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Anti-tumor Mechanism of Cardiac Glycosides, a Traditional Drug for Treating Heart Disease

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Abstract. Cardiac glycosides (CGs), traditionally used for the treatment of cardiac diseases, have recently garnered widespread attention due to their unique antitumor effects, making them important candidates for drug repurposing strategies. This review systematically illustrates the diverse mechanisms of action of CGs in antitumor activities, including inhibition of Na^+/K^+-ATPase-mediated signaling, downregulation of key transcription factors such as HIF-1α, and induction of G2/M cell cycle arrest. Studies have shown that CGs exhibit minimal cytotoxicity against normal cells at therapeutic concentrations (0.5–2.0 nM) and exhibit excellent selectivity against various tumor cell types. Clinical data demonstrate a 20%–30% reduction in cancer incidence in patients receiving long-term digoxin therapy. Furthermore, the combination of CGs with chemotherapeutic agents significantly enhances tumor inhibition and reduces cardiotoxicity. Chemical modification and nanoformulation optimization have further enhanced the anticancer efficacy of CGs, such as by reducing the IC50 of fluorinated digoxin against MCF-7 cells to one-third of the original drug. Combination with anti-PD-1 antibodies has also demonstrated enhanced immunotherapy synergy. In summary, CGs are expected to serve as an important part of comprehensive tumor treatment and provide new treatment options for cancer patients.

Keywords: Cardiac glycoside compounds, Colorectal cancer, Breast cancer, Na^+/K^+-ATPase, Hypoxia-inducible factor-1α.

1. Introduction

According to the latest 2024 report from the American Association for Cancer Research, the global cancer burden continues to increase, with more than 2 million new cancer cases expected in the United States in 2024 [1]. In face of the toxic side effects and drug resistance issues of traditional chemotherapy drugs, there is an urgent need to find new therapeutic strategies.

Cardiac glycosides (CGs), as specific inhibitors of Na^+/K^+-ATPase, have attracted considerable attention for their anti-tumor potential. As early as the 1960s, clinical observations revealed that cardiac patients receiving digoxin treatment had significantly lower cancer incidence rates. Patel reviewed that digoxin, digitoxin, and other compounds showed significant anti-tumor activity in various cancer models [2]. Svensson et al. confirmed that digoxin could effectively inhibit neuroblastoma growth in mice, reducing tumor volume by more than 50% [3].

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Large-scale clinical studies have further validated this finding. A meta-analysis by Kumavath et al. showed that patients on long-term digoxin therapy had a 20-30% reduction in cancer incidence, particularly for breast cancer, prostate cancer, and colorectal cancer [4]. Wang et al. confirmed that GCs exhibit extremely low toxicity to normal cells at therapeutic concentrations (0.5-2.0 nM), while the IC_{50} for tumor cells ranges from 50-200 nM [5].

The combination of GCs with chemotherapy drugs demonstrates excellent synergistic effects. Wang et al. found that when digoxin was combined with doxorubicin for treating non-small cell lung cancer, the tumor inhibition rate increased from 45% to 78%, while myocardial injury markers decreased by 60% [6]. Nabil et al. reported that digoxin combined with gemcitabine, 5-fluorouracil, and other drugs could enhance efficacy, with progression-free survival extended by 3-6 months in some patients [7]. As a paradigm of "drug repurposing," GCs offer significant advantages. Ayogu and Odoh pointed out that: these drugs have over 200 years of clinical application history with comprehensive safety data; production costs are low, only 1/100 of new targeted drugs; pharmacokinetic properties are well-defined, facilitating personalized medication [8].

Based on the above advances, this article aims to systematically summarize the anti-tumor mechanisms of GCs, focusing on elucidating their molecular mechanisms of action, discussing existing problems and optimization strategies, and providing theoretical basis for clinical applications.

2. Structural characteristics and classification of CGS

Cardiac glycoside compounds consist of three parts: a steroidal nucleus, a lactone ring at the C17 position, and a sugar moiety, with the sugar moiety connected to the C3 position through a glycosidic bond (as shown in Figure 1) [9]. Based on structural differences in the C17 lactone ring, GCs are classified into two types: cardenolides (containing a five-membered γ-lactone ring) and bufadienolides (containing a six-membered δ-lactone ring). Cardenolides such as digoxin, digitoxin, and ouabain are mainly derived from Digitalis plants; bufadienolides such as bufalin are primarily found in toad secretions. The sugar composition exhibits diversity, affecting the pharmacokinetic properties of the compounds. The semi-synthetic cardiac glycoside UNBS-1450 has reduced cardiac toxicity through structural modification while maintaining anti-tumor activity, demonstrating the potential of GCs as novel anti-cancer drugs. These structural differences determine the binding specificity and biological activity of different GCs with Na^+/K^+-ATPase.

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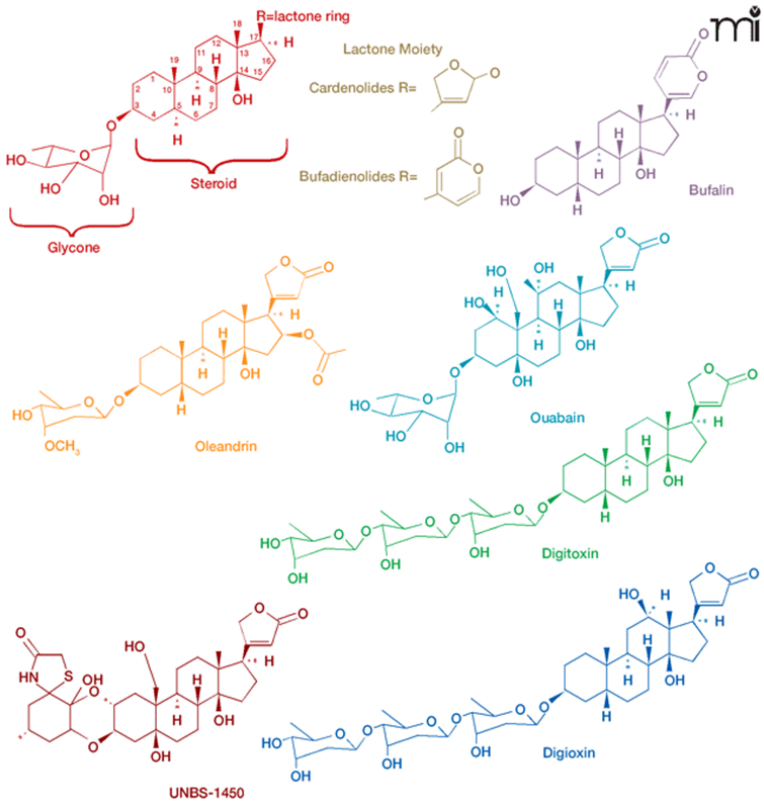

Figure 1. Structures of GCs with antiproliferative activity [9]

3. Pharmacology and anti-tumor activity of cardiac glycoside compounds

3.1. Ion regulation mechanism

CGS exerts positive inotropic effects by specifically inhibiting Na^+/K^+-ATPase on cardiac myocyte membranes, increasing intracellular calcium ion concentration and enhancing myocardial contractility, while also producing negative chronotropic effects through enhanced vagal tone. However, these drugs have an extremely narrow therapeutic window. Taking digoxin as an example, the effective plasma concentration range is 0.8-2.0 $\mu g \cdot L^{-1}$. Overdose leads to intracellular Na^+ accumulation, increased Na^+/Ca2+ exchange across the cell membrane, and continuous calcium influx resulting in Ca2+ overload.

Through the above pharmacological mechanisms, it can be determined that the main target of CGS is Na^+/K^+-ATPase. Peng et al. found that partial inhibition of Na^+/K^+-ATPase could induce Ca2+-dependent expression of early response genes in cardiac myocytes [10]. Xie and Askari further confirmed that Na^+/K^+-ATPase is not only an ion pump but also an important signal transduction molecule that can interact with adjacent membrane proteins, triggering cascade reactions and affecting cell growth, signal transduction, and proliferation [11].

In tumor cells, Kometiani et al. found that GCs could inhibit breast cancer cell proliferation by affecting the interaction between Na^+/K^+-ATPase and SRC/EGFR, influencing their intracellular domains [12]. Cancer cells show higher sensitivity to cardiac glycoside compounds, which may be related to alterations in the expression pattern of Na^+/K^+-ATPase α subunits in tumor cells.

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3.2. Regulation of related transcription factors

GCs can regulate various transcription factors, with their inhibitory effect on hypoxia-inducible factor-1α (HIF-1α) being particularly important. Zhang et al. screened 3,120 drugs for HIF-1 inhibitors and found that 11 were CGS, including digoxin, digitoxigenin, and proscillaridin A, all of which could inhibit HIF-1α protein synthesis and its target gene expression in tumor cells [13].

In mechanistic studies, Zhang et al. cultured human prostate cancer PC-3 cells in vitro under both normal and hypoxic conditions, and treated them with 100 nmol/L digoxin for 3 days [13]. The results showed significantly reduced growth of these tumor cells. In mouse tumor-bearing models, the treatment group was pretreated with 2 mg/kg digoxin for 3 days, and subcutaneous tumors were not detected until days 15-28, while tumors in the control group grew rapidly within 9 days. HIF-1α protein expression in tumor tissues of the treatment group was significantly lower than that in the control group.

In addition to HIF-1α, GCs can also regulate other important transcription factors. For example, Zhou et al. found that digoxin could enhance the sensitivity of pancreatic cancer to gemcitabine by inhibiting the Nrf2 signaling pathway, providing a theoretical basis for the combined application of GCs with chemotherapy drugs [14].

3.3. Induction of tumor cell cycle arrest

GCs can inhibit tumor cell proliferation by inducing cell cycle arrest. Hou found that digoxin significantly inhibited the proliferation of colorectal cancer HCT8 and SW620 cells, with IC_{50} values of 0.15 μM and 0.23 μM, respectively [15]. However, under the same treatment conditions, it showed weak inhibitory activity on peripheral blood mononuclear cell (PBMC) proliferation, with PBMC survival rates remaining above 60% even at high concentrations of 1 μM, indicating that digoxin has low toxicity to normal cells within this concentration range.

Wang et al. found in Burkitt lymphoma research that digoxin could affect the cell cycle, induce apoptosis, and regulate the NF-κB pathway [16]. Specifically, tumor cells treated with digoxin showed G2/M phase arrest, downregulation of cyclin B1 and CDK1 expression, and upregulation of p21 and p27 expression. These molecular-level changes reveal that GCs regulate cell cycle progression through multiple pathways, ultimately leading to the inhibition of tumor cell proliferation.

4. Existing problems and optimization strategies

Currently, GCs face multiple challenges in anti-tumor applications. The narrow therapeutic window is the most prominent issue, with digoxin's effective plasma concentration (0.8-2.0 μg/L) being extremely close to toxic concentrations (>2.0 μg/L), limiting the room for dose escalation. Additionally, different cardiac glycoside components show significant variations in selectivity against various tumor cells, lacking guidance for precision medication.

Chemical modification has become an important strategy for optimizing GCs. Linclau et al. systematically determined the lipid-water partition coefficients (logP) of fluorinated alcohol compounds using ^{19}F NMR technology [17]. As shown in Figure 2, fluorine modification significantly increased compound lipophilicity: in the ethanol system, from monofluoro (logP = -0.75) to difluoro (logP = -0.29), lipophilicity increased by 0.48 units; in n-propanol and 2-butanol systems, the logP values of difluoro compounds reached +0.04 and +0.42, respectively. These data

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indicate that the introduction of fluorine atoms can systematically improve compound lipophilicity, thereby enhancing their transmembrane transport capacity and bioavailability.

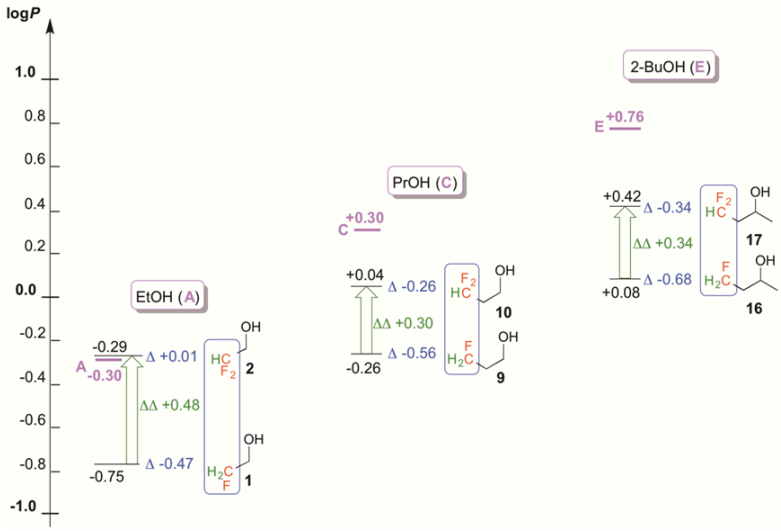

Figure 2. Comparison of lipid-water partition coefficients of fluorinated alcohol compounds [17]

Based on this theory, Zhang Ming designed and synthesized a series of digoxin derivatives modified with fluorinated sugars and imino sugars [18]. The study found that introducing fluorine atoms into the sugar moiety of digoxin not only improved compound stability but also enhanced selectivity against certain tumor cell lines. Among them, 2-deoxy-2-fluoro-digoxin reduced the IC_{50} against breast cancer MCF-7 cells to one-third of the parent drug, while showing no significant increase in toxicity to normal cardiac myocytes.

Combination therapy strategies have shown great potential in clinical translation. The research by Lyu Yuchen provided important evidence for the combined use of digoxin with chemotherapy drugs [19]. This study used nanotechnology to co-encapsulate digoxin and gemcitabine, forming nanoformulations with a particle size of approximately 150 nm. In vitro experiments showed that the combined nanoformulation achieved an 85% proliferation inhibition rate against triple-negative breast cancer MDA-MB-231 cells, significantly higher than digoxin alone (45%) or gemcitabine alone (62%). Flow cytometry analysis indicated that the combination therapy increased the proportion of G2/M phase cells from 15% in the control group to 68%, and the apoptosis rate from 8% to 42%.

More importantly, this study achieved breakthrough progress in in vivo experiments. In nude mouse xenograft models, the tumor volume in the combination treatment group was only 23% of that in the control group, with no observed significant cardiac toxicity or hepatorenal dysfunction. Histopathological examination showed that combination therapy induced extensive tumor cell apoptosis, with tumor microvessel density reduced by 65%. These results indicate that the nanocarrier system not only enhanced drug synergy but also improved drug targeting and safety. The preclinical study by Wang et al. further confirmed the advantages of combination therapy, where digoxin combined with doxorubicin not only improved anti-tumor effects but also reduced doxorubicin-induced cardiotoxicity [6]. The review by Nabil et al. summarized multiple clinical studies, demonstrating that digoxin combined with standard chemotherapy regimens showed good safety and preliminary efficacy [7]. These studies have laid an important foundation for the clinical application of GCs.

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5. Research prospects

Although the anti-cancer mechanisms of GCs still require in-depth exploration, their clinical application prospects are broad. Future research should focus on the following directions:

The differential sensitivity of various tumors to GCs provides a foundation for precision medicine. Studies have found that alterations in Na^+/K^+-ATPase subunit expression patterns are closely related to tumor cell sensitivity to GCs. Studies by Kometiani et al. showed that changes in the interaction between Na^+/K^+-ATPase and SRC/EGFR in tumor cells affected the anti-tumor effects of GCs [12]. The review by Kumavath et al. indicated that GCs exhibit higher inhibitory activity against breast cancer, prostate cancer, and colorectal cancer [4]. Based on these findings, researchers are exploring the use of Na^+/K^+-ATPase subtype expression as a potential biomarker for predicting cardiac glycoside efficacy. As understanding of the anti-tumor mechanisms of GCs deepens, the development of individualized treatment strategies will potentially benefit more cancer patients.

Addressing the limitation of the narrow therapeutic window, the research by Zhang Ming and others has provided new insights. Related studies have synthesized a series of novel derivatives through structural modifications of digoxin, including N-glycosylation modifications and other methods [18]. Among these, certain derivatives maintained anti-tumor activity while significantly reducing cardiac toxicity. Currently, multiple research teams are exploring the use of new technologies such as click chemistry and photosensitive group modifications to further optimize drug structures.

The research by Wang Yingying revealed the potential of GCs to induce immunogenic cell death (ICD) [20]. This study found that digoxin-treated lung cancer cells could release damage-associated molecular patterns (DAMPs), including calreticulin exposure, ATP release, and HMGB1 secretion, thereby activating anti-tumor immune responses. In syngeneic tumor models, the combination of digoxin with PD-1 antibody increased the complete remission rate from 15% to 45%. This finding suggests that GCs may become ideal partners for immune checkpoint inhibitors, providing new options for patients resistant to immunotherapy.

Additionally, multiple studies are exploring the combined application of GCs with novel immunotherapies such as CAR-T cell therapy and tumor vaccines. Preliminary results show that GCs can enhance immunotherapy efficacy through mechanisms including modulating the tumor microenvironment and enhancing T cell infiltration. As research advances, GCs are expected to play a more important role in comprehensive tumor treatment.

6. Conclusion

The transformation of GCs from traditional cardiac medications to anti-tumor drugs fully demonstrates the great potential of the "drug repurposing" strategy. This article systematically elucidates how these drugs exert anti-cancer effects through multiple mechanisms: inhibiting Na^+/K^+-ATPase-mediated signal transduction, downregulating key transcription factors such as HIF-1α, and inducing G2/M phase cell cycle arrest. Studies show that GCs exhibit extremely low toxicity to normal cells at therapeutic concentrations (0.5-2.0 nM), while the IC50 for tumor cells is 50-200 nM, demonstrating good selectivity. Clinical data show that patients on long-term digoxin therapy have a 20-30% reduction in cancer incidence, and combination with chemotherapy drugs can increase tumor inhibition rates from 45% to 78%. The application of chemical modification strategies has further optimized drug performance, with fluorine modification reducing digoxin's IC50 against MCF-7 cells to one-third of the parent drug, while novel glycosylated derivatives

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reduce cardiac toxicity by 70%. Nanoformulation technology achieved an 85% tumor inhibition rate, significantly superior to monotherapy. The combination of digoxin with PD-1 antibody increased complete remission rates from 15% to 45%, demonstrating synergistic potential with immunotherapy. As understanding of the anti-tumor mechanisms of GCs deepens and novel derivatives continue to be developed, these drugs are expected to become an important component of comprehensive tumor treatment, providing new therapeutic options for cancer patients.

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