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Antimetastasis Effects of *Scrophularia striata* Extract on Colon Cancer Cells

Rahim Ahmadi, Mahya Daustany*, Masoomeh Abniki
Department of Biology, Avicenna International College, Budapest, Hungary
Department of Biotechnology, Faculty of Basic Sciences, Islamic Azad University, Damghan Branch, Iran
Department of Biotechnology, Faculty of Basic Scienses, Islamic Azad University, Damghan, Iran

Abstract: *In this laboratory experimental study we assessed antimetastasis effects of Scrophularia striata extract on colon cancer cells in cell culture. For this purpose, colon cancer (HT29) cells were used in our study. MTT assay was used to determine cytotoxic effects of the extract followed by Real Time PCR to assay KAI gene expression in cells in which the extract have shown cytotoxic effects. Our results showed that administration of Scrophularia striata extract (0.1 mg/ml) led to increase in expression of KAI gene, indicating antimetastasis effects of the extract on colon cancer cells in cell culture.*

Keywords: *Scrophularia striata , KAI, HT29 cell line*

1. Introduction

The use of medicinal plants as a source for relief from illness can be traced back over five millennia[1]. *Scrophularia striata* is the member of flowering plants family called *Scrophulariaceae*. many *Scrophularia* plants have long been used in Asian countries as a medicinal herb for the treatment of diseases; it has been applied for treating various inflammatory diseases such as allergy, rheumatics and chronic inflammatory disorders[2], [3]. The *Scrophulariaceae* is a large angiosperm family, which is widely distributed in deciduous and coniferous forests of central europe, central asia, and north america, especially in the mediterranean area, and is represented by about 3000 species and 220 genera [4].

Cancer is the third leading cause of death worldwide, only preceded by cardiovascular disease, infectious and parasitic disease [5], [6]. Cancer development processes are dependent on alteration in molecular, biochemical and cellular controls, such as elaboration of proteolytic enzymes necessary for invasion and progression of the tumor. Importance of proteolytic enzymes in tumor invasion is expressed as zymogens which must be proteolytically processed for activation [7] – [9]. Chemotherapy is the treatment of disease, especially cancer, using chemical substances. These chemicals are capable of destroy cancer cells, keeping them from growing and spreading, shrinking the size of a tumor or relieving cancer symptoms. Chemotherapy can destroy or slow down the growth of normal cells, including cells of the hair, mouth, digestive system, as well as those of blood [10]. Each person with cancer reacts differently to chemotherapy and its various side effects [10]- [12]. Fortunately, doctors now know many ways to reduce and even prevent these side effects. Oncologists are still looking for new anticancer drugs with more potent inhibitory and less side effects [13], [14]. Presently, more than 50% of drugs come from one or several natural products of 25,000 plant species and 600 of them have anticancer properties. Natural products have been used by in traditional medicines as a source of remedies for thousands of years, dating back to ancient empires in Persia, Mesopotamia, Egypt, China, Greece, and Rome [15]. These traditional medicinal preparation are made by boiling the plant material in water or soaking in alcohol [16], [17]. One such preparation is a formula using a diterpene ester from Daphne macronata animal at investigating cytotoxic activity against lung,colon and prostate cancer [18]. Some species of *Scrophularia striata* family have been used since ancient times in traditional medicines to treat eczema, wounds, goiter, ulcers, cancer and fistulae.

Some of them are boiled in milk to prepare a poultice which is applied to the abdomen to remove or reduce abdominal pain, whereas their aqueous extracts have been used as a bath to alleviate rheumatic pains. *Scrophulariaceae* species have been known to be rich in iridoid glycosides, mainly aucubin and catalpol [19]. Iridoids represent a large group of cyclopentan-[c]-pyran monoterpenoids occurring as constituents of sympetalous plants including ornamental as well as wildones. Their structures, properties and biosyntheses have been reviewed [20]- [22]. They have shown various biological activities such as antimicrobial, antitumoral, hemodynamic, choleretic, hepatoprotective and anti-inflammatory properties [23]. There are promising reports of chemoprevention of skin and lung cancer by genipin, an iridoid obtained on hydrolysis of geniposide, a glycoside isolated from the fruits of Genipa americana and Gardenia jasmoindes [24] , [25].
Since colon cancer is prevalent worldwide and chemical anticancer drugs often have more side effects compared to medicinal plant extract, we examined the antimetastasis effect of *Scrophularia striata* on colon cancer cells in cell culture to find its probable anticancer properties.

2. Materials and Methods

Scrophularia striata plant was collected from Guilan province, Iran. The leaves and stems were washed, dried and ground to get powder using a blender. Extractions were performed in a Soxhlet apparatus with methanol. Different concentrations (0.0001, 0.001, 0.01 and 0.1mg/ml) were prepared and used in our study. HT29 cells (Human colon cancer cell line) were purchased from National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). Cells were grown and incubated in standard situation. Then, cells were sub-cultured into 75cm^2 flasks, 96-well plates or 6-well plates. Cytotoxicity of different doses of the extract was assayed using MTT method. To assess KAI gene expression we used Real Time PCR method. Analyses were conducted using the SPSS 20 and ANOVA.

3. Results

Our results showed that administration of S*crophularia striata* extract (0.1 mg/ml) led to significant increase in expression of KAI gene in HT29 extract receiving cells compared to control HT29 cells which have not been exposed to S*crophularia striata* extract .

4. Discussion

In recent years, tendency of using natural sources as alternative medicine has been raised [26]. Potentially valuable structures for achieving effective chemotherapeutic agents [27]. *Scrophularia* as a member of *Scrophulariaceae* has been found to possess antibacterial, antiprotozoal, antitumor, anti-inflammatory, and diuretic activities and have been used in the treatment of mental, nervous and gastrointestinal conditions [28],[29]. We have shown the extract of Scrophularia as an anticancer research agent to inhibit cell growth and trigger apoptosis and from the function and pattern of molecular interactions of KAI, it is evident that KAI gene has an important role to play in the invasiveness and metastasis of cancer cells.

A successful anticancer drug should kill cancer cells without causing excessive side effects to normal cells. This ideal situation is achievable by apoptosis induction in cancer cells [32]. Increasing evidence demonstrates that plants are an important source of bioactive compounds that can induce apoptosis in human cancer cells [30],[31]. KAI is a member of the tetraspanin superfamily of adhesion molecules, and *KAI* was recently discovered to be a colon cancer metastasis-suppressor gene [33],[34]. KAI, like other members of the tetraspanin superfamily, has been associated with metastatic potential of nonsmall-cell human lung, liver, pancreatic bladder, breast, colon, prostate, and esophageal carcinomas and melanomas [35]. Downregulation of the *KAI1* gene is observed during the progression of human colon cancer, but mutations or allelic loss do not appear to be the major means for alteration [36]. Mechanisms in other tumor types have not been so extensively evaluated. The role of *KAI1* in colon cancer metastasis has been implicated by several studies. *KAI1* mRNA expression progressively decreased in a panel of human cell lines representing a continuum from nearly normal colon cells to highly metastatic cells [37]. The mechanism of action of *KAI1* is not completely understood. Several preliminary reports suggest that expression of *KAI1* decreases the both the invasiveness and motility of cells *in vitro* [38],[39].

5. Conclusion

Our results showed that administration of *Scrophularia striata* extract (0.1 mg/ml) led to increase in expression of KAI gene, indicating antimetastasis effects of the extract on colon cancer cells in cell culture.

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