Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Review

Phenolic compounds, saponins and alkaloids on cancer progression: emphasis on p53 expression and telomere length

Somayeh Afsah Vakili[1], Ajay George[2], Seyed Abdulmajid Ayatollahi[3,4], Miquel Martorell[5,6], Elise Adrian Ostrander[7], Bahare Salehi[8*], Natália Martins[9,10*], Javad Sharifi-Rad[11*]

[1] Department of Pharmacology, Visveswarapura Institute of Pharmaceutical Sciences, Bangalore-560070, Karnataka, India
[2] Department of Pharmacology, St. Johns Pharmacy college, Bangalore-560104, Karnataka, India
[3] Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4] Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5] Department of Nutrition and Dietetics, Faculty of Pharmacy, University of Concepcion, Concepcion 4070386, Chile
[6] Universidad de Concepción, Unidad de Desarrollo Tecnológico, UDT, Concepción 4070386, Chile
[7] Medical Illustration, Kendall College of Art and Design, Ferris State University, Grand Rapids, MI, USA
[8] Student Research Committee, School of Medicine, Bam University of Medical Sciences, Bam, Iran
[9] Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal
[10] Institute for Research and Innovation in Health (i3S), University of Porto, 4200-135 Porto, Portugal
[11] Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[*]**Correspondence to:** bahar.salehi007@gmail.com; ncmartins@med.up.pt; javad.sharifirad@gmail.com

Received April 3, 2020; **Accepted** April 30, 2020; **Published** June 25, 2020

Doi: http://dx.doi.org/10.14715/cmb/2020.66.4.15

Abstract: Telomere length is correlated with cell proliferation, and cancer cells are characterized by an uncontrolled cell cycle. Being apoptosis one of the checks and balances incorporated into cells cycle, due to its characteristics, cancer cells are able to overcome this process. In particular, the tumour suppressor protein p53 loss or inactivation can lead to activation of telomerase enzyme, which can make cells unable to detect DNA damages that spurs apoptosis. Some bioactive compounds, in particular phenolic compounds, saponins and alkaloids have revealed good abilities to affect p53 expression and indirectly control the telomere length. In this sense, this review gives a key emphasis to the ability of these compounds in blocking cancer progression by acting on p53 expression and controlling telomere length. As main findings, phenolic compounds, saponins and alkaloids interfere with cancer progression by stimulating p53 expression, which can cause pro-apoptotic onset and restrict the anti-apoptotic activity, in addition to preventing telomerase enzyme activity.

Key words: Telomerase; p53; Phytochemical; Apoptosis; Phenolic compound; Saponin; Alkaloid.

Introduction

Telomeres are placed at end of chromosome and become shorted during each cell cycle (1). Telomere acts as an internal molecular clock that can decline the cell's division cycles number. Telomeres length is correlated with cell proliferation, in other hands, the telomerase activation and telomeres elongation can enhance cell's ability to proliferate (2, 3). One of the tumour suppressor mechanisms in eukaryotes cells is the replicative senescence, produced by the shortening of telomeres (4). The replicative immortality of cancer and stem cells is due to telomerase activation to sustain telomere length. Most cancer cells are able to skip this mechanism by activating telomerase, that can enhance the 3′- ends of telomeres. Telomerase enzyme can solve the end replication problem in cancer cells, promoting replicative senescence in normal somatic cells. This enzyme is used as an interesting biomarker in cancer detection and treatment, as it is mostly related to carcinogenesis (2, 3, 5). On the other side, the tumour suppressor protein p53 binds to the sub-telomere section and increases telomeres stability. So, p53 loss or inactivation makes cells unable to sense DNA damages that spurs apoptosis. Then, p53 deficiency can lead to telomerase activation, thus accelerating carcinogenesis (6).

Some bioactive compounds, such as phenolic compounds, saponins and alkaloids have shown to affect p53 expression and indirectly control telomere length (1, 7-9). Thus, this review specially addresses the ability of some bioactive molecules, namely phenolic compounds, saponins and alkaloids, to block cancer progression, specifically acting on p53 expression and telomere length.

Telomere and telomere shortening

Telomeres are regions of repetitive sequences (same short DNA sequences repeated over and over) at each 3′-end of eukaryotic chromosomes (3). In human beings, the telomeres sequence is TTAGGG. They are nucleoprotein protective caps over distal part of two sister chromatids in chromosome (3). Telomeres protect chromosome from degradation and entanglement with other

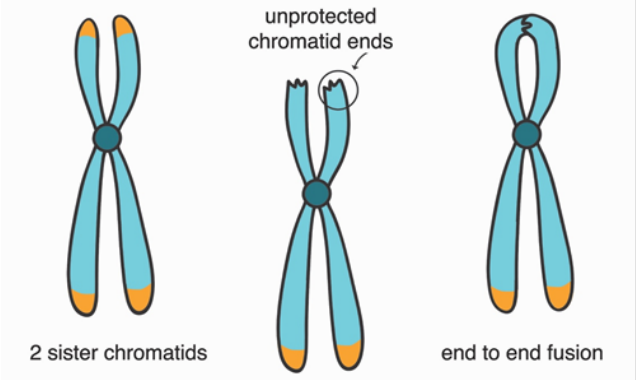

Figure 1. Mechanisms of breakage-fusion-bridge cycles.

chromosomes. In addition, they restrain fusion of two sister chromatid together (end to end fusion) (Figure 1) (3).

On average, each eukaryotic cell can divide 50-70 times before death, and the shortening of telomeres has been seen as a regulatory mechanism capable of controlling the replicative capacity. With successive replications, the cell's telomeres become smaller and smaller, until they become so short that cell division is blocked and cell goes into replicative senescence (Figure 2) (2, 3).

Shelterin complex and telomerase retain telomere length

Shelterin complex are telomeres-associated proteins, composed of three core shelterin subunits: telomere repeat factor-1 (TRF1) and -2 (TRF2), and protection of telomeres 1 (POT1) that identify and bind duplex TTAGGG repeat sequences and pinpoints and ties with single-stranded TTAGGG overhangs, respectively. In mammalian cells, telomeres length is controlled by telomerase and six telomere-associated proteins, known as "shelterin complex or telosome" that consist of TRF1, TRF2, POT1, Ras-proximate-1 (RAP1), TERF1-interacting nuclear factor 2 (TIN2) and tripeptidyl peptidase 1 (TPP1) (Figure 3). TRF1 and TRF2 are essential proteins for direct telomere duplex DNA binding (10).

TRF1 are assumed to check telomere length. TRF2, by contrast, assist to stabilize telomeric loop (t-Loop), inserting the chromosome end back into the chromosome DNA (10, 11). POT1 confers protection to telomere-1 protein, a negative regulator of telomerase activity (telomerase inhibition). POT1 conserve telomeres from rapid degradation by interconnecting with telomeres either through 3′ over hang G-strand DNA binding or TRF1 interplay (12). RAP1 is a Ras-related protein which interacts with TRF2 and enhances telomere binding. TIN2 is crucial for the association between TPP1/POT1 and TRF1/TRF2 that can stabilize TRF1 and TRF2. TPP1 is situated between TIN2 and POT1 that relates activity of TRF1 to POT1 (10). Telomerase is a ribonucleoprotein that conveys its own RNA (Figure 4). In stem and cancer cells, it adds to telomere during cell division and maintains telomere length (prevent telomere shortening) and ensures telomeres stability as well as cancer cells immortality (11). So, the eternal proliferation of cancer cells is due to silent human TERT gene (hTERT, human telomerase reverse transcriptase) up-regulation, that encodes telomerase (10).

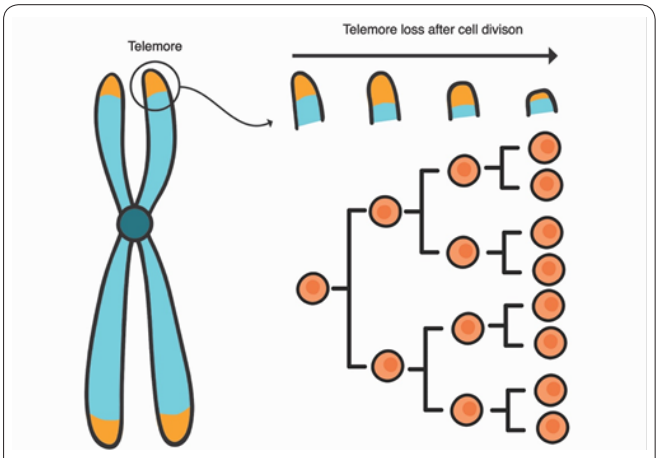

Figure 2. The Hayflick limit.

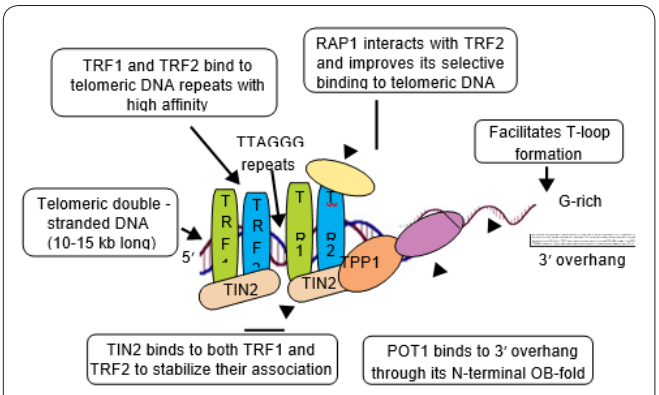

Figure 3. Shelterin complex or telosome.

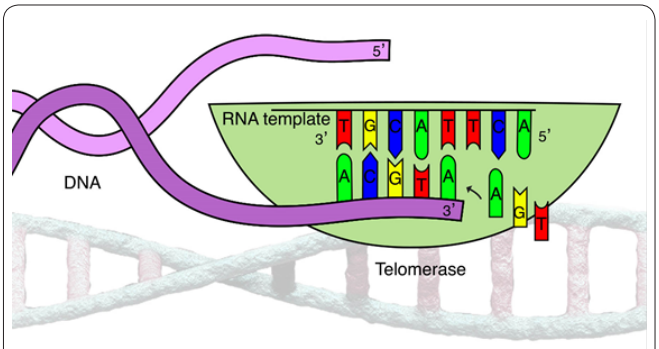

Figure 4. Telomerase protein components.

Cancer, apoptosis and P53 regulates telomerase activity

Apoptosis is a programmed cell death process, being even considered one of the checks and balances built into the cell cycle (13). Usually, when something goes wrong in a cell, it is rapidly impaired via apoptosis, which aids to exhibit cancer cells proliferation. On the other side, cancer cells are characterized by an uncontrolled proliferation cycle, and so, they are able to overcome apoptosis; however, without this process, these abnormal cells can subsist and form cancer cells. The tumour suppressor protein p53 loss or inactivation makes cells unable to sense DNA damages that spurs apoptosis. There are several checkpoints during cell division which allow for DNA repair; so, if the repair is done, the cycle continue to act normally; if not, p53 gene on p53 protein would trigger apoptosis (cell suicide). Hence, p53 prevents tumour formation that is why it is called as "tumour suppressor". Thus, p53 in-

activation is involved in cancer cells immortalization. Briefly, p53 binds to the sub-telomere section and thus increases telomeres stability. Consequently, telomeres can protect chromosome from end-to-end fusion. The fused chromosomes may lead to chromosome fusion-bridge-breakage cycles, fuelling cancer onset. Furthermore, p53 down-regulates telomerase activity through interaction with the telomerase RNA moiety and regulating protein phosphorylation. Ergo, p53 deficiency can lead to telomerase enzyme activation, which can accelerate carcinogenesis (6).

Dysfunctional telomere promotes activation of P53

Telomere dysfunction and telomere uncapping itself lead to chromosome end to end fusion which assists to DNA break. This chromosomal fusion bridge breakage initiates Ataxia-telangiectasia-mutated/Ataxia telangiectasia and Rad3-related (ATM/ATR) signalling pathway (14) which can impact DNA repair response and supply a barrier to tumour growth, inducing senescence, cell cycle division arrest (G1 arrest) and apoptosis (15). ATM and ATR proteins are prime DNA damage response (DDR) regulators, and sustain cell's genomic integrity. Briefly, ATM directly phosphorylates p53 and Chk2 checkpoint kinase that further stimulates p53 phosphorylation (15). ATR directly phosphorylates p53 (16). p53 activation influences p21 stimulation, another suppressor gene (Downstream transcription gene) that can hinder cyclin/cyclin dependent kinase complexes substrate pRb phosphorylation in G1 phase, which leads to prevent their activity as they are crucial elements in cell cycle division. Accordingly, it causes cell cycle arrest at G1; so, it renders time for repairing DNA (17). Promyelocytic leukemia (PML) gene supplies a protein that acts on tumour suppression. PML protein with p21 can block cell proliferation and induce apoptosis (14). p53 stimulation can influence on pro-apoptotic Bax activation and also promote p53 unregulated modulator of apoptosis (PUMA) and Noxa activity, components bind to Bcl-2 family (antiapoptotic) and block their action, leading to apoptosis (14).

Role of p53 on telomere stability

Tutton and Lieberman (18) reported that p53 protein is an essential tumour suppressor that activates the various transcription genes involved in cell stress response. p53 direct binding at human sub-telomeres quadrates with activation of transcription and magnifies telomere stability when exists DNA damage (18). Consequently, telomerase inhibitors (or telomere shortening) are now being considered as potential anticancer drugs. Strategies to inhibit telomerase activity have initiated a vigorous prevention of hTERT, that can assist to decrease the number of telomeres and ultimately the death of cancer cells (10). Telomere perpetuation is vital to corroborate the correct organs' size and function. Flores and Blasco (19) reported that p53-dependent response restrains epidermal stem cell function and organismal size in mice with telomere dysfunction. p53 initiates the cell response to various types of DNA damage, such as telomere damage, being able to distinguish telomere dysfunction in stem/progenitor cell populations; so, it can assist to enormous apoptosis of germ cells. Sequentially, p53 activity eradication impedes the shorting of telomeres leading to tumour genesis in telomerase-deficient mice without p53 (19). Artandi and Attardi (14) described pathways that connect telomeres and p53 in senescence, apoptosis, and cancer. p53 has a notable role in apoptotic responses to dysfunctional or damaging telomeres. p53 loss establishes an environment in which severely short telomeres are inadequately combined to create end-to-end chromosomal fusions, which result in chromosome fusion-bridge-breakage that can progress cancer predominantly in epithelial tissues, promoting gene changes (14). Eukaryotic cells proliferation relies on telomeres length and functions maintenance. Chin, Artandi (20) investigated the interaction between the dysfunction of telomeres and p53 in telomerase-deficient mice cells and organs. The simultaneous p53activation, telomeres shortening and genome instability terminates cells growth and induces apoptosis. On the other side, p53 deletion/deficiency partakes to stimulate the transformation process only in prior stage of genetic crisis which boosts carcinogenesis (20).

Expression of p53 prohibits telomerase activity

Zhang, Tu (21) evaluated p53 activation, retarded telomerase activity and estrogenic beta receptor promotion, which was related to the anti-proliferative effect of the combination of ovarian hormones and retinoid in sempiternal human epithelial cells. This research revealed that the anti-proliferative effect of estrogen/progesterone (E/P) and retinoid is p53 activation-dependent following stimulation of p21 expression. The up-regulation of estrogenic beta receptor and obstruction of telomerase activity are also linked to E/P and retinoid mediated growth prohibition (21). The role of biomarkers, such as p53, Bcl-2 and telomerase activity was scrutinized on breast cancer patients in Egypt (22), in order to appraise the prognostic relevance of such markers implied in apoptosis and carcinogenesis. A notable expression of Bcl-2, mutant p53 proteins and a considerable telomerase activity were stated in patients with malignant breast cancer when compared to benign ones. The analysis divulged that p53 and telomerase activity are the only biomarkers indicating a significant correlation with each other, thus might predict tumour recurrence. Additionally, telomerase activity was more pronouncedly exhibited in late than in early stages. Bcl-2 did not reveal any direct association with telomerase activity. Hence, telomerase activity seems to be independent of Bcl-2 protein expression. This lack of correlation could be ascribed to the fact that p53 can directly affect Bax activity in apoptosis process. Bcl-2 overexpression increases angiogenesis, particularly enhancing the vascular endothelial cell growth factor (VEGF) protein secretion level. Mutant p53 expression was related with enhanced VEGF expression level, which has been recommended to be the major angiogenic factor in human tumours (22). p53 inactivation and telomerase activation take place in most human cancers, thus enhancing the possibility of a relation among both pathways. Shats, Milyavsky (23) reported that p53 protein overexpression lead to telomerase activity down-regulation in different cancer cell lines by suppressing

catalytic subunit transcription, hTERT, that was found to be cell type-specific. The absence of p53 binding to the hTERT promoter was also evaluated by south western and chromatin immune precipitation experiments, which increased the likelihood of an indirect restrain mechanism. Mutational analysis recognized a specific E2F (transcription factor) site responsible for p53-mediated repression, triggering hTERT promoter activity down-regulation abolishment. Moreover, it was found that hTERT prevention through p53 is p21 activation-mediated, which can inhibit Rb (retinoblastoma) family activity using viral oncoproteins or RNA interference (23). Stampfer, Garbe (24) demonstrated a novel effect of p53 loss on immortal transformation, comparing human mammary epithelial cell (HMEC) lines immortally transformed without functional p53. This research discloses that dysfunctional p53 can directly influence malignancy through effects on progression conversion that enhance telomerase activity stimulation in human mammary epithelial cell lines of unlimited lifespan. This investigation also supplied evidence that the role of p53 in preventing telomerase enzyme activity is distinguishable from its function of cell cycle checkpoint (24). Gonzalez-Suarez, Flores (25) described the cooperation between p53 mutation and elevated telomerase transgenic expression in the spontaneous development of cancer. The incidence of aging/spontaneous cancer in mice with transgenic telomerase expression was assessed in a wide range of adult somatic tissues and K5-Tert mice. K5-Tert mice exhibited a reduction in life span compared to wild-type cohorts related to a higher frequency of pre-neoplastic and neoplastic lesions in various types of tissues. In K5-Tert mice, neoplasias coexisted with transgene expression in the affected tissues. These data recommended that high telomerase activity may contribute to genetic changes that occur with age to boost carcinogenesis. Furthermore, cancer development and the decreased K5-Tert mice viability were more severe a in a p53 mutation, suggesting that telomerase enzyme cooperates with decreased p53 function in promoting carcinogenesis. Based on the data, high telomerase activity levels cause a decrease in life span, associated with an increase in neoplasias as the body ages (25). Li, Cao (26) elucidated the molecular interactions *in vitro* between telomerase and p53, and found a direct interaction between telomerase enzyme and mutant p53 in the nuclear lysates of human breast cancer cells and with recombinant human p53. The obtained results demonstrated that the carboxyl-terminal region of both p53 types interacts directly with human telomerase associated protein 1 (hTEP1), causing impediment to telomerase activity (26).

Bioactive compounds on p53 expression and telomeres length

Phenolic compounds activate p53

Phenolic compounds are naturally-occurring biomolecules characterized by a structure containing at least one benzene ring and one hydroxyl group substituted. Structurally, they can be classified into 2 main groups: flavonoids and non-flavonoids. Flavonoids are often found in fruits, vegetables, seeds, spices, herbs, tea, cocoa, and wine, and are divided into 6 subclasses: anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and isoflavonoids. Non-flavonoids can be subclassified into phenolic acids (benzoic and cinnamic acids), tannins, stilbenes and curcuminoids, such as curcumin, obtained mainly from dried turmeric and curry powder. Etienne-Selloum, Dandache (7) provided information obtained from current investigation concerning phenolic compounds-induced apoptosis through tumour suppressor (mainly p53 and p73) regulation. Under physiological conditions, p53 down-regulation can be done by three mechanisms: i) action of the mouse's double minute protein (MDM2) causes ubiquitin-mediated protosomal degradation, ii) reduction of nuclear export-induced nuclear level, and iii) suppression of chromatin transcriptional. Phenolic compounds can inhibit MDM2 expression, involved p53 overexpression. Moreover, p53 stabilization and activation is linked to serine/threonine residues phosphorylation, and polyphenols can also regulate the p53 phosphorylation. The role of p53 and p73 is also controlled through acetylation on different lysine residues, and polyphenols also monitor and maintain acetylation (7). Gupta, Thakur (27) reported that green tea polyphenols were able to trigger p53-dependent and p53-independent apoptosis in prostate cancer cells by 2 distinct mechanisms: i) p53 stabilization and p21/waf1 and Bax activation, mainly in LNCaPshV cells, simultaneously up-regulating Fas by c-jun-N-terminal kinase activation, thus activating caspase-8 and apoptosis in LNCaPshV and LNCaP-shp53 cells, also activating Bid as pro-apoptotic protein; and ii) Akt and BAD deactivation as antiapoptotic protein, mainly in LNCaPshp53 cells. Then, it triggers mitochondrial transmembrane potential loss and cytochrome-c release and terminal caspases activation (27). The interaction between three types of flavonoid compounds (e.g., wogonin, apigenin and baicalein) and p53 genes in an ovarian cancer cell line was also assessed (28). The flavonoid compounds were packed in biodegradable nanoparticles to enhance their bioavailability, and the assessment of their anticancer activity on cellular function was done through *in vitro* assays (i.e. cell viability, growth curve and cell cycles). Ovarian cancer cell line (OVCAR4 cell line) was treated using nanoparticles containing wogonin, apigenin or baicalein for 48 h. The p53 gene functions in cells were controlled by transfecting cells with human papillomavirus (HPV) E6 oncogene (HPV16 E6) cloned into a pCMV plasmid, which degraded the p53 protein through the ubiquitin dependent proteolytic pathway, consequently diminishing p53 gene production. Cells without transfection were considered as control group; then, both transfected and non-transfected cells were X-ray exposed. X-ray radiation led to DNA damages in cells promoting p53 expression. The p53 and p21 genes expression was estimated by RT-PCR. Cells with HPV16 E6 transfection exhibited no significant rise in p53 expression, demonstrating that transfections retarded p53 production, but non-transfected cells revealed an increase in p53 expression upon exposure to X-ray. The above results were attained due to reaction of cells to DNA damage by p53 and p21 overexpression. Wogonin exhibited potential anticancer activity by reducing cells viability and growth rate compared to other flavonoids. Moreover, wogonin increased the prevention of HPV16 E6 trans-

fected cells division in G0/G1 phases (28). Moghtaderi, Sepehri (29) displayed the combination of gallic acid and curcumin as natural phenolic compounds on MDA-MB-231 breast cancer cells by assessing their cytotoxic potential and ability to induce apoptosis. Mitochondrial membrane potential, cell cycle analysis, MTT assay, fluorescence microscopy, nitrite detection, reactive oxygen species (ROS) and glutathione levels, annexin V assay, RT-PCR and Western blotting methods were used. The fluorescent staining and Annexin V/PI assay displayed that apoptotic cells were remarkably enhanced in the group treated with the combination of gallic acid and curcumin and also crucially declined Bcl-2 level, while improved p53 expression and followed up in enhancing in Bax expression and caspase-3 and poly-ADP-ribose-polymerase (PARP) levels in MDA-MB-231 cells (29).

Alkaloids activate p53

Avtanski, Nagalingam (9) reported that indolo-pyrido-isoquinolin-based alkaloid prevents breast cancer cells growth, invasion and migration through p53-miR34a axis activation. As tumour cells are sensitive to p53 activation, new targeted therapies have been searched acting on tumour suppressor p53, which has a vital function in restraining cancer growth and proliferation through several biological processes (*i.e.* apoptosis, inhibition of cells growth, prevention of angiogenesis, obstruction of migration and metastasis). The current research investigates synthesis of various indolo-pyrido-isoquinolin-based alkaloids to promote p53 function and evaluated their therapeutic efficacy via NCI-60 screening. Molecular verification issued that 11-methoxy-2,3,4,13-tetrahydro-1H-indolo[2',3':3,4] pyrido[1,2-b]isoquinolin-6-ylium-bromide (termed P18/NSC-768219) suppressed proliferated cancer cells survival. P18 was still able to intercede on p53 activation, thus enhancing nuclear localization and raising p53 target genes expression. The assessment in isogenic cancer cells with p53 deficiency revealed crucial p53 effects in mesenchymal and epithelial genes changes and metastasis suppression in cancer cells p18-mediated. Additionally, p18 enhanced miR-34a expression in a p53-dependent way which may be essential for the interceded p18 obstruction of growth, metastasis and mammosphere formation in breast cancer cells (9). Hammerova, Uldrijan (30) showed that the benzo[c] phenanthridine alkaloids have marked anti-proliferative effects in malignant melanoma cells despite their p53 status. In this research, the anticancer activity of the five benzo[c]phenanthridine alkaloids (e.g. sanguinarine, chelerythrine, chelidonine, sanguilutine and chelilutine) was evaluated by assessing the protein level, DNA, apoptotic activity and cell response to alkaloid remedy in a p53-dependent way. The above mentioned types of alkaloids, in spite of other alkaloids, demonstrated strong anti-proliferative activity in a p53-independent manner. Chelilutine, chelerythrine and sanguinarine triggered apoptosis, diminishing the antiapoptotic protein (*e.g.* Bcl-xL, Mcl-1, X1AP) levels and increasing caspase 3 release and poly (ADP-ribose) polymerase cleavage (30).

Aaptamine is a spongean alkaloid which enables p21 promoter activation in a p53-independent fashion (31). p21 is also called cyclin dependent kinase inhibitor (CDKI) recognized for targeting p53 protein. It also acts as a negative regulator of cell cycle division. Thus, p21 expression is regulated by diverse mechanisms in a p53-dependent manner. Previous investigation displayed evidence regarding mutant p53 manifestation in many human cancer cells, whereas p21 mutation was infrequently reported. Ergo, the compounds can enhance p21 expression in a p53-independent way, thus enabling to suppress or remedy cancers. Aaptamine exhibited ability to dose-dependently activate p21 transfected in MG63 cells at doses of 20-50 μM. This activation was performed by Sp1 sites between 82 and 50 bp independently of p53. Hence, MG63 cell cycle division was inhibited at the G2/M phase within 48 h (31). Shaer (32) revealed that the crude alkaloids extract of *Rhazya stricta* Decne. was able to induce apoptosis in pancreatic cancer cells (PANC-1and AsPC-1), through assessment of p53 and Bcl-2 expression, as apoptotic and antiapoptotic markers, respectively. The obtained data displayed that the alkaloid extract at dose of 10 and 100 μg/ml can enhance the p53 mRNA expression, while Bcl-2 mRNA expression diminished, leading to cancer cells apoptosis (32). Habartova, Havelek (33) revealed that scoulerine, an isoquinoline alkaloid, can affects the microtubules structure, arrest cell cycle division and suppress cancer cells proliferation; therefore, it lead to cancer cells apoptotic death. The assessment was done for apoptosis activation through measuring DNA fragmentation using TUNEL assay, determining caspase activity and p53 expression in MOLT-4 cells. Inclusively, scoulerine exhibited ability to activate caspases-3/7, -8 and -9, recommending the association of an extrinsic and intrinsic pathway on programmed cell death (33). Rattanawong, Payon (34) revealed the anticancer potential of cepharanthine, a biscoclurine alkaloid, isolated from *Stephania cephalantha* Hayata, in p53 mutated colorectal cancer cells through p21 up-regulation. The previous investigation revealed that p53 mutation can apply chemo-resistance through several mechanisms, such as drug efflux, cell cycle regulation disruption, apoptosis avoidance and DNA repair up-regulation. That study was designed to assess the anticancer activity of cepharanthine in p53 mutant versus p53 wild-type colorectal cancer cells and to determine its mode of action. Cepharanthine displayed a higher efficiency in growth prevention towards the p53 mutant colorectal cancer cell lines (e.g. HT-29 and SW-620) than in p53 wild-type colorectal cancer cell lines (e.g. COLO-205 and HCT-116). Data revealed that cepharanthine can restrain cell cycle division and apoptosis via enhancing p21waf1/Cip1 expression in p53 mutant cell line. Moreover, cepharanthine treatment led to a decline in Bcl-2 and cyclin A expression. Thus, these findings revealed that cepharanthine is an alkaloid that can be suitable for recommending novel anticancer compounds against p53 mutant colorectal cells, resistant to chemotherapeutic agents (34). Aloperine, a quinolizidine alkaloid, has been shown to arrest cell cycle division at G2/M phase and induce apoptosis in HCT116 human colon cancer cells. Thus, in a study, this molecule was isolated from *Sophora alopecuroides* L. leaves, a plant traditionally used as anti-inflammatory, anti-allergic, antitumor, and antiviral. This alkaloid induced cell cycle suppression through ascending p21, p53 and Bax protein expression

and reducing cyclin D1, B1 and Bcl-2. In addition, aloperine prevented phosphatidylinositol 3-kinase/Akt and JAK/Stat3 pathways (35). The alkaloid fraction of jarong (*Achyranthes aspera* L.) leaf triggered apoptosis in breast cancer cells via p53 pathways, without necrosis stimulation, thus suggesting the anticancer potential of this plant, through safely inducing programmed cells death without stimulation of tissue inflammation. The process of apoptosis occurs through two pathways. The first one involves the role of telomere in chromosome protection, where telomerase enzyme can control telomere formation. Telomerase enzyme restriction can suppress telomere formation, causing chromosome break and cells death. The second pathway can be done through enhancing the activation of p53 and pro-apoptotic protein, such as Bax, that can stimulate mitochondria to release cytochrome-c. Cytochrome-c can stimulate apoptosis factor 1 (APAF1) followed by caspase-9 activation. Later on, activation of caspase 9 with caspase-8 can stimulate caspase-3 formation. Caspase-3 activation promotes DNase activity to fragment DNA, and consequently, die. In addition, apoptosis is related to cycline dependent kinases (CDK) (*i.e.* CDK1, CDK2, CDK4 and CDK6) activation. Thus, that study revealed that the alkaloid obtained from *A. aspera* leaf can induce apoptosis especially by increasing p53 gene expression and suppressing cycline dependent kinases. Moreover, it was also shown that the alkaloid extract obtained from *A. aspera* had better anticancer benefit over cyclophosphamide (36).

Saponin activates p53

One of the bioactive compounds with prominent anticancer effects are saponin, compounds constituted of sugar (glucose, galactose, rhamnose or xylose, etc.) and aglycones (sapogenin) on hydrolysis. Xu, Li (8) described that triterpenoid saponins (ginsenosides; ginsenoside Rg3, ginsenoside Rh2, other ginsenosides and saikosaponins A and D) and steroid saponins (dioscin, polyphyllin D, and timosaponin) isolated from Chinese medicinal plants reveal anticancer effects, by exerting anti-proliferative, anti-metastasis, anti-angiogenesis, anti-multidrug resistance, and autophagy regulation effects. Furthermore, Chinese scientists elucidated the signalling pathways and target proteins responsible for the anticancer effects of saponins (8). Two saponin fractions (4A3 and 4A4) isolated from *Securidaca longipedunculata* Fresen. were recognized and reported as triterpenoid glycosides, being enable to induce apoptosis in cervical tumor cell lines. In addition, as human papillomavirus (HPV) E6 oncogene has crucial role on cervical cells, the authors also assessed the possible pathways for apoptosis transmission. One of the suggested ideas was that saponin fractions 4A3 and 4A4 can induce late apoptosis and early apoptosis, respectively, by targeting E6 molecular activity which assist on p53 restoration follow by activation of pro-apoptotic proteins, such as Bax, Bak and Cyt, and also enhancing PUMA activity (37, 38).

One of the considerable causes of cancer death among men is lung cancer. Some naturally-occurring bioactive compounds have been shown to be beneficial for lung cancer. Samarakoon, Ediriweera (39) elucidated the cytotoxic and apoptotic potential of a triterpenoid saponin, 3-*O*-α-L-arabinosyl oleanolic acid, 3-O-L-AO, isolated from *Schumacheria castanifolia* Vahl in human non-small-cell lung cancer (NCI-H292) cells. The 3-O-L-AO produced more potential cytotoxicity in NCI-H292 cells than in normal lung (MRC-5) cells. The 3-O-L-AO exerted dose-dependent Bax and p53 up-regulation and survivin down-regulation in NCI-H292 cells. For bye, caspase 3/7 activation and the morphological features related to apoptosis proved that 3-O-L-AO induced apoptosis. Caspases activity were regulated by Bax and p53 (39). Zhang, Men (40) reported the antitumor effect of triterpene acid compounds divided into lupane, oleanane type, ursane type, cork type and lanostane type triterpene acid. Triterpene acid is one of the triterpene acid type which is to be in unbound state, and that can be classified into tetracyclic triterpene and pentacyclic triterpene. Pentacyclic triterpenes are the most commonly used in traditional Chinese medicine(41). Among the above mentioned triterpene acid compounds, lanostane belongs to the tetracyclic triterpenes(42, 43). These compounds can repress tumour proliferation by caspase-3 activation which induces apoptosis through up-regulating the pro-apoptotic protein Bax and p53 tumour suppression and down-regulating the antiapoptotic protein Bcl-2 expression. They are able to block tumour cell cycle growth in G1 phase through activation of MAPK/EKR signalling pathway and to up-regulate the CDK inhibitor p16 or G2/M phase by diminishing cyclin Bi/cdc2 activity. Furthermore, they can inhibit cell entering into the S phase of cell cycle division process through preventing cyclin A expression. These compounds can retard the expression of angiogenic factors, VEGF-A and bFGF, also down-regulating sonic hedgehog (SHH), STAT3, Akt and p70S6K pathways. Ergo, they can restrain angiogenesis, achieving the anticancer goal. These compounds can also inhibit metastasis and tumour cell invasion by reducing cell's adhesion to laminin; so, it can decrease cathepsin B secretion and finally decline migration or induce matrix metalloproteinase (MMP) blockage and down-regulate intracellular junctional adhesion molecule (JAM). Moreover, they can enhance cancer cell adhesion to extracellular matrix by exhibiting the potent sialyltransferase inhibitor (38, 40, 44). Escobar-Sánchez, Sánchez-Sánchez (45) reported that steroidal saponins have ability to initiate apoptosis. Various studies have been performed to assess the anticancer activity of steroid saponins obtained from different plants, viz *Panax ginseng* C.A.Mey., *Solanum chrysotrichum* Schltdl., *Dillenia suffruticosa* (Griff.) Martelli, *Withania somnifera* (L.) Dunal, *Asparagus officinalis* L., *Paris polyphylla* Sm., *Allium flavum* L., *Allium macrostemon* Bunge, *Rohdea chinensis* (Baker) N.Tanaka, against different types of cancer cell lines. The *in vitro* evaluation of the anticancer potential was done on breast (MCF-7, MDA, MB-231 and HT-9) and colorectal (Caco2, SW480) cancer cell lines, glima (C6), hepatocarcinoma (HepG2, SMMC-7721), leukaemia (HL-60), lung cancer (A549) and melanoma (B16). The *in vivo* assessments were carried out on lung cancer bearing -C57BL/6 mice. It was shown that apoptosis can be initiated through two pathways: 1) extrinsic pathway that includes the cytoplasmic membrane receptor participation, and 2) intrinsic pathway involving pro-apoptotic proteins delivery by mitochondria. Steroi-

dal saponins revealed to be able to activate both routes. The extrinsic pathway can stimulate cell death receptors (Death receptor 5) present in cell's cytoplasmic membrane. The saponins' molecular activity was found to be related to their structural composition and that the hetero-sugar moiety leads to steroidal saponins heteropolarity, prompting to different membrane permeability and selectivity in the bioactivity of the compounds. Steroidal saponins can initiate apoptosis by activation of the intrinsic pathway. Saponins can target the mitochondria and endoplasmic reticulum, promoting cytochrome-c release from mitochondria. In addition, the cytotoxic effect of steroidal saponins can be influenced at distinct molecular levels, including internal transcription factors, such as NF-κB, in addition to exerting p53 tumour suppression and p38 MAPK signalling pathway that increase caspases level (45). *P. polyphylla*-derived saponins were assessed for their ability to inhibit bladder cancer cells growth, through mutant p53 degradation induction and up-regulation of cyclin dependent kinase inhibitor A1 (CDKN1A) expression. Total steroidal saponins were obtained from *P. polyphylla* var. *yunnanensis* (Franch.) Hand.-Mazz. by ethanol extraction. These saponins were used to remedy of bladder cancer cells line, such as HT1197 and J82, which contain mutant p53. Gene expression was ascertained using qPCR and immunoblotting, and cell cycle using flow cytometry. DNA damage response activation was analysed through immunofluorescence staining. The results revealed that steroidal saponins lead to a dose-dependent reduction in both HT1197 and J82 cancer cells number. Cell growth was ceased at G2/M phase by apoptosis activation. Thus, it was shown that the anticancer effect of total steroidal saponins derived from *P. polyphylla* var. *yunnanensis* might induce mutant p53 degradation and concurrently activate CDKN1A gene transcription. As previously referred, CDKN1A gene is known as p21, and p53 is a prime regulator of the CDKN1A expression. Thus, p21 is a key target of p53 activity and, thus, is linked to DNA damage which lead to cell cycle arrest by preventing cyclin/CDK complexes formation (46).

Role of bioactive compounds to control telomere length

Phenolic compounds

Shin, Zoh (1) demonstrated that phenolic extracts from walnut diminish telomere length and telomerase activity in a model of colon cancer stem cell (CSC). $CD133^{+}CD44^{+}$ cells obtained from the human colon cancer cell line HCT116 were sorted by FACS, and treated with 10, 20 and 40 μg/mL of walnut phenolic extract for 6 days. Telomeres length was evaluated by qRT-PCR, and RT-PCR was also applied to determine hTERT and c-MYC. The obtained results manifested a dose-dependent decline in telomere length, which was correlated with a reduction in hTERT and c-MYC transcriptions and telomerase activity. Hence, walnut phenolic extract could diminish cancer cells viability (1). Savelyev, Baykuzina (47) already evinced through a comprehensive analysis of the gallotannin effects on telomerase inhibition. Gallotannin is a tannic acid compound obtained from plants, such as *Caesalpinia spinosa* (Molina) Kuntze, *Rhus chinensis* Mill. and *Quercus infectoria* G.Olivier. It can suppress cell growth and the activity of various DNA-polymerases, such as telomerase. Current research suggested that telomerase inhibition could happen due to reduction in the quantity of telomerase components without any changes in hTR and hTER number in HEK293T cells, while the observed results obtained showed a decline in the level of hTERT inhibitor NF-κB expression in A549 cells treated with gallotannin. Indeed, gallotannin has revealed to have a role poly (ADP-ribose) glycohydrolase suppression, which can split poly(ADP-ribose) branches by poly (ADP-ribose) polymerase synthesis. This modification can supply proteins covalently joined to negative charged and branched poly(ADP-ribose) polymers that can affect the interaction between protein and nucleic acid. The telomerase complex competence is crucial for telomerase activity regulation that can be target for discovering novel telomerase inhibitors. Telomerase gathering can be influenced by inhibitor interaction with telomerase components or with supplementary cellular proteins, modulating telomerase complex formation. Inadequate collection of telomerase complexes, such as dyskerin mutations, can diminish the level of the active telomerase enzyme in cells (47). Kaewtunjai, Wongpoomchai (4) described the effect of *Zingiber officinale* Roscoe (ginger) extract in elevating telomere shortening and cell division suppression in A549 lung cancer cells. The extract had telomerase inhibitory effect, restraining hTERT expression that could promote the decrease in hTERT protein level and telomerase activity. The data obtained by authors proved that *Z. officinale* extract can initiate telomere shortening and cell senescence in A549-treated cells over 60 days. The bioactive compounds paradols and shogaols, of various chain lengths, were found in *Z. officinale* extract. Ergo, pure 6-paradol and 6-shogaol could inhibit hTERT expression and telomerase activity, besides to reveal anticlastogenic activity on diethylnitrosamine-induced liver micronucleus formation in rats. This data proved the non-toxic effect of compounds; so, ginger extract can be recommended for dietary cancer prevention (4). Abliz, Mijit (48) demonstrated the anti-carcinogenic effects of the phenolic-rich extract from abnormal Savda Munziq (ASMq), a traditional uyghur medicine, by assessing its cytotoxic effect, potential to induce apoptosis and on telomerase activity in human cervical cancer cells (SiHa). ASMq preparation was done mixing Pobumuguo (*Cordia dichotoma* G. Forst fruits), Niushecao (*Anchusa italica* Retz.), Gancao (*Glycyrrhiza uralensis* Fisch. root), Tiexianjue (*Adiantum capillus-veneris* L.), Dijincao (*Euphorbia humifusa* Willd.), Hongzao (*Ziziphus jujuba* Mill. fruits), Xunyicao (*Lavandula angustifolia* Mill. aerial parts), Xiaohuixiang (*Foeniculum vulgare* Mill. fruits), Mifenghua (*Melissa officinalis* L.) and Citang (*Alhagi pseudoalhagi* Desv. sugar secretion). Results revealed that the phenolic-rich extract significantly restrain cells viability and proliferation at concentrations of 75-175 μg/mL in a dose-dependent manner. Apoptosis induction also revealed to be time-dependent, in the same way that it can be correlated with anti-apoptotic Bcl-2 expression down-regulation and telomerase and survivin expression. Furthermore, phenolic-rich extracts led to a dose-dependent expression of fragile histidine triad protein (48).

Alkaloids

Kazemi Noureini, Fatemi (49) reported the effect of benzylisoquinoline alkaloid chelidonine on telomere shortening in breast cancer cells (MCF7) and found that the compound triggered cell death through different ways at same time that down-regulated telomerase enzyme activity. Breast cancer MCF7 cells were treated with low doses of chelidonine (0.01 or 0.05 μM) for 48 h after each passage. Assessment was done by monochrome multiplex qPCR and q-TRAP to estimate telomere length and telomerase activity, respectively. MCF7 cell growth was absolutely suppressed after third times treatments (0.1 μM chelidonine), while telomere length diminished to approximately 10% when compared to the untreated control. Furthermore, chelidonine restrained the telomerase activity in a dose- and time-dependent fashion, via changing hTERT correlation towards non-enzyme coding isoform of the transcript. Ergo, data manifested that chelidonine can prevent breast cancer cells growth by affecting telomere length, telomere stability and microtubule formation (49). Zhao and Wink (5) reported that β-carboline alkaloid harmine blocks telomerase activity in MCF-7 cells through hTERT mRNA expression down-regulation. DNA intercalation also seemed to be a beneficial pharmacological characters of the indole alkaloid harmine, which can promote frame shift mutations. The authors alos found that harmine exerted anti-proliferative activity in MCF-7 cells via cytotoxic activity, telomerase activity suppression and senescence phenotype administration, through over-expression of the p53/p21 pathway elements. Harmine was also able to affect DNA, RNA, or associated enzymes, as it intercalates DNA leading to DNA mutation and damage followed by anti-proliferation and cell death. Furthermore, cycline dependent kinases (CDK) suppression, such as CDK2 and CDK5 influenced harmine cytotoxicity and inhibited P450 plus DNA topoisomerase activity (5). Kazemi Noureini, Kheirabadi (50) evinced telomerase inhibition by a new synthetic derivative of the aporphine alkaloid boldine. Nowadays, predominant investigation programs in anticancer drug discovery are being undertaken on telomerase enzyme activity due to its involvement on cell immortality. Boldine is aporphine alkaloid obtained from *Peumus boldus* Molina and known to suppress telomerase at non-toxic concentration. N-benzylsecoboldine hydrochloride (BSB), a synthetic derivative of boldine, was assessed for cytotoxicity via MTT assay in MCF7 and MDA-MB231 cells. Docking and molecular dynamics analysis were applied to determine the crystal structure of TERT. The qTRAP-ligand data expressed the IC_{50} value of 0.17μM for BSB, which was 400 times stronger than boldine. Although both compounds binds to the active site, molecular dynamics revealed a second binding site, where BSB interacts through two hydrogen bonds, more strongly than boldine. BSB manifested more potential hydrophobicity and flexibility than boldine, which can prove structural evidence to prevent telomerase activity at non-toxic concentrations (50).

Saponins

Cycloastragenol is a type of triterpenoid saponin compound obtained from *Astragalus propinquus* Schischkin. Yu, Zhou (51) recommended cycloastragenol as a novel candidate for age-associated diseases. Cycloastragenol had more potential for wound healing in elderly mice than three other saponins obtained from *Astragalus* species, such as astragaloside IV, cyclocephaloside I, and cyclocanthoside E. Cycloastragenol showed to be an efficacious compound in improving wound healing, being able to initiate human keratinocyte proliferation and migration at 1 ng/mL, through telomerase activity stimulation. Moreover, the wound healing properties of cycloastragenol demonstrated recovery of brain injuries in both *in vitro* and *in vivo* experiments. In addition, cycloastragenol enhanced neural stem cells proliferation and increased survival and counters the effects of oxygen-glucose deprivation injury *in vitro.* Cycloastragenol was also capable to decline neuron apoptosis along with improvement in nerve functional scoring, decrease cerebral infraction volume in a rat model with cerebral ischemia reperfusion injury. These effects are correlated with increased TERT expression. Ergo, telomere length and stimulation of telomerase activity are correlated with tissue recovery, which is telomere dependent; so they are compelling for remedy targets and exhibit tissue recovery (51). Platycodin D (PD), a triterpenoid bidesmoside isolated from *Platycodon grandiflorus* (Jacq.) A.DC. roots, was assessed for antitumor effects in different human leukemia cell lines (U937, THP-1 and K562) (52). A dose-dependent cytotoxic effect was seen, as was inhibition of telomerase activity through down-regulating hTERT. In-PD treated cells, c-Myc and Sp1 protein levels (involved in hTERT transcription regulation) decreased together with their DNA binding activity. PD also down-regulated Akt activation, and thus reduced hTERT phosphorylation and nuclear translocation. These findings indicate that PD exerts cytotoxic effects by suppressing telomerase activity secondary to transcriptional and posttranslational suppression of hTERT (52).

Conclusion

Nautrally-occurring bioactive compounds, namely phenolic compounds, saponins and alkaloids have received an exponential interest in the couple years, given their prominent antioxidant and anti-inflammatory potentialities. Anyway, recent evidences have also pointed their remarkable antitumor abilities. Indeed, it has been shown that they can effectively prevent cancer by stimulation of p53 expression, which can trigger the pro-apoptotic and restrain the anti-apoptotic activity, besides to prevent telomerase enzyme activity by inhibiting hTERT, which can assist in the progressive telomere shortening and eventually death of cancer cells. In short, and although the current advances, further studies are needed to find more bioactives from plant origin with great activity on cancer prevention and treatment.

Acknowledgements

This work was supported by CONICYT PIA/APOYO CCTE AFB170007.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Shin PK, Zoh Y, Choi J, Kim MS, Kim Y, Choi SW. Walnut phenolic extracts reduce telomere length and telomerase activity in a colon cancer stem cell model. Nutrition research and practice. 2019;13(1):58-63.
2. Counter CM. The roles of telomeres and telomerase in cell life span. Mutation research. 1996;366(1):45-63.
3. Aksenova AY, Mirkin SM. At the Beginning of the End and in the Middle of the Beginning: Structure and Maintenance of Telomeric DNA Repeats and Interstitial Telomeric Sequences. Genes (Basel). 2019;10(2):118.
4. Kaewtunjai N, Wongpoomchai R, Imsumran A, Pompimon W, Athipornchai A, Suksamrarn A, et al. Ginger Extract Promotes Telomere Shortening and Cellular Senescence in A549 Lung Cancer Cells. ACS Omega. 2018;3(12):18572-81.
5. Zhao L, Wink M. The beta-carboline alkaloid harmine inhibits telomerase activity of MCF-7 cells by down-regulating hTERT mRNA expression accompanied by an accelerated senescent phenotype. PeerJ. 2013;1:e174.
6. Roblick RR. The P53 pathway: role of telomerase and identification of novel targets: acts of a master regulator of tumor suppression. [Thesis] Stockholm, Sweden: Karolinska Institute. 2007.
7. Etienne-Selloum N, Dandache I, Sharif T, Auger C, B V. Polyphenolic Compounds Targeting p53-Family Tumor Suppressors: Current Progress and Challenges. Future Aspects of Tumor Suppressor Gene, Yue Cheng, IntechOpen. 2013.
8. Xu X-H, Li T, Fong CMV, Chen X, Chen X-J, Wang Y-T, et al. Saponins from Chinese Medicines as Anticancer Agents. Molecules. 2016;21(10):1326.
9. Avtanski DB, Nagalingam A, Tomaszewski JE, Risbood P, Difillippantonio MJ, Saxena NK, et al. Indolo-pyrido-isoquinolin based alkaloid inhibits growth, invasion and migration of breast cancer cells via activation of p53-miR34a axis. Mol Oncol. 2016;10(7):1118-32.
10. Xin H, Liu D, Songyang Z. The telosome/shelterin complex and its functions. Genome Biol. 2008;9(9):232-.
11. Jafri MA, Ansari SA, Alqahtani MH, Shay JW. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Med. 2016;8(1):69-.
12. Kelleher C, Kurth I, Lingner J. Human protection of telomeres 1 (POT1) is a negative regulator of telomerase activity in vitro. Molecular and cellular biology. 2005;25(2):808-18.
13. Mishra AP, Salehi B, Sharifi-Rad M, Pezzani R, Kobarfard F, Sharifi-Rad J, et al. Programmed Cell Death, from a Cancer Perspective: An Overview. Molecular Diagnosis and Therapy. 2018;22(3):281-95.
14. Artandi SE, Attardi LD. Pathways connecting telomeres and p53 in senescence, apoptosis, and cancer. Biochemical and biophysical research communications. 2005;331(3):881-90.
15. Awasthi P, Foiani M, Kumar A. ATM and ATR signaling at a glance. Journal of cell science. 2015;128(23):4255-62.
16. Tibbetts RS, Brumbaugh KM, Williams JM, Sarkaria JN, Cliby WA, Shieh SY, et al. A role for ATR in the DNA damage-induced phosphorylation of p53. Genes & development. 1999;13(2):152-7.
17. Pellegata NS, Antoniono RJ, Redpath JL, Stanbridge EJ. DNA damage and p53-mediated cell cycle arrest: a reevaluation. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(26):15209-14.
18. Tutton S, Lieberman PM. A role for p53 in telomere protection. Molecular & cellular oncology. 2017;4(6):e1143078.
19. Flores I, Blasco MA. A p53-dependent response limits epidermal stem cell functionality and organismal size in mice with short telomeres. PloS one. 2009;4(3):e4934.
20. Chin L, Artandi SE, Shen Q, Tam A, Lee SL, Gottlieb GJ, et al. p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. Cell. 1999;97(4):527-38.
21. Zhang J, Tu Y, Smith-Schneider S. Activation of p53, inhibition of telomerase activity and induction of estrogen receptor beta are associated with the anti-growth effects of combination of ovarian hormones and retinoids in immortalized human mammary epithelial cells. Cancer cell international. 2005;5(1):6.
22. Swellam M, Ismail M, Eissa S, Hamdy M, Mokhtar N. Emerging role of p53, bcl-2 and telomerase activity in Egyptian breast cancer patients. IUBMB life. 2004;56(8):483-90.
23. Shats I, Milyavsky M, Tang X, Stambolsky P, Erez N, Brosh R, et al. p53-dependent down-regulation of telomerase is mediated by p21waf1. The Journal of biological chemistry. 2004;279(49):50976-85.
24. Stampfer MR, Garbe J, Nijjar T, Wigington D, Swisshelm K, Yaswen P. Loss of p53 function accelerates acquisition of telomerase activity in indefinite lifespan human mammary epithelial cell lines. Oncogene. 2003;22(34):5238-51.
25. Gonzalez-Suarez E, Flores JM, Blasco MA. Cooperation between p53 mutation and high telomerase transgenic expression in spontaneous cancer development. Molecular and cellular biology. 2002;22(20):7291-301.
26. Li H, Cao Y, Berndt MC, Funder JW, Liu JP. Molecular interactions between telomerase and the tumor suppressor protein p53 in vitro. Oncogene. 1999;18(48):6785-94.
27. Gupta K, Thakur VS, Bhaskaran N, Nawab A, Babcook MA, Jackson MW, et al. Green tea polyphenols induce p53-dependent and p53-independent apoptosis in prostate cancer cells through two distinct mechanisms. PloS one. 2012;7(12):e52572.
28. Yu L, Zheng S, Chen Y, Luo J. Assessing the interactions between p53 gene and three types of flavonoid therapeutic compound in ovarian cancer cell line. Int J Chin Exp Med. 2017;10(3):4764-71.
29. Moghtaderi H, Sepehri H, Delphi L, Attari F. Gallic acid and curcumin induce cytotoxicity and apoptosis in human breast cancer cell MDA-MB-231. Bioimpacts. 2018;8(3):185-94.
30. Hammerova J, Uldrijan S, Taborska E, Slaninova I. Benzo[c] phenanthridine alkaloids exhibit strong anti-proliferative activity in malignant melanoma cells regardless of their p53 status. Journal of dermatological science. 2011;62(1):22-35.
31. Aoki S, Kong D, Suna H, Sowa Y, Sakai T, Setiawan A, et al. Aaptamine, a spongean alkaloid, activates p21 promoter in a p53-independent manner. Biochemical and biophysical research communications. 2006;342(1):101-6.
32. Shaer NA. Can crude alkaloids extract of Rhazya stricta induce apoptosis in pancreatic cancer: In vitro study? Pathophysiology : the official journal of the International Society for Pathophysiology. 2019;26(1):97-101.
33. Habartova K, Havelek R, Seifrtova M, Kralovec K, Cahlikova L, Chlebek J, et al. Scoulerine affects microtubule structure, inhibits proliferation, arrests cell cycle and thus culminates in the apoptotic death of cancer cells. Scientific reports. 2018;8(1):4829.
34. Rattanawong A, Payon V, Limpanasittikul W, Boonkrai C, Mutirangura A, Wonganan P. Cepharanthine exhibits a potent anticancer activity in p53-mutated colorectal cancer cells through upregulation of p21Waf1/Cip1. Oncology reports. 2018;39(1):227-38.
35. Zhang L, Zheng Y, Deng H, Liang L, Peng J. Aloperine induces G2/M phase cell cycle arrest and apoptosis in HCT116 human colon cancer cells. International journal of molecular medicine. 2014;33(6):1613-20.
36. Adnyana D, Meles ID, Meles W. Alkaloid fraction of jarong (Achyranthes aspera Linn) leaf induced apoptosis breast cancer cell through p53 pathways. Advances in Natural and Applied Sciences. 2012;6(2):124-7.

37. Obasi TC, Braicu C, Iacob BC, Bodoki E, Jurj A, Raduly L, et al. Securidaca-saponins are natural inhibitors of AKT, MCL-1, and BCL2L1 in cervical cancer cells. Cancer management and research. 2018;10:5709-24.
38. Cheng L, Xia T-S, Wang Y-F, Zhou W, Liang X-Q, Xue J-Q, et al. The apoptotic effect of D Rhamnose β-hederin, a novel oleanane-type triterpenoid saponin on breast cancer cells. PloS one. 2014;9(6):e90848-e.
39. Samarakoon SR, Ediriweera MK, Nwokwu CDU, Bandara CJ, Tennekoon KH, Piyathilaka P, et al. A Study on Cytotoxic and Apoptotic Potential of a Triterpenoid Saponin (3-O-alpha-L-Arabinosyl Oleanolic Acid) Isolated from Schumacheria castaneifolia Vahl in Human Non-Small-Cell Lung Cancer (NCI-H292) Cells. BioMed research international. 2017;2017:9854083.
40. Zhang W, Men X, Lei P. Review on anti-tumor effect of triterpene acid compounds. Journal of cancer research and therapeutics. 2014;10 Suppl 1:14-9.
41. Choudhary MI, Hussain A, Ali Z, Adhikari A, Sattar SA, Ayatollahi SAM, et al. Diterpenoids including a novel dimeric conjugate from salvia leriaefolia. Planta Medica. 2012;78(3):269-75.
42. Shamsabadipour S, Ghanadian M, Saeedi H, Reza Rahimnejad M, Mohammadi-Kamalabadi M, Ayatollahi SM, et al. Triterpenes and steroids from euphorbia denticulata lam. with anti-herpes symplex virus activity. Iranian Journal of Pharmaceutical Research. 2013;12(4):759-67.
43. Mesaik MA, Halim SA, Ul-Haq Z, Choudhary MI, Shahnaz S, Ayatollahi SAM, et al. Immunosuppressive activity of buxidin and E-buxenone from buxus hyrcana. Chemical Biology and Drug Design. 2010;75(3):310-7.
44. Zafar M, Sarfraz I, Rasul A, Jabeen F, Samiullah K, Hussain G, et al. Tubeimoside-1, Triterpenoid Saponin, as a Potential Natural Cancer Killer. Natural Product Communications. 2018;13(5):1934578X1801300530.
45. Escobar-Sánchez ML, Sánchez-Sánchez L, Sandoval-Ramírez J. Steroidal Saponins and Cell Death in Cancer. In: Tobias M, editor. Autophagy, Apoptosis and Necrosis: Ntuli, IntechOpen; 2015.
46. Guo Y, Liu Z, Li K, Cao G, Sun C, Cheng G, et al. Paris Polyphylla-Derived Saponins Inhibit Growth of Bladder Cancer Cells by Inducing Mutant P53 Degradation While Up-Regulating CDKN1A Expression. Current urology. 2018;11(3):131-8.
47. Savelyev N, Baykuzina P, Dokudovskaya S, Lavrik O, Rubtsova M, Dontsova O. Comprehensive analysis of telomerase inhibition by gallotannin. Oncotarget. 2018;9(27):18712-9.
48. Abliz G, Mijit F, Hua L, Abdixkur G, Ablimit T, Amat N, et al. Anti-carcinogenic effects of the phenolic-rich extract from abnormal Savda Munziq in association with its cytotoxicity, apoptosis-inducing properties and telomerase activity in human cervical cancer cells (SiHa). BMC Complementary and Alternative Medicine. 2015;15(1):23.
49. Kazemi Noureini S, Fatemi L, Wink M. Telomere shortening in breast cancer cells (MCF7) under treatment with low doses of the benzylisoquinoline alkaloid chelidonine. PloS one. 2018;13(10):e0204901.
50. Kazemi Noureini S, Kheirabadi M, Masoumi F, Khosrogerdi F, Zarei Y, Suarez-Rozas C, et al. Telomerase Inhibition by a New Synthetic Derivative of the Aporphine Alkaloid Boldine. International journal of molecular sciences. 2018;19(4).
51. Yu Y, Zhou L, Yang Y, Liu Y. Cycloastragenol: An exciting novel candidate for age-associated diseases. Experimental and therapeutic medicine. 2018;16(3):2175-82.
52. Kim MO, Moon DO, Choi YH, Shin DY, Kang HS, Choi BT, et al. Platycodin D induces apoptosis and decreases telomerase activity in human leukemia cells. Cancer letters. 2008;261(1):98-107.