ATPase by cardiac glycosides triggers the formation of the signalosome complex, contributing to the initiation of signaling cascades that favor cell death [25-27].

It is well documented that apoptosis generally occurs through two main pathways: the mitochondrial-dependent and death receptor-dependent pathways [46]. Gan and colleagues have reported that oleandrin induces cervical cell apoptosis through the mitochondrial cell death mechanism [47]. This compound significantly stimulates the caspase-dependent pathway by triggering the cleavage of caspase-3/7, -6, and -9 and by upregulating the proapoptotic factor Bim. Similarly, data reported by Elbaz and colleagues support the hypothesis that digitoxin mediates the induction of the mitochondrial apoptotic pathway via caspase-9

Cardiac glycoside	Mechanism
Digitoxin	Induction of cell cycle arrest in G2/M phase through the downregulation of cyclin B1, cdc2 and survivin [38] Increase in the intracellular Ca ²⁺ concentration [40]
Digoxin	Increase in the intracellular Ca ²⁺ concentration [40] Inhibition of DNA topoisomerases I and II and an increase in the intracellular Ca ²⁺ concentration [41] Induction of cell cycle arrest through the upregulation of HIF-1α [42]
Ouabain	Depletion of Na ⁺ /K ⁺ -ATPase and upregulation of p21 [39] Increase in the intracellular Ca ²⁺ concentration [40] Inhibition of DNA topoisomerases I and II and increase in the intracellular Ca ²⁺ concentration [41]
Oleandrin	Attenuation of NF-kB, JNK and AP-1 (nuclear transcription factors) activation [43,44]
Bufalin	Induction of cell cycle arrest in G2/M phase through the upregulation of p21 WAF1 and p53 and the downregulation of cyclin D [34,35] Inhibition of DNA topoisomerases I and II [45]
Proscillaridin A	Inhibition of DNA topoisomerases I and II and an increase in the intracellular Ca ²⁺ concentration [41]

Table 2. Antiproliferative effects of cardiac glycosides

activation [38]. This study demonstrated not only a cell growth inhibitory effect but also an apoptotic induction effect for digitoxin.

Fas and TNF-related apoptosis-inducing ligand (TRAIL) are important mediators of the death receptor pathway, and the deregulation of their expression is a major cause of chemoresistance and immune escape in cancers [48]. Recently, Sreenivasna and colleagues investigated whether oleandrin triggers the expression of the Fas receptor to potentiate apoptosis in cancer cells without affecting normal primary cells [49]. Additionally, oleandrin has been found to be able to attenuate the NF-kB pathway, which is a key pathway with antiapoptosis and pro-proliferative effects. Cardiac glycosides including oleandrin, bufalin, digitoxin, and digoxin also initiate apoptosis through Apo2L/TRAIL by elevating the levels of death receptors 4 and 5 in non-small cell lung cancer cells [50]. Interestingly, both Fas and Apo2L/TRAIL induce apoptosis in cancer cells but have little to no effect on normal cells. Furthermore, our recent work has demonstrated that ouabain was able to increse TRAIL-mediated lung cancer cell death through anti-apoptosis Mcl-1 down-regulation [51]. Because of these results, cardiac glycosides are of great interest in the field of cancer research.

A growing number of studies have indicated that the disruption of the oxidative state inside cancer cells, due to either the suppression of the antioxidant system or the introduction of reactive oxygen species, can lead to cell death [52]. In androgen-independent prostate cancer cells, ouabain triggers apoptosis by interfering with mitochondrial function [53]. Because the mitochondria are a major source of reactive oxygen species, the application of ouabain causes a steady increase in the level of these species, which leads to apoptosis. This study also

indicated that a low dose of ouabain was able to upregulate prostate apoptosis response 4, which is required to reach the desired level of apoptotic cell death.

Other mechanisms of cardiac glycoside-induced apoptosis have also been reported (Fig 2). Mitogen-activated protein kinases (MAPKs) have been reported to be targeted in bufalin-induced human leukemia cell apoptosis [54]. JNK and AP-1 are transcription factors that activate the transcription of various genes, including apoptosis-related genes [55,56]. In response to bufalin treatment, the MAPK signaling pathway is triggered, leading to a notable elevation in the activities of c-Jun N-terminal protein kinase (JNK) and AP-1.

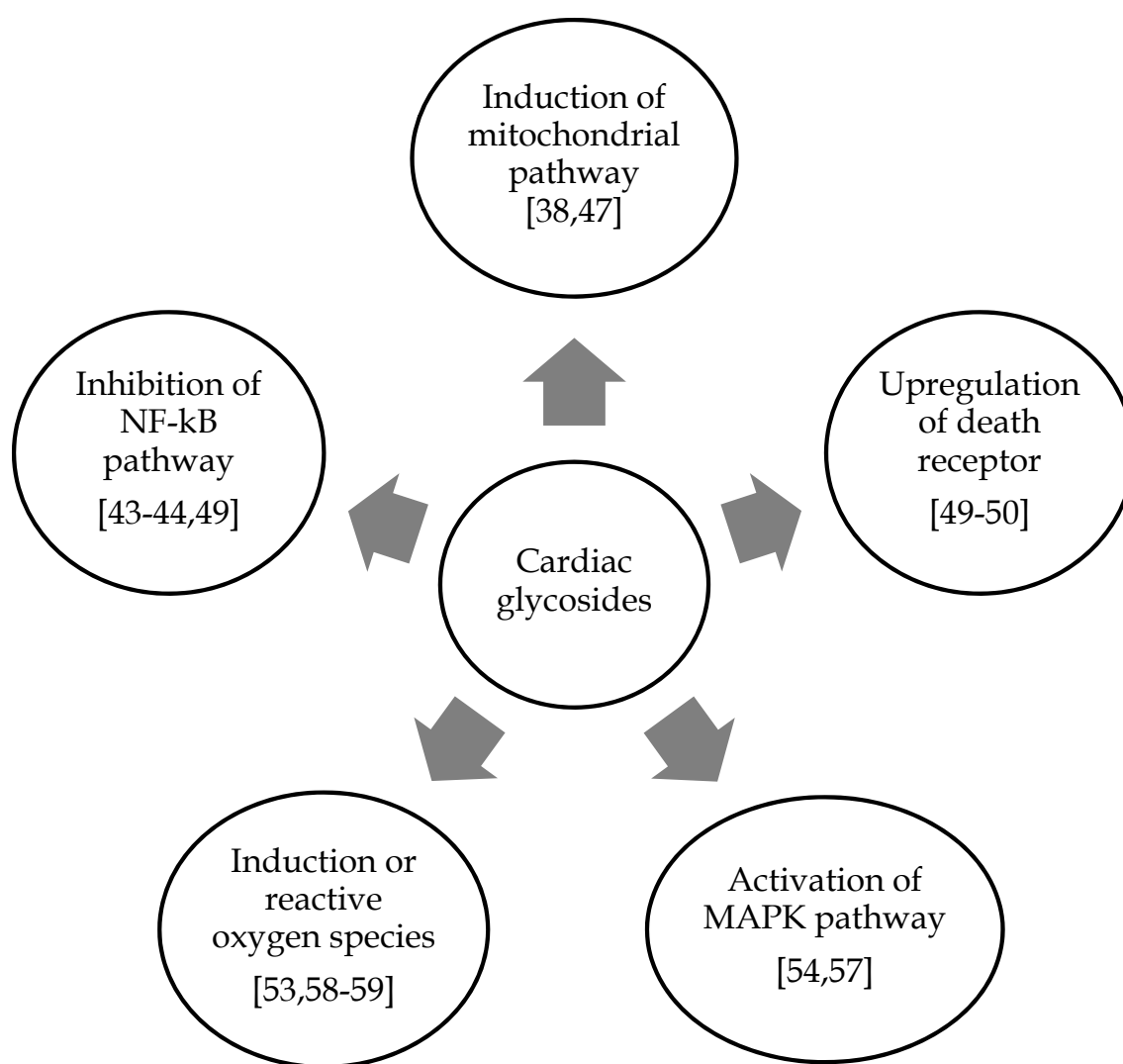


Figure 2. Molecular mechanisms of cardiac glycoside-induced apoptosis

4.3. Sensitization to chemotherapy and enhancement of radiotherapy sensitivity

The susceptibility of a given cancer to chemotherapy often appears to decrease after several rounds of chemotherapy. Resistance to drug-induced cell death is therefore a critical problem in cancer therapy. Combination therapy may be initiated as an alternative approach to overcome this problem. Furthermore, the use of combination therapy increases the cytotoxicity of anticancer agents and reduces their serious side effects on normal cells by reducing the dosage required for each individual agent. Cardiac glycosides have beneficial effects when used as part of combination therapies. Felth and colleagues have investigated the cytotoxicities of cardiac glycosides alone and in combination with various clinically relevant anticancer drugs [60]. Of the glycosides tested, convallatoxin, oleandrin, and proscillaridin A have been shown to be the most potent inducers of colon cancer cell death. Furthermore, co-treatment with cardiac glycosides, including digoxin, digitoxin, oleandrin, and digitonin, and other anticancer drugs, namely 5-fluorouracil, oxaliplatin, cisplatin, and irinotecan, was shown to result in a substantial increase in cancer cell death. However, this study was only a primary screen of the effects of these compounds, and the mechanisms responsible for these effects have not been elucidated.

It is significant that the members of the ATP binding cassette family of transporters, including ABCC7 (CFTR), ABCB1 (P-glycoprotein), and ABCC1 (MRP1), play critical roles in pumping a broad range of drugs out of cells and that these transporters are obviously overexpressed in several tumors [61]. Ouabain has been identified in a recent study to be able to regulate both the expression and activity of ABCC1 in an embryonic kidney cell line. The impairment of ABCC1 following ouabain treatment suggests that this compound might be able to prevent the reduction of the therapeutic concentration inside target cells.

Radiotherapy is a traditional approach used to destroy localized and unresectable tumor cells and to prevent these cells from metastasizing. The combination of chemotherapy and radiation limits the aggressiveness of cancers and increases the patient survival rate. The basic concept underlying chemoradiation is that chemotherapeutics are administered to make cancer cells more susceptible to radiation. Unfortunately, most cancers develop chemoresistance, and anticancer agents have serious side effects in normal cells. The administration of potent anticancer agents with less toxicity against normal cells to sensitize the tumor cells to radiotherapy is a promising strategy. Cardiac glycosides are substances that exhibit selectivity and significant activity against cancer cell lines; thus, the addition of these compounds to existing chemoradiation regimens has been investigated. Huachansu, which is extracted from the skin glands of *Bufo bufo gargarizans*, exhibits a radiosensitizing effect on human lung cancer cells [62]. This Chinese medicine contains a group of steroidal cardiac glycosides and substantially increases radiation-mediated cell death via a p53-dependent pathway. The underlying mechanism involves the cleavage of caspase-3 and poly-(ADP-ribose) polymerase (PARP) concurrent with the downregulation of the antiapoptotic protein Bcl-2 and the inhibition of DNA repair.

The ability of ouabain to sensitize cancer cells to radiotherapy has also been established. Transformed fibroblasts and tumor cells exposed to gamma radiation undergo apoptosis in the presence of ouabain [63-65]. In addition, the recovery of cells is clearly delayed when the

cells are exposed to ouabain after irradiation. These events are the results of the inhibitory effect of ouabain on the G2/M phase of the cell cycle.

4.4. The selectivity and sensitivity of cardiac glycosides for cancer cells

The ideal anticancer agent would not only be effective but also selective against tumor cells. As emphasized above, cardiac glycosides have beneficial anticancer effects but do not affect normal cells. Oleandrin attenuates the activation of nuclear transcription factor- κ B and activator protein-1 and mediates ceramide-induced apoptosis [43]. These effects are apparently specific to human tumor cells. Consistent with the above findings, bufalin selectively kills leukemia cells, whereas normal leukocytes remain largely unharmed [66, 67]. Furthermore, cardiac glycosides have also been shown to exhibit selectivity in sensitizing cancer cells to apoptosis during radiation treatment. Large numbers of tumor cells and transformed cells die in response to radiation following ouabain pretreatment, but normal cells do not [63, 65]. These studies support the hypothesis that cardiac glycosides have selective anticancer effects, suggesting that these compounds have potential clinical uses.

This selective killing effect has received attention in the search for the fundamental differences between cancer cells and normal cells that modulate the survival pathway. One attempt to identify such differences demonstrated that the subunit composition of Na^+/K^+ -ATPase is dissimilar in rodent and human cancer cells, affecting the sensitivity to apoptosis induced by cardiac glycosides [68]. The Na^+/K^+ -ATPase consists of two main subunits, the catalytic α subunit and the glycosylated β subunit. It is well known that the α subunit serves as a binding site for cardiac glycosides, Na^+ , K^+ and ATP, whereas the β subunit plays a role in the regulation of heterodimer assembly and insertion into the plasma membrane [69, 70]. Recent data indicate that the $\alpha 1$ and $\alpha 3$ subunits are commonly expressed in human tumor cells, whereas only $\alpha 1$ can be found in rodent tumor cell lines [71,72]. It has also been suggested that the lack of the $\alpha 3$ subunit in rodent cancer cells causes resistance to apoptosis mediated by cardiac glycosides. This finding strengthens the hypothesis that normal cells might have lower $\alpha 3$ subunit expression levels than cancer cells, accounting for the selective anticancer effects of cardiac glycosides. Furthermore, it has been demonstrated that the biological activity of cardiac glycosides results from the binding of these compounds with all α subunits, but the $\alpha 3$ subunit is a favorable target [73]. Ouabain, for example, has a 1000-fold stronger interaction with the $\alpha 3$ isoform than the $\alpha 1$ isoform [74].

Expanding on the above findings, that the expression of the $\alpha 3$ subunit has been shown to increase concurrent with the decrease in $\alpha 1$ subunit expression in human colorectal cancer and colon adenoma cell lines, whereas no significant alteration of $\alpha 3$ subunit expression is detected in normal kidney and renal cells [75]. These results indicate that the overexpression of the $\alpha 3$ subunit is associated with responsiveness to cardiac glycosides. Because all α subunits are commonly expressed at a basal level in cancers, the $\alpha 3/\alpha 1$ ratio might be a marker of cell sensitivity to cardiac glycosides, and this ratio could be determined in tumor biopsy samples taken prior to treatment with cardiac glycosides [76]. A lower $\alpha 3/\alpha 1$ ratio may indicate unresponsiveness to cardiac glycosides; conversely, cardiac glycoside treatment may improve the clinical outcomes of patients who have tumor tissues with higher ratios.

It has been established that the $\alpha 1$ isoform of the Na^+/K^+ -ATPase plays a critical role in the progression of non-small cell lung cancer. The suppression of $\alpha 1$ subunit expression by RNA interference attenuates the invasiveness of cancer, reducing both migration and proliferation [77]. In addition, an increase the $\alpha 1$ subunit level enhances sensitivity to cardiac glycosides. In more than half of glioblastoma samples, the level of Na^+/K^+ -ATPase $\alpha 1$ mRNA was markedly elevated, up to 10 times greater than that in normal samples [78]. Similarly, significant upregulation of the $\alpha 1$ isoform was observed in metastatic melanoma cell lines and melanoma tissue samples [79, 80]. These results indicate that the responsiveness of either cancer cells or normal cells to cardiac glycosides based on the $\alpha 3/\alpha 1$ ratio is tissue specific. It is important to determine the differences in the expression levels of the α subunits between cancer cells and normal cells. Furthermore, the characterization of the specificity of each cardiac glycoside for each enzyme subunit is necessary to identify cancers with the appropriate $\alpha 3/\alpha 1$ expression pattern for treatment and to reduce the effect on normal cells, thus optimizing the effectiveness of cardiac glycosides as potent anticancer drugs.

5. Conclusion

Cancer remains a life-threatening disease that is typically characterized by frequently related to dysregulated cell growth and resistance to apoptosis. Within the past decade, cancer research has provided interesting insights with the potential to define the exact causes of cancer and to aid in the development of anticancer agents with enhanced effectiveness against and selectivity for cancer. Several plant-derived compounds were once used as ingredients of treatments for diseases without any established scientific evidence to support the claimed effects. Later, these compounds were found to exhibit relevant biological activities. Numerous studies have screened medicinal plants for compounds with anticancer activity, including cardiac glycosides. Generally, cardiac glycosides are recognized as antiarrhythmic drugs that function by inhibiting Na^+/K^+ -ATPase. These compounds have been reported to be therapeutically beneficial for the treatment of various tumor types because of their antiproliferative effects, ability to induce apoptosis, and ability to sensitize cells to chemo/radiotherapy-induced cell death.

As already emphasized, cardiac glycosides have a narrow therapeutic index, which could cause serious cardiovascular toxicity. Interestingly, it has been observed that the concentration required to treat cancer was lower than of that used to treat cardiac disorders. Furthermore, cardiac glycosides appear to exert a cancer-specific killing activity by targeting the Na^+/K^+ -ATPase α subunit in tumor cells. However, the expression pattern of the enzyme subunits and the target specificity of cardiac glycosides must be optimized. Synthetic cardiac glycosides have been designed to achieve the desired effects; these compounds include UNBS-1450 [81,82] and D6-MA [38,83]. Although cardiac glycosides have potential effects on cancer, at present, evidence supporting their usefulness is still needed, and the safety profile of cardiac glycosides as anticancer agents must be determined.

Acknowledgements

This work was supported by a Grant for Development of New Faculty Staff, Chulalongkorn University, and the Thailand Research Fund. The author wishes to thank Dr. Yon Rojanasakul and Dr. Pithi Chanvorachote.

Author details

Varisa Pongrakhananon

Cell-based Drug and Health Product Development Research Unit, Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

References

- [1] Mijatovic T, Ingrassia L, Facchini V, Kiss R. Na⁺/K⁺-ATPase alpha subunits as new targets in anticancer therapy. *Expert Opinion on Therapeutic Targets* 2008;12 1403-1417.
- [2] Böhm M. Digoxin in patients with heart failure. *The New England Journal of Medicine*. 1997;337 129-130.
- [3] Stenkvist B. Cardenolides and cancer. *Anticancer Drugs* 2001;12 635-638.
- [4] Stenkvist B. Is digitalis a therapy for breast carcinoma? *Oncology Report* 1999;6 493-496.
- [5] Gupta RS, Chopra A, Stetsko DK. Cellular basis for the species differences in sensitivity to cardiac glycosides (digitalis). *Journal of cellular physiology* 1986;127 197-206.
- [6] López-Lázaro M. Digitoxin as an anticancer agent with selectivity for cancer cells: possible mechanisms involved. *Expert Opinion on Therapeutic Targets* 2007;11 1043-1053.
- [7] Huang YT, Chueh SC, Teng CM, Guh JH. Investigation of ouabain-induced anticancer effect in human androgen-independent prostate cancer PC-3 cells. *Biochemical Pharmacology* 2004;67 727-733.
- [8] Yang P, Menter DG, Cartwright C, Chan D, Dixon S, Suraokar M, Mendoza G, Llansa N, Newman RA. Oleandrin-mediated inhibition of human tumor cell proliferation: importance of Na,K-ATPase alpha subunits as drug targets. *Molecular Cancer Therapeutic* 2009;8 2319-2328.

- [9] Winnicka K, Bielawski K, Bielawska A, Milyk W. Apoptosis-mediated cytotoxicity of ouabain, digoxin and proscillaridin A in the estrogen independent MDA-MB-231 breast cancer cells. *Archives of pharmacal research* 2007;30 1216-1224.
- [10] Jiang Y, Zhang Y, Luan J, Duan H, Zhang F, Yagasaki K, Zhang G. Effects of bufalin on the proliferation of human lung cancer cells and its molecular mechanisms of action. *Cytotechnology* 2010;62 573-83.
- [11] Haux J. Digitoxin is a potential anticancer agent for several types of cancer. *Medical Hypotheses* 1999;53 543-548.
- [12] Mijatovic T, Dufrasne F, Kiss R. Cardiotonic steroids-mediated targeting of the Na⁺/K⁺-ATPase to combat chemoresistant cancers. *Current Medical Chemistry* 2012;19 627-646.
- [13] Newman RA, Yang P, Pawlus AD, Block KI. Cardiac glycosides as novel cancer therapeutic agents. *Molecular Intervention* 2008;8 36-49.
- [14] Prassas I, Diamandis EP. Novel therapeutic applications of cardiac glycosides. *Nature reviews. Drug discovery* 2008;7 926-95.
- [15] Schönfeld W, Weiland J, Lindig C, Masnyk M, Kabat MM, Kurek A, Wicha J, Repke KR. The lead structure in cardiac glycosides is 5a,14a-androstane-3a14-diol. *Naunyn-Schmiedeberg's Archives of Pharmacology* 1985;329 414-426.
- [16] Steyn PS, van Heerden FR. Bufadienolides of plant and animal origin. *Natural Product Reports* 1998;15 397-413.
- [17] Mathews WR, DuCharme DW, Hamlyn JM, Harris DW, Mandel F, Clark MA, Ludens JH. Mass spectral characterization of an endogenous digitalislike factor from human plasma. *Hypertension* 1991;17 930-935.
- [18] Hamlyn JM, Blaustein MP, Bova S, DuCharme DW, Harris DW, Mandel F, Mathews WR, Ludens JH. Identification and characterization of a ouabain-like compound from human plasma. *Proceedings of the National Academy of Sciences of the United States of America* 1991;88 6259-6263.
- [19] Schneider R, Antolovic R, Kost H, Sich B, Kirch U, Tepel M, Zidek W, Schoner W. Proscillaridin A immunoreactivity: its purification, transport in blood by a specific binding protein and its correlation with blood pressure. *Clinical and Experimental Hypertension* 1998;20 593-599.
- [20] Goto A, Yamada K, Ishii M, Sugimoto T. Digitalis-like activity in human plasma: relation to blood pressure and sodium balance. *The American Journal of Medicine* 1990;89 420-426.
- [21] Bagrov AY, Fedorova OV, Dmitrieva RI, Howald WN, Hunter AP, Kuznetsova EA, Shpen VM. Characterization of a urinary bufodienolide Na⁺, K⁺-ATPase inhibitor in patients after acute myocardial infarction. *Hypertension* 1998;31 1097-1103.

- [22] Schoner W, Scheiner-Bobis G. Endogenous and exogenous cardiac glycosides: their roles in hypertension, salt metabolism, and cell growth. *American Journal of Physiology Cell Physiology* 2007;293 C509-36.
- [23] Aronson JK. (1986) An account of the foxglove and its medical uses 1785-1985. Oxford University Press.
- [24] Kaplan JH. Biochemistry of Na,K-ATPase. *Annual Review of Biochemistry* 2002;71 511-35.
- [25] Xie Z, Cai T. Na⁺/K⁺ ATPase-mediated signal transduction: from protein interaction to cellular function. *Molecular Intervention* 2003;3 157-168.
- [26] Haas M, Wang H, Tian J, Xie Z. Src-mediated inter-receptor cross-talk between the Na⁺/K⁺-ATPase and the epidermal growth factor receptor relays the signal from ouabain to mitogen-activated protein kinases. *Journal of Biological Chemistry* 2002;277 18694-702.
- [27] Aperia A. New roles for an old enzyme: Na,K-ATPase emerges as an interesting drug target. *Journal of Internal Medicine* 2007;261 44-52.
- [28] Stenkvist B. Cardiac glycosides and breast cancer. *Lancet* 1979;1 563.
- [29] Stenkvist B: Evidence of a modifying influence of heart glucosides on the development of breast cancer. *Analytical and Quantitative Cytology* 1980;2 49-54.
- [30] Goldin AG, Safa AR: Digitalis and cancer. *Lancet* 1984;1 1134.
- [31] Haux J, Klepp O, Spigset O, Tretli S. Digitoxin medication and cancer; case control and internal dose-response studies. *BMC Cancer* 2001;1 11.
- [32] Hanahan D, Weinberg RA. The Hallmarks of cancer. *Cell* 2000;1 57-70.
- [33] Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. *Nature* 2001;6835 342-348.
- [34] Yang P, Chan D, Vijjeswarapu M, Cartwright C, Cohen L, Meng Z, Liu L, Newman RA. Anti-proliferative activity of Huachansu, a Bufo toad skin extract, against human malignant melanoma cells. *Proceeding of American Association Cancer Research* 2006; 47
- [35] Jiang Y, Zhang Y, Luan J, Duan H, Zhang F, Yagasaki K, Zhang G. Effects of bufalin on the proliferation of human lung cancer cells and its molecular mechanisms of action. *Cytotechnology* 2010;62 573-83.
- [36] Pestell RG, Albanese C, Reutens AT, Segall JE, Lee RJ, Arnold A. The cyclins and cyclin-dependent kinase inhibitors in hormonal regulation of proliferation and differentiation. *Endocrine Review* 1999;20 501-534.
- [37] Baldin V, Lukas J, Marcote MJ, Pagano M, Draetta G. Cyclin D1 is a nuclear protein required for cell cycle progression in G1. *Genes & Development* 1993;7 812-21.

- [38] Elbaz HA, Stueckle TA, Wang HY, O'Doherty GA, Lowry DT, Sargent LM, Wang L, Dinu CZ, Rojanasakul Y. Digitoxin and a synthetic monosaccharide analog inhibit cell viability in lung cancer cells. *Toxicology and Applied Pharmacology* 2012;258 51-60.
- [39] Tian J, Li X, Liang M, Liu L, Xie JX, Ye Q, Kometiani P, Tillekeratne M, Jin R, Xie Z. Changes in sodium pump expression dictate the effects of ouabain on cell growth. *Journal of Biological Chemistry* 2009;284 14921-14929.
- [40] Yeh JY, Huang WJ, Kan SF, Wang PS. Inhibitory effects of digitalis on the proliferation of androgen dependent and independent prostate cancer cells. *Journal of Urology* 2001;166 1937-42.
- [41] Winnicka K, Bielawski K, Bielawska A, Surazyński A. Antiproliferative activity of derivatives of ouabain, digoxin and proscillaridin A in human MCF-7 and MDA-MB-231 breast cancer cells. *Biological and Pharmaceutical Bulletin* 2008;31 1131-1140.
- [42] Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR, Rey S, Hammers H, Chang D, Pili R, Dang CV, Liu JO, Semenza GL. Digoxin and other cardiac glycosides inhibit HIF-1 α synthesis and block tumor growth. *Proceedings of the National Academy of Sciences of the United States of America* 2008;105 19579-19586.
- [43] Sreenivasan Y, Sarkar A, Manna SK. Oleandrin suppresses activation of nuclear transcription factor-kB and activator protein-1 and potentiates apoptosis induced by ceramide. *Biochemical Pharmacology* 2003;66 2223-2239.
- [44] Manna SK, Sah NK, Newman RA, Cisneros A, Aggarwal BB. Oleandrin suppresses activation of nuclear transcription factor-kappaB, activator protein-1, and c-Jun NH2 terminal kinase. *Cancer Research* 2000;60 3838-3847.
- [45] Hashimoto S, Jing Y, Kawazoe N, Masuda Y, Nakajo S, Yoshida T, Kuroiwa Y, Nakaya K. Bufain reduces the level of topoisomerase II in human leukemia cells and affects the cytotoxicity of anticancer drugs. *Leukemia Research* 1997;21 875-883.
- [46] Lavrik IN, Golks A, and Krammer PH. Caspases: pharmacological manipulation of cell death. *The Journal of Clinical Investigation* 2005;115 2665-2672.
- [47] Gan N, Chen G, Zhang W, Zhou J. Oleanen induces apoptosis of cervical cancer cells by up-regulation of Bim. *International Journal of Gynecological Cancer* 2012;22 38-42.
- [48] Toillon RA, Descamps S, Adriaenssens E, Ricort JM, Bernard D, Boilly B, Le Bourhis X. Normal breast epithelial cells induce apoptosis of breast cancer cells via Fas signaling. *Experimental Cell Research* 2002;275 31-43.
- [49] Sreenivasan Y, Raghavendra PB, Manna SK. Oleandrin-mediated expression of Fas potentiates apoptosis in tumor cells. *Journal of Clinical Immunology* 2006;26 308-322.
- [50] Frese S, Frese-Schaper M, Anne-Catherine A, Miescher D, Zumkehr B, Schmid RA. Cardiac glycosides initiate Apo2L/TRAIL induced apoptosis in non-small cell lung

- cancer cells by up-regulation of death receptors 4 and 5. *Cancer Research* 2006;66 6867–5874.
- [51] Chanvorachote P, Pongrakhananon V. Ouabain down-regulates Mcl-1 and sensitizes lung cancer cells to TRAIL-induced apoptosis. *American Journal of Physiology-Cell Physiology* 2012; doi:10.1152/ajpcell.00225.2012.
- [52] Simon HU, Haj-Yehia A, Levi-Schaffer F. Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis* 2000;5 415-418.
- [53] Huang YT, Chueh SC, Teng CM, Guh JH. Investigation of ouabain-induced anticancer effect in human androgen-independent prostate cancer PC-3 cells. *Biochemical Pharmacology* 2004;67 727-733.
- [54] Watabe M, Ito K, Masuda Y, Nakajo S, Nakaya K. Activation of AP-1 is required for bufalin-induced apoptosis in human leukemia U937 cells. *Oncogene* 1998;16 779-787.
- [55] Ameyar M, Wisniewska M, Weitzman JB. A role for AP-1 in apoptosis: the case for and against. *Biochimie* 2003;85 747-52.
- [56] Okamoto K, Fujisawa K, Hasunuma T, Kobata T, Sumida T, Nishioka K. Selective activation of the JNK/AP-1 pathway in Fas-mediated apoptosis of rheumatoid arthritis synoviocytes. *Arthritis & Rheumatism* 1997;40 919-926.
- [57] Wang Z, Zheng M, Li Z, Li R, Jia L, Xiong X, Southall N, Wang S, Xia M, Austin CP, Zheng W, Xie Z, Sun Y. Cardiac glycosides inhibit p53 synthesis by a mechanism relieved by Src or MAPK inhibition. *Cancer Research* 2009;69 6556-6564.
- [58] Liu J, Tian J, Haas M, Shapiro JJ, Askari A, Xie Z. Ouabain interaction with cardiac Na⁺/K⁺-ATPase initiates signal cascades independent of changes in intracellular Na⁺ and Ca²⁺ concentrations. *Journal of Biological Chemistry* 2000; 275 27838–27844.
- [59] Newman RA, Yang P, Hittelman WN, Lu T, Ho DH, Ni D, Chan D, Vijeswarapu M, Cartwright C, Dixon S, Felix E, Addington C. Oleandrin-mediated oxidative stress in human melanoma cells. *Journal of Experimental Therapeutics & Oncology* 2006;5 167-181.
- [60] Felth J, Rickardson L, Rosén J, Wickström M, Fryknäs M, Lindskog M, Bohlin L, Gullbo J. Cytotoxic effects of cardiac glycosides in colon cancer cells, alone and in combination with standard chemotherapeutic drugs. *Journal of Natural Products* 2009;72 1969-1974.
- [61] Kunta JR, Sinko PJ. Intestinal drug transporters: in vivo function and clinical importance. *Current Drug Metabolism* 2004;5 109-24.
- [62] Wang L, Raju U, Milas L, Molkentine D, Zhang Z, Yang P, Cohen L, Meng Z, Liao Z. Huachansu, containing cardiac glycosides, enhances radiosensitivity of human lung cancer cells. *Anticancer Research* 2011;31 2141-2148.

- [63] Verheye-Dua FA, Böhm L. Na⁺, K⁺-ATPase inhibitor, ouabain accentuates irradiation damage in human tumour cell lines. *Radiation Oncology Investigations* 1998;6 109-119.
- [64] Verheye-Dua FA, Böhm L. Influence of ouabain on cell inactivation by irradiation. *Strahlentherapie und Onkologie* 1996;172 156-161.
- [65] Lawrence T.S. Ouabain sensitizes tumor cells but not normal cells to radiation. *International Journal of Radiation Oncology, Biology, Physics* 1998;15 953-958.
- [66] Numazawa S, Honna Y, Yamamoto T, Yoshida T, Kuroiwa YA. cardiogenic steroid bufalin-like factor in human plasma induces leukemia cell differentiation. *Leukemia Research* 1995;19 945-953.
- [67] Zhang L, Nakaya K, Yoshida T, Kuroiwa Y. Induction by bufalin of differentiation of human leukemia cells HL60, U937 and ML1 toward macrophage/monocyte-like cells and its potent synergistic effect on the differentiation of human leukemia cells in combination with other inducers. *Cancer Research* 1992;52 4634-4641.
- [68] Pathak S, Multani AS, Narayan S, Kumar V, Newman RA. Anvirzel, an extract of *Nerium oleander*, induces cell death in human but not murine cancer cells. *Anticancer Drugs* 2000;11 455-63.
- [69] Blanco G. Na,K-ATPase subunit heterogeneity as a mechanism for tissue-specific ion regulation. *Seminar in Nephrology* 2005;25 292-303.
- [70] Mobasher A, Avila J, Cózar-Castellano I, Brownleader MD, Trevan M, Francis MJ, Lamb JF, Martín-Vasallo P. Na/K-ATPase isozyme diversity; comparative biochemistry and physiological implications of novel functional interactions. *Bioscience Report* 2000;20 51-91.
- [71] Lin Y, Dubinsky WP, Ho DH, Felix E, Newman RA. Determinants of human and mouse melanoma cell sensitivities to oleandrin. *Journal of Experimental Therapeutics & Oncology*. 2008;7 195-205.
- [72] Lucchesi PA, Sweadner KJ. Postnatal changes in Na, K-ATPase isoforms expression in rat cardiac ventricle. conservation of biphasic ouabain affinity. *Journal of Biological Chemistry* 1991;266 9327-9331.
- [73] Noel F, Fagoo M, Godfraind T. A comparison of the affinities of rat (Na⁺,K⁺)-ATPase isozymes for cardioactive steroids, role of lactone ring, sugar moiety and KCl concentration. *Biochemical Pharmacology* 1990;40 2611-2616.
- [74] O'Brien WJ, Lingrel JB, Wallick ET. Ouabain binding kinetics of the rat alpha two and alpha 3 isoforms of the sodium-potassium adenosine triphosphate. *Archives of Biochemistry and Biophysics* 1994;310 32-39.

- [75] Sakai H, Suzuki T, Maeda M, Takahashi Y, Horikawa N, Minamimura T, Tsukada K, Takeguchi N. Up-regulation of Na(+),K(+)-ATPase alpha 3-isoform and down-regulation of the alpha1-isoform in human colorectal cancer. *FEBS Letters* 2004;563 151-154.
- [76] Yang P, Menter DG, Cartwright C, Chan D, Dixon S, Suraokar M, Mendoza G, Llansa N, Newman RA. Oleandrin-mediated inhibition of human tumor cell proliferation: importance of Na,K-ATPase alpha subunits as drug targets. *Molecular Cancer Therapeutics* 2009;8 2319-2328.
- [77] Mijatovic T, Roland I, Van Quaquebek, Nilsson EB, Mathieu A, Van Vynckt F, Darro F, Blanco G, Facchini V, Kiss R. The α 1 subunit of the sodium pump could represent a novel target to combat non-small cell lung cancers. *Journal of Pathology* 2007;212 170-179.
- [78] Lefranc F, Mijatovic T, Kondo Y, Sauvage S, Roland I, Debeir O, Krstic D, Vasic V, Gailly P, Kondo S, Blanco G, Kiss R. Targeting the α 1 subunit of the sodium pump to combat glioblastoma cells. *Neurosurgery* 2008;62 211-221.
- [79] Boukerche H, Su ZZ, Kang DC, Fisher PB. Identification and cloning of genes displaying elevated expression as a consequence of metastatic progression in human melanoma cells by rapid subtraction hybridization. *Gene* 2004;343 191-201.
- [80] Mathieu V, Pirker C, Vernier M, Mijatovic T, Berger W, Kiss R. New cardenolides, binders of the sodium pump, could represent interesting chemotherapeutical agents for melanoma treatment. American Association for Cancer Research (AACR) Annual Meeting, April 12-16, San Diego, USA; 2008.
- [81] Juncker T, Cerella C, Teiten MH, Morceau F, Schumacher M, Ghelfi J, Gaascht F, Schnekenburger M, Henry E, Dicato M, Diederich M. UNBS1450, a steroid cardiac glycoside inducing apoptotic cell death in human leukemia cells. *Biochemical Pharmacology* 2011;81 13-23.
- [82] Juncker T, Schumacher M, Dicato M, Diederich M. UNBS1450 from *Calotropis procera* as a regulator of signaling pathways involved in proliferation and cell death. *Biochemical Pharmacology* 2009;78 1-10.
- [83] Elbaz HA, Stueckle TA, Tse W, Rojanasakul Y, Dinu CZ. Digitoxin and its analogs as novel cancer therapeutics. *Experimental Hematology & Oncology* 2012; 1 4.

