ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
Food Science and Technology

DOI: http://dx.doi.org/10.1590/1678-457X.00917

Inhibitory effect of *Dendrobium officinale* polysaccharide on human gastric cancer cell xenografts in nude mice

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Abstract

This study investigated the inhibitory effect of *Dendrobium officinale* polysaccharide (DOPA) on human gastric cancer cell SGC-7901 xenografts in nude mice. The nude mice with SGC-7901 xenografts were randomly divided into model, 5-fluorouracil (5-Fu), low-dose DOPA, middle-dose DOPA and high-dose DOPA group. The later four groups were intragastrically administrated with 100, 200 and 400 mg·kg^{-1}·day^{-1} DOPA, 400 mg·kg^{-1}·day^{-1} 5-Fu and normal saline, respectively. After treatment for 20 days, the tumor inhibition rate of in high-dose DOPA group was basically equivalent to 5-Fu group. Compared with 5-Fu, DOPA had no obvious toxic side effect on spleen or thymus indexes, routine blood indexes or liver and kidney functions of nude mice. Compared with model group, the serum tumor necrosis factor-α and interleukin-2 levels in middle- and high-dose DOPA group were significantly increased ($P < 0.05$), Bax protein expression was significantly increased ($P < 0.05$), and Bcl-2 protein expression was significantly decreased ($P < 0.05$). DOPA can inhibit the growth of SGC-7901 cell xenografts in nude mice. The mechanism may be related to its increase of serum TNF-α and IL-2 levels, up-regulation of Bax protein expression and down-regulation of Bcl-2 protein expression.

Keywords: *Dendrobium officinale*; polysaccharide; gastric cancer; nude mice.

Practical Application: *Dendrobium officinale* polysaccharide has applicable value for treatment of human gastric cancer.

1 Introduction

Dendrobium officinale is a member of *Orchidales Dendrobium* plants, and is one of the precious traditional Chinese medicines in China (Li et al., 2008). It has the effect of nourishing *Yin*, clearing heat, reinforcing stomach fluid, and moistening lung (Lin et al., 2010). A variety of active ingredients have been isolated from *Dendrobium officinale*, including alkaloids, polysaccharides, sesquiterpenes, phenanthrene quinones, bibenzyls, fluorenones, steroids, phenols and volatile oils (Lv et al., 2013). The polysaccharide is the main component of *Dendrobium officinale*, which has immune-regulating (Liu et al., 2011), anti-oxidant (Luo et al., 2016), anti-tumor (Bao, 2008), antibacterial (Lei, 2011) and anti-hypoglycemic activity (Chen et al., 2003). Gastric cancer is one of the common malignant tumors, with high mortality (Leylabadlo et al., 2016). With the change of modern lifestyle, the incidence of gastric cancer is increasing year by year, which poses a great threat to people's health. The occurrence and development of gastric cancer is a process of carcinogenesis caused by multiple factors, multiple stages and polygenic variations (Eom et al., 2012). The single resection can be applied for gastric cancer, but the cure is only limited for early-stage gastric cancer without any metastasis. For early-stage gastric cancer with metastasis or advanced-stage gastric cancer, the medical treatment occupies an important position (Lepage et al., 2010). SGC-7901 cell line is one kind of the most common gastric cancer cells in human. The propagation speed of SGC-7901 cells directly determines the development of gastric cancer (Sun & Wang, 2003). At present, the application of *Dendrobium officinale* polysaccharide (DOPA) to treatment of SGC-7901 cells is rarely reported. This study investigated the inhibitory effect of DOPA on growth of SGC-7901 cell xenografts in nude mice, and analyzed the possible mechanism. The objective was to provide an experimental basis to further application of DOPA to treatment of human gastric cancer.

2 Materials and methods

2.1 Materials

DOPA was prepared in our laboratory by extracting from the stem of *Dendrobium officinale* (Yunnan Kim Gu Biological Technology Co., Ltd., Kunming, China). The content of polysaccharide was 80%. BALB/c nude mice (male, 4-6 weeks old, 18.32-22.45 g) were purchased from Shanghai Silaike Experimental Animal Co., Ltd (Shanghai, China). Human gastric cancer SGC-7901 cells were provided by Nanjing KeyGen Biotech. Co., Ltd. (Nanjing, China). 5-Fluorouracil (5-FU) injection (0.25 mg, 10 ml) was provided by Tianjin KingYork Amino Acid Co., Ltd. (Tianjin, China). Other reagents were purchased from Sigma-Aldrich Corp. (MO, USA).

Received 19 Jan., 2017
Accepted 21 Mar., 2017
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2.2 Establishment of SGC-7901 xenograft model of nude mice

SGC-7901 cells were cultured in vitro. The cells in logarithmic growth phase were collected, and the single cell suspension (1×10^8 cells/mL) was prepared. 0.2 mL of cell suspension was subcutaneously inoculated at the right dorsal side of nude mice. The nude mice were routinely reared. The nutritional status and daily activity were observed daily. The diameter of subcutaneous tumor was measured using vernier caliper every 2-3 days. When the tumor volume increased to about 50 mm^3, the nude mice with no bleeding, necrosis or infection were selected for further experiments.

2.3 Animal grouping and treatment

The modeled nude mice were randomly divided into model, 5-fluorouracil (5-Fu), low-dose DOPA, middle-dose DOPA and high-dose DOPA group, 10 rats in each group. The low-, middle-and high-dose DOPA group were intragastrically administrated with DOPA with dose of 100, 200 and 400 $mg \cdot kg^{-1} \cdot day^{-1}$, respectively. The 5-Fu group was intragastrically administrated with 5-Fu ($400\ mg \cdot kg^{-1} \cdot day^{-1}$). The model group was given with the same volume of normal saline. The treatment was continued for 20 days. On the second day after stopping taking drugs, the nude mice were weighted, and the orbital blood of mice was taken. Then, the mice were sacrificed by cervical dislocation.

2.4 Detection of blood routine and liver and kidney functions

The blood routine indexes including red blood cell (RBC), white blood cell (WBC); hemoglobin (Hb) and platelet (PLT) liver and kidney function indexes including alanine aminotransferase (ALT), aspartate aminotransferase (AST) creatinine (CREA) and urea were detected using BC-5000 full automatic blood analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China).

2.5 Determination of tumor growth

After the mice were sacrificed, the complete tumor tissue was taken out. After stripping thin fiber film, the tumor tissue was weighted on the electronic balance. The tumor inhibition rate was calculate as follows: tumor inhibition rate (%) = (average tumor mass in model group - average tumor mass in experiment group) / average tumor mass in model group) × 100%. In addition, the spleen and thymus were separated, and were weighted. The spleen index and thymus index were calculated as follows: thymus index = thymus mass (mg) / body mass (g); spleen index = spleen mass (mg) / body mass (g).

2.6 Determination of serum tumor necrosis factor-α and interleukin-2 levels

The orbital blood of nude mice was centrifuged at 3000 r/min for 5 min. The serum levels of tumor necrosis factor-α (TNF-α) and interleukin-2 (IL-2) were detected by ELISA (Ajami et al., 2014).

2.7 Determination of B-cell lymphoma-2 and Bcl-2 associated X protein expression in tumor tissue

The paraffin sections of tumor were prepared. The immunohistochemical SP staining was performed to detect the expression of B-cell lymphoma-2 (Bcl-2) and Bcl-2 associated X (Bax) protein. PBS instead of primary antibody was used as negative control. The brown staining was judged as positive, with tawny staining as weakly positive. 10 tumor tissue sections were selected for each index, and 3 fields of vision were selected for each section. The images were analyzed using BI-2000 image analysis system (Chengdu Taimeng Software Co. Ltd., Chengdu, China), and the integrated option density (IOD) of each section was calculated.

2.8 Statistical analysis

All statistical analysis was carried out using SPSS17.0 software (SPSS Inc., Chicago, IL, USA). Each experiment was repeated for three times, and the data were presented as mean±SD. The difference between two groups was analyzed using t test. $P < 0.05$ was considered as statistically significant.

3 Results

3.1 Effects of DOPA on growth of SGC-7901 tumor in nude mice

As shown in Table 1, the tumor masses in model, 5-Fu, low-dose DOPA, middle-dose DOPA and high-dose DOPA groups were 1.0 ± 0.1, 0.5 ± 0.1, 0.8 ± 0.1, 0.6 ± 0.1 and 0.5 ± 0.1 g, respectively, and the inhibition rates of tumor in 5-Fu, low-dose DOPA, middle-dose DOPA and high-dose DOPA groups were 49 ± 7%, 21 ± 3%, 39 ± 6% and 47 ± 8%, respectively. The inhibition rate in middle- and low-dose DOPA group was significantly lower than that in 5-Fu group, respectively ($P < 0.05$), but there was no significant difference between high-dose DOPA group and 5-Fu group ($P > 0.05$).

Table 1. Effects of DOPA on growth of SGC-7901 tumor in nude mice.

Group	n	Tumor mass (g)	Inhibition rate (%)
Model	10	1.0 ± 0.1	-
5-Fu	10	0.5 ± 0.1^{a}	49 ± 7
Low-dose DOPA	10	0.8 ± 0.1^{ab}	21 ± 3^{b}
Middle-dose DOPA	10	0.6 ± 0.1^{a}	39 ± 6^{c}
High-dose DOPA	10	0.5 ± 0.1^{ad}	47 ± 8^{d}

[a]$P < 0.05$ compared with model group; [b]$P < 0.01$ compared with 5-Fu group; [c]$P < 0.05$ compared with 5-Fu group; [d]$P < 0.05$ compared with low-dose DOPA group. DOPA: *Dendrobium officinale* polysaccharide; 5-Fu: 5-fluorouracil.

3.2 Effects of DOPA on spleen and thymus indexes of nude mice

Table 2 showed that, compared with model group, the spleen and thymus index in 5-Fu group were significantly decreased, respectively ($P < 0.05$), but those in low-, middle- and high-dose DOPA group were significantly higher than 5-Fu group, respectively ($P < 0.05$). There was no significant difference of each index between each DOPA group and model ($P > 0.05$).

3.3 Effects of DOPA on routine blood indexes of nude mice

As shown in Table 3, the WBC and PLT concentrations in 5-Fu group were significantly lower than those in model group, respectively ($P < 0.05$), and those in low-, middle- and high-dose DOPA group were significantly higher than that in 5-Fu group, respectively ($P < 0.05$). There was no significant difference of WBC or PLT among low-, middle- and high-dose DOPA group and model groups ($P > 0.05$), and there was no significant difference of RBC or Hb concentration among 5 groups ($P > 0.05$).

3.4 Effects of DOPA on liver and kidney functions of nude mice

Table 4 showed that, the ALT level in 5-Fu group was significantly higher than that in model group, respectively ($P < 0.05$), and that in low-, middle- and high-dose DOPA group was significantly lower than that in 5-Fu group, respectively ($P < 0.05$). There was no significant difference of ALT level among low-, middle- and high-dose DOPA group and model groups ($P > 0.05$), and there was no significant difference of AST, CREA or urea level among 5 groups ($P > 0.05$).

3.5 Effects of DOPA on serum TNF-α and IL-2 levels of nude mice.

Compared with model group, the serum TNF-α and IL-2 levels of nude mice in 5-Fu, middle-dose DOPA and high-dose DOPA group were significantly increased, respectively ($P < 0.05$). In addition, the serum TNF-α and IL-2 levels in middle-dose and high-dose DOPA group were significantly higher than low-dose DOPA group, respectively ($P < 0.05$). There was no significant difference of TNF-α or IL-2 level between middle-dose DOPA and 5-Fu group or between high-dose DOPA and 5-Fu group ($P < 0.05$) (Table 5).

3.6 Effects of DOPA on expression of Bax and Bcl-2 protein

As shown in Table 6, compared with model group, the IOD value of Bax protein expression in 5-Fu, middle-dose DOPA and high-dose DOPA group were significantly increased, respectively ($P < 0.05$), the IOD value of Bcl-2 protein expression in 5-Fu, middle-dose DOPA and high-dose DOPA group were significantly decreased, respectively ($P < 0.05$). The IOD value of Bcl-2 and

Table 2. Effects of DOPA on spleen and thymus indexes of nude mice.

Group	n	Spleen index (mg/g)	Thymus index (mg/g)
Model	10	5 ± 1	34 ± 17
5-Fu	10	3 ± 1[a]	2 ± 1[a]
Low-dose DOPA	10	5 ± 1[b]	3 ± 1[b]
Middle-dose DOPA	10	5 ± 1[b]	3 ± 1[b]
High-dose DOPA	10	5 ± 1[b]	4 ± 1[b]

[a]$P < 0.05$ compared with model group; [b]$P < 0.05$ compared with 5-Fu group. DOPA: *Dendrobium officinale* polysaccharide; 5-Fu: 5-fluorouracil.

Table 3. Effects of DOPA on routine blood indexes of nude mice.

Group	n	RBC ($\times 10^{12}$/L)	WBC ($\times 10^{9}$/L)	Hb (g/L)	PLT ($\times 10^{9}$/L)
High-dose DOPA	10	8 ± 3	113 ± 36[b]	148 ± 20	1145 ± 201[b]
Middle-dose DOPA	10	8 ± 2	10 ± 3[b]	153 ± 18	1123 ± 231[b]
Low-dose DOPA	10	8 ± 2	10 ± 2[b]	147 ± 24	1172 ± 178[b]
5-Fu	10	9 ± 2	7 ± 1[a]	138 ± 19	934 ± 100[a]
Model	10	8 ± 2	11 ± 4	146 ± 17	1211 ± 189

[a]$P < 0.05$ compared with model group; [b]$P < 0.05$ compared with 5-Fu group; DOPA: *Dendrobium officinale* polysaccharide; 5-Fu: 5-fluorouracil; RBC: red blood cell; WBC: white blood cell; Hb: hemoglobin; PLT: platelet.

Table 4. Effects of DOPA on liver and kidney functions of nude mice.

Group	n	ALT (U/L)	AST (U/L)	CREA (μmol/L)	Urea (mmol/L)
Model	10	62 ± 6	486 ± 79	8 ± 1	6 ± 2
5-Fu	10	85 ± 8[a]	490 ± 56	8 ± 2	78 ± 1
Low-dose DOPA	10	61 ± 7[b]	472 ± 89	7 ± 2	6 ± 2
Middle-dose DOPA	10	68 ± 9[b]	446 ± 67	7 ± 21	6 ± 2
High-dose DOPA	10	62 ± 8[b]	445 ± 78	8 ± 2	6 ± 2

[a]$P < 0.05$ compared with model group; [b]$P < 0.05$ compared with 5-Fu group. DOPA: *Dendrobium officinale* polysaccharide; 5-Fu: 5-fluorouracil; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CREA: creatinine.

Table 5. Effects of DOPA on serum TNF-α and IL-2 levels of nude mice.

Group	n	TNF-α (ng/L)	IL-2 (ng/L)
Model	10	38 ± 4	616 ± 9
5-Fu	10	64 ± 5[a]	153 ± 20[a]
Low-dose DOPA	10	49 ± 6[b]	70 ± 7[b]
Middle-dose DOPA	10	62 ± 7[ac]	143 ± 16[ac]
High-dose DOPA	10	60 ± 8[ac]	165 ± 19[ac]

[a]P < 0.05 compared with model group; [b]P < 0.05 compared with 5-Fu group; [c]P < 0.05 compared with low-dose DOPA group. DOPA: *Dendrobium officinale* polysaccharide; 5-Fu: 5-fluorouracil; TNF-α: tumor necrosis factor-α; IL-2: interleukin-2.

Table 6. Effects of DOPA on expression of Bax and Bcl-2 protein (IOD).

Group	n	Bax	Bcl-2
Model	10	32367 ± 5623	79159 ± 9567
5-Fu	10	57443 ± 6892[a]	38221 ± 4563[a]
Low-dose DOPA	10	37755 ± 4785	64536 ± 7834
Middle-dose DOPA	10	52535 ± 6456[a]	42987 ± 7345[a]
High-dose DOPA	10	61174 ± 7345[b]	42545 ± 7346[a]

[a]P < 0.05 compared with model group; [b]P < 0.01 compared with model group. DOPA: *Dendrobium officinale* polysaccharide; 5-Fu: 5-fluorouracil; IOD: integrated option density; Bax: Bcl-2 associated X protein; Bcl-2: B-cell lymphoma-2.

Bax protein had no significant difference between middle-dose DOPA and 5-Fu group and between high-dose DOPA and 5-Fu group, respectively (P < 0.05).

4 Discussion

Dendrobium is a kind of precious medicinal plants in China, which has high medicinal value. It is found that *Dendrobium* has obvious anti-tumor effect. He et al. (2007) have studied the inhibition effect of DOPA on mouse hepatoma H22 cells and find that, DOPA with dose of 50 mg/kg can significantly inhibit the growth of H22 cells, and the inhibition rate is 28.6%. Bao (2008) has investigated the inhibition effect of water extracts from 4 kinds of *Dendrobium* on human cervical cancer HelaS3 cells and human hepatoma HepG2 cells. Results show that, all the water extracts from *Dendrobium huoshanense*, *Dendrobium officinale*, *Dendrobium nobile* and *Dendrobium fimbriatum* have inhibitiory effect on HeLaS3 and HepG2 cells. The DOPA has the best inhibitory effect on HelaS3 cells.

This study has established the model of SGC-7901 xenograft in nude mice, and investigated the inhibitory effect of DOPA on the growth of SGC-7901 tumor. Results showed that, the inhibition rate of SGC-7901 tumor in high-dose DOPA group was basically equivalent to that in 5-Fu group. Generally, the drug over a certain concentration will produce toxic side effects to the body, which are mainly manifested in the blood system and liver and kidney function (Boisdron-Celle et al., 2007). In this study, compared with model group, the spleen and thymus indexes of nude mice in 5-Fu group were significantly decreased (P < 0.05). In addition, the WBC and PLT concentrations in 5-Fu group were significantly lowered (P < 0.05), and the ALT level in 5-Fu group was significantly increased (P < 0.05). This indicates that, 5-Fu can inhibit the growth of SGC-7901 tumor in nude mice, but it has certain toxicity to the blood and liver and kidney system. Compared with 5-Fu group, the spleen and thymus indexes in low-, middle- and high-dose DOPA group were significantly increased (P < 0.05), the WBC and PLT concentration was significantly improved (P < 0.05), and ALT level was significantly decreased (P < 0.05). This indicates that, DOPA can obviously inhibit the growth of SGC-7901 tumor in nude mice, and has no obvious toxic side effect on the blood system and liver and kidney function of nude mice.

Cytokines are the protein polypeptides secreted and mediated by immune cells and stromal cells, which regulate the immune function and inflammatory response. Cytokines play an important role in the occurrence and development of tumors and their therapy (Ardestani et al., 1999). IL-2 and TNF-α were the important cytokines in anti-tumor aspect. IL-2 is a growth factor with pleiotropic activity. It constitutes a cytokine network together with other cytokines, and participates in the self-tolerance and autoimmunity. IL-2 is an important index to reflect the immune function, and plays an important role in tumor therapy (Carrier et al., 2007; Dooms & Abbas, 2010). TNF-α also has direct cytotoxic effect and growth-inhibition effect on tumor cells. It can directly inhibit and kill tumor cells and induce the cell apoptosis, mediate the immune response, cause the tumor microvascular injury and inhibit the tumor angiogenesis, thus exerting the anti-tumor function (Wang et al., 2000b; Hammam et al., 2013). Results of this study showed that, compared with model group, the serum TNF-α and IL-2 levels of nude mice in 5-Fu, middle-dose DOPA and high-dose DOPA group were significantly increased, respectively (P < 0.05). This indicates that, DOPA may inhibit the growth of SGC-7901 tumor in nude mice by up-regulating the serum TNF-α and IL-2 levels. This is the same with the previous studies (Zhang et al., 2004; Tang et al., 2013).

The maintenance of homeostasis of cell proliferation and apoptosis is the premise of the stability of body function. Once the homeostasis is broken, the cell proliferation exceeds the apoptosis, and the tumor will occur (Lai et al., 2005). For tumor cells, if the apoptosis exceeds the proliferation, they will be inhibited. Bcl-2 is an oncogene, of which the expression is

related to the differentiation degree and proliferation ability of tumor cells. Bcl-2 is over-expressed in tumor cells with low differentiation and strong proliferation ability (Wang et al., 2000a). Bax protein is a water-soluble protein homologous to Bcl-2 protein. It can promote the tumor cell apoptosis. The over-expression of Bax protein can antagonize the protective effect of Bcl-2 and cause the apoptosis of cells (Deichman et al., 2007). Bcl-2 and Bax form an apoptotic regulatory system, of which the Bcl-2/Bax ratio determines whether the cells tend to apoptosis. Bcl-2 protein is distributed in the cell membrane and cytoplasm, and controls the permeability of mitochondrial outer and inner membranes. It can inhibit the reduction of mitochondrial membrane potential, release CytC and apoptosis initiation factor, and activate Caspase, thus inhibiting the cell apoptosis (Sentman et al., 1991). Bax protein is located in the cytoplasm, and has the function of promoting cell apoptosis (Nutt et al., 2002). In this study, compared with model group, the IOD value of Bax protein expression in 5-Fu, middle-dose DOPA and high-dose DOPA group were significantly increased, respectively ($P < 0.05$), the IOD value of Bcl-2 protein expression in 5-Fu, middle-dose DOPA and high-dose DOPA group were significantly decreased, respectively ($P < 0.05$). This indicates that, the inhibitory effects of DOPA on SGC-7901 tumor may be related to its up-regulation of Bax protein expression and down-regulation of Bcl-2 protein expression.

5 Conclusions

DOPA can inhibit the growth of SGC-7901 cell xenografts in nude mice. The possible mechanism may be that, DOPA can up-regulate the serum TNF-α and IL-2 levels. In addition, the inhibitory effects may be related to its up-regulation of Bax protein expression and down-regulation of Bcl-2 protein expression. DOPA has no has no obvious toxic side effect on the blood system and liver and kidney function of nude mice. This study has provided an experimental basis for application of DOPA to treatment of human gastric cancer. Of course, whether there are other mechanisms of DOPA action and whether there are correlations among the present indexes need to be further investigated.

References

Ajami, A., Abedian, F., Hamzeh Hosseini, S., Akbarian, E., Alizadeh-Navaei, R., & Taghipour, M. (2014). Serum TNF-α, IL-10 and IL-2 in schizophrenic patients before and after treatment with risperidone and clozapine. *Iranian Journal of Immunology*, 11(3), 200-209. PMid:25265997.

Ardestani, S. K., Inserra, P., Solkoff, D., & Watson, R. R. (1999). The role of cytokines and chemokines on tumor progression: A review. *Cancer Detection and Prevention*, 23(3), 215-225. PMid:10337000. http://dx.doi.org/10.1046/j.1525-1500.1999.99021.x.

Bao, L. J. (2008). Inhibitory effects of water extracts from four species of Dendrobiums on HelaS3 cells and HepG2 cells. *Journal of Anhui Agricultural Sciences*, 36(36), 15968-15970.

Boisdron-Celle, M., Remaud, G., Traore, S., Poirier, A. L., Gamelin, L., Morel, A., & Gamelin, E. (2007). 5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. *Cancer Letters*, 249(2), 271-282. PMid:17064846. http://dx.doi.org/10.1016/j.canlet.2006.09.006.

Carrier, Y., Yuan, J., Kuchroo, V. K., & Weiner, H. L. (2007). Th3 cells in peripheral tolerance. II. TGF-beta-transgenic Th3 cells rescue IL-2-deficient mice from autoimmunity. *Journal of Immunology (Baltimore, MD.: 1950)*, 178(1), 172-180. PMid:17182552. http://dx.doi.org/10.4049/jimmunol.178.1.172.

Chen, Y., He, G., Zhang, M., & Li, H. (2003). Hypoglycemic effect of the polysaccharide from *Dendrobium moniliforme (L.) Sw. Journal of Zhejiang University*, 30(6), 693-696.

Deichman, G. I., Dyakova, N. A., Matveeva, V. A., & Kashkina, L. M. (2007). Alternative production of Bcl-2 and Bax by tumor cells determines the rates of in vivo tumor progression: suggested mechanisms. *Journal of Cellular Biochemistry*, 101(5), 1148-1212. PMid:17295204. http://dx.doi.org/10.1002/jcb.21242.

Dooms, H., & Abbas, A. K. (2010). Revisiting the role of IL-2 in autoimmunity. *European Journal of Immunology*, 40(6), 1538-1578. PMid:20458706. http://dx.doi.org/10.1002/eji.201040617.

Eom, B. W., Lee, J. H., Choi, I. J., Kook, M. C., Nam, B. H., Ryu, K. W., & Kim, Y. W. (2012). Pretreatment risk factors for multiple gastric cancer and missed lesions. *Journal of Surgical Oncology*, 105(8), 813-820. PMid:22006627. http://dx.doi.org/10.1002/jso.22124.

Hammam, O., Mahmoud, O., Zahran, M., Sayed, A., Salama, R., Hosny, K., & Farghly, A. (2013). A possible role for TNF-α in coordinating inflammation and angiogenesis in chronic liver disease and hepatocellular carcinoma. *Gastrointestinal Cancer Research : GCR*, 6(4), 107-121. PMid:24147158.

He, T. G., Yang, L. T., Li, Y. R., Wang, C. Q., & Huang, D. P. (2007). Physicochemical properties and antitumor activity of polysaccharide DCPP1a-1 from suspension-cultured protocorms of Dendrobium candidum. *Natural Product Research & Development*, 19(3), 410-414.

Lai, Z. C., Wei, X., Shimizu, T., Ramos, E., Rohrbaugh, M., Nikolaidis, N., Ho, L. L., & Li, Y. (2005). Control of cell proliferation and apoptosis by mob as tumor suppressor, mats. *Cell*, 120(5), 675-760. PMid:15766530. http://dx.doi.org/10.1016/j.cell.2004.12.036.

Lei, L. I. (2011). Study on the antibacterial effects of two Dendrobium polysaccharides. *Medicinal Plant*, (2), 38-52.

Lepage, C., Sant, M., Verdecchia, A., Forman, D., Esteve, J., & Faivre, J. (2010). Operative mortality after gastric cancer resection and long-term survival differences across Europe. *British Journal of Surgery*, 97(2), 235-244. PMid:20069605. http://dx.doi.org/10.1002/bjs.6865.

Leylabadlo, H. E., Kafil, H. S., & Yousefi, M. (2016). Gastric cancer mortality in a high-incidence area (Ardabil Province, Northwest Iran): what risk factors are causative? *European Journal of Cancer Prevention*, 25(6), 573-577. PMid:27662226. http://dx.doi.org/10.1097/CEJ.0000000000000237.

Li, X., Ding, X., Chu, B., Zhou, Q., Ding, G., & Gu, S. (2008). Genetic diversity analysis and conservation of the endangered Chinese endemic herb Dendrobium officinale Kimura et Migo (Orchidaceae) based on AFLP. *Genetica*, 133(2), 159-166. PMid:17805978. http://dx.doi.org/10.1007/s10709-007-9196-8.

Lin, X., Sze, S. C. W. ; Tong, Y.; Zhang, Z.; Feng, Y.; Chen, J. P.; Ng, T. B. ;Lin, X; Shaw, PC; Zhang, KY. (2010). Protective effect of Dendrobium officinale polysaccharides on experimental Sjogren's syndrome. *Journal of Complementary and Integrative Medicine*, 7(1), 1.

Liu, X. F., Zhu, J., Ge, S. Y., Xia, L. J., Yang, H. Y., Qian, Y. T., & Ren, F. Z. (2011). Orally administered Dendrobium officinale and its polysaccharides enhance immune functions in BALB/c mice. *Natural Product Communications*, 6(6), 867-870. PMid:21815428.

Luo, Q. L., Tang, Z. H., Zhang, X. F., Zhong, Y. H., Yao, S. Z., Wang, L. S., Lin, C. W., & Luo, X. (2016). Chemical properties and antioxidant activity of a water-soluble polysaccharide from Dendrobium officinale. *International Journal of Biological Macromolecules*, 89, 219-227. PMid:27131730. http://dx.doi.org/10.1016/j.ijbiomac.2016.04.067.

Lv, G. Y., Yan, M. Q., & Chen, S. H. (2013). Review of pharmacological activities of Dendrobium officinale based on traditional functions. *China Journal of Chinese Materia Medica*, 38(4), 489-493. PMid:23713270.

Nutt, L. K., Pataer, A., Pahler, J., Fang, B., Roth, J., McConkey, D. J., & Swisher, S. G. (2002). Bax and Bak promote apoptosis by modulating endoplasmic reticular and mitochondrial Ca^{2+} stores. *The Journal of Biological Chemistry*, 277(11), 9219-9244. PMid:11741880. http://dx.doi.org/10.1074/jbc.M106817200.

Sentman, C. L., Shutter, J. R., Hockenbery, D., Kanagawa, O., & Korsmeyer, S. J. (1991). Bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. *Cell*, 67(5), 879-967. PMid:1835668. http://dx.doi.org/10.1016/0092-8674(91)90361-2.

Sun, L., & Wang, X. (2003). Effects of allicin on both telomerase activity and apoptosis in gastric cancer SGC-7901 cells. *World Journal of Gastroenterology*, 9(9), 1930-1934. PMid:12970878. http://dx.doi.org/10.3748/wjg.v9.i9.1930.

Tang, C. L., Hao, B., Zhang, G. X., Shi, R. H., & Cheng, W. F. (2013). Helicobacter pylori tumor necrosis factor-α inducing protein promotes cytokine expression via nuclear factor-κB. *World Journal of Gastroenterology*, 19(3), 399-403. PMid:23372364. http://dx.doi.org/10.3748/wjg.v19.i3.399.

Wang, J. L., Liu, D., Zhang, Z. J., Shan, S., Han, X., Srinivasula, S. M., Croce, C. M., Alnemri, E. S., & Huang, Z. (2000a). Structure-based discovery of an organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. *Proceedings of the National Academy of Sciences of the United States of America*, 97(13), 7124-7133. PMid:10860979. http://dx.doi.org/10.1073/pnas.97.13.7124.

Wang, R., Alam, G., Zagariya, A., Gidea, C., Pinillos, H., Lalude, O., Choudhary, G., Oezatalay, D., & Uhal, B. D. (2000b). Apoptosis of lung epithelial cells in response to TNF-alpha requires angiotensin II generation de novo. *Journal of Cellular Physiology*, 185(2), 253-262. PMid:11025447. http://dx.doi.org/10.1002/1097-4652(200011)185:2<253::AID-JCP10>3.0.CO;2-#.

Zhang, J. H., Wan, M. X., Yuan, J. Y., & Pan, B. R. (2004). Construction and identification of recombinant vectors carrying herpes simplex virus thymidine kinase and cytokine genes expressed in gastric carcinoma cell line SGC7901. *World Journal of Gastroenterology*, 10(1), 26-30. PMid:14695763.