JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2020/24474
RESEARCH ARTICLE

Changes in the Protein Profile of Cervical Cancer Mice Xenograft Model in Response to *Streblus asper* Treatment

Muhammad Nabil[1,2*], Azman Seeni[1], Wan Ismahanisa Ismail[2], Mohd Hafiz Mail[3] and Nurhidayah Ab. Rahim[2]

[1]Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia; nabilfikrimail@gmail.com

[2]Faculty of Health Science, Universiti Teknologi MARA, Cawangan Pulau Pinang, Kampus Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia

[3]Malaysian Institute of Pharmaceuticals and Neutraceuticals, National Institute of Biotechnology Malaysia

Abstract

Cervical cancer is the third most prevalent cancer in females (2018) with an estimation of 569,847 incidences and 311,365 deaths worldwide despite the rapid advancement of current technology in treating cervical cancer. Radiotherapy and chemotherapy pose side effects and subsequently hinder treatment efficacy. Therefore, taken together with the previous reports of the plants' ability in treating cancers, *Streblus asper* is suggested to be a potential candidate for cervical cancer. This study was conducted to investigate the anti-cervical cancer potential of *Streblus asper* through the identification of key proteins and their expression that are regulated in the treatment using mice xenograft model. By employing the use of Liquid Chromatography Mass Spectrometry (LCMS), several proteins associated with cancer growth mechanisms were successfully identified. Four-hundred and fifty-two proteins common to both groups were identified, and 122 proteins were found able to be quantified. Among those proteins, 52 proteins were expressed more than 2-fold changes and 12 proteins were selected based on its established relationship with cancers, including annexin A2, 14-3-3 protein, transgelin-2, galectin-1, keratin, heat shock protein 10 and 70, glucose regulated protein (78kDa), gelsolin, alpha enolase, cofilin-1, vimentin, and calreticulin. All these proteins were downregulated upon treatment of cervical cancer tumour by *Streblus asper*. Pathway enrichment analysis revealed 40 related pathways which include among others, metabolism of protein, post-translational protein modification, cellular responses to external stimuli and stress, cell cycle, and apoptosis. These analyses may improve our molecular insight of the mechanisms involved in the treatment of cervical cancer tumour by *Streblus asper* extract.

Keywords: Anticancer, Cervical Cancer, *In vivo*, Proteomics, *Streblus asper*, Xenograft

1. Introduction

With an estimation of 569,847 incidences and 311,365 deaths in 2018 worldwide, cervical cancer is the third most prevalent cancer in females[1]. In Malaysia alone, about 1,682 new cases were recorded in the same year[1]. Histologically, there are two subtypes of cervical cancer: Squamous Cell Carcinoma (SCC) and adenocarcinoma. Ninety percent of all cervical cancer cases are SCC while adenocarcinoma constitutes another 10% of the cases[2]. Adenocarcinomas are associated with a poorer prognosis compared to SCC[3]. The existing preventive measures that have been established for cervical cancer are vaccination and precancerous screening.

The most significant contributor to cervical cancer is Human Papillomavirus (HPV)[4]. With over 80 types of well-categorized HPVs, type 16 and 18 have been seen responsible for about 70% of cervical cancer

*Author for correspondence

Article Received on: 27.11.2019 Revised on: 17.07.2020 Accepted on: 17.07.2020

cases[5]. In response to this, vaccines were developed and used in approximately 70 countries across the globe[6]. However, weaknesses within its implementation such as unbearable cost and lack of knowledge on HPV vaccination have been found to hold back its effectiveness especially in low and middle-income countries[7–10]. Similar challenges are faced during the precancerous screening strategy upon its introduction. Precancerous screening which is also known as Pap smear seems to have several unique challenges as identified by previous studies, including failure to identify the eligible population, difficulties in access that leads to demotivation of participation, weakness in screening operation, insufficiency in monitoring and follow-up upon non-responders, and inadequacy of systematic monitoring of treatment[11].

Despite current advancement, the ability to treat cervical cancer and provide complete remission on advanced cases is still limited. The unavoidable side effects of radiotherapy and chemotherapy have been the major setbacks that hinder treatment efficacy. Radiotherapy is often associated with urologic complications which include radiation cystitis, lower urinary tract dysfunction, stricture disease, fistula formation, and the development of second primary cancer[12]. While chemotherapy is commonly linked to toxicities, it often leads to an increased risk of ovarian dysfunction in the elderly[13]. The anti-angiogenic agent can produce adverse effects like hypertension, left ventricular dysfunction and congestive heart failure, acute vascular event, and bleeding tendencies[14].

Streblus asper, from the family of *Moraceae*[15], is a tree that mostly occurs in tropical countries, including Sri Lanka, Malaysia, Thailand, the Philippines, and India. Traditionally, *Streblus asper* is renowned for its potential in treating various kinds of maladies such as ulcers, epilepsy, obesity, toothache, fever dysentery, diarrhoea, and stomachache[15]. *Streblus asper* extract has been found to possess anti-fungal[16,17] anti-filarial[18], anti-microbial[19], anti-viral[20–22], anti-inflammatory[23], anti-hyperglycemic[24], and anti-diabetic properties[25]. On top of that, many previous findings have proved its ability to inhibit cancers like mouse lymphocytic leukaemia (P388 cells)[26], osteosarcoma (HOS cells)[27], tongue carcinoma (SCC-15 cells)[27] and human nasopharyngeal epidermoid carcinoma (KB cells)[15]. In our previous study, we have successfully revealed the modulation of protein expression upon *Streblus asper* treatment in HeLa cervical cancer cells. *Streblus asper* has been found to regulate several important key proteins in HeLa cells growth. These include galectin-1, 10 kDa heat shock protein, dermcidin, keratin, type I cytoskeletal 9, tropomyosin, myristoylated alanine-rich C-kinase, tumour protein D52, folate receptor alpha, and parathymosin[28]. In addition, it was also found that *Streblus asper* likely to induce apoptosis via Tumour Necrosis Factor (TNF) signalling[29]. Unlike the past, the present study employs the use of *in vivo* model to investigate the capability of *Streblus asper* to exhibit positive effects on cervical cancer tumour as it does *in vitro* study. The xenograft model on immunocompromised animals becomes a model of choice to establish tumour growth for protein analysis because it is deemed as more cost effective than genetically engineered animal and is much safer in terms of handling procedure than using carcinogen. This immunocompromised animal is a type of non-obese diabetic (NOD) and severe combined immunodeficient (SCID) mice which are known to have impaired T- and B-cell development, deficiency of natural killer (NK) cell function, reduced levels of activated macrophage, an absence of haemolytic complement, and abnormal function of dendritic cell. These weaknesses increase the engraftment ability of the model for solid transplantable human cancers[30].

2. Materials and Methods

2.1 Plant Extract

The *Streblus asper* plant was purchased from a nursery in Tasek Gelugor, Penang. The plant was authenticated by the Malaysian Institute of Pharmaceuticals and Nutraceuticals (FRIM394(T)/TU490/5/1[Sub1] KH). The roots were left to dry for 2 weeks at room temperature. Then, the roots were ground into powder. The powder sample was boiled in distilled water for 30 minutes. The solution was filtered using a 0.75mm filter and freeze-dried the filtered solution. Upon usage, the weight of the freeze-dried powder was measured accordingly and diluted with double deionized distilled water.

2.2 Animal Protocol

The Animal Ethics Committee of Universiti Sains Malaysia (USM) (ref. USM/Animal Ethics Approval/2015/682) granted ethical approval on 7th October 2015. Ten female mice of type Severe Combined Immunodeficient (SCID) aged 5 to 6 weeks were purchased from Prima Nexus Sdn. Bhd. Health certificate was supplemented along with all purchased mice. Five of the mice were grouped as untreated. While another five mice were grouped as treated. We carefully placed all mice in individually ventilated cage (IVC) system with autoclaved woodchips for bedding and controlled conditions of 22±2°C temperature, 55±10% humidity and 12h light/dark lighting. All mice had unrestricted access to sterilized food and water. Upon arrival, all mice were undergoing acclimatization process for 2 weeks. At week 3, HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 1% sodium pyruvate and 1% penicillin-streptomycin, and incubated in 37°C humidified CO^2 incubator, with 5 % CO^2 and 95% of air. The medium was replaced every 72 hours until it reached 80% to 90% of cell confluency before sub-culturing was done. Three to ten of cell passages were used in the experiment. The cells were sub-cultured in the ratio of 1:6 in T-25 flask. Cells were seeded and prepared with a concentration of $1x10^7$cells for animal usage. Then, it was injected at the right flank of mice subcutaneously using the restraining method. We followed the US guidelines (NIH publication 85-23, revised in 1985) for laboratory animal use and care while performing the xenograft model. The untreated group was left to live freely without any treatment given. As for the treated group, *Streblus asper* treatment at a dose of 200 mg/kg of body weight was given at week 14 onwards in an approximately 0.4 ml of sterilized water. The treatment was administered through oral gavage on the alternate day. We determined the dosage following the guidelines by OECD (organization of economic corporation and development's) as detailed by Oghenesuvwe[30]. The mice's weight, water and food consumption were monitored and recorded every three days. Any clinical signs and symptoms of health-related problems such as respiratory, skin and growth problems were carefully monitored. Any observed endpoint signs like weight loss exceeding 20% of the body weight, ulceration or infection at the tumour site, and localised tumour invading the surrounding tissues would subject the mice to early euthanasia. The post-mortem was performed on both groups at week 16. All mice were sacrificed using carbon dioxide gas. The harvested tumour tissues were kept in liquid nitrogen for protein analysis.

2.3 Protein Digestion

Tissues collected during post-mortem were kept in liquid nitrogen and cut into smaller pieces. Then, the tissues were mixed up with 500ul of prepared RIPA buffer (1ml of RIPA buffer with 9 ml of double deionized distilled water) in falcon tubes. The tissues were homogenised and sonicated for 60 minutes in ice. Lysed tissues were centrifuged at 15000rpm for 2 minutes and collected the supernatant into a new microtube regarded as a sample tube. Eighty percent cold acetone was added six times of the sample volume to the sample tube. The tube three was inverted three times and incubated at −20°C overnight. Then, the sample tubes were centrifuged at 6000x g for 10 minutes, decanted the acetone, and dried the pellet in a speed vacuum. The pellet was re-suspended in ammonium bicarbonate 50 mM, pH 8.0. The tissue lysates were stored at -80°C prior to further analysis and peptide digestion.

2.4 Peptide Digestion

Samples with total protein of 100 ug were resuspended in 100 ul of ammonium bicarbonate (NH_4HCO_3). Each sample was added with 100 ul of 0.05% Rapigest^{TM}SF. Samples were mixed using Vortex. Then the Vivaspin column MWCO 3000 was used to make the samples more concentrated to a volume of 100 µl. The samples were centrifuged at 14,000 rpm (20800 x g) for 10 – 15 minutes and heated on a thermomixer at 80°C for 15 minutes. Then to each mixture 5 µl of 100 mM DTT was added and incubated them at 37°C for 30 minutes in a thermomixer. Then the samples were added with 5 µl of 200 mM Iodoacetamide and incubated at room temperature for 45 minutes. 5 µl trypsin (0.2 µg/µl) was added to the reaction and incubated each mixture overnight at 37°C. The trypsin digestion reaction was inhibited by adding 1µl of concentrated trifluoroacetic acid (TFA), incubated at 37°C for 20 minutes and centrifuged the mixtures at 14,000 rpm for 10 minutes.

The supernatants were stored at -80°C prior to LCMS analysis.

2.5 LCMS Analysis

LCMS analysis was conducted by using Orbitrap Fusion mass spectrometer coupled with Dionex 3000 Ultimate RSLCnano (Thermo Fisher Scientific) liquid chromatography system. We used the EASY-Spray Column Acclaim PepMap™ C18 (100 A0, 2 µm particle size, 50 µm id x 15 cm) as the analytical column and the Easy column C18 (2 cm, 0.1 mm i.d., 5 µm) as the pre-column. Pre-column was calibrated at a flow rate of 3 ul/min for 15 µl and the analytical column at a flow rate of 250 nL/min for 4 µl, and set the column temperature at 35°C. Then, chromatographically separated 2 µl of samples was injected to the column at a flow rate of 250 nL/min. 0.1% formic acid in deionized water, and 0.1% formic acid in acetonitrile was used as the running buffers. The peptides were eluted by using the following gradient: 91 min at 5 - 40% solvent B, 2 min at 95% solvent B, 6 min at 95% solvent B, and back to 5% solvent B in 2 min. The instrument was operated in the data-dependent mode. The full-scan spectra were collected by using the Orbitrap MS (OTMS); the parameters were: scan range of 310–1800m/z, resolving power of 120000, AGC target of 4.0 e5 (400 000), and maximum injection time of 50ms. This method consisted of 3 sec Top Speed Mode where precursors were selected for a maximum of 3 sec cycle and we only further analysed precursors with an assigned monoisotopic m/z and a charge state of 2–7 for MS2. We filtered all precursors using a 20 sec dynamic exclusion window and intensity threshold of 5000 and analysed the MS2 spectra by ion trap MS (ITMS) using the following parameters: rapid scan rate with a resolving power of 60000, AGC target of 1.0e2 (100), 1.6 m/z isolation window, and a maximum injection time of 250ms. The precursors were fragmented by using Collision Induced Dissociation (CID) and high-energy collision dissociation (HCD) at normalised collision energy of 30% and 28%. Each sample was analysed thrice.

2.6. Peptide Identification

The result of protein analysis produced by LCMS was in the form of MS/MS raw data. This data was used to make peptide identification. We carried out the process of peptide identification using Thermo Scientific™ Proteome Discoverer™ Software Version 2.1. We improved the vast quality of this data prior to *de novo* sequencing which employs the peptide sequence from the tandem mass spectrum without the use of sequence database. Based on the peptide sequence obtained, we searched the database, assisted by the Proteome Discoverer™ which was built-in with a result validation using an enhanced target-decoy approach to estimate the False Discovery Rate (FDR), calculated as the percentage of positive hits in the decoy database versus the target database both for proteins and peptides using a decoy fusion approach to ensure that only valid results were reported. Uniprot_*homo sapiens* database was downloaded on 27th October 2017 in the database matching against peptide sequence obtained and the following constraints for the searches and filters: carbamidomethylation and oxidation were set as fixed and variable modifications, respectively; maximum missed cleavage was set as two; a maximum of one nonspecific cleavage site of trypsin cleavage; mass tolerance of 0.1 Da for-parent ions and 0.8 Da for MS/MS fragment ions; FDR was set at < 1%; and significant score (-10 lgP) for protein was set as ≥ 20 with at least one unique peptide. The label-free quantification method was used based on the relative intensities of peptide features detected in multiple samples by matching the retention time and mass over charge ratio (m/z). The high-performance retention time alignment algorithm reliably aligned the features of the same peptide from different samples. Mass error tolerance and retention time shift tolerance was set at 0.2 Da and 1 minute, respectively.

2.7. Bioinformatic Analysis

We carried out the analysis of the peptide identified from raw data using Thermo Scientific™ Proteome Discoverer™, leading to the production of protein information from different forms like peptide-spectrum matches (PSM), peptide group, protein groups and proteins. These data were further analysed using three types of bioinformatics tools, including Perseus, Panther and Reactome. Perseus provided a list of protein with its expression and its Uniprots ID. Panther (Protein Analysis Through Evolutionary Relationships) software Version 13.0 (http://pantherb.org/) on the

other hand classified the proteins based on their families and their functionally related subfamilies. The biological function, cellular localisation, and molecular function of identified protein were obtained using this tool. The next tool used was Reactome version 64 (http://reactome.org). This tool was used to reveal comprehensive analysis and interpretation of pathway information. Significant pathways were chosen from the analysis according to the nature of the pathways and its relationship with cancers.

3. Results

3.1 Protein Identification

34317 peptide-spectrum matches (PSM), 2909 peptide group, 639 protein groups and 2058 proteins were identified at the same FDR setting in the untreated samples; and 31938 PSM, 2401 peptide group, 601 protein groups and 1577 proteins in the *Streblus asper*-treated samples (Figure 1).

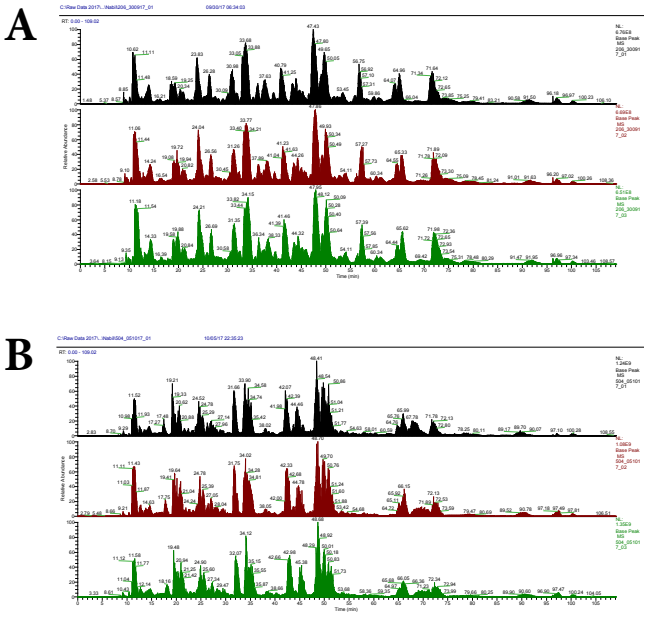

Figure 1. Total ion chromatograms (TICs) plot for the(A) untreated group and (B) treated group.

A total of 1240 proteins were further analysed n both groups (untreated and treated) using Panther to obtain the information on protein classification based on its biological process, molecular function, and cellular localisation.

As shown in Figure 2A, based on their biological process, most of the proteins in untreated group were involved in the cellular process (31.9%), followed by metabolic process (27.0%), cellular component organization or biogenesis (13.5%), localization (7.1%), biological regulation (5.3%), response to stimulus (4.9%), developmental process (3.6%), multicellular organismal process (3.1%), biological adhesion (1.4%), immune system process (1.2%), locomotion (0.7%), reproduction (0.2%). Few of them were involved in growth (0.1%). In the treated group, most of the protein was involved in cellular process (30.5%), followed by metabolic process (26.0%), cellular component organization or biogenesis (13.3%), localization (6.9%), biological regulation (5.8%), response to stimulus (5.7%), developmental process (4.6%), multicellular organismal process (3.3%), biological adhesion (1.7%), immune system process (1.1%), locomotion (0.8%) and reproduction (0.2%). Few of them were involved in growth (0.1%).

As shown in Figure 2B, based on their molecular function, most of the proteins in the untreated group were involved in binding activity (38.1%), followed by catalytic activity (36.8%), structural molecule activity (16.1%), transporter activity (2.6%), antioxidant activity (2.2%), translation regulator activity (2.0%), receptor activity (1.3%). The least group of protein were involved in signal transducer activity (0.9%). In the treated group, most of the proteins were involved in binding activity (40.7%), followed by catalytic activity (37.0%), structural molecule activity (12.8%), antioxidant activity (2.5%), translation regulator activity (1.9%), signal transducer activity (1.7%) and transporter activity (1.7%). Few of the proteins were involved in receptor activity (1.6%).

As shown in Figure 2C, based on their cellular component, most of the proteins in untreated group were involved in cell part (40.4%), followed by organelle (29.4%), macromolecular complex (19.7%), membrane (5.7%), extracellular region (2.6%), extracellular matrix (1.1%) and cell junction (0.6%). Few of the proteins were involved in synapse (0.4%). In the treated group, most of the proteins were involved in cell part (40.4%), followed by organelle (27.9%), macromolecular complex (19.5%), membrane (7.1%), extracellular region (2.9%) and extracellular matrix (1.4%). Few of

the proteins were involved in synapse (0.5%) and cell junction (0.5%).

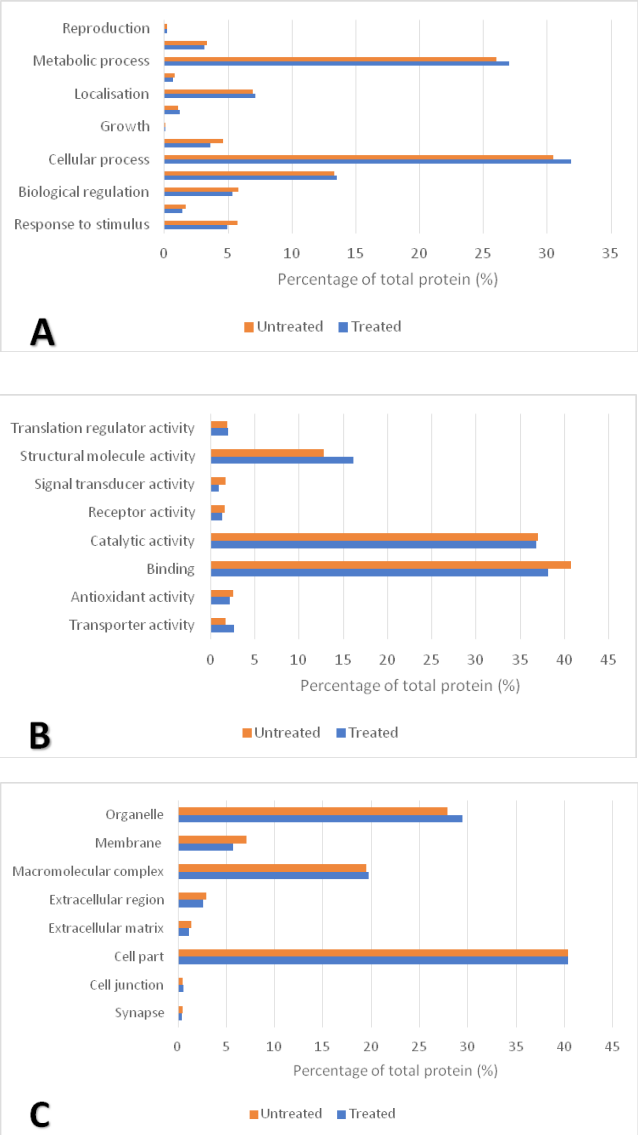

Figure 2. GO analysis illustrates classes of proteins differing between untreated and treated tumour tissues. Protein with significant differences between the two groups was subjected to GO classification in terms of **(A)** biological process, **(B)** molecular function, and **(C)** cellular localisation.

3.2. Protein Quantification

Only 452 proteins were common to both groups when compared to the 639 proteins identified in the untreated group, and 601 proteins identified in *Streblus asper* treated group (as illustrated in Figure 3). We further analysed these identified proteins using Perseus to identify the differentially expressed proteins in both groups. The $\log_2$ ratios of normalised volume obtained from the Thermo Scientific™ Proteome Discoverer™ Software Version 2.1 represented the quantitative variations in protein abundance per injection between the protein group of the untreated and treated samples.

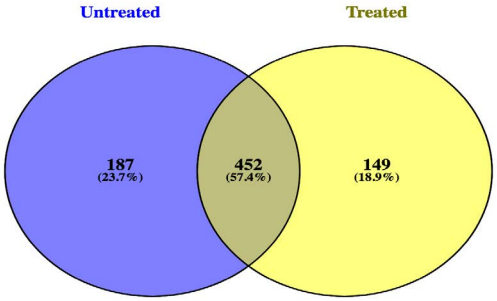

Figure 3. A Venn diagram comparing the unique and common proteins found in the untreated and treated group.

Upon analysis, 122 proteins were able to be quantified. Of that, 1 protein was up-regulated, 36 proteins had no changes, and 85 proteins were down-regulated. Fifty-two proteins were expressed with more than 2-fold changes (as shown in Table 1).

3.3 Pathways Analysis

Pathway enrichment analysis was carried out on common proteins with at least two peptides found in untreated and treated samples. The analysis was done using Reactome, an online bioinformatics tool. From that analysis, 40 pathways that played a meaningful role in cancer were selected (as illustrated in Table 2).

4. Discussion

The present study focused on differentially expressed proteins that were discovered based on a comparison of protein expression between *Streblus asper* treated and untreated groups. Throughout the study protocol, no mice were subjected to early euthanasia due to reaching the signs of endpoint. Of the 1240 proteins identified from both groups, 452 proteins (57.4%) were common

Table 1. Differentially expressed proteins with at least 2-fold changes

No	Protein Description	MW [kDa]	Regulation
1	Isoform 2 of Annexin A2	40.386	Down
2	Proteasome subunit alpha type-4	29.465	Down
3	Peptidyl-prolyl cis-trans isomerase B	23.728	Down
4	Small nuclear ribonucleoprotein Sm D1	13.273	Down
5	14-3-3 protein beta/alpha	28.065	Down
6	Macrophage-capping protein	38.474	Down
7	Isoform 2 of Transgelin-2	24.438	Down
8	Isoform 3 of Malate dehydrogenase, Cytoplasmic	38.603	Down
9	Galectin-1	14.706	Down
10	60S ribosomal protein L12	17.808	Down
11	SH3 domain-binding glutamic acid-rich-like protein 3	10.431	Down
12	Keratin, type I cytoskeletal 18	48.029	Down
13	Myosin regulatory light chain 12B	19.767	Down
14	Proteasome subunit beta type-3	22.933	Down
15	10 kDa Heat shock protein, mitochondrial	10.925	Down
16	14-3-3 protein zeta/delta	27.728	Down
17	Histone H2A type 1-B/E	14.127	Down
18	Gelsolin	85.644	Down
19	14-3-3 protein theta	27.747	Down
20	Actin-related protein 3	47.341	Down
21	Fibrinogen gamma chain	51.479	Down
22	ERO1-like protein alpha	54.358	Down
23	rho GDP-dissociation inhibitor 1	23.193	Down
24	Isoform Sap-mu-9 of Prosaposin	58.445	Down
25	Elongation factor 2	95.277	Down
26	Beta-enolase	46.957	Down
27	Elongation factor 1-alpha 1	50.109	Down
28	Cofilin-1	18.491	Down
29	Heterogeneous nuclear ribonucleoprotein D0	38.41	Down
30	Alpha-enolase	47.139	Down
31	Isoform 2 of Heterogeneous nuclear Ribonucleoprotein K	50.996	Down
32	Ran-specific GTPase-activating protein	23.296	Down
33	Catalase	59.719	Down
34	Heat shock cognate 71 kDa protein	70.854	Down
35	78 kDa Glucose-regulated protein	72.288	Down
36	Peptidyl-prolyl cis-trans isomerase A	18.001	Down
37	Phosphoglycerate mutase 1	28.786	Down
38	Fatty acid-binding protein, adipocyte	14.709	Down
39	Proteasome subunit alpha type-5	26.394	Down
40	Triosephosphate isomerase	30.772	Down

41	60S Acidic ribosomal protein P2	11.658	Down
42	Protein disulfide-isomerase	57.081	Down
43	Vimentin	53.619	Down
44	14-3-3 protein gamma	28.285	Down
45	Calreticulin	48.112	Down
46	Isoform 2 of Glucose-6-phosphate isomerase	64.284	Down
47	Carbonic anhydrase 3	29.539	Down
48	Isoform 2 of Fructose-bisphosphate aldolase A	45.232	Down
49	Actin, cytoplasmic 1	41.71	Down
50	Serum albumin	69.321	Down
51	Ubiquitin-40S ribosomal protein S27a	17.953	Down
52	Creatine kinase M-type	43.074	Down

Table 2. Pathways to which the common proteins found were annotated

No	Pathway Name	Number of Proteins	P-Value
1	Metabolism of proteins	46	3.42E-06
2	Post-translational protein modification	33	3.78E-05
3	Cellular responses to external stimuli	25	2.03E-10
4	Cellular responses to stress	24	3.43E-11
5	Gene expression (Transcription)	23	0.04906431
6	Cell Cycle	17	7.03E-04
7	Apoptosis	16	1.49E-10
8	G2/M Checkpoints	14	2.53E-09
9	MAPK family signaling cascades	14	9.84E-06
10	MAPK1/MAPK3 signaling	13	1.07E-05
11	Metabolism of carbohydrates	13	1.97E-05
12	Deubiquitination	12	9.83E-05
13	Signaling by WNT	11	6.18E-04
14	Transcriptional Regulation by TP53	11	0.003259565
15	G2/M Transition	10	9.36E-05
16	Ub-specific processing proteases	10	1.29E-04
17	Glycolysis	9	2.70E-07
18	TP53 Regulates Metabolic Genes	9	9.00E-07
19	MAPK6/MAPK4 signaling	9	9.87E-07
20	Gluconeogenesis	8	7.17E-09
21	Detoxification of Reactive Oxygen Species	8	2.05E-08
22	Regulation of activated PAK-2p34 by proteasome mediated degradation	8	1.35E-07
23	Ubiquitin-dependent degradation of Cyclin D1	8	1.81E-07
24	p53-Independent G1/S DNA damage checkpoint	8	2.09E-07
25	Ubiquitin Mediated Degradation of Phosphorylated Cdc25A	8	2.09E-07

26	Oxygen-dependent proline hydroxylation of Hypoxia-inducible Factor Alpha	8	1.07E-06
27	Activation of NF-kappaB in B cells	8	1.19E-06
28	Regulation of RAS by GAPs	8	1.33E-06
29	Regulation of PTEN stability and activity	8	1.48E-06
30	Cdc20: Phospho-APC/C mediated degradation of Cyclin A	8	2.03E-06
31	CDK-mediated phosphorylation and removal of Cdc6	8	2.03E-06
32	Cyclin E associated events during G1/S transition	8	5.70E-06
33	Cyclin A: Cdk2-associated events at S phase entry	8	6.76E-06
34	Metabolism of lipids	7	0.751564145
35	Cellular response to heat stress	6	7.31E-04
36	Chk1/Chk2(Cds1) mediated inactivation of Cyclin B: Cdk1 complex	5	4.83E-07
37	Activation of BAD and translocation to mitochondria	5	9.70E-07
38	Activation of BH3-only proteins	5	2.71E-05
39	MAP2K and MAPK activation	5	1.04E-04
40	Signaling by RAS mutants	5	4.12E-04

or identical to both groups. Among these common proteins, only 52 of them were differentially expressed. Twelve of them (including those derived from the same family) were selected based on their known establishment and relationship with cancers, including annexin A2, 14-3-3 protein, transgelin-2, galectin-1, keratin, heat shock protein, glucose regulated protein (78kDa), gelsolin, alpha enolase, cofilin-1, vimentin, and calreticulin. All these proteins were found downregulated by at least 2-fold in cervical cancer tumour tissue upon treatment with *Streblus asper*.

Annexin A2, found in various types of tumour cells, is a cytoskeletal protein produced from 40kb gene on the long arm of chromosome 15[31]. It is overexpressed in cancers like breast, liver, prostate, and pancreas[32–35] and involved in cancers via signal transduction, angiogenesis, tumour invasion, metastasis, and apoptosis[36]. The upregulation of annexin A2 expression occurs in cervical carcinoma[37,38], explaining how *Streblus asper* treatment was capable of downregulating its expression in cervical cancer according to our findings.

The 14-3-3 protein is a phosphoserine/phosphothreonine binding protein consisting of 7 isoforms (β, γ, ε, η, ζ, σ and τ/θ)[39] and imperative in many cellular processes, including cell cycle progression, DNA repair, apoptosis inhibition, cell differentiation and senescence control, and cell adhesion and motility regulation[40]. Many studies have indicated that increasing 14-3-3 protein expression enhances cancer growth. For instance, the increased expression of 14-3-3 sigma, theta, and epsilon isoforms promote cell survival, invasion and metastasis in prostate, breast, and liver cancer cells respectively[41–43]. As for beta isoform, the increased expression is associated with progression of liver cancer[44]. The suppression of 14-3-3 zeta expression leads to inhibition of lung cancer cells growth[45]. The sigma isoform, on the other hand, is upregulated in cervical cancer tumour and the increase is correlated with cervical intraepithelial lesion (CIN) grades[46]. However, few isoforms of 14-3-3 proteins have been characterised in cervical cancer.

Transgelin-2, a member of actin-binding protein, is imperative in cell differentiation, apoptosis, proliferation, and migration[47,48]. It regulates the cytoskeletal RNA transcription and processing, chromatin remodelling, and nuclear transport[48]. The expression of transgelin-2 is higher in tumour tissue in comparison to healthy tissue. Its overexpression is associated with tumorigenesis[49]. Besides that, the elevation of its expression is proportionate to the tumour progression. Thus, it was previously suggested

as a tumour biomarker[47]. Transgelin-2 was observed to be highly expressed in cervical SCC while its suppression inhibits cell migration and secretion of matrix metalloproteinases[50,51]. We found that cervical cancer tissue treated with *Streblus asper* resulted in the downregulation of transgelin-2 expression compared to the untreated cancerous tissues. Based on the previous findings, transgelin-2 is likely to possess a different sets of expression levels in tumourigenesis and malignancy transformation process.

Galectins are group of proteins that occur in different subtypes (1,2,3,4,5,7,8,9,10,11,12,13,14 and 15). These subtypes are determined according to their structure which comprises of tandem, dimeric and chimeric[52]. Galectins are able to bind β-galactoside sugar. In breast cancer, galectin-1 has been found contributing to disease progression and metastasis[53]. Besides that, it also contributes to the progression of prostate cancer[54]. A study has associated the overexpression of galectin-1 with poor prognosis of lung cancer[55]. The invasiveness of cervical cancer has been observed to correspond with the expression of galectin-1[56]. Based on this, *Streblus asper* seems to be effective against cervical cancer by downregulating the expression of galectin-1.

Keratins are the structural proteins of the epithelial cells which line multiple organs and cavities in the body. Keratins play a crucial role in the determination of cell size, control of the translational process, cell proliferation, cell type-specific, transportation of organelle, transformation of malignancy and various stress responses[57]. Keratins are expressed in cancers like breast, cervix, colon, liver, kidney, lung, pancreas, prostate[58]. Given its functional roles in normal epithelial cells, keratins are also actively involved in cancer cell progression, metastasis, and response to treatments[59,60]. Keratin 18 has been found to be overexpressed in breast cancer and correlated with poor prognosis of the disease[61]. In the present study, *Streblus asper* exhibited downregulation of keratin 18 in cervical cancer. Therefore, it is suggested that *Streblus asper* could contribute to the improvement of cervical cancer prognosis.

Heat shock proteins (HSPs) are a protein family that facilitates protein protection against degradation, hypoxia, thermal destruction and oxidative stress[62]. HSPs are present in different types depending on their relative molecular sizes, including HSP27, HSP40, HSP60, HSP70, HSP90, and family of large HSPs (HSP110 and HSP170)[63–65]. In cancer cells, HSPs protect the oncoprotein associated with proliferation, differentiation, and progression. HSP10, a chaperone protein located in the mitochondria, is overexpressed in tissues with carcinogenesis. This has been discovered in exocervical carcinoma, large bowel, and serous ovarian carcinoma[66–68]. HSP70 is critical in the inhibition of apoptosis. It facilitates the inhibition of caspases activity and blockage of TNF alpha mediated apoptosis[69]. *Streblus asper* treatment seems capable of downregulating the expression of HSP10 and HSP70, potentially leading to suppression of carcinogenesis and induction of apoptosis. Nevertheless, the detailed explanation for this remains underexplored.

Glucose-regulated protein 78kDa (GRP78), a stress-inducible molecular chaperone, usually resides in the endoplasmic reticulum (ER). The endoplasmic reticulum-GRP plays significant roles in protein folding, and secretion, degradation, apoptosis suppressor, calcium homeostasis, cytokine and chemokine secretion, and ER signalling control and ER structural integrity[70]. It has been proven to be elevated in various cancer cells[70]. In the xenograft tumour model, knockdown of GRP78 resulted in the metastasis process being inhibited[71]. Meanwhile, *in vitro* analysis revealed that the knockdown of GRP78 suppresses cell invasion[72]. Consistent with our data, *Streblus asper* treatment may cause inhibition of cervical cancer tumour by downregulating the expression of GRP78.

Gelsolin is another actin-binding protein crucial in numerous activities pertaining to actin cytoskeletal function, including motility, maintenance of cell morphology and polarity, intracellular trafficking and cell division[73]. The upregulation of this protein in oral SCC cell line may promote cell proliferation[74]. In other analyses, gelsolin was found overexpressed in the liver cancer cell line (HepG2). The study indicated the relationship between gelsolin and p53 expression whereby the gelsolin might be able to inhibit apoptosis through the p53 suppression. In other words, it negatively regulated the p53 expression[75]. Overexpression of gelsolin was also discovered in cervical cancer cases. This was proven in tissue and plasma samples. The increase was correlated with cellular proliferation and

migration abilities[76]. In accordance with that, our result suggests that *Streblus asper* downregulated the expression of gelsolin in cervical cancer tumour and thus reflected the reduction of tumour proliferation.

Alpha enolase, a glycolytic enzyme responsible for ATP generation in cells, is a candidate marker for cancer prognosis since it promotes cell growth, invasion, and migration in various cancers [77,78]. In gastric cancer, it promotes cell proliferation and metastasis by modulating AKT signalling cascade[79]. It also regulates similar pathways to encourage cell growth migration and invasion in non-small cell lung cancer[77]. The upregulation of alpha enolase occurs in various tumour cells compared to normal cells[77–80]. These overexpression patterns suggest that highly proliferative cells require higher glycolytic activity as it is understood in nature to be more aggressive than normal cells.

Cofilin-1 is essential in the regulation of actin filaments[81]. Generally, cofilin expression is upregulated in many types of cancer cells, including lung, pancreatic, breast, oral, renal, ovarian, and liver[82–88]. Cofilin-1, a member of the cofilin family, is overexpressed in cancers like colorectal, bladder, pancreatic, oral and cervical cancer[89–93]. The overexpression of cofilin-1 in SCC and adenocarcinoma of the gallbladder is correlated to the disease progression[94]. Furthermore, it promotes cell growth, proliferation, cell cycle progression, migration, invasion, adhesion as well as inhibiting apoptosis[92]. Concordant with our data, cofilin-1 expression was initially high in untreated cervical cancer and it decreased upon treatment with *Streblus asper*. However, the underlying mechanism of cofilin-1 in cancer is yet to be determined.

Vimentin is involved in maintaining cellular integrity and providing resistance against stress. It is an intermediate filament family of protein that serves as a potential biomarker for cancers[95]. Studies have shown that vimentin expression is upregulated in many kinds of cancer cells like prostate, gastric, colorectal, pancreatic, oesophageal, breast, and cervical cancers[96–102]. Most of the studies showed that the expression of vimentin is highly associated with cancer invasiveness. The suppression of vimentin leads to a reduction in cancer invasion potential[103,104]. In our study, *Streblus asper* treatment seems to be effective against cervical cancer tumour as it downregulates vimentin expression by more than 2-fold changes.

Streblus asper downregulates the expression of calreticulin in cervical cancer, indicating the positive effects of *Streblus asper* on cervical cancer since the overexpression of calreticulin enhances proliferation and metastasis of cancers[105–107]. Calreticulin is overexpressed in cancers like oral, breast, pancreatic, oesophageal, gastric, colorectal, and bladder[105,108–113]. On the contrary, the knockdown of calreticulin resulted in the inhibition of cancer proliferation and metastasis[106,114,115]. As discussed by Lu and co-researchers (2015), calreticulin plays multiple roles in cell physiological and pathological responses. Its main functions include homeostasis of calcium ion and protein chaperoning[116]. However, the possible relationships between its roles and cancer mechanisms especially proliferation and metastasis remain elusive.

5. Conclusion

In this study, several proteins have been identified from the *Streblus asper* treated and untreated cervical cancer tumour tissues. Twelve proteins were selected as they have been reported to have relationships directly or indirectly with cancers. Of these, twelve of the proteins were observed to support our hypothesis while two seem to be contradictory. Based on these, it is likely that *Streblus asper* demonstrated anticancer activities on cervical cancer tumour tissue *in vivo* through the downregulation of annexin A2, 14-3-3 protein, transgelin-2, galectin-1, keratin, heat shock protein 10 and 70, glucose regulated protein (78kDa), gelsolin, alpha enolase, cofilin-1, vimentin, and calreticulin. Hence, further validation of the profile and expression pattern will significantly contribute to the establishment of therapeutic biomarker of cervical cancer targeted by *Streblus asper*. Besides that, it will also provide a comprehensive understanding of the *Streblus asper* effects at cellular and molecular levels.

6. Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

7. Acknowledgement

We would like to thank the Malaysian Ministry of Education for funding the study under the National Research Acculturation Grant Scheme (RAGS) (Ref no.: RAGS/1/2015/SKK08/UITM/03/1). In addition, we gratefully thank Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia (USM) and Universiti Teknologi MARA (UiTM) Penang Branch, for supportively providing spaces and sufficient facilities throughout the study process.

8. References

1. Bruni L, Barrionuevo-Rosas L, Albero G, Aldea M, Serrano B, Valencia S, et al. Human papillomavirus and related diseases in Malaysia. ICO/IARC Inf Cent HPV Cancer (HPV Inf Centre); 2018 Dec.
2. Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality rates and trends--An update. Cancer Epidemiology, Biomarkers & Prevention. 2016 Jan; 25(1):16–27. https://doi.org/10.1158/1055-9965.EPI-15-0578. PMid:26667886
3. Kleine W, Rau K, Schwoeorer D, Pfleiderer A. Prognosis of the adenocarcinoma of the cervix uteri: A comparative study. Gynecologic Oncology. 1989; 35(2):145–9. https://doi.org/10.1016/0090-8258(89)90032-2
4. Rizzo AE, Feldman S. Update on primary HPV screening for cervical cancer prevention. Current Problems in Cancer. 2018 ;0:1–14.
5. Ramakrishnan S, Partricia S, Mathan G. ScienceDirect overview of high-risk HPV ' s 16 and 18 infected cervical cancer: Pathogenesis to prevention. Biomedicine & Pharmacotherapy. 2015; 70:103–10. https://doi.org/10.1016/j.biopha.2014.12.041. PMid:25776487
6. Sankaranarayanan R, Joshi S, Muwonge R, Esmy PO, Basu P, Prabhu P, et al. Can a single dose of human papillomavirus (HPV) vaccine prevent cervical cancer? Early findings from an Indian study. Vaccine. 2018 Aug; 36(32 Pt A):4783–91. https://doi.org/10.1016/j.vaccine.2018.02.087. PMid:29551226
7. Bonanni P, Zanella B, Santomauro F, Lorini C, Bechini A, Boccalini S. Safety and perception: What are the greatest enemies of HPV vaccination programmes? Vaccine. 2018; 36(36):5424–9. https://doi.org/10.1016/j.vaccine.2017.05.071. PMid:28610824
8. Laurent J, Luckett R, Feldman S. HPV vaccination and the effects on rates of HPV-related cancers. Current Problems in Cancer. 2018; 42(5):493–506. https://doi.org/10.1016/j.currproblcancer.2018.06.004. PMid:30041818
9. Levin A, Wang SA, Levin C, Tsu V, Hutubessy R. Costs of introducing and delivering HPV vaccines in low and lower middle income countries: Inputs for GAVI policy on introduction grant support to countries. PLoS One. 2014; 9(6). https://doi.org/10.1371/journal.pone.0101114. PMid:24968002. PMCid:PMC4072768
10. Nickel B, Dodd RH, Turner RM, Waller J, Marlow L, Zimet G, et al. Factors associated with the human papillomavirus (HPV) vaccination across three countries following vaccination introduction. 2017; 8(May):169–76. https://doi.org/10.1016/j.pmedr.2017.10.005. PMid:29062681. PMCid:PMC5645176
11. Priaulx J, de Koning HJ, de Kok IMCM, Széles G, McKee M. Identifying the barriers to effective breast, cervical and colorectal cancer screening in thirty one European countries using the Barriers to Effective Screening Tool (BEST). Health Policy (New York). 2018. https://doi.org/10.1016/j.healthpol.2018.08.004. PMid:30177278
12. Lobo N, Kulkarni M, Hughes S, Nair R, Khan MS, Thurairaja R. Urological complications following pelvic radiotherapy. Urology. 2018. https://doi.org/10.1016/j.urology.2018.07.017. PMid:30036617
13. Overbeek A, van den Berg MH, van Leeuwen FE, Kaspers GJL, Lambalk CB, van Dulmen-den Broeder E. Chemotherapy-related late adverse effects on ovarian function in female survivors of childhood and young adult cancer: A systematic review. Cancer Treatment Reviews. 2017; 53:10–24. https://doi.org/10.1016/j.ctrv.2016.11.006. PMid:28056411
14. Gunderson CC, Matulonis U, Moore KN. Management of the toxicities of common targeted therapeutics for gynecologic cancers. Gynecologic Oncology. 2018; 148(3):591–600. https://doi.org/10.1016/j.ygyno.2018.01.010. PMid:29395304
15. Rastogi S, Kulshreshtha DK, Rawat AKS. Streblus asper Lour. (Shakhotaka): A Review of its Chemical, Pharmacological and Ethnomedicinal Properties. Evidence-Based Complementary and Alternative Medicine. 2006 Jun; 3(2):217–22. https://doi.org/10.1093/ecam/nel018. PMid:16786051. PMCid:PMC1475940
16. Taweechaisupapong S, Choopan T, Singhara S, Chatrchaiwiwatana S, Wongkham S. In vitro inhibitory effect of Streblus asper leaf-extract on adhesion of Candida albicans to human buccal epithelial cells. Journal of Ethnopharmacology. 2005 Jan; 96(1–2):221–6. https://doi.org/10.1016/j.jep.2004.09.010. PMid:15588674
17. Taweechaisupapong S, Klanrit P, Singhara S, Pitiphat W, Wongkham S. Inhibitory effect of Streblus asper leaf-extract on adhesion of Candida albicans to denture acrylic. Journal of Ethnopharmacology. 2006 Jul; 106(3):414–7. https://doi.org/10.1016/j.jep.2006.01.021. PMid:16529890
18. Chatterjee RK, Fatma N, Murthy PK, Sinha P, Kulshrestha DK, Dhawan BN. Macrofilaricidal activity of the stembark of Streblus asper and its major active constituents. Drug Development Research. 1992; 26(1):67–78. https://doi.org/10.1002/ddr.430260106

19. Taweechaisupapong S, Wongkham S, Chareonsuk S, Suparee S, Srilalai P, Chaiyarak S. Selective activity of Streblus asper on Mutans streptococci. Journal of Ethnopharmacology. 2000 Apr; 70(1):73–9. https://doi.org/10.1016/S0378-8741(99)00140-3
20. Chen H, Li J, Wu Q, Niu XT, Tang MT, Guan XL, et al. Anti-HBV activities of Streblus asper and constituents of its roots. Fitoterapia. 2012; 83(4):643–9. https://doi.org/10.1016/j.fitote.2012.01.009. PMid:22305944
21. Li J, Huang Y, Guan X-L, Li J, Deng S-P, Wu Q, et al. Anti-hepatitis B virus constituents from the stem bark of Streblus asper. Phytochemistry. 2012 Oct; 82:100–9. https://doi.org/10.1016/j.phytochem.2012.06.023. PMid:22818524
22. Li L-Q, Li J, Huang Y, Wu Q, Deng S-P, Su X-J, et al. Lignans from the heartwood of Streblus asper and their inhibiting activities to hepatitis B virus. Fitoterapia. 2012 Mar; 83(2):303–9. https://doi.org/10.1016/j.fitote.2011.11.008. PMid:22119765
23. Sripanidkulchai B, Junlatat J, Wara-aswapati N, Hormdee D. Anti-inflammatory effect of Streblus asper leaf extract in rats and its modulation on inflammation-associated genes expression in RAW 264.7 macrophage cells. Journal of Ethnopharmacology. 2009 Jul; 124(3):566–70. https://doi.org/10.1016/j.jep.2009.04.061. PMid:19439173
24. Kumar RBS, Kar B, Dolai N, Bala A, Haldar PK. Evaluation of antihyperglycemic and antioxidant properties of Streblus asper Lour against streptozotocin-induced diabetes in rats. Asian Pacific Journal of Tropical Disease. 2012; 2(2):139–43. https://doi.org/10.1016/S2222-1808(12)60032-2
25. Choudhury MK, Venkatraman S, Upadhyay L. Phytochemical analysis and peripheral glucose utilization activity determination of Steblus asper. Asian Pacific Journal of Tropical Disease. 2012; 2(2):s656–61. https://doi.org/10.1016/S2221-1691(12)60291-3
26. Phutdhawong W, Donchai A, Korth J, Pyne SG, Picha P, Ngamkham J, et al. The components and anticancer activity of the volatile oil from Streblus asper. Flavour and Fragrance Journal. 2004; 19(5):445–7. https://doi.org/10.1002/ffj.1342
27. Seeni A, Ayunie N, Abdul Wahab R. Apoptosis Inducer from Streblus asper Extracts for Cancer Chemoprevention. In: Novel Apoptotic Regulators in Carcinogenesis; 2012. p. 1–25. https://doi.org/10.1007/978-94-007-4917-7_1
28. Nabil M, Seeni A, Ismail WI, Rahim NA. Proteomic analysis of anti-cancer effects of streblus asper root extract on HeLa Cancer Cells. 2019; 12(Sep):1263–77. https://doi.org/10.13005/bpj/1755
29. Nabil M, Seeni A, Ismail WI, Ab N. Induction of apoptotic mechanism by streblus asper root extract on cervical cancer using in vitro and in vivo models. 2019; 12(Dec):1661–73. https://doi.org/10.13005/bpj/1796
30. Oghenesuvwe EE, Nwoke E, Lotanna AD. Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. 2014; 4(18):100–6.
31. Huebner K, Cannizzaro LA, Frey AZ, Hecht BK, Hecht F, Croce CM, et al. Chromosomal localization of the human genes for lipocortin I and lipocortin II. Oncogene Research. 1988; 2(4):299–310.
32. Inokuchi J, Narula N, Yee DS, Skarecky DW, Lau A, Ornstein DK, et al. Annexin A2 positively contributes to the malignant phenotype and secretion of IL-6 in DU145 prostate cancer cells. International Journal of Cancer. 2009; 124(1):68–74. https://doi.org/10.1002/ijc.23928. PMid:18924133
33. Mohammad HS, Kurokohchi K, Yoneyama H, Tokuda M, Morishita A, Jian G, et al. Annexin A2 expression and phosphorylation are up-regulated in hepatocellular carcinoma. International Journal of Oncology. 2008; 33(6):1157–63.
34. Sharma MR, Koltowski L, Ownbey RT, Tuszynski GP, Sharma MC. Angiogenesis-associated protein annexin II in breast cancer: selective expression in invasive breast cancer and contribution to tumor invasion and progression. Experimental and Molecular Pathology. 2006; 81(2):146–56. https://doi.org/10.1016/j.yexmp.2006.03.003. PMid:16643892
35. Vishwanatha JK, Chiang Y, Kumble KD, Hollingsworth MA, Pour PM. Enhanced expression of annexin II in human pancreatic carcinoma cells and primary pancreatic cancers. Carcinogenesis. 1993; 14(12):2575–9. https://doi.org/10.1093/carcin/14.12.2575. PMid:8269629
36. Mussunoor S, Murray GI. The role of annexins in tumour development and progression. Pathological Society of Great Britain and Ireland. 2008; 216(2):131–40. https://doi.org/10.1002/path.2400. PMid:18698663
37. Bae SM, Lee C-H, Cho YL, Nam KH, Kim YW, Kim CK, et al. Two-dimensional gel analysis of protein expression profile in squamous cervical cancer patients. Gynecologic Oncology. 2005 Oct; 99(1):26–35. https://doi.org/10.1016/j.ygyno.2005.05.041. PMid:16051329
38. Hellman K, Alaiya AA, Becker S, Lomnytska M, Schedvins K, Steinberg W, et al. Differential tissue-specific protein markers of vaginal carcinoma. British Journal of Cancer. 2009; 100(8):1303. https://doi.org/10.1038/sj.bjc.6604975. PMid:19367286. PMCid:PMC2676541
39. Khorrami A, Sharif Bagheri M, Tavallaei M, Gharechahi J. The functional significance of 14-3-3 proteins in cancer: Focus on lung cancer. Hormone Molecular Biology and Clinical Investigation. 2017 Aug; 32(3). https://doi.org/10.1515/hmbci-2017-0032. PMid:28779564
40. Wilker E, Yaffe MB. 14-3-3 Proteins--a focus on cancer and human disease. Journal of Molecular and Cellular Cardiology. 2004 Sep; 37(3):633–42. https://doi.org/10.1016/j.yjmcc.2004.04.015. PMid:15350836
41. Liu T-A, Jan Y-J, Ko B-S, Liang S-M, Chen S-C, Wang J, et al. 14-3-3ε overexpression contributes to epithelial-mesenchymal transition of hepatocellular carcinoma. PLoS One. 2013 Mar 6; 8(3):e57968. https://doi.org/10.1371/journal.pone.0057968. PMid:23483955 PMCid:PMC3590290

42. Pulukuri SM, Estes N, Rao JS. 14-3-3 sigma promotes cell survival in human prostate cancer cells. Cancer Research. 2005; 65(9 Supplement):229.
43. Xiao Y, Lin VY, Ke S, Lin GE, Lin F-T, Lin W-C. 14-3-3τ Promotes breast cancer invasion and metastasis by inhibiting RhoGDIα. Molecular and Cellular Biology. 2014; 34(14):2635–49. https://doi.org/10.1128/MCB.00076-14. PMid:24820414. PMCid:PMC4097670
44. Liu T, Jan Y, Ko B, Hung Y, Hsu C, Shen T, et al. Increased Expression of 14-3-3 β Promotes Tumor Progression and Predicts Extrahepatic Metastasis and Worse Survival in Hepatocellular Carcinoma. 2011; 179(6):2698–708. https://doi.org/10.1016/j.ajpath.2011.08.010. PMid:21967815. PMCid:PMC3260858
45. Li Z, Zhao J, Du Y, Park HR, Sun S-Y, Bernal-Mizrachi L, et al. Down-regulation of 14-3-3ζ suppresses anchorage-independent growth of lung cancer cells through anoikis activation. Proc Natl Acad Sci. 2008; 105(1):162–7. https://doi.org/10.1073/pnas.0710905105. PMid:18162532. PMCid:PMC2224179
46. Zhang W, Shen Q, Chen M, Wang Y, Zhou Q, Tao X, et al. The role of 14-3-3 proteins in gynecological tumors CANCER 3. 1. Changes in 14-3-3 proteins in cervical carcinogenesis 3. 2. Roles of 14-3-3 proteins in the treatment of cervical cancer. Frontiers in Bioscience. 2015; 934–45. https://doi.org/10.2741/4348. PMid:25961534
47. Dvorakova M, Nenutil R, Bouchal P. Transgelins, cytoskeletal proteins implicated in different aspects of cancer development. Expert Review of Proteomics. 2014 Apr; 11(2):149–65. https://doi.org/10.1586/14789450.2014.860358. PMid:24476357
48. Kristo I, Bajusz I, Bajusz C, Borkuti P, Vilmos P. Actin, actin-binding proteins, and actin-related proteins in the nucleus. Histochemistry and Cell Biology. 2016 Apr; 145(4):373–88. https://doi.org/10.1007/s00418-015-1400-9. PMid:26847179
49. Meng T, Liu L, Hao R, Chen S, Dong Y. Transgelin-2: A potential oncogenic factor. Tumor Biolody. 2017; (277).https://doi.org/10.1177/1010428317702650. PMid:28639888
50. Fukushima C, Murakami A, Yoshitomi K, Sueoka K, Nawata S, Nakamura K, et al. Comparative proteomic profiling in squamous cell carcinoma of the uterine cervix. Proteomics – Clinical Applications. 2011 Apr 1; 5(3–4):133–40. https://doi.org/10.1002/prca.201000077. PMid:21365771
51. Yakabe K, Murakami A, Kajimura T, Nishimoto Y, Sueoka K, Sato S, et al. Functional significance of transgelin-2 in uterine cervical squamous cell carcinoma. Journal of Obstetrics and Gynaecology Research. 2016 May; 42(5):566–72. https://doi.org/10.1111/jog.12935. PMid:26891454
52. Ebrahim AH, Alalawi Z, Mirandola L, Rakhshanda R, Dahlbeck S, Nguyen D, et al. Galectins in cancer: Carcinogenesis, diagnosis and therapy. Annals of Translational Medicine. 2014 Sep; 2(9):88.
53. Dalotto-Moreno T, Croci DO, Cerliani JP, Martinez-Allo VC, Dergan-Dylon S, Mendez-Huergo SP, et al. Targeting galectin-1 overcomes breast cancer-associated immunosuppression and prevents metastatic disease. Cancer Research. 2013 Feb; 73(3):1107–17. https://doi.org/10.1158/0008-5472.CAN-12-2418. PMid:23204230
54. Compagno D, Gentilini LD, Jaworski FM, Perez IG, Contrufo G, Laderach DJ. Glycans and galectins in prostate cancer biology, angiogenesis and metastasis. Glycobiology. 2014 Oct; 24(10):899–906. https://doi.org/10.1093/glycob/cwu055. PMid:24939371
55. Carlini MJ, Roitman P, Nuñez M, Pallotta MG, Boggio G, Smith D, et al. Clinical relevance of galectin-1 expression in non-small cell lung cancer patients. Lung Cancer. 2014; 84(1):73–8. https://doi.org/10.1016/j.lungcan.2014.01.016. PMid:24560493
56. Kim H-J, Do I-G, Jeon H-K, Cho YJ, Park YA, Choi J-J, et al. Galectin 1 expression is associated with tumor invasion and metastasis in stage IB to IIA cervical cancer. Human Pathology. 2013; 44(1):62–8. https://doi.org/10.1016/j.humpath.2012.04.010. PMid:22939954
57. Magin TM, Vijayaraj P, Leube RE. Structural and regulatory functions of keratins. Experimental Cell Research. 2007 Jun; 313(10):2021–32. https://doi.org/10.1016/j.yexcr.2007.03.005. PMid:17434482
58. Karantza V. Keratins in health and cancer: more than mere epithelial cell markers. Oncogene. 2011 Jan; 30(2):127–38. https://doi.org/10.1038/onc.2010.456. PMid:20890307. PMCid:PMC3155291
59. Alix-Panabières C, Vendrell J-P, Slijper M, Pellé O, Barbotte E, Mercier G, et al. Full-length cytokeratin-19 is released by human tumor cells: a potential role in metastatic progression of breast cancer. Breast Cancer Research. 2009 Jun; 11(3):R39. https://doi.org/10.1186/bcr2326. PMid:19549321. PMCid:PMC2716508
60. Ding S-J, Li Y, Tan Y-X, Jiang M-R, Tian B, Liu Y-K, et al. From Proteomic Analysis to Clinical Significance. Molecular & Cellular Proteomics. 2004; 3(1):73–81. https://doi.org/10.1074/mcp.M300094-MCP200. PMid:14593079
61. Somiari RI, Sullivan A, Russell S, Somiari S, Hu H, Jordan R, et al. High-throughput proteomic analysis of human infiltrating ductal carcinoma of the breast. Proteomics. 2003 Oct; 3(10):1863–73. https://doi.org/10.1002/pmic.200300560. PMid:14625848
62. Jia H, Halilou AI, Hu L, Cai W, Liu J, Huang B. Heat shock protein 10 (Hsp10) in immune-related diseases: One coin, two sides. International Journal of Biochemistry and Molecular Biology. 2011; 2(1):47–57.
63. Ciocca DR, Calderwood SK. Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones. 2005 Jun; 10(2):86–103. https://doi.org/10.1379/CSC-99r.1. PMid:16038406. PMCid:PMC1176476
64. Wang J-T, Ding L, Jiang S-W, Hao J, Zhao W-M, Zhou Q, et al. Folate deficiency and aberrant expression of DNA methyltransferase 1 were associated with cervical cancerization. Current Pharmaceutical Design. 2014; 20(11):1639–46.

65. Wu J, Liu T, Rios Z, Mei Q, Lin X, Cao S. Heat Shock Proteins and Cancer. Trends in Pharmacological Sciences. 2017 Mar 1; 38(3):226–56. https://doi.org/10.1016/j.tips.2016.11.009. PMid:28012700
66. Cappello F, Bellafiore M, David S, Anzalone R, Zummo G. Ten kilodalton heat shock protein (HSP10) is overexpressed during carcinogenesis of large bowel and uterine exocervix. Cancer Letters. 2003 Jun; 196(1):35–41. https://doi.org/10.1016/S0304-3835(03)00212-X
67. Cappello F. HSP60 and HSP10 as diagnostic and prognostic tools in the management of exocervical carcinoma. Vol. 91, Gynecologic Oncology. United States; 2003. p. 661. https://doi.org/10.1016/j.ygyno.2003.08.009. PMid:14675699
68. Tetu B, Popa I, Bairati I, L'Esperance S, Bachvarova M, Plante M, et al. Immunohistochemical analysis of possible chemoresistance markers identified by micro-arrays on serous ovarian carcinomas. Modern Pathology features diagnostic anatomic pathology Inc. 2008 Aug; 21(8):1002–10. https://doi.org/10.1038/modpathol.2008.80. PMid:18500265
69. Kumar S, Stokes J, Singh UP, Gunn KS, Acharya A, Manne U, et al. Targeting Hsp70: A possible therapy for cancer. Cancer Letters. 2017; 374:156–66. https://doi.org/10.1016/j.canlet.2016.01.056. PMid:26898980. PMCid:PMC5553548
70. Lee AS. Glucose-regulated proteins in cancer: molecular mechanisms and therapeutic potential. Nature Reviews Cancer. 2014 Apr; 14(4):263–76. https://doi.org/10.1038/nrc3701. PMid:24658275. PMCid:PMC4158750
71. Dong D, Stapleton C, Luo B, Xiong S, Ye W, Zhang Y, et al. A critical role for GRP78/BiP in the tumor microenvironment for neovascularization during tumor growth and metastasis. Cancer Research. 2011; 71(8):2848–57. https://doi.org/10.1158/0008-5472.CAN-10-3151. PMid:21467168. PMCid:PMC3078191
72. Li Z, Zhang L, Zhao Y, Li H, Xiao H, Fu R, et al. Cell-surface GRP78 facilitates colorectal cancer cell migration and invasion. International Journal of Biochemistry & Cell Biology. 2013; 45(5):987–94. https://doi.org/10.1016/j.biocel.2013.02.002. PMid:23485528
73. Winder SJ, Kathryn R, Winder SJ, Ayscough KR. Actin-binding proteins Actin-binding Proteins. 2005; 2005(L):651–4. https://doi.org/10.1242/jcs.01670. PMid:15701920
74. Deng R, Hao J, Han W, Ni Y, Huang X, Hu Q. Gelsolin regulates proliferation, apoptosis, migration and invasion in human oral carcinoma cells. Oncology Letters. 2015 May; 9(5):2129–34. https://doi.org/10.3892/ol.2015.3002. PMid:26137026. PMCid:PMC4467278
75. An J-H, Kim J-W, Jang S-M, Kim C-H, Kang E-J, Choi K-H. Gelsolin negatively regulates the activity of tumor suppressor p53 through their physical interaction in hepatocarcinoma HepG2 cells. Biochemical and Biophysical Research Communications. 2011 Aug; 412(1):44–9. https://doi.org/10.1016/j.bbrc.2011.07.034. PMid:21801713
76. Liao C-J, Wu T-I, Huang Y-H, Chang T-C, Wang C-S, Tsai M-M, et al. Overexpression of gelsolin in human cervical carcinoma and its clinicopathological significance. Gynecologic Oncology. 2011 Jan; 120(1):135–44. https://doi.org/10.1016/j.ygyno.2010.10.005. PMid:21035170
77. Fu Q, Liu Y, Fan Y, Hua S, Qu H, Dong S, et al. Alpha-enolase promotes cell glycolysis, growth, migration, and invasion in non-small cell lung cancer through FAK-mediated PI3K / AKT pathway. 2015; 1–13. https://doi.org/10.1186/s13045-015-0117-5. PMid:25887760. PMCid:PMC4359783
78. Song Y, Luo Q, Long H, Hu Z, Que T, Zhang X, et al. Alpha-enolase as a potential cancer prognostic marker promotes cell growth, migration, and invasion in glioma. 2014; 1–12. https://doi.org/10.1186/1476-4598-13-235. PMid:25600072. PMCid:PMC4464720
79. Sun L, Lu T, Tian K, Zhou D, Yuan J, Wang X, et al. Alpha-enolase promotes gastric cancer cell proliferation and metastasis via regulating AKT signaling pathway. European Journal of Pharmacology. 2019 Feb; 845:8215. https://doi.org/10.1016/j.ejphar.2018.12.035. PMid:30582908
80. Sun L, Guo C, Cao J, Burnett J, Yang Z, Ran Y, et al. Over-expression of alpha-enolase as a prognostic biomarker in patients with pancreatic cancer. International Journal of Medical Sciences. 2017; 14(7):655–61. https://doi.org/10.7150/ijms.18736. PMid:28824297. PMCid:PMC5562116
81. Bamburg JR, Mcgough A. Putting a new twist on actin: ADF / cofilins modulate actin dynamics. 1999; 9(Sep):364–70. https://doi.org/10.1016/S0962-8924(99)01619-0
82. Ding S, Li Y, Shao X, Zhou H, Zeng R, Tang Z, et al. Proteome analysis of hepatocellular carcinoma cell strains, MHCC97-H and MHCC97-L, with different metastasis potentials. Proteomics. 2004; 4(4):982–94. https://doi.org/10.1002/pmic.200300653. PMid:15048980
83. Dowling P, Meleady P, Dowd A, Henry M, Glynn S, Clynes M. Proteomic analysis of isolated membrane fractions from superinvasive cancer cells. Biochim Biophys Acta (BBA)-Proteins Proteomics. 2007; 1774(1):93–101. https://doi.org/10.1016/j.bbapap.2006.09.014. PMid:17085086
84. Keshamouni VG, Michailidis G, Grasso CS, Anthwal S, Strahler JR, Walker A, et al. Differential protein expression profiling by iTRAQ– 2DLC– MS/MS of lung cancer cells undergoing epithelial-mesenchymal transition reveals a migratory/invasive phenotype. Journal of Proteome Research. 2006; 5(5):1143–54. https://doi.org/10.1021/pr050455t. PMid:16674103
85. Martoglio A-M, Tom BDM, Starkey M, Corps AN, Charnock-Jones DS, Smith SK. Changes in tumorigenesis-and angiogenesis-related gene transcript abundance profiles in ovarian cancer detected by tailored high density cDNA arrays. Molecular Medicine. 2000; 6(9):750. https://doi.org/10.1007/BF03402191
86. Sinha P, Hütter G, Köttgen E, Dietel M, Schadendorf D, Lage H. Increased expression of epidermal fatty acid binding

protein, cofilin, and 14-3-3-σ (stratifin) detected by two-dimensional gel electrophoresis, mass spectrometry and microsequencing of drug-resistant human adenocarcinoma of the pancreas. Electrophoresis. 1999; 20(14):2952–60. https://doi.org/10.1002/(SICI)1522-2683(19991001)20:14<2952::AID-ELPS2952>3.0.CO;2-H

87. Turhani D, Krapfenbauer K, Thurnher D, Langen H, Fountoulakis M. Identification of differentially expressed, tumor-associated proteins in oral squamous cell carcinoma by proteomic analysis. Electrophoresis. 2006; 27(7):1417–23. https://doi.org/10.1002/elps.200500510. PMid:16568407
88. Unwin RD, Craven RA, Harnden P, Hanrahan S, Totty N, Knowles M, et al. Proteomic changes in renal cancer and co-ordinate demonstration of both the glycolytic and mitochondrial aspects of the Warburg effect. Proteomics. 2003; 3(8):1620–32. https://doi.org/10.1002/pmic.200300464 PMid:12923786
89. Mousavi S, Safaralizadeh R, Hosseinpour-Feizi M, Azimzadeh-Isfanjani A, Hashemzadeh S. Study of cofilin 1 gene expression in colorectal cancer. Journal of Gastrointestinal Oncology. 2018 Oct; 9(5):791–6.
90. Pappa KI, Lygirou V, Kontostathi G, Zoidakis J, Makridakis M, Vougas K, et al. Proteomic analysis of normal and cancer cervical cell lines reveals deregulation of cytoskeleton-associated proteins. Cancer Genomics Proteomics. 2017; 14(4):253–66. https://doi.org/10.21873/cgp.20036. PMid:28647699. PMCid:PMC5572303
91. Polachini GM, Sobral LM, Mercante AMC, Paes-Leme AF, Xavier FCA, Henrique T, et al. Proteomic approaches identify members of cofilin pathway involved in oral tumorigenesis. PLoS One. 2012; 7(12):e50517. https://doi.org/10.1371/journal.pone.0050517. PMid:23227181. PMCid:PMC3515627
92. Wang F, Wu D, Fu H, He F, Xu C, Zhou J, et al. Cofilin 1 promotes bladder cancer and is regulated by TCF7L2. Oncotarget. 2017 Sep 6; 8(54):92043–54. https://doi.org/10.18632/oncotarget.20664. PMid:29190896. PMCid:PMC5696162
93. Wang Y, Kuramitsu Y, Ueno T, Suzuki N, Yoshino S, Iizuka N, et al. Differential expression of up-regulated cofilin-1 and down-regulated cofilin-2 characteristic of pancreatic cancer tissues. Oncology Reports. 2011; 26(6):1595–9. https://doi.org/10.3892/or.2011.1447
94. Yang Z-L, Miao X, Xiong L, Zou Q, Yuan Y, Li J, et al. CFL1 and Arp3 are biomarkers for metastasis and poor prognosis of squamous cell/adenosquamous carcinomas and adenocarcinomas of gallbladder. Cancer Investigation. 2013; 31(2):132–9. https://doi.org/10.3109/07357907.2012.756113. PMid:23320827
95. Satelli A, Li S. Vimentin in cancer and its potential as a molecular target for cancer therapy. Cellular and Molecular Life Sciences. 2011 Sep; 68(18):3033–46. https://doi.org/10.1007/s00018-011-0735-1. PMid:21637948. PMCid:PMC3162105
96. Gilles C, Polette M, Piette J, Delvigne A, Thompson EW, Foidart J, et al. Vimentin expression in cervical carcinomas: association with invasive and migratory potential. Journal of Pathology. 1996; 180(2):175–80. https://doi.org/10.1002/(SICI)1096-9896(199610)180:2<175::AID-PATH630>3.0.CO;2-G
97. Gilles C, Polette M, Mestdagt M, Nawrocki-Raby B, Ruggeri P, Birembaut P, et al. Transactivation of vimentin by β-catenin in human breast cancer cells. Cancer Research. 2003; 63(10):2658–64. https://doi.org/10.1136/ijgc-00009577-200303001-00219. PMid:12750294
98. Hong S-H, Misek DE, Wang H, Puravs E, Hinderer R, Giordano TJ, et al. Identification of a specific vimentin isoform that induces an antibody response in pancreatic cancer. Biomark Insights. 2006; 1. https://doi.org/10.1177/117727190600100006
99. Jin H, Morohashi S, Sato F, Kudo Y, Akasaka H, Tsutsumi S, et al. Vimentin expression of esophageal squamous cell carcinoma and its aggressive potential for lymph node metastasis. Biomedical Research. 2010; 31(2):105–12. https://doi.org/10.2220/biomedres.31.105. PMid:20460738
100. Ngan CY, Yamamoto H, Seshimo I, Tsujino T, Man-i M, Ikeda JI, et al. Quantitative evaluation of vimentin expression in tumour stroma of colorectal cancer. British Journal of Cancer. 2007; 96(6):986. https://doi.org/10.1038/sj.bjc.6603651. PMid:17325702. PMCid:PMC2360104
101. Takemura K, Hirayama R, Hirokawa K, Inagaki M, Tsujimura K, Esaki Y, et al. Expression of vimentin in gastric cancer: a possible indicator for prognosis. Pathobiology. 1994; 62(3):149–54. https://doi.org/10.1159/000163895. PMid:7945921
102. Wu M, Bai X, Xu G, Wei J, Zhu T, Zhang Y, et al. Proteome analysis of human androgen-independent prostate cancer cell lines: Variable metastatic potentials correlated with vimentin expression. Proteomics. 2007; 7(12):1973–83. https://doi.org/10.1002/pmic.200600643. PMid:17566973
103. Vuoriluoto K, Haugen H, Kiviluoto S, Mpindi JP, Nevo J, Gjerdrum C, et al. Vimentin regulates EMT induction by Slug and oncogenic H-Ras and migration by governing Axl expression in breast cancer. Oncogene. 2011; 30(12):1436. https://doi.org/10.1038/onc.2010.509. PMid:21057535
104. Walsh N, O'Donovan N, Kennedy S, Henry M, Meleady P, Clynes M, et al. Identification of pancreatic cancer invasion-related proteins by proteomic analysis. Proteome Science. 2009; 7(1):3. https://doi.org/10.1186/1477-5956-7-3. PMid:19216797. PMCid:PMC2646716
105. Chen C-N, Chang C-C, Su T-E, Hsu W-M, Jeng Y-M, Ho M-C, et al. Identification of calreticulin as a prognosis marker and angiogenic regulator in human gastric cancer. Annals of Surgical Oncology. 2009; 16(2):524–33. https://doi.org/10.1245/s10434-008-0243-1. PMid:19050968
106. Sheng W, Chen C, Dong M, Zhou J, Liu Q, Dong Q, et al. Overexpression of calreticulin contributes to the development and progression of pancreatic cancer. Journal of Cellular Physiology. 2014; 229(7):887–97. https://doi.org/10.1002/jcp.24519. PMid:24264800

107. Vaksman O, Davidson B, Tropé C, Reich R. Calreticulin expression is reduced in high-grade ovarian serous carcinoma effusions compared with primary tumors and solid metastases. Human Pathology. 2013; 44(12):2677–83. https://doi.org/10.1016/j.humpath.2013.07.009. PMid:24060004
108. Alfonso P, Núñez A, Madoz-Gurpide J, Lombardia L, Sánchez L, Casal JI. Proteomic expression analysis of colorectal cancer by two-dimensional differential gel electrophoresis. Proteomics. 2005; 5(10):2602–11. https://doi.org/10.1002/pmic.200401196. PMid:15924290
109. Du X-L, Hu H, Lin D-C, Xia S-H, Shen X-M, Zhang Y, et al. Proteomic profiling of proteins dysregulted in Chinese esophageal squamous cell carcinoma. Journal of Molecular Medicine. 2007; 85(8):863–75. https://doi.org/10.1007/s00109-007-0159-4. PMid:17318615
110. Harada K, Takenawa T, Ferdous T, Kuramitsu Y, Ueyama Y. Calreticulin is a novel independent prognostic factor for oral squamous cell carcinoma. Oncology Letters. 2017 Jun; 13(6):4857–62. https://doi.org/10.3892/ol.2017.6062. PMid:28599487. PMCid:PMC5452987
111. Kageyama S, Isono T, Iwaki H, Wakabayashi Y, Okada Y, Kontani K, et al. Identification by proteomic analysis of calreticulin as a marker for bladder cancer and evaluation of the diagnostic accuracy of its detection in urine. Clinical Chemistry. 2004; 50(5):857–66. https://doi.org/10.1373/clinchem.2003.027425. PMid:14764641
112. Matsukuma S, Yoshimura K, Ueno T, Oga A, Inoue M, Watanabe Y, et al. Calreticulin is highly expressed in pancreatic cancer stem-like cells. Cancer Science. 2016 Nov 1; 107(11):1599–609. https://doi.org/10.1111/cas.13061. PMid:27561105. PMCid:PMC5132278
113. Zamanian M, Qader Hamadneh LA, Veerakumarasivam A, Abdul Rahman S, Shohaimi S, Rosli R. Calreticulin mediates an invasive breast cancer phenotype through the transcriptional dysregulation of p53 and MAPK pathways. Cancer Cell International. 2016; 16(1):56. https://doi.org/10.1186/s12935-016-0329-y. PMid:27418879. PMCid:PMC4944499
114. Opas M, Szewczenko-Pawlikowski M, Jass GK, Mesaeli N, Michalak M. Calreticulin modulates cell adhesiveness via regulation of vinculin expression. Journal of Cell Biology. 1996; 135(6):1913–23. https://doi.org/10.1083/jcb.135.6.1913. PMid:8991101. PMCid:PMC2133944
115. Lu Y-C, Chen C-N, Wang B, Hsu W-M, Chen S-T, Chang K-J, et al. Changes in tumor growth and metastatic capacities of J82 human bladder cancer cells suppressed by down-regulation of calreticulin expression. American Journal of Pathology. 2011; 179(3):1425–33. https://doi.org/10.1016/j.ajpath.2011.05.015. PMid:21723245. PMCid:PMC3157280
116. Lu Y-C, Weng W-C, Lee H. Functional roles of calreticulin in cancer biology. BioMed Research International. 2015.