Physcion and Physcion 8-*O*-*β*-D-glucopyranoside: Natural Anthraquinones with Potential Anti-cancer Activities

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Abstract

Nature has provided prodigious reservoirs of pharmacologically active compounds for drug development since times. Physcion and physcion 8-*O*-*β*-D-glucopyranoside (PG) are bioactive natural anthraquinones which exert anti-inflammatory and anti-cancer properties with minimum or no adverse effects. Moreover, physcion also exhibits anti-microbial and hepatoprotective properties while PG is known to have anti-sepsis as well as ameliorative activities against dementia. This review aims to highlight the natural sources and anti-cancer activities of physcion and PG along with associated mechanisms of actions. On the basis of the literature, physcion and PG regulate multitudinous cell signaling pathways through the modulation of various regulators of cell cycle, protein kinases, microRNAs, transcriptional factors, and apoptosis linked proteins resulting in the effective killing of cancerous cells *in vitro* as well as *in vivo*. Both compounds effectively suppress metastasis, furthermore, physcion acts as inhibitor of 6PGD and also play an important role in chemosensitization. This review article suggests that physcion and PG are potent anti-cancer drug candidates but further investigations on their mechanism of action and pre-clinical trials are mandatory in order to comprehend the full potential of these natural cancer killers in anti-cancer remedies.

Keywords: Physcion, physcion 8-*O*-*β*-D-glucopyranoside, natural products, anthraquinone, anticancer

1. Introduction

Natural products (NPs) are biologically active chemical substances produced by living organisms having molecular weight lower than 3000 Da [1]. NPs have been proven to be valuable source for drug development [2-3]. They can attain the same level of effectiveness as synthetic drugs, are safer to use for prolonged period of time and are reported to have fewer side effects [4-5]. The earliest records of plant-based medicinal system date back to 2600 BC, which reports the use of around 1000 plant-derived drugs in Mesopotamia. The Ebers Papyrus also records the utilization of more than 700 drugs, majority of which belongs to plants. The Chinese Materia Medica and Indian Ayurveda system have been reported extensively over centuries for their medicinal values [6]. Historically, all the medicinal preparations were acquired through NPs. Recently natural products have provided number of lead compounds for development of anti-microbial and anti-cancer agents. From 1981-2010, about 34% of the drugs sanctioned by the FDA were either natural products or their derivatives [7].

Natural products play vital role in the discovery of new anti-cancer agents. Most of the natural anti-cancer agents have a wide spectrum of molecular targets as compared to the mono-targeted synthetic anti-cancer drugs [8]. Almost 50% of the approved anti-cancer drugs between 1940-2014 are either directly acquired from natural sources or their synthetic derivatives [9]. Vincristine, vinblastine, etoposide phosphate, vinflunine, paclitaxel, homoharringtonine, vincristine sulfate, and topotecan are some examples of anti-cancer drugs from plant origin [10-12] while anthracyclines, bleomycin, dactinomicine and mitomycin are microbial-derived anti-cancer agents [13]. Citarabine, aplidine, bryostatin-1, ET-743, and dolastatin 10 are examples of anti-cancer agents obtained from marine sources [14-15], all of which highlights the immense significance of NPs in drug discovery.

Nature derived phenolic compounds such as flavonoids, carotenoids and tannins are bioactive entities with versatile pharmacological properties [16-17]. Anthraquinones are the derivatives of 1,8-dihydroxyanthrone which belongs to the class of phenolic compounds [18]. Anthraquinone enriched plants like rhubarb have been utilized in folk medicine for centuries. Anthraquinone derivatives exhibit several pharmacological activities such as antifungal, anti-viral, anti-bacterial, anti-oxidant, anti-platelet, and anti-cancer. They also have the capability to cure multiple sclerosis and malaria [19-21].

To date, no review is available on the anti-cancer potential of natural anthraquinones, physcion and physcion 8-*O*-*β*-D-glucopyranoside (PG). This review aims to summarize the studies relevant to the pharmacological efficacy of physcion and PG against different cancer types. It is speculated that the accumulated data will pave a path for researchers towards the development of these natural compounds into pharmacological drugs in the future. The literature was collected through various e-sites such as Elsevier Science Direct, Springer Link, Scopus, PubMed, and other medical linked journals. Keywords used for searching were "Natural products", "Physcion", "Physcion 8-*O*-*β*-D-glucopyranoside (PG)", "Anti-cancer activity" and "Antitumor effect".

2. Natural sources of physcion and PG

Physcion has been isolated from *Rheum tanguticum* [22], *Rheum palmatum* [23] or Radix et Rhizoma Rhei [24-26], *Rheum emodi* [27], *Rhamnus sphaerosperma* var. *pubescens* [28], *Polygonum multiflorum* [29], *Frangula rupestris* [21], *Muehlenbeckia hastulata* [30], *Reynoutria elliptica* [31], *Anethum sowa* [32], *Vismia rubescens* [33], *Ventilago madraspatana* Gaertn. [34], *Cassia tora* [35], *Cassia alata* [36], *Cassia javanica, Cassia accutifolia, Cassia biflora, Cassia italic, Cassia spectabilis, Cassia sophera, Cassia renigera* [37], *Rumex crispus* [38], *Osmunda japonica* [39], marine fungus *Microsporum* sp. [19, 29, 40], and sponge associated fungus *Eurotium cristatum* [41]. Along with many important herbs like *Vitis vinifera*, *Plantagines lanceolatae* and *Rhizoma graminis*, physcion is also found in many vegetables such as beans, cabbage lettuce and peas [42] (Table 1) (Figure 1).

Table 1. Natural sources of physcion and their parts used

Name of Plants		Parts used	References
Scientific Name	**Common Name**		
Anethum sowa	Dill	Roots	[32]
Polygonum multiflorum	He shou wu, Tuber fleece flower	Dried roots	[29]
Rheum emodi	Revand-chini, Himalayan rhubarb	Rhizome	[27]
Rheum palmatum	Turkey rhubarb, Chinese rhubarb	Rhizome	[23-26]
Rheum tanguticum	Rhubarb	Rhizome	[22]
Rheum dentatus	Toothed dock	Roots	[43]
Ventilago madraspatana	Red creeper	Stem bark	[34]
Muehlenbeckia hastulata	Wirevine	Leaves	[30]
Rhamnus sphaerosperma var. *pubescens*	Fruto-de-pombo, Arracacho, Cangica	Dried stem	[28]
Cassia javanica	Pink shower	Leaves	[37]

Cassia tora	Sickle Senna	Seeds	[35, 37]
Cassia alata	Candle bush	Roots	[36-37]
Rumex crispus	Curly dock	---	[38]
Osmunda japonica	Asian royal fern	---	[39]
Frangula rupestris	---	Stem bark	[21]
Vismia rubescens	---	Stem bark	[33]
Eurotium cristatum	---	---	[41]
Reynoutria elliptica	Asian knotweed	---	[31]
Microsporum sp	---	---	[19, 29, 40]
Vitis vinifera	Grape vine	Leaves	[42]
Latuca sativa var. capitate	Cabbage lettuce	Leaves	[42]

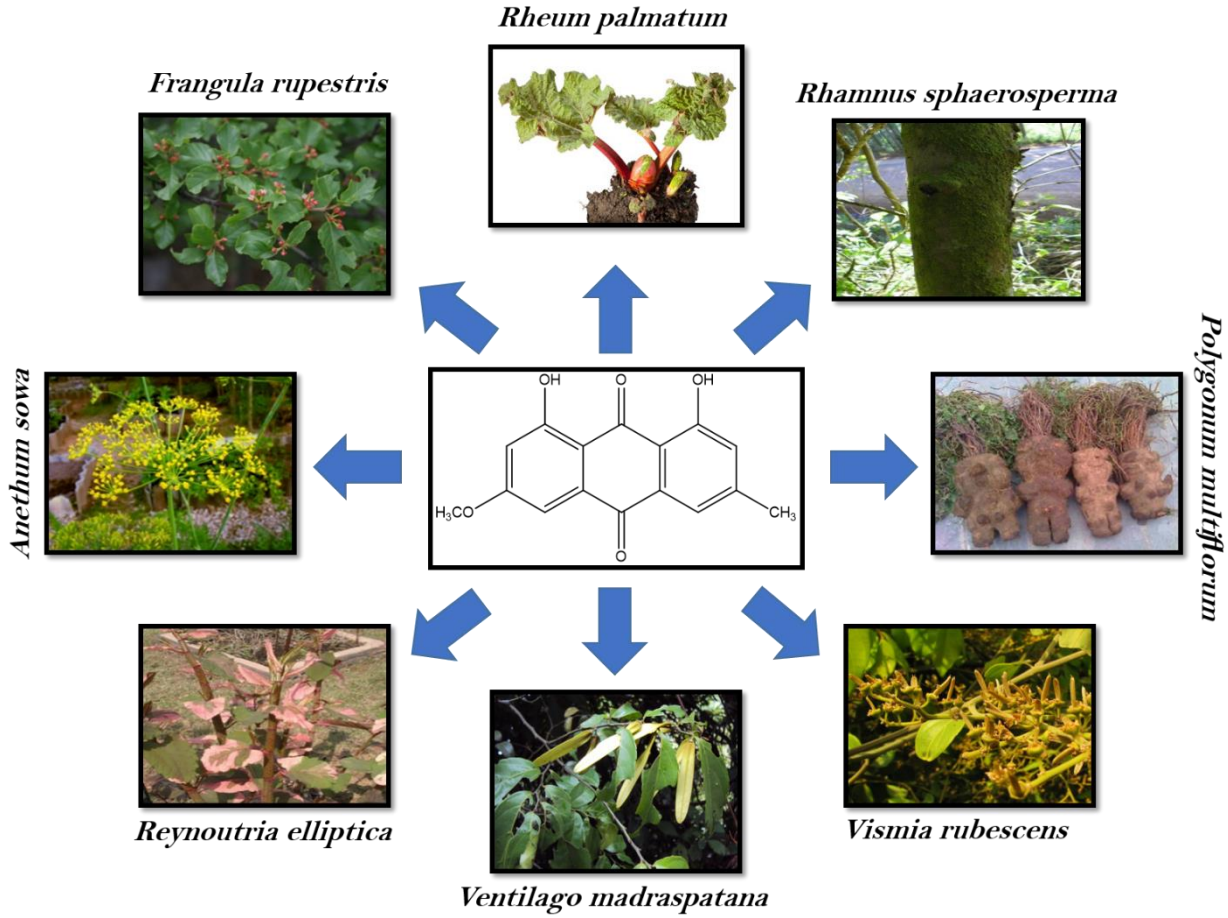

Figure 1. Natural sources and chemical structure of physcion

PG is a biologically active component of *Rheum palmatum[44], Rheum officinale*, *Polygonum multiflorum, Polygonum reynoutria* [45], *Polygonum cuspidatum* [46], *Reynoutria sachalinensis* [47], *Fallopia sachalinensis* [48] and *Rumex japonicus* Houtt (Figure 2) [49-52].

Table 2. Natural sources of PG and their parts used

Name of Plants		Parts used	References
Scientific Name	**Common Name**		
Rheum palmatum	Turkey rhubarb, Chinese rhubarb	Roots	[44]
Rheum officinale	Chinese rhubarb, Indian rhubarb	Roots	[45]
Polygonum multiflorum	He shou wu, Tuber fleece flower	Roots	[45]
Polygonum reynoutria	Japanese knotweed	Roots	[45]
Polygonum cuspidatum	Peashooter plant, Huzhang	Roots	[46]
Reynoutria sachalinensis	Giant knotweed	Flowers	[47]
Fallopia sachalinensis	Giant knotweed	Rhizome	[48]
Cassia obtusifolia	Sicklepod	Seeds	[37]
Cassia occidentalis	Coffee senna	Seeds	[37]
Rumex japonicus Houtt	Goat-hoof	Whole plant	[49-52]

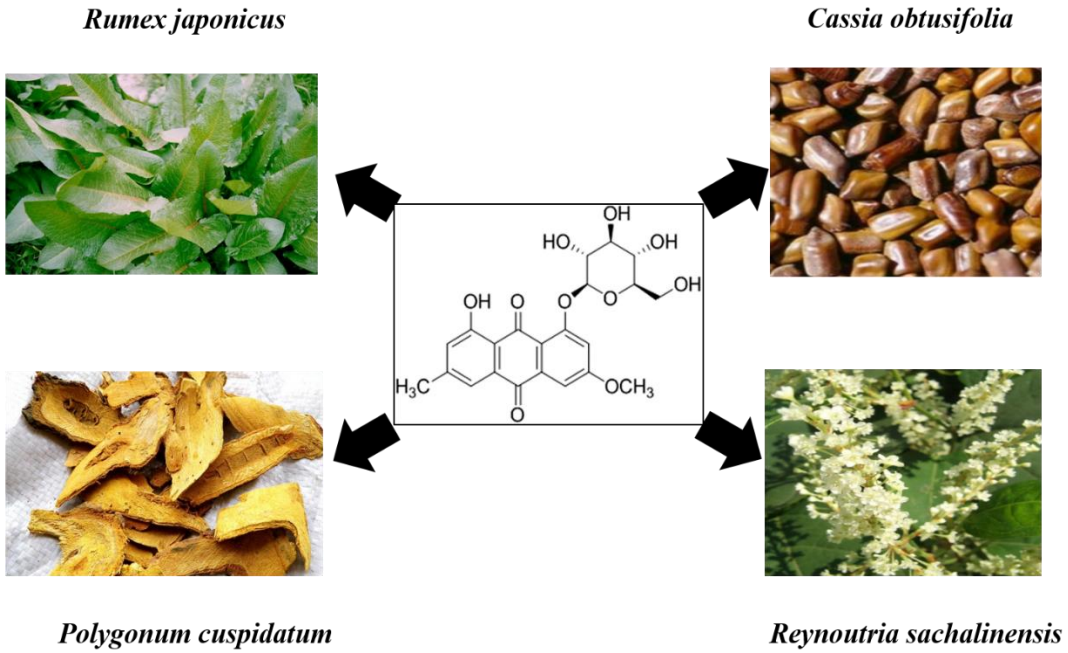

Figure 2. Natural sources and chemical structure of PG

3. Biological activities of physcion and PG

The biologically and pharmacologically active anthraquinones, physcion and PG have been well-known for their potential anti-cancer [19, 22-23, 28, 49, 51] and anti-inflammatory activities [34, 53]. Moreover, physcion also shows anti-microbial [33] and hepatoprotective properties [54] while PG displays ameliorative effects against dementia [46] and have anti-sepsis properties [55]. This review spotlights on the mechanism by which both these compounds act to combat cancer progression.

3.1 Anti-cancer activity

Cancer is a multifaceted malady encompassing genetic, signaling and metabolic aberrations that remarkably disturb cellular homeostasis [56]. It is the 2nd leading cause of mortalities worldwide with approximately 9.6 million cancer-associated deaths reported in 2018 [57]. Currently available treatments for cancer are based on chemotherapeutic drugs which have limited clinical effectiveness due to high toxicity and poor selectivity which arouse the ultimate demand for safer, reliable and cheap anti-cancer drugs [58]. NPs have been utilized since ancient times for the management of several ailments including cancer. Therapeutic potential of NPs against cancer is associated with prohibiting angiogenesis, halting metastasis, inducing cell cycle arrest, activating tumor suppressor genes as well as regulating cell signaling pathways [59-61]. Chemoprevention by naturally occurring entities specifically phytochemicals has turned up as auspicious and pragmatic strategy to decrease the risk of cancer and this area of research is gaining attention [62]. These natural bioactive entities are multi-targeted having promising competency to halt cancer growth and development as compared to monotarget-synthetic drugs, [63].

Anthraquinones comprise a large class of natural bioactive compounds with their broad spectrum of applications in the biological systems [18]. Approximately 700 varied anthraquinone derivatives have been reported, out of which 200 belongs to medicinal plants. Anthraquinone monomers like rhein, emodin, chrysophanol, physcion, hypericin, and aloe-emodin are gaining attentiveness due to their excellent medicinal competency against pathological conditions such as cancer. The anti-cancer properties of many anthraquinones are influenced by the substituents present on anthraquinone ring. For single anthraquinone, the position of OH-group (C-8 and -5) may play a vital role in its anti-tumor activity and hydroxylation at C-1 position can subsequently increase its cytotoxicity suggesting that the phenolic OH-groups contribute towards

the anti-cancer potential of anthraquinones [20]. Anthraquinones including physcion and PG exert their anti-cancer effects by suppressing the growth of cancerous cells, reprograming cancer cell metabolism, inhibiting metastasis, triggering apoptosis, inducing cell cycle arrest, regulating various signaling pathways, and improving efficacy of chemo drugs in drug resistance cancer cells [18, 20, 64].

3.1.1 Induction of cell cycle arrest

Cell cycle progression from one phase towards the next is predominantly regulated by cyclins and cyclin-dependent kinases (CDKs). The amount of CDKs throughout the cell cycle remains relatively stable, whereas cyclins' concentration rises or falls during different stages of the cell cycle [65]. Cyclin H binds to CDK7 which serves as a CDK activating kinase. Cyclin D1/D2/D3 bind to CDK4 and 6 and this CDK-cyclin D network is indispensable for entrance into the G1 phase. Cyclin E binds to CDK2 and controls advancement from G1 into S-phase. For S-phase, Cyclin A-CDK2 complex is essential. Cyclin A binds with CDK1 during late G2 and initial mitotic phase to promote entry into it. Cyclin B in complex with CDK1 further regulates mitosis [66].

Physcion and PG have been reported to halt cell cycle at G0/G1 and G2/M phases in many cancer cells. Physcion arrested HCT116 cells at G0/G1 phase via downregulating cyclin D1 and E [67]. Physcion treatment led to the reduction in cyclin A/D/E, cyclin-dependent kinases 2 and 4, PCNA, elevation of p21 and suppression of c-Myc and p-Rb, ultimately arresting the cell cycle at G0/G1 in MDA-MB-231 breast cancerous cells [22]. SGC-7901 cells treated with physcion reduced cyclin A and B1 expression causing cell cycle arrest at G2/M phase [68]. Physcion led CNE2 cells to G1 phase arrest via enhanced p21/27 expression and reduced cyclin D1 and cyclin E expression [26]. It repressed HOXA5 (Homeobox A5) expression which resulted in cyclin D1 reduction and cell cycle arrest at G1 phase in NALM6 and SUPB15 cells [25]. Physcion up-regulated the level of p21 in HeLa cells which ultimately leads to cell cycle arrest [19].

In NALM6 and SupB15 cells, PG caused cell cycle arrest at G0/G1 phase due to SphK1 suppression and downregulating cyclin A and cyclin D1 while upregulating p27 and p21 expression [69]. G1 phase arrest in HepG2 cell line by PG was linked with a decrease in the level of cyclin D1 and cyclin E and elevated p21 and p27 expression [70]. PG possesses the capacity to halt cell cycle at G1 phase in glioblastoma U87 and U251 cells [51]. PG significantly lowered

the level of cyclin D1, CDK4 and CDK6 eventually arresting cells at G1/G0 phase in A549 and H358 cells [71]. PG arrested A549 lung cancer cells at G2/M phase by decreasing the levels of cyclin B1 and cdc2 [72] while in case of renal cancer cells by decreasing cyclin B1 and increasing CDK2 level [73]. Although physcion and PG has been reported to halt cell cycle at G2/M or G0/G1 phase, however, whether at G2, M, G0 or G1 needs to be investigated. Furthermore, whether physcion and PG directly targets p21 or via its upstream mediator, p53, also needs to be studied in future investigations.

3.1.2 Induction of apoptosis

In response to various stimuli, cells undergo a programmed death or apoptosis [74]. Apoptosis is designated by distinctive biochemical and morphological alterations involving chromatin condensation, shrinkage of nucleus, plasma membrane blebbing, and oligonucleosomal DNA fragmentation [75-76]. A number of pathophysiological and physiological stimuli can induce apoptosis like TNF-α (tumor necrosis factor-α) [77], CD95 (cluster of differentiation 95) [78], UV radiations [79], oxidative stress [80], ceramide treatment [81], growth factors [82], bacteria [83] as well as cytotoxic drugs [84-86].

Two significant pathways are mainly involved in the activation of apoptosis, classified as extrinsic and intrinsic pathways [76]. These pathways stimulate activation of pioneer caspases (caspase-8, -9, and -12) that in turn activate executioner caspases which cleave target proteins leading to the cell death [87-88]. Physcion has been documented to trigger apoptosis in different cancer cell lines including MDA-MB-231 [22], HeLa [19], HCT116 [67], SGC-7901 [68], NALM6, SUPB15 [25], Huh7, Bel7402 [40], SMMC7721, Hep3B [23], and in CNE2 cells [26] (Table 2a). PG has been documented to stimulate apoptosis in several cancerous cells such as MG-63 [52], NALM6, SupB15 [69], SMMC7721, HepG2 [24], SMMC7721, BEL7402 [89], H358, A549 [58, [72], RCC4, ACHN [73], and in KB cells [90] (Table 2b).

3.1.2.1 Intrinsic apoptotic pathway

Mitochondria plays key role in induction of apoptosis through modulation of Bcl-2 (B-cell lymphoma 2) family members [91]. The role of Bcl-2 family is significant in apoptosis as it contains pro-apoptotic and anti-apoptotic proteins. The ratio of Bcl-2 to Bax (Bcl-2-associated x protein) has a critical role in determining the fate of cell [92]. Apoptosis resistance and tumor cell survival are correlated in many cancers due to an increased proportion of anti- to pro-apoptotic Bcl-2 proteins [93]. Controlling the discharge of cytosolic cytochrome c from

mitochondria has been the primary mechanism of Bcl-2 family members for apoptosis regulation. Elevated expression of anti-apoptotic proteins forbids the loss of cytochrome c while pro-apoptotic proteins induce cytochrome c release [91]. The stimuli result in mitochondria outer membrane permeabilisation (MOMP) leading towards the discharge of cytochrome c. Cytochrome c oligomerises APAF-1 (apoptotic protease activating factor 1) to generate apoptosome that activates procaspase-9 and then activates effector caspase-3/-7. These effector caspases in turn cleave many substrates causing cell death. Cancer cells prevent MOMP via up-regulating the level of Bcl-2 by various mechanisms like microRNAs down-regulation or transcriptional up-regulation by oncogenic signaling [94]. Moreover, Smac/DIABLO (second mitochondria-derived activator of caspase/direct inhibitor of apoptosis protein (IAP)-binding protein with low PI) released from the mitochondria to cytosol interact with anti-apoptotic XIAP (X-linked inhibitor of apoptosis) protein antagonizing its effect, which normally binds with caspases-3, -7 and -9 via IAP repeat domain and prevent heedless activation of caspases [95]. Physcion results in the down-regulation of Bcl-2 [19, 22-23, 25] and Ras [96], up-regulation of Bax [19, 23, 25, 96], loss of MMP (mitochondrial membrane potential) [19, 40, 67-68], opening of MPT (mitochondrial permeability transition) pore [40], release of cytochrome c to the cytosol [26, 40, 67-68], enhanced expression of activated caspase-3 and -9 [19, 22-23, 25, 40, 67-68, 96], and cleaved PARP (Poly (ADP-ribose) polymerase) [25-26, 40] resulting in apoptosis. PG causes up-regulation of Bax and decrease in Bcl-2 level [69, 72], loss of MMP, cytosolic release of cytochrome c [52, 70, 90], increased caspase-9 and -3 activation [52, 69-72, 89-90], increased level of cleaved caspase-7 [72], and PARP activation [52, 69, 71, 89] leading to apoptosis. Whether physcion and PG targets other members of Bcl-2 family proteins such as Bak and Bad to induce apoptosis should be investigated in future researches.

3.1.2.2 Extrinsic apoptotic pathway

Death ligands from the family of TNF bind to their associated receptors at the surface of cell to initiate extrinsic apoptotic pathway. Upon binding, they form a death inducing signaling network which activates caspase-8 and then caspase-8 cleaves procaspase-3 ensuing apoptosis [97]. In addition to intrinsic pathway, physcion also induces apoptosis in nasopharyngeal CNE2 cell line via extrinsic pathway which is obvious by increased expression of Fas, DR5 (death receptor-5), TRAIL (TNF-related apoptosis-inducing ligand), and caspase-8 [26]. Down-regulation of Bid (BH3 interacting domain death agonist) and increased caspase-8 activity by physcion is also

found in MDA-MB-231 and PC3 cells along with increased caspase-9 expression [22, 96]. Together with intrinsic pathway activation, PG activates extrinsic apoptotic pathway in HepG2 cells evident from caspase-8 activation and increased DR4 and TRAIL expression although the levels of Fas and DR5 remain the same [70].

3.1.2.3 Role of ROS and microRNAs in physcion and PG induced apoptosis

Various studies have documented that excess ROS production causes oxidative stress which disturbs intracellular redox status and leads to mitochondria mediated apoptosis in cancer cells [98-99]. In cancerous cells, a slight escalation in ROS production can increase drug resistance, cell survival and growth, nevertheless, excessive increase of ROS can cause cell cycle arrest and apoptosis [100]. The role of ROS in regulating Sp1 (specificity protein 1) expression through multiple signaling pathways is evident from several reports. High ROS level modulates Sp1 expression via activating ERKI/2 (extracellular-signal-regulated kinase) signaling in breast cancer [101] and primary human diploid fibroblast cells [102], while activating p38 MAPK (mitogen-activated protein kinase) signaling in liver cancerous cells [103]. Suppression of Sp1 by regulation of miR-27a has also been reported [104].

MicroRNAs work either as tumor suppressors or oncogenes. They control different cellular processes such as proliferation, differentiation, apoptosis, and invasion via suppressing target genes expression [105]. Besides apoptosis, miRNAs have a critical role in regulating autophagy [106]. miR-370 serves as the tumor suppressor in gastric cancer [107], acute myeloid leukemia [108] and laryngeal squamous cell carcinoma [109] while have an oncogenic role in wilms tumor [110]. miR-124 is documented to be down-regulated in various cancers such as liver [111], breast [112] and gastric [113] cancers.

Physcion caused up-regulation of miR-370 leading to mitochondrial apoptosis in SMMC7721 and HepG2 cells [24]. Physcion increased intracellular ROS and mediated the miR-27a/ZBTB10 (zinc finger and BTB domain containing 10) axis by decreasing miR-27a and elevating ZBTB10 expressions which ultimately down-regulated Sp1 at both mRNA and protein levels in nasopharyngeal cancer [26]. In osteosarcoma MG-63 cells, PG treatment resulted in ROS production, which reduced the level of miR-27a and in turn up-regulated Sp-repressor ZBTB10. miR-27a is found to have a regulatory effect on Sp1. Down-regulation of miR-27a decreased Sp1 expression which subsequently suppressed EMMPRIN (extracellular matrix metalloproteinase inducer) suggesting miR-27a role in PG induced pro-apoptotic effect [52]. PG also decreased the

expression of miR-21 in KB cells which then up-regulated PTEN (phosphatase and tensin homolog) expression and reduced p-Akt and p-GSK3β (Ser9) (glycogen synthase kinase 3 beta), thus, inactivating Akt/GSK3β signaling and decreasing survivin expression that led towards apoptosis [90]. Several studies have shown that down-regulation of survivin also inhibits metastasis [114-116], so, possibly PG may have anti-metastatic role in KB cells which needs to be investigated. miR-124, which has been down-regulated in malignant melanomas, acts as a tumor suppressor gene and RLIP76 (Ral binding protein) mediates its anti-neoplastic effect [117]. Over-expression of RLIP76 is reported in various tumors and its down-regulation results in the regression of tumor in many models [118]. PG exerts its antitumor effects in A375 and SK-MEL-1 cancer cells via up-regulating miR-124 and down-regulating mRNA and protein of RLIP76 dose dependently [117]. RLIP76 is also needed for the angiogenesis of solid tumors [119], thus, miR-124 may have suppressive function on angiogenesis along with cell proliferation and invasion which should be examined in future studies.

3.1.3 Inhibition of metastasis by physcion and PG

The metastasis of cancer is a sequential process that includes cancer cells invasion in the nearby tissues, their continuance in circulatory system, penetration in blood or/and lymphatic vessel's wall, and macroscopic secondary tumor growth in the far-away organs [120]. Natural products have turned up as potential reservoirs of anti-metastatic agents. Thus, inhibition of migration and invasion capability of malignant cells with natural phytochemicals may provide new avenues to cure different types of cancer [121].

EMT (epithelial–mesenchymal transition) is a major step in metastasis of cancer and β-catenin nuclear translocation is the main feature of EMT [122]. After treating HCT116 and MDA-MB-231 cells with PG, researchers found aggregation of β-catenin in the cell membrane suggesting EMT inhibition by PG [49-50]. PG reduced the level of transcriptional repressors (Snail, Slug and Twist) and mesenchymal markers (N-cadherin, fibronectin, vimentin, α-SMA) in addition to the enhanced expression of epithelial marker (E-cadherin) in MDA-MB-231 [49], HepG2 [70] and HCT116 cells [50]. These transcriptional repressors have important role in regulation of mesenchymal and epithelial markers [123]. PG regulates HIF-1α (hypoxia-inducible factor-1α) through PTEN/Akt signaling. It caused dose dependent increase in PTEN and inhibited Akt activation which in turn down-regulated the protein level of HIF-1α and EMMPRIN in HCT116 cells [50].

Physcion prohibited metastasis in colorectal SW620 cancer cells by enhancing E-cadherin expression and down-regulating the levels of fibronectin, vimentin, N-cadherin, and α-SMA along with transcriptional repressors Slug, Twist and Snail [124]. Physcion causes the suppression of migration in SMMC7721 and Hep3B cells in a dose responsive way by decreasing the level of MMP-2/-9 (matrix metalloproteinases) and increasing that of TIMP3 (tissue inhibitor of metallopeptidase inhibitor 3) [23]. Whether physcion and PG directly interact with Snail, Twist and Slug to inhibit their activity or targets their nuclear transport machinery, further needs to be investigated.

3.1.4 Role of AMPK in physcion and PG induced apoptosis and metastasis inhibition

AMPK (AMP-activated protein kinase) signaling acts as a central switch between apoptosis and cell survival. AMPK can inhibit cancer by regulating cell proliferation, cell polarity, cell growth, autophagy, and stress feedbacks [125]. Inhibition of proliferation in several cancerous cells has been documented by elevating p53, p21Cip and p27Kip through AMPK activation [126]. In colorectal HCT116 cells, physcion enhanced AMPK phosphorylation, thus, activating AMPK signaling which in turn down-regulated HIF1-α expression and subsequently suppressed EMMPRIN, causing increased apoptosis. As physcion represses HIF1-α, it has potential to thwart aggressive behavior of tumor cells due to hypoxia and invasion [67]. EMMPRIN is enriched at the surface of several malignant cancerous cells and acts as an adhesion molecule that promotes invasion and metastasis by inducing secretion of matrix metalloproteinases. Along with invasion and metastasis, the link between apoptosis and EMMPRIN has been reported in various tumor cells [127-128]. HIF-1α up-regulates EMMPRIN by binding at hypoxia responsive element in the promoter region of EMMPRIN [129]. Physcion increased p-AMPK which then induced endoplasmic reticulum stress in Bel7402 and Huh7 carcinoma cells. Endoplasmic reticulum stress was evident by the increase in p-PERK (protein kinase R (PKR)-like endoplasmic reticulum kinase), GRP78 (78-kDa glucose-regulated protein), CHOP (C/EBP homologous protein), eIF2a (eukaryotic translation initiation factor 2A), ATF6 (activating transcription factor 6), and GRP94 (94 kDa glucose-regulated protein) levels along with elevated expression of activated caspase-12 (a key indicator of ER stress) proposing that physcion triggered intrinsic apoptosis by ER stress via activating AMPK signaling cascade [40]. Physcion treatment has potential to up-regulate ROS production in gastric SGC-7901 cells which activates AMPK leading to apoptosis [68]. Most of the human genome methylation including anomalous

methylation in cancers occurs by DNMT1 (DNA methyltransferase 1) [130]. DNMT1 overexpression has been documented in various tumors such as pancreatic [131], hepatocellular [132] and non-small cell lung carcinomas [133]. In, SMMC7721 and HepG2 cells, AMPK activation by physcion decreases Sp1 expression both at mRNA and protein levels which in turn suppress DNMT1 and leads to apoptosis [24]. In glioblastoma U87 and U251 cells, PG exerts its anti-tumor effects (apoptotic and anti-invasive) by repressing Skp2 (S-phase kinase-associated protein expression) and results in the subsequent up-regulation of p21/p27 which are the downstream targets of Skp2. PG treatment enhanced ROS generation with AMPK activation and mTOR suppression which indicates the regulation of Skp2 expression by PG via ROS generation and modulation of AMPK/mTOR. Either compound C (AMPK inhibitor) or NAC (ROS inhibitor) remarkably abolished the suppressing effect of PG on Skp2 expression, showing that it regulated the Skp2 expression via modulation of ROS/AMPK/mTOR signaling [51].

Several investigations have proven the conflicting role of AMPK in metastasis where AMPK signaling inhibits metastasis in some cancers and promotes metastasis in other. AMPK activation by metformin in melanoma resulted in the prohibition of invasion and migration [121] while lysophosphatidic acid induced AMPK activation enhanced metastasis in ovarian cancer [134]. Metastatic potential in SW620 cells is halted by physcion via the down-regulation of SOX2 (Sex determining region Y-box 2) which is carried out by the activation of AMPK/GSK3β signaling due to intracellular ROS production predominately of hydrogen peroxide [124]. In HepG2 cells, PG treatment enhanced intracellular level of ROS and activated AMPK. AMPK activation decreased expression of Sp1 which subsequently suppressed DNMT1 expression. Down-regulation of DMNTI induced apoptosis and suppressed metastasis [57]. MDA-MB-231 cells treated with PG led to the inhibition of AMPK which in turn decreased the level of Sp1 protein and subsequently reduced the expression of DNMT1. Down-regulation of DMNTI reduced mesenchymal markers expression and boosted the E-cadherin expression, thus, suppressed metastasis [49, 70]. In contrast, DNMT1 is as an upstream target of Sp1 in bladder cancerous cells [135]. PG suppresses metastasis by AMPK activation in HepG2 and SW620 cells while AMPK inhibition in MDA-MB-231 and this conflicting role of AMPK in metastasis might be associated with the difference in cancer but further researches are mandatory to fully understand AMPK signaling role in cancer. Anti-cancer activities of physcion and PG are summarized in Table 2(a) and Table 2(b), respectively.

Table 3 (a) Anti-cancer activities of physcion

Cancer type	Cell line	No. of cells	Treatment time	EC_{50}/Dose	Molecular targets	Cell cycle arrest	References
Liver	Hep3B, SMMC7221	5×10^3 cells/well	24h, 48h	5, 10 μM	↑Cleaved caspase-3, ↑Bax, ↓Bcl-2, ↑LC3B II, ↑Beclin-1, ↑Atg5, ↓p62, ↓MMP-2/-9 ↑TIMP3, ↓p-JAK2, ↓p-STAT3, ⊥JAK2/STAT3 pathway	---	[23]
	Huh7, Bel7402	---	24h, 48 h, 72h	5, 10 μM	↑GRP78/94, ↑CHOP, ↑Caspase-3, ↑PARP, ↑Caspase-9, ↑Caspase-12, ↑p-PERK, ↑BIP, ↑p-eIF2a, ↑ATF6, ↑p-AMPK	---	[40]
	SMMC7721 and HepG2	1×10^5 cells/ml	24h, 48 h	10, 20 μM	↑Caspase-3/-9, ↑miR-370, ↑p-AMPK, $AMPK^{Act}$,↓Sp1, ↓DNMT1	G1	[24]
Leukemia	NALM6, SuPB15	5×10^5 cells/well	---	---	↑Caspase-3, ↑PARP, ↓HOXA5, ↑Bax, ↓Bcl-2, ↓cyclin D1	G1	[25]
Breast	MDA-MB-231	5×10^4 cells/ml	72 h	45.4 μM	↓Cyclin A/D/E, ↓CDK2, ↑CDK4, ↓PCNA, ↑p21, ⊥c-Myc, ⊥Rb, ↑caspase-3/-8/-9, ↑cleavage of PARP, ↓Bcl-2, ↓Bid	G0/G1	[22]
Nasopharyn-geal	CNE2	5.0×10^3 cells/well	24h, 48h	10, 20 μM	↓Cyclin D1,↓Cyclin E, ↑p21, ↑p27, ↑Caspase-3, ↑Cleavage of PARP,↑caspase-9, ↑TRAIL, ↑Fas, ↑DR5, ↓Sp1, ↑ROS, ↑LC3B-II, ↓p62, ↑ZBTB10, ↓miR-27a	G_1	[26]
Colorectal	SW620	5×10^3 cells/well	---	---	⊥EMT, ↑E-cadherin, ↓Vimentin, ↓αSMA,	---	[124]

					↓N-cadherin, ⊥Snail, ⊥Twist, ⊥Slug, ↑ROS, ↑p-AMPK, ↑GSK3β, ↓SOX2		
	HCT116	5×10^3 cells/well	24h, 48 h, 72h	1.25, 2.5, 5 μM	↓ Cyclin D1, ↓Cyclin E, ↑Caspase-9/-3, ↑p-AMPK, $AMPK^{Act}$, ↓HIF-1α, ↓EMMPRIN	G0/G1	[67]
Cervical	HeLa	5×10^3 cells/well	24h	---	↑ROS, ↑Caspase-3 and -9, ↑p53, ↑p21, ↑Bax, ↓Bcl-2	---	[19]
	SiHa, C33A	5×10^3 cells/well	24h	12.5 to 50 μg/ml	↑TBARS, ↑Micronuclei formation, ↑PARP-1	---	[28]
Oral	HSC-3	5×10^3 cells/well	12h	12.5 to 50 μg/ml	↑Caspase-3, ↓p-Akt,	---	[28]
Gastric	SGC-7901	---	24h, 48h, 72h	1.25, 2.5, 5 μM	↓Cyclin A, ↓Cyclin B1, ↑Caspase-3, ↑ROS , ↑p-AMPKa1, ⊥mTOR	G2/M	[68]
Neuro-blastoma	SK-N-BE(2)-C	---	---	40 μM	↑JNK, ↑p-ERK, ↑p38 MAPK, ↑hST8Sia VI	---	[29]
Prostate	PC3	5×10^4 cells/ml	72 h	0 to 100 μM	↑Bax, ↓Bcl-2, ↓Ras, ↓Bcl-xL,↑Caspase-3, ↑Caspase-8, ↑Caspase-9		[81]

Table 3 (b). Anti-cancer activities of PG

Cancer type	Cell line	No. of cells	Treatment time	EC_{50}/Dose	Molecular targets	Cell cycle arrest	References
Liver	SMMC7221, Bel-7402	---	---	---	↑Caspase-3, ↑PARP, ↑Caspase-9, ↓PIM1, ↓Bcl-2, ↓Bcl-xL, ↑Bax	---	[89]
	HepG2	5×10^3 cells/well	24h, 48 h, 72h	---	↓Cyclin D1, ↓Cyclin E, ↑p21/27, ↑Caspase-3, ↑PARP, ↑Caspase-8, ↑Caspase-9, ↓N-cadherin, ↑E-cadherin, ↓DNMT1, ↓Sp1, ↑ROS, ↑p-AMPK	G1	[70]
Lungs	A549, H358	1×10^5 cells/ml	24h, 48h	10, 20 μM	↑Caspase-3, ↑Cleaved PARP, ↓CDK4, ↓CDK6, ↓Cyclin D1, ↑PPARγ	G0/G1	[71]
	A549	1×10^4/0.2ml	24 h, 48 h	53.01 μg/ml at 24h, 27.31 μg/ml at 48h	↓p-Cdc2 (Tyr15), ↑Bax, ↑Caspase-3/-7, ↓Bcl-2, ↓Cyclin B1,	G2/M	[72]
Leukemia	NALM6, SuPB15	---	24h, 48h, 72h	5, 10 μM	↑Caspase-3, ↑PARP, ↓Bcl-2, ↑Bax, ↓Cyclin D1, ↓Cyclin A, ↑p21/27, ⊥SphK1 at translational level, ↓S1P, ↑ceramide	G0/G1	[69]
Renal	RCC4, ACHN	---	24h, 48h	10, 20μM	↓Ki-67 protein, ↑CDK2, ↓Cyclin B1, ↑Cleaved Caspase-9, ↑Caspase-3, ↓HK2	G2/M	[73]
Glioblastoma	U87, U251	1×10^4cells	---	---	↓Skp2, ↑p21, ↑p27, ↑ROS, $AMPK^{Act}$	G1	[51]

Breast	MDA-MB-231	1×10^4 cells/well	24h, 48h, 72h	10, 20, 50 µg/ml	⊥EMT, ↑E-cadherin, ↓N-cadherin, ↓Vimentin, ↓α-SMA, ↓Fibronectin, ↓Snail, ↓Slug, ↓Twist, ⊥DNMT1, ↓Sp1, ↓EMMPRIN, ↑p-AMPK	---	[49]
Colorectal	HCT116 cells	1.2×10^5 cells/ml	---	---	↑E-cadherin, ↓Vimentin, ↓N-cadherin, ⊥EMT, ↓Fibronectin, ↓Snail, ↓Slug, ↓αSMA, ↓Twist, ↑PTEN, $\downarrow Akt^{Act}$, ↓HIF1α, ↓EMMPRIN	---	[50]
Osteosarcoma	MG-63	3×10^3 cells	24h, 48h, 72h	50, 100 µg/ml	↑Caspase-3, ↑Caspase-9, ↑PARP, ↑ROS, ↓miR-27a , ↑ZBTB10, ↓Sp1, ↓EMMPRIN	---	[52]
Melanoma	SK-MEL-1, A375	$5x10^3$ cells/well	---	---	↑miR-124,↓RLIP76	---	[117]
Oral	KB	3×10^4	24h, 48h or 72 h	10, 20, 50 µg/ml	↑Bax, ↓Bcl-2, ↓miR-21, ↑Caspase-3/-9, ↑PTEN, ↓p-GSK3β, ↓p-Akt, ↓miR-21, ↓Survivin	---	[90]

Up-regulation ↑, Down-regulation ↓, Inhibition ⊥, Activation ^{Act}

3.1.5 Induction of autophagy by physcion

Autophagy is defined as type-II programmed cell death and is distinguished by the production of autophagy vacuoles in the cytoplasm. It is a response of cancerous cells towards different anti-cancer treatments. Recent evidences have shown that natural bioactive compounds are involved in modulating autophagy via inhibiting or inducing various transcriptional factors and cellular signaling pathways [136-137]. Physcion induced autophagy in SMMC7721 and Hep3B cells which was evident by the decreased level of p62 and increased level of autophagy markers Atg5 (Autophagy related 5), Beclin1 and LC3B-II, autolysosomes and autophagosomes. Physcion induced autophagy involves JAK2/STAT3 (Janus kinase 2/signal transducer and activator of transcription 3) signaling. Physcion reduced phosphorylation of JAK2 and STAT3, thus, inactivating JAK2/STAT3 pathway.

Regarding metastasis, autophagy can have pro-metastatic or anti-metastatic action. Blocking autophagy abrogated the anti-metastatic effects of physcion in liver cancer cells, indicating its anti-metastatic role [23]. Physcion also stimulated autophagy in CNE2 nasopharyngeal cells which is noticeable by increased LC3B-II and decreased p62 levels [26]. Pro-autophagic effects on osteosarcoma cells have been reported by ROS generating agents [138-139] and PG is known to induce ROS mediated apoptosis in osteosarcoma, so, research in this regard is needed that PG might also inhibit proliferation of osteosarcoma cells via inducing autophagy.

3.1.6 Synergistic effects of physcion

Currently, mono-drug therapy is not a conventional therapeutic approach for treating diseases with intricate pathophysiology such as AIDS and cancer where single drug proves less effective, so, the conservative approach of “one disease, one drug, one target” has been shifted towards multi-drug therapy [140]. Sorafenib’s anti-proliferative effects in Huh and HepG2 cells were enhanced by physcion treatment. Physcion with sorafenib down-regulated Notch 3 expression which in turn decreased the level of p-Akt, thus, repressing Notch 3/Akt signaling [141]. Leukemic K562/ADM cells are resistant to adriamycin and have significantly down-regulated miR-146a expression associated with elevated expression of CXCR4 (C-X-C chemokine receptor type 4) (miR-146a target). Drug resistance to adriamycin was reversed by physcion via elevating the expression of miR-146a hence, down-regulating CXCR4 expression. Physcion also impeded binding between CXCR4 and CXCR12 to impair migration [142].

3.1.7 Physcion as inhibitor of 6-phosphogluconate dehydrogenase (6PGD)

Reprogrammed cell metabolism generally exists in different types of cancerous cells. These abnormal changes in metabolism provide energy as well as metabolic intermediates which are essential for the increased proliferation of cancer cells. Various investigations have revealed that cancer cells overexpress oxidative pentose phosphate pathway enzymes to support cellular growth and survival. 6-phosphogluconate dehydrogenase (6PGD) is the 3rd enzyme in the oxidative PPP (pentose phosphate pathway) that converts 6-phosphogluconate to ribulose-5-phosphate (Ru-5-P) and generates NADPH to control redox status in the mammalian cells [143]. Over-expression of 6PGD has been documented to be involved in several cancers such as lung, thyroid, cervical intraepithelial neoplasia, and colorectal cancers. Prohibition of 6PGD causes decreased cellular proliferation and H_2O_2-induced cell death, probably due to scarcity of formation of reducing power NADPH. Thus, 6PGD acts as a novel promising target to overcome chemotherapeutic resistance [144-147].

Physcion and its derivative S3 acted as 6PGD inhibitors with IC_{50} values of about 17.8 μM and 38.5 μM, respectively, while K_d value of interaction between S3-6PGD and physcion-6PGD were found to be 17.1 μM and 26.0 μM, respectively. Molecular docking showed that physcion attaches to a pocket near 6-PG binding site surrounded by residues Lys 76, Met 15, His 452, and Lys 261 of 6PGD and creates hydrophobic interaction with them as well as with Asn 103 forms hydrogen bond via its 10 keto group. Treatment of K562 cells with physcion and H1299 cells with S3 for 12 h decreased ratio of NADPH/$NADP^+$, levels of Ru-5-P, biosynthesis of lipids, RNA and PPP oxidative flux while increased intracellular level of 6-PG without triggering apoptosis. Enhanced p-AMPK, p-ACCA1 (Acetyl-coenzyme A carboxylase carboxyl transferase), intracellular levels of ATP, lactate formation, and LKB1 (liver kinase B1) complex disruption was found in H1299 cells treated with physcion or S3. LKB1-deficient A549 cells showed decreased expression of Ru-5-P and 6PGD activity without affecting lipogenesis and phosphorylation of AMPK. HFF and HaCaT cells reduced the levels of Ru-5-P and 6PGD activity upon physcion treatment while lipogenesis was decreased along with enhanced p-AMPK [146]. Inhibition of 6PGD by physcion in HCC cells reduced cellular growth and triggered apoptosis. Furthermore, physcion also considerably increased the pro-apoptotic and anti-proliferative effects of cisplatin, doxorubicin and paclitaxel in HCC cells [148]. Physcion suppressed 6PGD activity in CaLo and CaSki cervical cells. Inhibition of 6PGD enhanced apoptosis while migration and proliferation were significantly down-regulated in cervical

carcinoma cells. Physcion activated AMPK which suppressed 6PGD and in turn decreased the activity of Rac1 and RhoA, both having cell migratory roles [149]. Inhibition of 6PGD by physcion sensitized the response of breast cancer to chemotherapeutic agents as evident from increased death and reduced growth of breast cancer cells upon exposure to combined treatment with physcion and doxorubicin or paclitaxel as compared to single drug treatment. Inhibition of 6PGD by physcion was brought about by the activation of AMPK which in turn inhibited ACC1 by phosphorylating it at S79. Prohibition of ACC1 results in significantly reduced lipid biosynthesis [150]. It is noted that AMPK inhibition in HCC, breast and cervical cancer cells attenuated the physcion induced effects suggesting prohibition of 6PGD acts on HCC, breast and cervical cancer cells by activation of AMPK. [148-150]. DHA and physcion synergistically reduced proliferation and triggered apoptosis in K562 cells without causing hemolysis. Halting the viability of leukemia cells was neither due to induced oxidative stress nor by COX2 (cyclooxygenase-2) inhibition. Rather, physcion and DHA activated AMPK which in turn inhibited ACC1 by phosphorylating it and resulted in reduced cellular proliferation and attenuated biosynthesis of lipids [147]. Ru-5-P formation by 6PGD prevents activation of AMPK through disruption of the LKB1 active complex [146]. Physcion and DHA treated cells showed reduced level of Ru-5-P and elevated activity of LKB1 suggesting LKB1 as the upstream AMPK activator with synergistic drug treatment and the effect of physcion and DHA was mainly mediated via ribulose-5-phosphate dependent regulation of LKB1 in cells [147]. Thus, treatment combined with physcion and DHA may serve as a treatment strategy against leukemia with no hemolysis. Thus, future studies are recommended to further investigate the role of combinatorial treatment of physcion with chemo-drugs in halting metastasis and chemo-sensitization of cancer cells as 6PGD is an important mediator of metastatic transformations and drug resistance in cancer. Being a nutraceutical, intake of dietary supplements of physcion with chemotherapy might have potential to reduce the side effects of chemotherapy and enhance the efficacy of cancer drugs.

3.1.8 Other molecular targets associated with anti-cancer activity of physcion and PG

Physcion triggered necrosis and caspase independent apoptosis in cervical C33A and SiHa as well as oral HSC-3 carcinoma cells by inducing various cellular events. Treatment with physcion caused high oxidative stress and induced irreversible DNA injury during cell cycle in cervical cancer cells. Physcion inhibited Akt in HSC-3 cells as compared to cervical cancer cells.

Oxeiptosis and many other cell deaths have been linked with increased oxidative stress. Reactive oxygen and nitrogen species interact with physcion to alter the concentration of •NO and H_2O_2, therefore, affecting the activity of enzymes such as superoxide dismutase and NADPH oxidase 1 [28]. In the case of limited DNA damage, PARP-1 repairs DNA by catalyzing ADP-ribosylation using NAD^+ which is then regenerated using adenosine triphosphate. If DNA is not repaired, cell undergoes apoptosis via caspase dependent pathway. On the contrary, extensive DNA damage may lead to necrosis as continuous PARP-1 activation exhaust ATP and NAD^+ stocks so, ATP dependent caspase pathways cannot be initiated [151]. Physcion treatment led to the down-regulation of HOXA5 in NALM6 and SUPB15 cells which then increased Bax and decreased Bcl-2 level leading to mitochondrial apoptosis [25]. SphK1 (sphingosine kinase 1), an enzyme involved in the regulation of the ceramide/S1P balance, is inhibited at translational level by PG, thus, significant increase in the ceramide/S1P ratio results when intracellular level of ceramide increases while that of SIP1 decreases which resulted in enhanced apoptosis in human SUPB15 and NALM6 cells [69]. As the up-regulation of miR-124 by PG is evident in melanoma [117] and SphK1 is documented as a direct target of miR-124 in various cancerous cells [113, 152], therefore, research in this regard is needed whether SphK1 expression by PG is down-regulated due to increased expression of miR-124 or not. PG anti-neoplastic effects in Bel7402 and SMMC7721 cells were linked with dose dependent suppression of mRNA expression and protein level of PIM1 (Proto-oncogene serine/threonine-protein kinase 1) along with its downstream targets Bcl-2 and Bcl-xL [89]. Furthermore, PG activates apoptosis in lung cancer cells via mitochondrial pathway by up-regulation of PPARγ (Peroxisome proliferator-activated receptor gamma) at transcriptional and translational levels [71]. In ACHN and RCC4 cells, PG inhibited glycolysis and induced apoptosis via suppressing HK2 (hexokinase 2) expression. Glycolysis inhibition was evident from the decreased lactate production and glucose consumption. PG decreased HK2/VDAC (voltage dependent anion channel) binding and former's mitochondrial fraction by enhancing its dissociation from mitochondrial membrane and increasing its cytosolic fraction, thus, redistributing HK2 [73]. The molecular targets and mechanisms of action of physcion and PG have been displayed in Figure 3 and Figure 4, respectively.

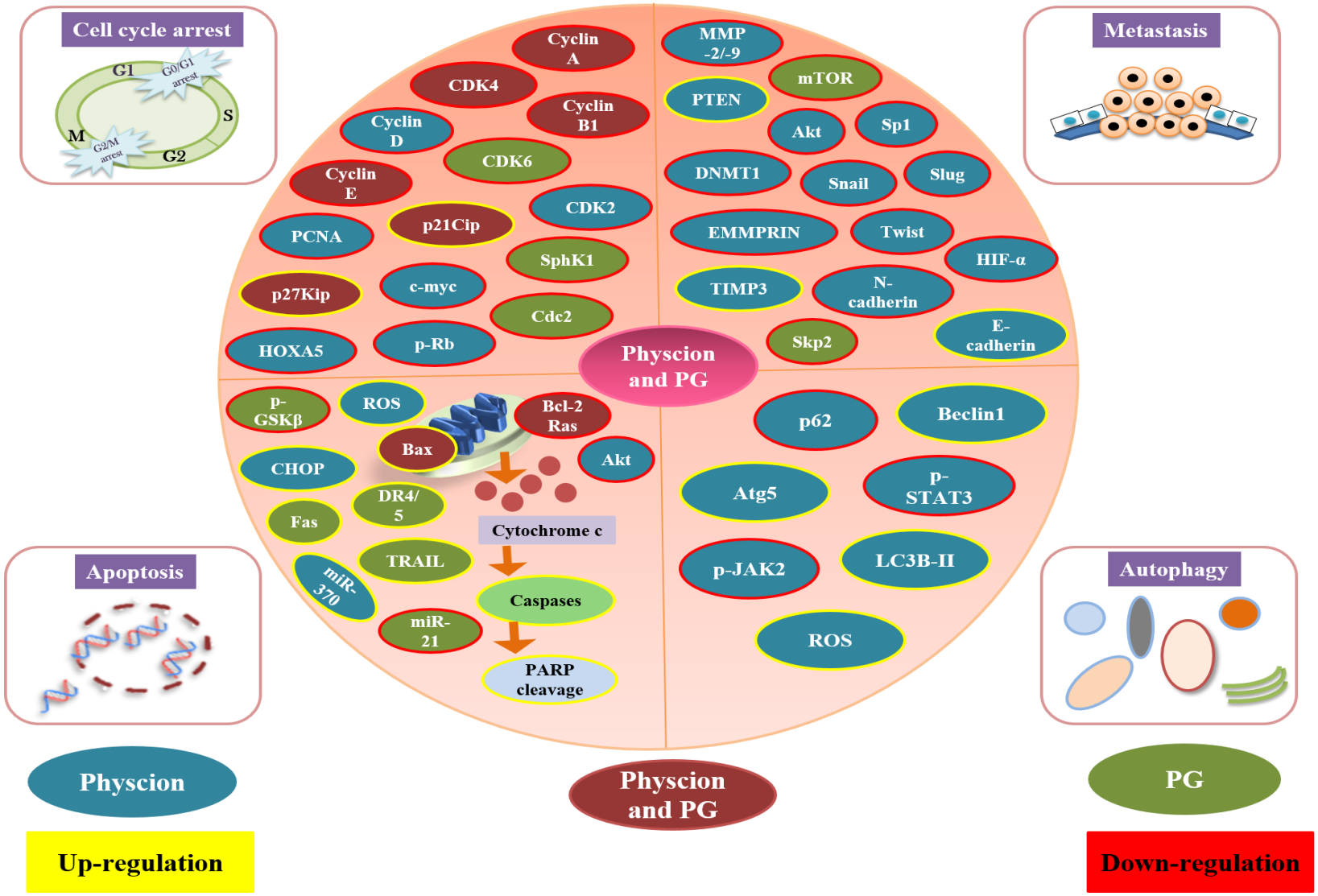

Figure 3. A diagrammatic representation of molecular targets for anti-cancer activity of physcion and P

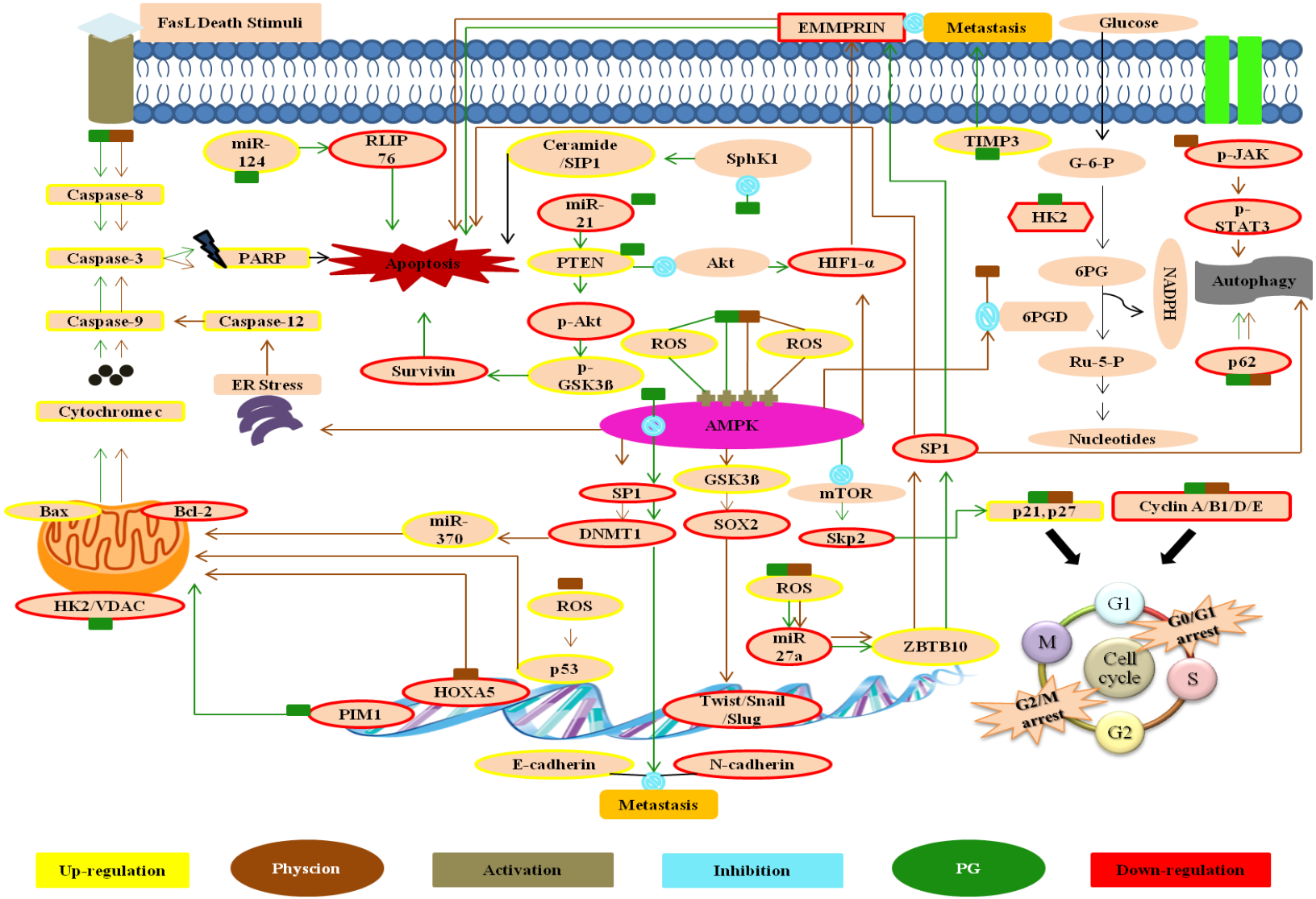

Figure 4. Representation of molecular mechanisms of actions associated with anti-cancer activity of physcion and PG

3.2 *In vivo* studies and safety profiling

Physcion treatment at the dosage of 20 and 40 mg/kg/day resulted in apoptotic cell death dose dependently in xenograft model which was established by injecting Huh7 cells into left flanks. Physcion treatment didn't affected total body mass of the xenograft mouse model. The mechanism of action of physcion involves activation of ER stress via reduced level of Ki-67 along with enhanced level of activated caspases-3/-12 and CHOP [40]. Physcion inhibited tumor growth and stimulated apoptosis at the dosage of 20 and 40 mg/kg/day in BALB/c xenograft mice model established by inoculation of HepG2 cells. Physcion treatment caused up-regulation of miR-370, increased p-AMPK while significantly decreased Sp1 and DNMT1 expression suggesting the antitumor efficacy of physcion via modulating AMPK/Sp1/DNMT1 signaling pathway [24]. Results from *in vivo* investigation on pulmonary metastasis model of hepatocellular carcinoma showed that physcion treatment decreased tumor size, tumor weight and pulmonary metastasis in a dose dependent mode (20 and 40 mg/kg/ day) without affecting total body weight and normal body tissues [23]. In CNE2 injected xenograft mouse model, physcion suppressed tumor growth without influencing total body weight and caused autophagy and apoptotic cell death as evident by increased level of LC3B-II and cleaved caspase-3. Inhibition of tumor growth was linked to reduced miR-27a and SP1 expressions [26]. In another *in vivo* investigation, combinatorial treatment of physcion and sorafenib enhanced caspase-3 activity and repressed Notch/Akt signaling as compared to sorafenib alone [141]. Physcion in combination with adriamycin reversed drug resistance in K562/ADM cells. Physcion enhanced antitumor effect of adriamycin by upregulating miR-146a expression which then suppressed the expression of CXCR4 [142]. Physcion in combination with paclitaxel inhibited tumor growth in xenograft mouse model (established by inoculation of MDA-MB-231 cancer cells) more effectively as compared to physcion or paclitaxel alone [150]. Treatment with S3 (physcion derivative) at dose of 20 mg kg^{-1} d^{-1} reduced 6PGD activity, Ki-67 expression and tumor growth without causing toxic effects in xenograft models injected with H1299 cells, Tu212 cells and K562 cells which further validates the *in vivo* efficacy S3 as a metabolic inhibitor [146]. Human K562 cells inoculated-xenograft model showed significantly reduced tumor growth when treated with DHA (dihydroartemisinin) and S3 at dose of 2.5 mg/kg/day and 5 mg/kg/day respectively for 15 days. Intraperitoneal administration of this dose caused reduced tumor growth accomplished by activation of AMPK without any adverse effect on hematopoietic properties of

xenograft model as no alterations in RBC and hemoglobin count were found. Moreover, histological studies show that DHA + S3 administration did not induce any noticeable damage to heart, kidney, liver or bone marrow tissues of mouse model. More interestingly, physcion + DHA treatment remarkably reduced the growth of primary leukemia cells from a leukemia patient while this treatment didn't exerted any effect on the viability of WBCs and RBCs from a healthy human donor which further validates its anti-leukemia potential [147]. Leukemia's are most common and deadliest forms of cancer, thus, physcion's potential against leukemia should be further investigated in patient-derived cancer xenografts and preclinical trials in combination with chemo-drugs.

PG has been investigated in lung metastatic mouse model established through injecting MDA-MB-231 cells by tail vein of 3-4 weeks old female nude mice. Histological analysis showed that PG lowered the metastatic pulmonary tumor amount and micro-metastatic nodules number per field at both 20 and 50 mg/kg dosage after 3 weeks of injection and this anti-metastatic effect was linked by p-AMPK along with SP1 and DNMT1 down-regulation, thus, supporting *in vitro* studies [49]. It displayed anti-leukemic activity in xenograft model of mouse generated by inoculating NALM6 cells into 2 months old NSI mice through angular veins. PG prolonged survival of xenograft model and resulted in tumor reduction evident by reduced weight of spleen at both 20 and 40 mg/kg [69]. PG at a dose of 40 and 80 mg/kg delayed tumor growth, increased activation of caspase-3 and repressed PIM1 expression in tumor tissues suggesting apoptosis induction via PIM1 modulation in xenograft mice model using HCC cells, thus, *in vivo* results correlate with *in vitro* findings [89]. Investigation of PG efficacy against NSCLC has shown delayed tumor growth, dose dependent increase in apoptosis with significant increase in caspase-3 and PPARγ, activation as revealed via IHC assay [71]. The *in vivo* anti-ccRCC effect of PG demonstrated significant tumor growth suppression at a dose of 40-80 mg/kg without changing body weight of murine model indicating its general safety. *In vitro* results are supported by the fact of increased apoptosis associated with reduction in HK2 expression in the tumor tissues [73]. PG prohibited tumor progression in nude mice grafted with KB cells in dose dependent way (10, 20, 40 mg/kg/day). The inhibitory effect was linked with marked downregulation of survivin and miR-21 expression and significant up-regulation of PTEN [90].

4. Conclusions and future recommendations

This review highlights the anti-cancer research progress of both physcion and PG in many *in vitro* and *in vivo* models of cancer. Data obtained from various investigations have shown that physcion and PG are potential anti-cancer agents. They trigger apoptosis, cause cell cycle arrest and inhibit metastasis in various cancers via interfering with different cellular mechanisms which are pivotal in the development of cancer and its progression. Physcion induced autophagy acts as both pro-apoptotic and pro-survival factor in different cancers. Physcion acts as potent inhibitor of 6PGD and can be used synergistically with chemo-drugs such as adriamycin to enhance their anti-cancer effects and to prevail over drug resistance. *In vivo* studies on physcion indicated its general safety as non-toxic compound. However, safety profiling was largely focused on their effect on body weight of animal while very few studies provide histological analysis of tissues from various organs, thus, future researches should focus on the exploration of hepatotoxicity, nephrotoxicity and cardiotoxicity of physcion as well as its effects on serum chemistry. Various studies report the anti-cancer potential of physcion in cell-line derived xenografts, thus, physcion and its derivatives should also be explored in patient-derived cancer xenografts in future studies. Further research should be conducted to determine the safe dosage of physcion and PG for therapeutic implications. Natural dietary sources of physcion should also be explored for the preparation of nutraceutical supplements for cancer patients. Furthermore, pharmacokinetics, pharmacodynamics, drug metabolism and bioavailability of physcion and its derivatives also needs to be investigated. Although physcion and PG possess good potential against various cancers, however, preclinical and clinical trials are yet mandatory to completely figure out their potential in order to establish these compounds as lead candidates for cancer therapies.

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Conflict of Interest

Authors have no conflict of interest.

References

[1] Fatima, I.; Kanwal, S.; Mahmood, T.; Natural Products Mediated Targeting of Virally Infected Cancer. *Dose Response,* **2019,** *17* (1), 1559325818813227.

[2] Ram, V. J.; Kumari, S.; Natural products of plant origin as anticancer agents. *Drug News Perspect,* **2001,** *14* (8), 465-82.

[3] Christen, P.; Cuendet, M.; Plants as a source of therapeutic and health products. *Chimia (Aarau),* **2012,** *66* (5), 320-3.

[4] Banerjee, J.; Das, A.; Sinha, M.; Saha, S.; Biological Efficacy of Medicinal Plant Extracts in Preventing Oxidative Damage. *Oxid Med Cell Longev,* **2018,** *2018*, 7904349.

[5] Kumar, N.; Yadav, N.; Amarnath, N.; Sharma, V.; Shukla, S.; Srivastava, A.; Prasad, P.; Kumar, A.; Garg, S.; Singh, S.; Sehrawat, S.; Lochab, B.; Integrative natural medicine inspired graphene nanovehicle-benzoxazine derivatives as potent therapy for cancer. *Mol Cell Biochem,* **2019,** *454* (1-2), 123-138.

[6] Cragg, G. M.; Newman, D. J.; Natural products: a continuing source of novel drug leads. *Biochim Biophys Acta,* **2013,** *1830* (6), 3670-95.

[7] Harvey, A. L.; Edrada-Ebel, R.; Quinn, R. J.; The re-emergence of natural products for drug discovery in the genomics era. *Nature reviews. Drug discovery,* **2015,** *14* (2), 111-29.

[8] Qin, J. J.; Li, X.; Hunt, C.; Wang, W.; Wang, H.; Zhang, R.; Natural products targeting the p53-MDM2 pathway and mutant p53: Recent advances and implications in cancer medicine. *Genes Dis,* **2018,** *5* (3), 204-219.

[9] Ren, Y.; Kinghorn, A. D.; Natural Product Triterpenoids and Their Semi-Synthetic Derivatives with Potential Anticancer Activity. *Planta Med,* **2019**.

[10] Atanasov, A. G.; Waltenberger, B.; Pferschy-Wenzig, E. M.; Linder, T.; Wawrosch, C.; Uhrin, P.; Temml, V.; Wang, L.; Schwaiger, S.; Heiss, E. H.; Rollinger, J. M.; Schuster, D.; Breuss, J. M.; Bochkov, V.; Mihovilovic, M. D.; Kopp, B.; Bauer, R.; Dirsch, V. M.; Stuppner, H.; Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnol Adv,* **2015,** *33* (8), 1582-1614.

[11] Newman, D. J.; Cragg, G. M.; Natural Products as Sources of New Drugs from 1981 to 2014. *J Nat Prod,* **2016,** *79* (3), 629-61.

[12] Agarwal, G.; Carcache, P. J. B.; Addo, E. M.; Kinghorn, A. D.; Current status and contemporary approaches to the discovery of antitumor agents from higher plants. *Biotechnol Adv,* **2019**.

[13] Catassi, A.; Cesario, A.; Arzani, D.; Menichini, P.; Alama, A.; Bruzzo, C.; Imperatori, A.; Rotolo, N.; Granone, P.; Russo, P.; Characterization of apoptosis induced by marine natural products in non small cell lung cancer A549 cells. *Cell Mol Life Sci,* **2006,** *63* (19-20), 2377-86.

[14] Schwartsmann, G.; Da Rocha, A. B.; Mattei, J.; Lopes, R.; Marine-derived anticancer agents in clinical trials. *Expert Opin Investig Drugs,* **2003,** *12* (8), 1367-83.

[15] da Rocha, A. B.; Lopes, R. M.; Schwartsmann, G.; Natural products in anticancer therapy. *Curr Opin Pharmacol,* **2001,** *1* (4), 364-9.

[16] Huang, W. Y.; Cai, Y. Z.; Zhang, Y.; Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. *Nutrition and cancer,* **2010,** *62* (1), 1-20.

[17] Tungmunnithum, D.; Thongboonyou, A.; Pholboon, A.; Yangsabai, A.; Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines,* **2018,** *5* (3).

[18] Malik, E. M.; Muller, C. E.; Anthraquinones As Pharmacological Tools and Drugs. *Medicinal research reviews,* **2016,** *36* (4), 705-48.

[19] Wijesekara, I.; Zhang, C.; Van Ta, Q.; Vo, T. S.; Li, Y. X.; Kim, S. K.; Physcion from marine-derived fungus Microsporum sp. induces apoptosis in human cervical carcinoma HeLa cells. *Microbiol Res,* **2014,** *169* (4), 255-61.

[20] Li, Y.; Jiang, J. G.; Health functions and structure-activity relationships of natural anthraquinones from plants. *Food & function,* **2018,** *9* (12), 6063-6080.

[21] Kremer, D.; Kosalec, I.; Locatelli, M.; Epifano, F.; Genovese, S.; Carlucci, G.; Zovko Koncˇic, M.; Anthraquinone profiles, antioxidant and antimicrobial properties of Frangula rupestris (Scop.) Schur and Frangula alnus Mill. bark. *Food Chem,* **2012,** *131* (4), 1174-1180.

[22] Hong, J. Y.; Chung, H. J.; Bae, S. Y.; Trung, T. N.; Bae, K.; Lee, S. K.; Induction of Cell Cycle Arrest and Apoptosis by Physcion, an Anthraquinone Isolated From Rhubarb (Rhizomes of Rheum tanguticum), in MDA-MB-231 Human Breast Cancer Cells. *J Cancer Prev,* **2014,** *19* (4), 273-8.

[23] Pan, X.; Wang, C.; Li, Y.; Zhu, L.; Zhang, T.; Protective autophagy induced by physcion suppresses hepatocellular carcinoma cell metastasis by inactivating the JAK2/STAT3 Axis. *Life Sci,* **2018,** *214*, 124-135.

[24] Pan, X.; Wang, H.; Tong, D.; Wang, C.; Sun, L.; Zhao, C.; Li, Y.; Zhu, L.; Wu, D.; Physcion induces apoptosis in hepatocellular carcinoma by modulating miR-370. *Am J Cancer Res,* **2016,** *6* (12), 2919-2931.

[25] Gao, F.; Liu, W.; Guo, Q.; Bai, Y.; Yang, H.; Chen, H.; Physcion blocks cell cycle and induces apoptosis in human B cell precursor acute lymphoblastic leukemia cells by downregulating HOXA5. *Biomed Pharmacother,* **2017,** *94*, 850-857.

[26] Pang, M. J.; Yang, Z.; Zhang, X. L.; Liu, Z. F.; Fan, J.; Zhang, H. Y.; Physcion, a naturally occurring anthraquinone derivative, induces apoptosis and autophagy in human nasopharyngeal carcinoma. *Acta Pharmacol Sin,* **2016,** *37* (12), 1623-1640.

[27] Agarwal, S. K.; Singh, S. S.; Verma, S.; Kumar, S.; Antifungal activity of anthraquinone derivatives from Rheum emodi. *J Ethnopharmacol,* **2000,** *72* (1-2), 43-6.

[28] Moreira, T. F.; Sorbo, J. M.; Souza, F. O.; Fernandes, B. C.; Ocampos, F. M. M.; de Oliveira, D. M. S.; Arcaro, C. A.; Assis, R. P.; Barison, A.; Miguel, O. G.; Baviera, A. M.; Soares, C. P.; Brunetti, I. L.; Emodin, Physcion, and Crude Extract of Rhamnus sphaerosperma var. pubescens Induce Mixed Cell Death, Increase in Oxidative Stress, DNA Damage, and Inhibition of AKT in Cervical and Oral Squamous Carcinoma Cell Lines. *Oxid Med Cell Longev,* **2018,** *2018*, 2390234.

[29] Yoon, H. K.; An, H. K.; Ko, M. J.; Kim, K. S.; Mun, S. W.; Kim, D. H.; Kim, C. M.; Kim, C. H.; Choi, Y. W.; Lee, Y. C.; Upregulation of Human ST8Sia VI (alpha2,8-Sialyltransferase) Gene Expression by Physcion in SK-N-BE(2)-C Human Neuroblastoma Cells. *Int J Mol Sci,* **2016,** *17* (8).

[30] Mellado, M.; Madrid, A.; Peña-Cortés, H.; López, R.; Jara, C.; Espinoza, L.; Antioxidant activity of anthraquinones isolated from leaves of muehlenbeckia hastulata (J.E. SM.) Johnst. (polygonaceae). *J. Chil. Chem. Soc.,* **2013,** *58* (2), 1767-1770.

[31] Lee, G.; Choi, T. W.; Kim, C.; Nam, D.; Lee, S. G.; Jang, H. J.; Lee, J. H.; Um, J. Y.; Jung, S. H.; Shim, B. S.; Ahn, K. S.; Ahn, K. S.; Anti-inflammatory activities of Reynoutria elliptica through suppression of mitogen-activated protein kinases and nuclear factor-kappaB activation pathways. *Immunopharmacol Immunotoxicol,* **2012,** *34* (3), 454-64.

[32] Saleh, E. I. M. M.; Roy, A.; Al-Mansur, M. A.; Mahmood Hasan, C.; Rahim, M. M.; Sultana, N.; Ahmed, S.; Islam, M. R.; van Staden, J.; Isolation and in silico prediction of potential drug-like compounds from Anethum sowa L. root extracts targeted towards cancer therapy. *Comput Biol Chem,* **2019,** *78*, 242-259.

[33] Tamokou Jde, D.; Tala, M. F.; Wabo, H. K.; Kuiate, J. R.; Tane, P.; Antimicrobial activities of methanol extract and compounds from stem bark of Vismia rubescens. *J Ethnopharmacol,* **2009,** *124* (3), 571-5.

[34] Ghosh, S.; Das Sarma, M.; Patra, A.; Hazra, B.; Anti-inflammatory and anticancer compounds isolated from Ventilago madraspatana Gaertn., Rubia cordifolia Linn. and Lantana camara Linn. *J Pharm Pharmacol,* **2010,** *62* (9), 1158-66.

[35] Kim, Y. M.; Lee, C. H.; Kim, H. G.; Lee, H. S.; Anthraquinones isolated from Cassia tora (Leguminosae) seed show an antifungal property against phytopathogenic fungi. *Journal of agricultural and food chemistry,* **2004,** *52* (20), 6096-100.

[36] Fernand, V. E.; Dinh, D. T.; Washington, S. J.; Fakayode, S. O.; Losso, J. N.; van Ravenswaay, R. O.; Warner, I. M.; Determination of pharmacologically active compounds in root extracts of Cassia alata L. by use of high performance liquid chromatography. *Talanta,* **2008,** *74* (4), 896-902.

[37] Dave, H.; Ledwani, L.; A review on anthraquinones isolated from Cassia species and their applications *Indian Journal of natural product and resources,* **2012,** *3* (3), 291-319.

[38] Fan, J. P.; Zhang, Z. L.; [Studies on the chemical constituents of Rumex crispus]. *Zhong yao cai = Zhongyaocai = Journal of Chinese medicinal materials*, **2009,** *32* (12), 1836-40.

[39] Bowen, L.; Li, C.; Bin, L.; Ying, T.; Shijun, L.; Junxing, D.; Chemical constituents, cytotoxic and antioxidant activities of extract from the rhizomes of Osmunda japonica Thunb. *Natural product research,* **2020,** *34* (6), 847-850.

[40] Pan, X. P.; Wang, C.; Li, Y.; Huang, L. H.; Physcion induces apoptosis through triggering endoplasmic reticulum stress in hepatocellular carcinoma. *Biomed Pharmacother,* **2018,** *99*, 894-903.

[41] Ana Paula, A.; Tida, D.; Narong, S.; Raquel, L.; Maria Helena, V.; Madalena, P.; Anake, K.; The in vitro anticancer activity of the crude extract of the sponge-associated fungus Eurotium cristatum and its secondary metabolites. *J. Nat. Pharm,* **2010,** *1* (1), 25-29.

[42] Mueller, S. O.; Schmitt, M.; Dekant, W.; Stopper, H.; Schlatter, J.; Schreier, P.; Lutz, W. K.; Occurrence of emodin, chrysophanol and physcion in vegetables, herbs and liquors. Genotoxicity and anti-genotoxicity of the anthraquinones and of the whole plants. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association,* **1999,** *37* (5), 481-91.

[43] Liu, S. Y.; Sporer, F.; Wink, M.; Jourdane, J.; Henning, R.; Li, Y. L.; Ruppel, A.; Anthraquinones in Rheum palmatum and Rumex dentatus (Polygonaceae), and phorbol esters in Jatropha curcas (Euphorbiaceae) with molluscicidal activity against the schistosome vector snails Oncomelania, Biomphalaria and Bulinus. *Tropical medicine & international health : TM & IH,* **1997,** *2* (2), 179-88.

[44] Zhang, C.; Li, L.; Xiao, Y. Q.; Tian, G. F.; Chen, D. D.; Wang, Y.; Li, Y. T.; Huang, W. Q.; Two new anthraquinone glycosides from the roots of Rheum palmatum. *Journal of Asian natural products research,* **2010,** *12* (12), 1026-32.

[45] Feng, J.; Ren, H.; Gou, Q.; Zhu, L.; Ji, H.; Yi, T.; Comparative analysis of the major constituents in three related polygonaceous medicinal plants using pressurized liquid extraction and HPLC-ESI/MS. . *Analytical Methods,* **2016,** *8* (7), 1557-1564.

[46] Xu, N. G.; Xiao, Z. J.; Zou, T.; Huang, Z. L.; Ameliorative effects of physcion 8-O-beta-glucopyranoside isolated from Polygonum cuspidatum on learning and memory in dementia rats induced by Abeta1-40. *Pharm Biol,* **2015,** *53* (11), 1632-8.

[47] Zhang, X.; Thuong, P. T.; Jin, W.; Su, N. D.; Sok, D. E.; Bae, K.; Kang, S. S.; Antioxidant activity of anthraquinones and flavonoids from flower of Reynoutria sachalinensis. *Archives of pharmacal research,* **2005,** *28* (1), 22-7.

[48] Oleszek, M.; Kowalska, I.; Oleszek, W.; Phytochemicals in bioenergy crops. *Phytochemistry Reviews* **2019,** *18*, 893-927.

[49] Chen, X.; Guo, H.; Li, F.; Fan, D.; Physcion 8-O-beta-glucopyranoside suppresses the metastasis of breast cancer in vitro and in vivo by modulating DNMT1. *Pharmacol Rep,* **2017,** *69* (1), 36-44.

[50] Ding, Z.; Xu, F.; Tang, J.; Li, G.; Jiang, P.; Tang, Z.; Wu, H.; Physcion 8-O-beta-glucopyranoside prevents hypoxia-induced epithelial-mesenchymal transition in colorectal cancer HCT116 cells by modulating EMMPRIN. *Neoplasma,* **2016,** *63* (3), 351-61.

[51] Li, W.; Li, F.; Zhu, Y.; Song, D.; Physcion 8-O-beta-glucopyranosideregulates cell cycle, apoptosis, and invasion in glioblastoma cells through modulating Skp2. *Biomed Pharmacother,* **2017,** *95,* 1129-1138.

[52] Wang, Z.; Yang, H.; EMMPRIN, SP1 and microRNA-27a mediate physcion 8-O-beta-glucopyranoside-induced apoptosis in osteosarcoma cells. *Am J Cancer Res,* **2016,** *6* (6), 1331-44.

[53] Geng, Q.; Wei, Q.; Wang, S.; Qi, H.; Zhu, Q.; Liu, X.; Shi, X.; Wen, S.; Physcion 8Obetaglucopyranoside extracted from Polygonum cuspidatum exhibits antiproliferative and antiinflammatory effects on MH7A rheumatoid arthritisderived fibroblastlike synoviocytes through the TGFbeta/MAPK pathway. *Int J Mol Med,* **2018,** *42* (2), 745-754.

[54] Zhao, Y. L.; Wang, J. B.; Zhou, G. D.; Shan, L. M.; Xiao, X. H.; Investigations of free anthraquinones from rhubarb against alpha-naphthylisothiocyanate-induced cholestatic liver injury in rats. *Basic Clin Pharmacol Toxicol,* **2009,** *104* (6), 463-9.

[55] Fu, W. J.; Tang, J. J.; Wang, H.; Wei, H. Y.; Cai, S. M.; Zeng, Z. H.; Chen, H.; Chen, Z. Q.; In vivo and in vitro anti-sepsis effects of physcion 8-O-beta-glucopyranoside extracted from Rumex japonicus. *Chin J Nat Med,* **2017,** *15* (7), 534-539.

[56] Khan, M.; Maryam, A.; Zhang, H.; Mehmood, T.; Ma, T.; Killing cancer with platycodin D through multiple mechanisms. *Journal of cellular and molecular medicine,* **2016,** *20* (3), 389-402.

[57] Ferlay, J.; Colombet, M.; Soerjomataram, I.; Mathers, C.; Parkin, D. M.; Pineros, M.; Znaor, A.; Bray, F.; Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer,* **2019,** *144* (8), 1941-1953.

[58] Rahmani, A. H.; Alzohairy, M. A.; Khan, M. A.; Aly, S. M.; Therapeutic Implications of Black Seed and Its Constituent Thymoquinone in the Prevention of Cancer through Inactivation and Activation of Molecular Pathways. *Evid Based Complement Alternat Med,* **2014,** *2014,* 13.

[59] Reddy, L.; Odhav, B.; Bhoola, K. D.; Natural products for cancer prevention: a global perspective. *Pharmacol Ther,* **2003,** *99* (1), 1-13.

[60] Pumiputavon, K.; Chaowasku, T.; Saenjum, C.; Osathanunkul, M.; Wungsintaweekul, B.; Chawansuntati, K.; Wipasa, J.; Lithanatudom, P.; Cell cycle arrest and apoptosis induction by methanolic leaves extracts of four Annonaceae plants. *BMC Complement Altern Med,* **2017,** *17* (1), 294.

[61] Sarwar, M. S.; Zhang, H. J.; Tsang, S. W.; Perspectives of Plant Natural Products in Inhibition of Cancer Invasion and Metastasis by Regulating Multiple Signaling Pathways. *Curr Med Chem,* **2018,** *25* (38), 5057-5087.

[62] Zafar, M.; Sarfraz, I.; Rasul, A.; Jabeen, F.; Samiullah, K.; Hussain, G.; Riaz, A.; Ali, M.; Tubeimoside-1, triterpenoid saponin, as a potential natural cancer killer. *Nat. Prod. Commun,* **2018,** *13* (0).

[63] Khan, M.; Maryam, A.; Qazi, J. I.; Ma, T.; Targeting Apoptosis and Multiple Signaling Pathways with Icariside II in Cancer Cells. *Int J Biol Sci,* **2015,** *11* (9), 1100-12.

[64] Huang, Q.; Lu, G.; Shen, H. M.; Chung, M. C.; Ong, C. N.; Anti-cancer properties of anthraquinones from rhubarb. *Med Res Rev,* **2007,** *27* (5), 609-30.

[65] Mullany, L. E.; Herrick, J. S.; Sakoda, L. C.; Samowitz, W.; Stevens, J. R.; Wolff, R. K.; Slattery, M. L.; miRNA involvement in cell cycle regulation in colorectal cancer cases. *Genes Cancer,* **2018,** *9* (1-2), 53-65.

[66] Vermeulen, K.; Van Bockstaele, D. R.; Berneman, Z. N.; The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. *Cell Prolif,* **2003,** *36* (3), 131-49.

[67] Chen, X.; Gao, H.; Han, Y.; Ye, J.; Xie, J.; Wang, C.; Physcion induces mitochondria-driven apoptosis in colorectal cancer cells via downregulating EMMPRIN. *Eur J Pharmacol,* **2015,** *764*, 124-33.

[68] Xiong, Y.; Ren, L.; Wang, Z.; Hu, Z.; Zhou, Y.; Anti-proliferative Effect of Physcion on Human Gastric Cell Line via Inducing ROS-Dependent Apoptosis. *Cell Biochem Biophys,* **2015,** *73* (2), 537-543.

[69] Han, J.; Zhao, P.; Shao, W.; Wang, Z.; Wang, F.; Sheng, L.; Physcion 8-O-beta-glucopyranoside exhibits anti-leukemic activity through targeting sphingolipid rheostat. *Pharmacol Rep,* **2018,** *70* (5), 853-862.

[70] Wang, Q.; Wang, Y.; Xing, Y.; Yan, Y.; Guo, P.; Zhuang, J.; Qin, F.; Zhang, J.; Physcion 8-O-beta-glucopyranoside induces apoptosis, suppresses invasion and inhibits epithelial to mesenchymal transition of hepatocellular carcinoma HepG2 cells. *Biomed Pharmacother,* **2016,** *83*, 372-380.

[71] Du, Y.; Lv, Z.; Sun, D.; Li, Y.; Sun, L.; Zhou, J.; Physcion 8-O-beta-Glucopyranoside Exerts Anti-Tumor Activity Against Non-Small Cell Lung Cancer by Targeting PPARgamma. *Anat Rec (Hoboken),* **2019,** *302* (5), 785-793.

[72] Xie, Q. C.; Yang, Y. P.; Anti-proliferative of physcion 8-O-beta-glucopyranoside isolated from Rumex japonicus Houtt. on A549 cell lines via inducing apoptosis and cell cycle arrest. *BMC Complement Altern Med,* **2014,** *14*, 377.

[73] Wang, Q.; Yan, Y.; Zhang, J.; Guo, P.; Xing, Y.; Wang, Y.; Qin, F.; Zeng, Q.; Physcion 8-O-beta-glucopyranoside inhibits clear-cell renal cell carcinoma bydownregulating hexokinase II and inhibiting glycolysis. *Biomed Pharmacother,* **2018,** *104*, 28-35.

[74] Lee, S. H.; Ryu, B.; Je, J. Y.; Kim, S. K.; Diethylaminoethyl chitosan induces apoptosis in HeLa cells via activation of caspase-3 and p53 expression. *Carbohydr. Polym,* **2010,** *84* (1), 571-578.

[75] Chiang, J. H.; Yang, J. S.; Ma, C. Y.; Yang, M. D.; Huang, H. Y.; Hsia, T. C.; Kuo, H. M.; Wu, P. P.; Lee, T. H.; Chung, J. G.; Danthron, an anthraquinone derivative, induces DNA damage and caspase cascades-mediated apoptosis in SNU-1 human gastric cancer cells through mitochondrial permeability transition pores and Bax-triggered pathways. *Chem Res Toxicol,* **2011,** *24* (1), 20-9.

[76] Hengartner, M. O.; The biochemistry of apoptosis. *Nature,* **2000,** *407* (6805), 770-6.

[77] Ashkenazi, A.; Targeting death and decoy receptors of the tumour-necrosis factor superfamily. *Nat Rev Cancer,* **2002,** *2* (6), 420-30.

[78] Janssen, O.; Qian, J.; Linkermann, A.; Kabelitz, D.; CD95 ligand--death factor and costimulatory molecule? *Cell Death Differ,* **2003,** *10* (11), 1215-25.

[79] Zhang, Y.; Xing, D.; Liu, L.; PUMA promotes Bax translocation by both directly interacting with Bax and by competitive binding to Bcl-X L during UV-induced apoptosis. *Mol Biol Cell,* **2009,** *20* (13), 3077-87.

[80] Meng, S. J.; Yu, L. J.; Oxidative stress, molecular inflammation and sarcopenia. *Int J Mol Sci,* **2010,** *11* (4), 1509-26.

[81] Gu, X.; Song, X.; Dong, Y.; Cai, H.; Walters, E.; Zhang, R.; Pang, X.; Xie, T.; Guo, Y.; Sridhar, R.; Califano, J. A.; Vitamin E succinate induces ceramide-mediated apoptosis in head and neck squamous cell carcinoma in vitro and in vivo. *Clin Cancer Res,* **2008,** *14* (6), 1840-8.

[82] Letai, A.; Growth factor withdrawal and apoptosis: the middle game. *Mol Cell,* **2006,** *21* (6), 728-30.

[83] Lancellotti, M.; Pereira, R. F.; Cury, G. G.; Hollanda, L. M.; Pathogenic and opportunistic respiratory bacteria-induced apoptosis. *Braz J Infect Dis,* **2009,** *13* (3), 226-31.

[84] Solary, E.; Droin, N.; Bettaieb, A.; Corcos, L.; Dimanche-Boitrel, M. T.; Garrido, C.; Positive and negative regulation of apoptotic pathways by cytotoxic agents in hematological malignancies. *Leukemia,* **2000,** *14* (10), 1833-49.

[85] Ahsan, H.; Reagan-Shaw, S.; Breur, J.; Ahmad, N.; Sanguinarine induces apoptosis of human pancreatic carcinoma AsPC-1 and BxPC-3 cells via modulations in Bcl-2 family proteins. *Cancer Lett,* **2007,** *249* (2), 198-208.

[86] Ola, M. S.; Nawaz, M.; Ahsan, H.; Role of Bcl-2 family proteins and caspases in the regulation of apoptosis. *Mol Cell Biochem,* **2011,** *351* (1-2), 41-58.

[87] Zaman, S.; Wang, R.; Gandhi, V.; Targeting the apoptosis pathway in hematologic malignancies. *Leuk Lymphoma,* **2014,** *55* (9), 1980-92.

[88] Creagh, E. M.; Martin, S. J.; Caspases: cellular demolition experts. *Biochem Soc Trans,* **2001,** *29* (Pt 6), 696-702.

[89] Wang, Q.; Jiang, Y.; Guo, R.; Lv, R.; Liu, T.; Wei, H.; Ming, H.; Tian, X.; Physcion 8-O-beta-glucopyranoside suppresses tumor growth of Hepatocellular carcinoma by downregulating PIM1. *Biomed Pharmacother,* **2017,** *92,* 451-458.

[90] Liu, M. D.; Xiong, S. J.; Tan, F.; Liu, Y.; Physcion 8-O-beta-glucopyranoside induces mitochondria-dependent apoptosis of human oral squamous cell carcinoma cells via suppressing survivin expression. *Acta Pharmacol Sin,* **2016,** *37* (5), 687-97.

[91] Desagher, S.; Martinou, J. C.; Mitochondria as the central control point of apoptosis. *Trends Cell Biol,* **2000,** *10* (9), 369-77.

[92] Oltvai, Z. N.; Milliman, C. L.; Korsmeyer, S. J.; Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell,* **1993,** *74* (4), 609-19.

[93] Fulda, S.; Evasion of Apoptosis as a Cellular Stress Response in Cancer. *Int. J. Cell Biol,* **2010,** *2010*.

[94] Lopez, J.; Tait, S. W.; Mitochondrial apoptosis: killing cancer using the enemy within. *Br J Cancer,* **2015,** *112* (6), 957-62.

[95] Salvesen, G. S.; Duckett, C. S.; IAP proteins: blocking the road to death's door. *Nat Rev Mol Cell Biol,* **2002,** *3* (6), 401-10.

[96] Ding, S. Y.; Kim, W. S.; Park, S. J.; Kim, S. K.; Apoptotic effect of physcion isolated from marine fungus Microsporum sp. in PC3 human prostate cancer cells. *Fish Aquat Sci,* **2018,** *21* (22).

[97] Duiker, E. W.; van der Zee, A. G.; de Graeff, P.; Boersma-van Ek, W.; Hollema, H.; de Bock, G. H.; de Jong, S.; de Vries, E. G.; The extrinsic apoptosis pathway and its prognostic impact in ovarian cancer. *Gynecol Oncol,* **2010,** *116* (3), 549-55.

[98] Yuan, L.; Wang, J.; Xiao, H.; Xiao, C.; Wang, Y.; Liu, X.; Isoorientin induces apoptosis through mitochondrial dysfunction and inhibition of PI3K/Akt signaling pathway in HepG2 cancer cells. *Toxicol Appl Pharmacol,* **2012,** *265* (1), 83-92.

[99] Raza, H.; John, A.; Benedict, S.; Acetylsalicylic acid-induced oxidative stress, cell cycle arrest, apoptosis and mitochondrial dysfunction in human hepatoma HepG2 cells. *Eur J Pharmacol,* **2011,** *668* (1-2), 15-24.

[100] Trachootham, D.; Alexandre, J.; Huang, P.; Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat Rev Drug Discov,* **2009,** *8* (7), 579-91.

[101] Choudhary, S.; Sood, S.; Donnell, R. L.; Wang, H. C.; Intervention of human breast cell carcinogenesis chronically induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. *Carcinogenesis,* **2012,** *33* (4), 876-85.

[102] Kim, H. S.; Lim, I. K.; Phosphorylated extracellular signal-regulated protein kinases 1 and 2 phosphorylate Sp1 on serine 59 and regulate cellular senescence via transcription of p21Sdi1/Cip1/Waf1. *J Biol Chem,* **2009,** *284* (23), 15475-86.

[103] Li, K.; Gao, B.; Li, J.; Chen, H.; Li, Y.; Wei, Y.; Gong, D.; Gao, J.; Zhang, J.; Tan, W.; Wen, T.; Zhang, L.; Huang, L.; Xiang, R.; Lin, P.; Wei, Y.; ZNF32 protects against oxidative stress-induced apoptosis by modulating C1QBP transcription. *Oncotarget,* **2015,** *6* (35), 38107-26.

[104] Jutooru, I.; Guthrie, A. S.; Chadalapaka, G.; Pathi, S.; Kim, K.; Burghardt, R.; Jin, U. H.; Safe, S.; Mechanism of action of phenethylisothiocyanate and other reactive oxygen species-inducing anticancer agents. *Mol Cell Biol,* **2014,** *34* (13), 2382-95.

[105] Ventura, A.; Jacks, T.; MicroRNAs and cancer: short RNAs go a long way. *Cell,* **2009,** *136* (4), 586-91.

[106] Chen, Y.; Fu, L. L.; Wen, X.; Liu, B.; Huang, J.; Wang, J. H.; Wei, Y. Q.; Oncogenic and tumor suppressive roles of microRNAs in apoptosis and autophagy. *Apoptosis,* **2014,** *19* (8), 1177-89.

[107] Feng, Y.; Wang, L.; Zeng, J.; Shen, L.; Liang, X.; Yu, H.; Liu, S.; Liu, Z.; Sun, Y.; Li, W.; Chen, C.; Jia, J.; FoxM1 is overexpressed in Helicobacter pylori-induced gastric carcinogenesis and is negatively regulated by miR-370. *Mol Cancer Res,* **2013,** *11* (8), 834-44.

[108] Zhang, X.; Zeng, J.; Zhou, M.; Li, B.; Zhang, Y.; Huang, T.; Wang, L.; Jia, J.; Chen, C.; The tumor suppressive role of miRNA-370 by targeting FoxM1 in acute myeloid leukemia. *Mol Cancer,* **2012,** *11*, 56.

[109] Yungang, W.; Xiaoyu, L.; Pang, T.; Wenming, L.; Pan, X.; miR-370 targeted FoxM1 functions as a tumor suppressor in laryngeal squamous cell carcinoma (LSCC). *Biomed Pharmacother,* **2014,** *68* (2), 149-54.

[110] Cao, X.; Liu, D.; Yan, X.; Zhang, Y.; Yuan, L.; Zhang, T.; Fu, M.; Zhou, Y.; Wang, J.; Stat3 inhibits WTX expression through up-regulation of microRNA-370 in Wilms tumor. *FEBS Lett,* **2013,** *587* (6), 639-44.

[111] Furuta, M.; Kozaki, K. I.; Tanaka, S.; Arii, S.; Imoto, I.; Inazawa, J.; miR-124 and miR-203 are epigenetically silenced tumor-suppressive microRNAs in hepatocellular carcinoma. *Carcinogenesis,* **2010,** *31* (5), 766-76.

[112] Lv, X. B.; Jiao, Y.; Qing, Y.; Hu, H.; Cui, X.; Lin, T.; Song, E.; Yu, F.; miR-124 suppresses multiple steps of breast cancer metastasis by targeting a cohort of pro-metastatic genes in vitro. *Chin J Cancer,* **2011,** *30* (12), 821-30.

[113] Xia, J.; Wu, Z.; Yu, C.; He, W.; Zheng, H.; He, Y.; Jian, W.; Chen, L.; Zhang, L.; Li, W.; miR-124 inhibits cell proliferation in gastric cancer through down-regulation of SPHK1. *J Pathol,* **2012,** *227* (4), 470-80.

[114] Zhang, M.; Coen, J. J.; Suzuki, Y.; Siedow, M. R.; Niemierko, A.; Khor, L. Y.; Pollack, A.; Zhang, Y.; Zietman, A. L.; Shipley, W. U.; Chakravarti, A.; Survivin is a potential mediator of prostate cancer metastasis. *Int J Radiat Oncol Biol Phys,* **2010,** *78* (4), 1095-103.

[115] Kogo, R.; How, C.; Chaudary, N.; Bruce, J.; Shi, W.; Hill, R. P.; Zahedi, P.; Yip, K. W.; Liu, F. F.; The microRNA-218~Survivin axis regulates migration, invasion, and lymph node metastasis in cervical cancer. *Oncotarget,* **2015,** *6* (2), 1090-100.

[116] Dogan, M.; Cagli, S.; Yuce, I.; Bayram, A.; Somdas, M. A.; Karatas, D.; Cihan, M. C.; Yuksel, F.; Guney, E.; Survivin expression correlates with nodal metastasis in T1-T2 squamous cell carcinoma of the tongue. *Eur Arch Otorhinolaryngol,* **2015,** *272* (3), 689-94.

[117] Zhang, D.; Han, Y.; Xu, L.; Upregulation of miR-124 by physcion 8-O-beta-glucopyranoside inhibits proliferation and invasion of malignant melanoma cells via repressing RLIP76. *Biomed Pharmacother*, **2016,** *84*, 166-176.

[118] Lee, S.; Goldfinger, L. E.; RLIP76 regulates HIF-1 activity, VEGF expression and secretion in tumor cells, and secretome transactivation of endothelial cells. *FASEB J*, **2014,** *28* (9), 4158-68.

[119] Lee, S.; Wurtzel, J. G.; Singhal, S. S.; Awasthi, S.; Goldfinger, L. E.; RALBP1/RLIP76 depletion in mice suppresses tumor growth by inhibiting tumor neovascularization. *Cancer Res*, **2012,** *72* (20), 5165-73.

[120] Steeg, P. S.; Tumor metastasis: mechanistic insights and clinical challenges. *Nat Med*, **2006,** *12* (8), 895-904.

[121] AlQathama, A.; Prieto, J. M.; Natural products with therapeutic potential in melanoma metastasis. *Nat Prod Rep*, **2015,** *32* (8), 1170-82.

[122] Brabletz, T.; Hlubek, F.; Spaderna, S.; Schmalhofer, O.; Hiendlmeyer, E.; Jung, A.; Kirchner, T.; Invasion and metastasis in colorectal cancer: epithelial-mesenchymal transition, mesenchymal-epithelial transition, stem cells and beta-catenin. *Cells Tissues Organs*, **2005,** *179* (1-2), 56-65.

[123] Bhat, F. A.; Sharmila, G.; Balakrishnan, S.; Arunkumar, R.; Elumalai, P.; Suganya, S.; Raja Singh, P.; Srinivasan, N.; Arunakaran, J.; Quercetin reverses EGF-induced epithelial to mesenchymal transition and invasiveness in prostate cancer (PC-3) cell line via EGFR/PI3K/Akt pathway. *J Nutr Biochem*, **2014,** *25* (11), 1132-1139.

[124] Han, Y. T.; Chen, X. H.; Gao, H.; Ye, J. L.; Wang, C. B.; Physcion inhibits the metastatic potential of human colorectal cancer SW620 cells in vitro by suppressing the transcription factor SOX2. *Acta Pharmacol Sin*, **2016,** *37* (2), 264-75.

[125] Wang, W.; Guan, K. L.; AMP-activated protein kinase and cancer. *Acta Physiol (Oxf)*, **2009,** *196* (1), 55-63.

[126] Rattan, R.; Giri, S.; Singh, A. K.; Singh, I.; 5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside inhibits cancer cell proliferation in vitro and in vivo via AMP-activated protein kinase. *J Biol Chem*, **2005,** *280* (47), 39582-93.

[127] Thammasit, P.; Sangboonruang, S.; Suwanpairoj, S.; Khamaikawin, W.; Intasai, N.; Kasinrerk, W.; Tayapiwatana, C.; Tragoolpua, K.; Intracellular Acidosis Promotes Mitochondrial

Apoptosis Pathway: Role of EMMPRIN Down-regulation via Specific Single-chain Fv Intrabody. *J Cancer,* **2015,** *6* (3), 276-86.

[128] Gao, H.; Jiang, Q.; Han, Y.; Peng, J.; Wang, C.; shRNA-mediated EMMPRIN silencing inhibits human leukemic monocyte lymphoma U937 cell proliferation and increases chemosensitivity to adriamycin. *Cell Biochem Biophys,* **2015,** *71* (2), 827-35.

[129] Ke, X.; Fei, F.; Chen, Y.; Xu, L.; Zhang, Z.; Huang, Q.; Zhang, H.; Yang, H.; Chen, Z.; Xing, J.; Hypoxia upregulates CD147 through a combined effect of HIF-1alpha and Sp1 to promote glycolysis and tumor progression in epithelial solid tumors. *Carcinogenesis,* **2012,** *33* (8), 1598-607.

[130] Rhee, I.; Jair, K. W.; Yen, R. W.; Lengauer, C.; Herman, J. G.; Kinzler, K. W.; Vogelstein, B.; Baylin, S. B.; Schuebel, K. E.; CpG methylation is maintained in human cancer cells lacking DNMT1. *Nature,* **2000,** *404* (6781), 1003-7.

[131] Azizi, M.; Teimoori-Toolabi, L.; Arzanani, M. K.; Azadmanesh, K.; Fard-Esfahani, P.; Zeinali, S.; MicroRNA-148b and microRNA-152 reactivate tumor suppressor genes through suppression of DNA methyltransferase-1 gene in pancreatic cancer cell lines. *Cancer Biol Ther,* **2014,** *15* (4), 419-27.

[132] Mudbhary, R.; Hoshida, Y.; Chernyavskaya, Y.; Jacob, V.; Villanueva, A.; Fiel, M. I.; Chen, X.; Kojima, K.; Thung, S.; Bronson, R. T.; Lachenmayer, A.; Revill, K.; Alsinet, C.; Sachidanandam, R.; Desai, A.; SenBanerjee, S.; Ukomadu, C.; Llovet, J. M.; Sadler, K. C.; UHRF1 overexpression drives DNA hypomethylation and hepatocellular carcinoma. *Cancer Cell,* **2014,** *25* (2), 196-209.

[133] Brodie, S. A.; Li, G.; El-Kommos, A.; Kang, H.; Ramalingam, S. S.; Behera, M.; Gandhi, K.; Kowalski, J.; Sica, G. L.; Khuri, F. R.; Vertino, P. M.; Brandes, J. C.; Class I HDACs are mediators of smoke carcinogen-induced stabilization of DNMT1 and serve as promising targets for chemoprevention of lung cancer. *Cancer Prev Res (Phila),* **2014,** *7* (3), 351-61.

[134] Kim, E. K.; Park, J. M.; Lim, S.; Choi, J. W.; Kim, H. S.; Seok, H.; Seo, J. K.; Oh, K.; Lee, D. S.; Kim, K. T.; Ryu, S. H.; Suh, P. G.; Activation of AMP-activated protein kinase is essential for lysophosphatidic acid-induced cell migration in ovarian cancer cells. *J Biol Chem,* **2011,** *286* (27), 24036-45.

[135] Devanand, P.; Kim, S. I.; Choi, Y. W.; Sheen, S. S.; Yim, H.; Ryu, M. S.; Kim, S. J.; Kim, W. J.; Lim, I. K.; Inhibition of bladder cancer invasion by Sp1-mediated BTG2 expression via inhibition of DNA methyltransferase 1. *FEBS J,* **2014,** *281* (24), 5581-601.

[136] Wang, P.; Zhu, L.; Sun, D.; Gan, F.; Gao, S.; Yin, Y.; Chen, L.; Natural products as modulator of autophagy with potential clinical prospects. *Apoptosis,* **2017,** *22* (3), 325-356.

[137] Lin, S. R.; Fu, Y. S.; Tsai, M. J.; Cheng, H.; Weng, C. F.; Natural Compounds from Herbs that can Potentially Execute as Autophagy Inducers for Cancer Therapy. *Int J Mol Sci,* **2017,** *18* (7).

[138] Li, H. Y.; Zhang, J.; Sun, L. L.; Li, B. H.; Gao, H. L.; Xie, T.; Zhang, N.; Ye, Z. M.; Celastrol induces apoptosis and autophagy via the ROS/JNK signaling pathway in human osteosarcoma cells: an in vitro and in vivo study. *Cell Death Dis,* **2015,** *6*, e1604.

[139] Liu, Y.; Zhao, L.; Ju, Y.; Li, W.; Zhang, M.; Jiao, Y.; Zhang, J.; Wang, S.; Wang, Y.; Zhao, M.; Zhang, B.; Zhao, Y.; A novel androstenedione derivative induces ROS-mediated autophagy and attenuates drug resistance in osteosarcoma by inhibiting macrophage migration inhibitory factor (MIF). *Cell Death Dis,* **2014,** *5*, e1361.

[140] Zhou, X.; Seto, S. W.; Chang, D.; Kiat, H.; Razmovski-Naumovski, V.; Chan, K.; Bensoussan, A.; Synergistic Effects of Chinese Herbal Medicine: A Comprehensive Review of Methodology and Current Research. *Front Pharmacol,* **2016,** *7*, 201.

[141] Pan, X.; Wang, C.; Zhang, T.; Physcion Synergistically Enhances the Cytotoxicity of Sorafenib in Hepatocellular Carcinoma. *Anat Rec (Hoboken),* **2019**.

[142] Liu, W.; He, J.; Yang, Y.; Guo, Q.; Gao, F.; Upregulating miR-146a by physcion reverses multidrug resistance in human chronic myelogenous leukemia K562/ADM cells. *Am J Cancer Res,* **2016,** *6* (11), 2547-2560.

[143] Sarfraz, I.; Rasul, A.; Hussain, G.; Shah, M. A.; Zahoor, A. F.; Asrar, M.; Selamoglu, Z.; Ji, X. Y.; Adem, S.; Sarker, S. D.; 6-Phosphogluconate dehydrogenase fuels multiple aspects of cancer cells: From cancer initiation to metastasis and chemoresistance. *BioFactors,* **2020**.

[144] Zheng, W.; Feng, Q.; Liu, J.; Guo, Y.; Gao, L.; Li, R.; Xu, M.; Yan, G.; Yin, Z.; Zhang, S.; Liu, S.; Shan, C.; Inhibition of 6-phosphogluconate Dehydrogenase Reverses Cisplatin Resistance in Ovarian and Lung Cancer. *Front Pharmacol,* **2017,** *8*, 421.

[145] Liu, R.; Li, W.; Tao, B.; Wang, X.; Yang, Z.; Zhang, Y.; Wang, C.; Liu, R.; Gao, H.; Liang, J.; Yang, W.; Tyrosine phosphorylation activates 6-phosphogluconate dehydrogenase and promotes tumor growth and radiation resistance. *Nat Commun,* **2019,** *10* (1), 991.

[146] Lin, R.; Elf, S.; Shan, C.; Kang, H. B.; Ji, Q.; Zhou, L.; Hitosugi, T.; Zhang, L.; Zhang, S.; Seo, J. H.; Xie, J.; Tucker, M.; Gu, T. L.; Sudderth, J.; Jiang, L.; Mitsche, M.; DeBerardinis, R. J.; Wu, S.; Li, Y.; Mao, H.; Chen, P. R.; Wang, D.; Chen, G. Z.; Hurwitz, S. J.; Lonial, S.; Arellano, M. L.; Khoury, H. J.; Khuri, F. R.; Lee, B. H.; Lei, Q.; Brat, D. J.; Ye, K.; Boggon, T. J.; He, C.; Kang, S.; Fan, J.; Chen, J.; 6-Phosphogluconate dehydrogenase links oxidative PPP, lipogenesis and tumour growth by inhibiting LKB1-AMPK signalling. *Nat Cell Biol,* **2015,** *17* (11), 1484-96.

[147] Elf, S.; Lin, R.; Xia, S.; Pan, Y.; Shan, C.; Wu, S.; Lonial, S.; Gaddh, M.; Arellano, M. L.; Khoury, H. J.; Khuri, F. R.; Lee, B. H.; Boggon, T. J.; Fan, J.; Chen, J.; Targeting 6-phosphogluconate dehydrogenase in the oxidative PPP sensitizes leukemia cells to antimalarial agent dihydroartemisinin. *Oncogene,* **2017,** *36* (2), 254-262.

[148] Chen, H.; Wu, D.; Bao, L.; Yin, T.; Lei, D.; Yu, J.; Tong, X.; 6PGD inhibition sensitizes hepatocellular carcinoma to chemotherapy via AMPK activation and metabolic reprogramming. *Biomed Pharmacother,* **2019,** *111*, 1353-1358.

[149] Guo, H.; Xiang, Z.; Zhang, Y.; Sun, D.; Inhibiting 6-phosphogluconate dehydrogenase enhances chemotherapy efficacy in cervical cancer via AMPK-independent inhibition of RhoA and Rac1. *Clin Transl Oncol,* **2019,** *21* (4), 404-411.

[150] Yang, X.; Peng, X.; Huang, J.; Inhibiting 6-phosphogluconate dehydrogenase selectively targets breast cancer through AMPK activation. *Clin Transl Oncol,* **2018,** *20* (9), 1145-1152.

[151] Ethier, C.; Tardif, M.; Arul, L.; Poirier, G. G.; PARP-1 modulation of mTOR signaling in response to a DNA alkylating agent. *PLoS One,* **2012,** *7* (10), e47978.

[152] Zhou, Y.; Han, Y.; Zhang, Z.; Shi, Z.; Zhou, L.; Liu, X.; Jia, X.; MicroRNA-124 upregulation inhibits proliferation and invasion of osteosarcoma cells by targeting sphingosine kinase 1. *Hum Cell,* **2017,** *30* (1), 30-40.