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Review Article

# Proscillaradin A: From Cardiac Glycosides to Cancer Therapeutics

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**Abstract** | Drug repurposing (finding new uses of existing drugs) has offered a novel strategy for discovering new anticancer drugs from non-cancer drugs. Drug repurposing has attracted the attention of scientific community involved in anticancer drug discovery because it is cost effective and less time consuming compared to the conventional drug discovery strategy involving *de novo* identification, characterization and validation of new bioactive molecules. Proscillaridin A (PSD-A), is a natural cardiac glycoside molecule used to treat cardiac arrhythmia. Recently, it has been rediscovered for its potential anticancer activity. This review will mainly focus on the anticancer activity, cellular targets and anticancer mechanism of PSD-A which may help the further design and conduct of research and repositioning it for oncological clinic.

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## Introduction

Cancer remains the most devastating health problem and represents the second leading cause of death worldwide (Khan *et al.*, 2015). In 2012, 8.2 million people died because of cancer and 17.2 million deaths are predicted to occur by 2050, in the world as per WHO estimates (Khan *et al.*, 2016). Despite recent advancements in technologies and huge investment for developing anticancer drugs through conventional methods involving *de novo* identification, characterization, and validation of novel anticancer drugs, the number of new drugs made available in public domain has not significantly increased in the last decades. The conventional drug development process is not only costly but also time consuming.

The average time required to develop a drug through conventional method in United State and Europe was 9.7 years in 1990s, which has been increased to 13.7 years from 2000 onward (Shim *et al.*, 2014). These hurdles in developing new drugs could be overcome by shifting from conventional method to drug repurposing strategy.

Sildenafil (Viagra) and thalidomide are the two well known examples of drug repurposing. Viagra (inhibitor cGMP -specific phosphodiesterase type 5) was originally formulated for treating coronary artery disease in 1980. The penile erection function of Viagra was discovered accidently as a side effect during phase-1 clinical trials for patients suffering from hypertension and angina. After dropping in phase-II clinical trials, viagra was redirected to treat erectile dysfunction and ultimately approved by US-FDA in 1998 for the treatment of erectile dysfunctions (Shim *et al.*, 2014). Thalidomide was originally synthesized in 1954 and developed to reduce morning sickness in pregnant women by a German company in 1957 under the name Contergan. Soon after it was launched into market, it was found to induce severe birth defects in children and ultimately was dropped from clinic (McBride, 1977; Shim *et al.*, 2014). Later on it was rediscovered for its potential use in the treatment of cancer by various research groups (D'Amato *et al.*, 1994; Ning *et al.*, 2010; Singhal *et al.*, 1999) and finally approved by FDA for the treatment of multiple myeloma in combination with dexamethasone in 2006 (Shim *et al.*, 2014).

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Cardiac glycosides are natural cardiotonic steroids compounds which have a long history of use in the treatment of heart ailments such as cardiac arrhythmia and cardiac congestion. The cardiotonic effect of cardiac glycosides is triggered by their anti-$Na^+/K^+$ ATPase pump activity. Although originally prescribed for cardiac failure, the anticancer activity of cardiac glycosides has been recently rediscovered (Maryam *et al.,* 2018). A large body of literature evidence showed that cardiac glycosides exhibit remarkable cytotoxicity against multiple human cancers at extremely low concentrations through multiple mechanisms (Winnicka et al., 2007; Denicolai et al., 2014; Berges *et al.,* 2018; He *et al.,* 2018; Li *et al.*, 2018; Maryam *et al.,* 2018). Collective data from various research reports highlight the anticancer scope of cardiac glycoside and strongly support their potential use in cancer clinic. PSD-A is a cardiac glycoside isolated from *Urginea maritima* (Maryam *et al.,* 2018). The anticancer activity of PSD-A has recently been evaluated which generated a promising data. This review will discuss the anticancer activity, cellular targets and anticancer mechanism of PSD-A which would be helpful in design and conduct of preclinical and clinical trials for developing this promising anticancer cardiac glycoside into anticancer drug.

*Anticancer Mechanism of PSD-A*

The published data demonstrate that PSD-A is a potent antineoplastic agent that inhibits cancer cell proliferation and induces cell death by activating apoptosis via interacting with a wide range of cellular targets and signaling pathways that are vital for cancer development and progression. The cellular targets and anticancer mechanism of PSD-A in various cancers have been shown in Figure 1.

*Induction of Apoptosis*

Apoptosis is highly programmed mode of cell death which is initiated by various intracellular as well as extracellular stimuli and play vital role in tissue homeostasis (Khan *et al.,* 2015). Cancer cells being the most advised cells with evolutionary point of view, have evolved several cellular mechanisms to inhibit apoptosis which ultimately results in cancer progression and drug resistance (Khan *et al.,* 2015; Khan and Ma, 2017). Activation of apoptosis in cancer cells is considered a novel approach to effectively decommission the cancerous cells. It is well established now that induction of apoptosis takes place through two main pathways: the mitochondrial pathway (intrinsic pathway) and extrinsic pathway. Apoptosis is characterized by several hallmarks such as DNA fragmentation, phosphatidylserine (PS) exposure on the outer leaflet of plasma membrane, caspases (Cystein-aspartic proteases) activation and cleavage of PARP [poly (ADP ribose) polymerase] (Khan *et al.,* 2016).

PSD-A has been shown to induce both intrinsic and extrinsic apoptosis in several human cancers including lung cancer, breast cancer, prostate cancer and glioblastoma through multiple mechanisms. We (Maryam *et al.,* 2018) and Li *et al.* (2018) very recently reported that PSD-A is a potent anticancer compound and induces intrinsic apoptosis in A549, H1650 and H1975 non-small cell lung carcinoma cell lines significantly in a dose-dependent fashion at a concentration range of 12.5-100nM. We have further shown that cytotoxic effect of PSD-A was relatively low on normal lung cells. The underlying mechanism involved increase in intracellular $Ca^{++}$ release, increased Bax/Bcl-2 ratio, JNK activation, inhibition of survivin (Inhibitor of apoptosis), caspases (Caspase-9, -7, and -3) activation, suppressive effect on STAT3 activation and PARP cleavage. We have further shown that PSD-A could effectively induce oxidative stress as evident from increased intracellular ROS level, and reduction in reduced glutathione (GSH) and thioredoxin reductase 1 (TRXR1) and endoplasmic reticulum (ER) stress as depicted from activation of JNK and p38 MAPK activation, increased phosphorylation of eIF2α and upregulation of CHOP and ATF4. Both these events play vital role in induction of intrinsic apoptosis. In addition to intrinsic apoptosis, Li *et al.* (2018) have further provided evidence of extrinsic apoptosis in A549 and H1975 cells by PSD-A through up-regulation of death receptor 4 (DR4). We have further extended our study and investigated the anticancer effects of PSD-A on prostate cancer cells using androgen-dependent (LNCaP) and androgen-independent (DU145) prostate cancer cells (He *et al.,* 2018). Significant induction of apoptosis was observed in LNCaP cells at a concentration range of 25-50nM, however; DU145 cells at these concentrations were found insensitive. The major events associated with PSD-A mediated apoptosis in prostate cancer cells were disruption of mitochondrial membrane potential, higher Bax/Bcl-2 ratio, inhibition of STAT3 phosphorylation, caspase-3 activation and PARP cleavage. Moreover, PSD-A effectively augmented the efficacy of clinical drug doxorubicin in prostate cancer cells. Further studies have shown that PSD-A is able to effectively induce apoptosis in estrogen-dependent MCF-7 (Bielawski *et al.,* 2006) and estrogen-independent MDA-MB-231 (Winnicka *et al.,* 2007; Winnicka *et al.,* 2008) breast cancer cells. The collective data from these studies showed that intracellular $Ca^{++}$ release, caspase-3 activation (MDA-MB-231) and topoisomerase-I and –II inhibition are the major events associated with induction of apoptosis in breast cancer cells. Another study by Denicolai *et al.* (2014) demonstrated that apoptotic potential of PSD-A in U87-MG, U251-MG, GBM6 and GBM9 glioblastoma cell lines. PSD-A inhibited $Na^+/K^+$ ATPase pump, induced apoptosis, arrested cell cycle at G2/M phase and impaired cell renewal ability of GBM stem cells. Another study by same group investigated the anticancer mechanism of PSD-A in glioblastoma cells using 2D and 3D culture (Berges *et al.,* 2018). The data depict-

ed that PSD-A induced cytotoxicity in tumor and stem like cells and impaired metastatic capacity of tumor cells by activating GSK3β and inducing microtubule dynamics alterations. It is worth mentioning that PSD-A induced cytotoxicity in tumor cells while sparing normal astrocytes and oligodendrocytes on the same concentrations.

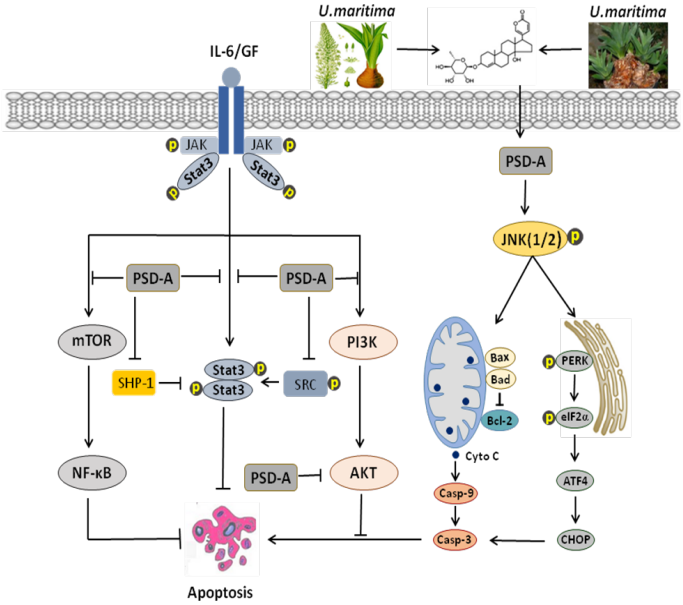


**Figure 1: A schematic presentation of cellular targets and anticancer mechanism of PSD-A.**

*Effect on Pro-Survival Signaling Pathways*

The normal cellular homoeostasis of a cell is regulated by a precise balance between pro-survival and pro-death signaling pathways (Portt *et al.*, 2011). Disruption of this balance results in severe pathological conditions. Pro-survival signaling pathways play vital role in cell proliferation, growth and cell survival. Constitutive activation of survival pathways leads to cancer initiation, progression and drug resistance. Cancer cells protect themselves from apoptosis by activating survival pathways (Flusberg and Sorger, 2015; Khan *et al.*, 2016). Targeting multiple survival pathways is rational approach to inhibit uncontrolled cell division and trigger apoptosis in cancer cells. Recent research reports suggested that PSD-A is a potential inhibitor of various survival pathways such as STAT3, PI3K/AKT/mTOR, and NF-κB pathways by interfering at multiple cellular levels.

*PSD-A as Inhibitor of STAT3 Signaling Pathway*

STAT3 signaling pathway constitutively overexpressed in a wide range of human cancers and implicated in various cellular processes such as cell proliferation, cell survival, angiogenesis, invasion, metastasis and chemo-resistance (Wu *et al.*, 2013; Khan et al., 2015). We have recently reported that PSD-A could act as a potent STAT3 inhibitor at a concentration range of 25-50nM. Using A549 lung adenocarcinoma and LNCaP and DU145 prostate cancer cell lines, we found that PSD-A inhibited both constitutive and inducible-STAT3 signaling through multiple mechanisms including inhibitory effects on JAK2 and Src activation, up-regulation of SHP-1 and direct binding of PSD-A with SH2 domain of STAT3.

*PSD-A as Inhibitor of PI3K/AKT/mTOR Signaling Pathway*

The PI3K/AKT/mTOR signaling pathway is a complex signaling pathways comprised of several bifurcating and converging cascades and is frequently overexpressed in multiple cancers. The aberrant activation of this pathway has been associated with several hallmarks of cancers including cell survival, proliferation, metabolism, and cancer progression. As this signaling pathway is composed of a large number of interconnected kinase cascades, it is considered target-rich pathway for chemotherapy (Ersahin *et al.*, 2015; Guo *et al.*, 2015). Very recently, Li *et al.* (2018) explored the effect of PSD-A on PI3K/AKT/mTOR signaling pathway using lung cancer cells. They found that PSD-A significantly inhibited phosphorylation of AKT and mTOR without affecting the total protein at a concentration range of 3-25nM. These *in vitro* effects were further validated using *in vivo* animal mouse model in the same study.

*PSD-A as Inhibitor of NF-κB Signaling Pathway*

NF-κB is a transcriptional factor involved in various cellular processes such as innate immunity, inflammation, and cancer development and progression. In mammalian cells five members of NF-κB family has been identified to date i.e. p65 (Rel A), Rel B, c-Rel, NF-κB1 (p105/50) and NF- κB2 (p100/52). Unlike Rel A, Rel B and c-Rel, NF-κB1and NF-κB2 are initially synthesized as pro-form (p105 & p100) and finally proteolytic cleavage of pro-form results in mature p50 and p52 (Tilbarghs *et al.*, 2017; Mehmood *et al.*, 2017). NF-κB family members can make various homodimer and heterodimer. P65/p50 is the most common heterodimer. In non-proliferating cells, these dimers are kept bound by inhibitors of NF-κB (IκB) and exist in inactive form in cytosol. Upon ligand binding such as TNFα, IκB kinase (IKK) induces phosphorylation of IκB which results in dissociation of IκB from dimer. After getting free from IκB, NF-κB dimer is translocated into nucleus where it controls transcription of various genes (Mehmood *et al.*, 2017; Tilbarghs et al., 2017). The effect of PSD-A on NF-κB pathway has been investigated recently in lung cancer cells. The data showed that PSD-A inhibited IKKβ, IκBα and p65 in A549 and H1975 cells.

*In vivo Anti-tumor Activity of PSD-A*

Aside from remarkable *in vitro* anticancer activity of PSD-A in various cancer cell lines, *in vivo* anti-tumor activity of PSD-A has also been explored in two different animal mouse models. In one study, the efficacy of PSD-A has been investigated in lung cancer xenograft model. Intraperitonial injection of 3mg/kg b.w. for 21 days

significantly suppressed the tumor growth. No apparent cytotoxicity has been observed in mice. Immunoblotting analysis of tumor tissues showed that PSD-A significantly inhibited phosphorylation of EGFR, mTOR, AKT and increased the expression of DR4. Further immunohistochemical analysis of tumor tissues demonstrated that PSD-A reduced the expression of proliferation marker Ki-67.

The anti-tumor activity of PSD-A has also been investigated in heterotropic and orthotropic xenotransplantation mouse models by Denicolai *et al*. (2014). Intraperitonial injection of 5mg/kg for 21 days significantly reduced tumor volume in both heterotropic and orthotropic gliblastoma mouse models. Immunostaining analysis of tumor tissues obtained from both models indicated that PSD-A significantly suppressed the expression of proliferation marker Ki-67. Taken together, the data revealed that PSD-A is a potent tumor suppressor both *in vitro* and *in vivo*.

## Concluding Remarks

This review highlights the therapeutic potential of PSD-A in cancer therapy and will help deciphering the antitumor mechanism of this cardiac glycoside in different human cancers. Collective data generated from multifarious *in vitro* and *in vivo* studies has provided convincing evidence for conducting pre-clinical and clinical trials for developing PSD-A into a promising anticancer drug. It is worth mentioning here that PSD-A effectively induces apoptosis in cancer cells at extremely low concentrations (nM) while less toxic to the normal cells at the same concentrations. This selective toxicity of PSD-A would be advantageous over existing chemotherapy.

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