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Pectic Oligosaccharide from tomato exhibiting anticancer potential on a gastric cancer cell line: Structure-function relationship

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ABSTRACT

Pectic Polysaccharide (PP) from dietary sources has been known to prevent cancer growth and hence impede cancer progression. We evaluated anticancer effect of Pectic-Oligosaccharide isolated from Sour Raw Tomato (SrTPO); its bioavailability and structure elucidated from purified fraction (SrTPO1). SrTPO1 inhibited galectin-3 activity with MIC of 0.25 ug/mL (100 fold better than standard galactose), inhibited the growth of AGS cells (IC_{50} 3.4 μg/mL) and induced apoptosis (70% inhibition at 30 μg/mL concentration). Normal– NIH 3T3 cells were not affected by SrTPO as opposed to doxorubicin, a known anticancer drug, which reduced 76% viability at equivalent dose. SrTPO1 was identified as Rhamnogalacturonanl-arabinogalactan (RGI-AG), where repeated alternative rhamnose and galacturonic acid residues were observed while arabinose in the branch point and β-1,4 linked galactose in the linear chain form. SrTPO was found to be bioavailable as evaluated by FITC labelled oligos inside the cell, which was in reciprocal proportion with apoptosis.

1. Introduction

Cancer belongs to the large group of complex, devastating diseases that entail uncontrolled cell growth with the potential to invade or affect any part of the body (WHO, 2015). Despite the availability of many anticancer drugs, pathogenicity disease profile convincingly evidenced that mortality of the affected patients is due to metastatic spread. So far, effective successful therapy is not available although some evidence exists from citrus pectin depicting that modified citrus pectin (MCP) effectually inhibits prostate cancer metastasis (Pienta et al., 1995; Guess et al., 2003). The reason why MCP was effective against metastasis was delineated by the fact that it has the ability to bind to galectin-3, a key molecule for metastasis. Our laboratory put efforts in validating the role of galectin-3 in metastasis employing human cancer tissues of the different degree from patients. Our systematic study supported the fact that galectin-3 is metastasis specific, since the trigger of this gene appears to push the cancer cell towards metastasis. Exclusive immunohisotological data followed by its estimation in the urine of cancer patients revealed that galectin- 3 is a universal molecule not only indexing metastasis; but initiating metastasis (Balasubramanian et al., 2009). Ever since this observation, our focus was directed towards looking for a strategic molecule from the food for the effective blockade of galectin-3.

Food and food derived molecules have a long history of exhibiting beneficial health properties. Unlike our previous thinking that diet can help in preventing disease occurrence, an array of studies from various organisations revealed that food derived molecules can function as a magic bullet to the complex disease like cancer also (Jayaram, Kapoor, & Dharmesh, 2015; Omenn et al., 1996). Numerous studies revealed that the action of cancer specific galectin-3 is via its binding to β-galactosides of the extracellular matrix of normal cell, hence transforming normal cell to cancer cell (Yang, Rabinovich, & Liu, 2008). In this context, our laboratory attempted to look for similar molecules from the dietary sources and galactose residues in multiple forms such as either linear chain galactan branch on the rhmnogalacturonic acid backbone or as units in arabinogalactan, etc. Pectins are abundant components in majority of the plant cell wall; plant being a source of food, pectins are also consumed and shown to possess several health beneficial

Abbreviations: PP, pectic polysaccharide; PO, pectic-oligosaccharide; GalA, galactouronic acid; GalpA, galactose; FBS, fetal bovine serum; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; DAPI, 4′,6-diaminidino-2-phenylindole; TUNEL, terminal deoxynucleotidyl transferase TdT mediated dUTP nick end labeling; FITC, fluorescein isothiocyanate; FTIR, fourier transform infrared spectroscopy; NMR, nuclear magnetic resonance; GLC, gas liquid chromatography; HPLC, high performance liquid chromatography; RIPA, radioimmunoprecipitation assay.

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effects. Our laboratory has shown its efficacy as an antiulcer agent, and the mechanism of antiulcer property was also investigated extensively (Harsha, Prakash, & Dharmesh, 2016).

Our attempts identified several potent sources for the blockade of galectin-3, including that from tomato (Kapoor & Dharmesh, 2016). Although several other food sources were identified as potent galectin-3 inhibitory property, our recent report on tomato triggered interest for further confirmation and exploration of this source for futuristic use against cancer/cancer metastasis. Our report suggested that low molecular weight oligosaccharide from specific variety (Mallika local vr, sour, raw form), showed better galectin-3 inhibitory property than that of the intact native pectin. The current study thus is designed to understand (1).Whether inhibition of galectin-3 result in inhibition of cancer cell growth; (2). If so, does it induce apoptosis; (3). If yes, what will be its effect on the normal cell; (4). What are the structural prerequisite to make low molecular weight oligosaccharides work better than the intact ones?

Gastric cancer is the 2nd leading cause of cancer mortality worldwide (Ferlay et al., 2010). Gastric cancer is closely associated with dietary factors and alterations in oxidant/antioxidant status, increased cell proliferation, angiogenesis, and dysregulation of apoptosis (Crew & Neugut, 2006). To throw light on the possible use of pectin oligosaccharide against gastric cancer, a human gastric adenocarcinoma cell line was used for the study.

Bioavailability of pectins is in debate. It is presumed so far that pectins are non-digestible fibre and get degraded through microbe's fermentation in the colon, thus producing short chain fatty acids, like butyric acid that can still be anti cancerous. Based on our repeated experience that pectins are effectively inhibiting cancer and cancer metastasis, it always raised a question, how these pectins are available to cancer cells in the body. A major drawback to study the turnover of pectin in either experimental animal model or in humans is its precise estimation. In other words, a specific reagent that detects pectins specifically is poorly available. Hence in the current study, we label the oligos with fluorescent probe and the same is followed in the AGS cells upon different time of ingestion of the compound in test. Results of the study are highlighted in this paper.

2. Materials and methods

Sour raw tomatoes were purchased from the local market, Mysore, India and processed for the preparation of pectics and pectic oligosaccharides for the study. Urine samples for galectin-3 mediated agglutination assay were collected from patients who were being monitored and tested for cancer at Bharath Hospital and Institute of Oncology, Mysore, India.

2.1. Chemicals

Ham's F-12, Dulbecco's modified eagle medium (DMEM), antibiotic solution, Fetal bovine serum (FBS), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) and trypsin were obtained from Hi-Media, Mumbai, India. Amberlite IR–120H$^+$ resin, 4′,6-diaminidino-2-phenylindole (DAPI), Goat anti-rabbit HRP conjugated secondary antibody, chemiluminescent peroxidase substrate and radioimmunoprecipitation assay (RIPA) lysis buffer, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Anti galectin-3 and anti β-actin rabbit polyclonal primary antibody were purchased from Abcam Pvt Ltd UK. TUNEL assay kit from Invitrogen Pvt Ltd. Other chemicals and solvents used were of analytical grade and procured from Sisco Research Laboratories, Mumbai, India.

2.2. Isolation of pectic-oligosaccharide (SrTPO)

Pectic-Oligosaccharide was isolated from pectic polysaccharide of Sour raw tomato as represented in Fig. 1 (Supplementary data) and described by Kapoor & Dharmesh (2016). Briefly, SrTPO was prepared by 2 N acetic acid treatment of sour raw tomato pectic polysaccharide (SrTPP) followed by ethanol precipitation. Excess acid in the supernatant was removed by co-distilling with water. After complete drying, the contents were suspended in the water and lyophilized to get pectic oligosaccharides (SrTPO).

2.3. Cell culture

AGS human gastric carcinoma cells and NIH-3T3 normal mouse embryonic fibroblast cells were obtained from the National Centre for Cell Sciences (NCCS) Pune, India; cultivated in Ham's F-12 and in DMEM medium containing L-glutamine and sodium bicarbonate and supplemented with 10% FBS respectively. The cells were grown in T 25 flasks in a regulated CO_2 incubator at 37 °C. After the cells were confluent, they were washed several times with PBS (pH 7.4) and harvested with 0.25% trypsin. AGS cells were analysed for galectin-3 expression, since it is intended to estimate galectin-3 inhibitory potential.

2.3.1. Determination of antiproliferative effect of SrTPO on AGS cells

Aliquots of 2×10^4 exponentially growing AGS cells were assessed for antiproliferative activity and NIH-3T3 cells were seeded in 96-well plates to check the sample toxicity. After 6 h of incubation at 37 °C in 5% CO_2, the growth medium was replaced with media containing different concentrations of SrTPO and known anticancer drug, doxorubicin (10, 20 and 30 μg/mL); the plates were incubated for 48 h again. The reaction was terminated by adding 25 mL of MTT solution (5 mg/mL) to each well, and the cells were incubated at 37 °C for 4 h. Then 100 μL of DMSO was added and measured at 570 nm in a microplate reader (Spectra Max – 340, Molecular Devices, Germany). Absorbance of the untreated wells (triplicates) was considered as 100%, and relative percent values are expressed for cells in wells treated with different doses of SrTPO and doxorubicin (Jayaram, Kapoor, & Dharmesh, 2015).

2.3.2. Determination of galectin-3 and inhibition of galectin-3 expression by SrTPO in AGS cells

Expression of galectin-3 and Inhibition of expression of galectin-3 was determined by immunoblotting method. Briefly, AGS cells were seeded into 60 mm-dishes (2×10^5cells/dish) overnight and treated with SrTPO (10, 20 and 30 μg/ mL) and incubated for 48 h. After harvesting the cells with trypsin treatment, the cells were lysed with RIPA buffer containing the protease inhibitor. Equal quantity of proteins (50 μg) of each lysate was resolved by 12% SDS-PAGE and electroblotted onto polyvinylidene difluoride membrane. Membranes were quenched in a solution of PBS containing 5% skim milk protein and 0.1% Tween 20 for 2 h. Blots were incubated with galectin-3 antibody at 1:1000 (v/v) dilution for 2 h. After washing three times in quench solution, membranes were incubated with 1:5000 (v/v) dilution of Goat anti- rabbit HRP conjugated secondary antibody, and then visualised by exposure to a freshly prepared chemiluminescent peroxidase substrate (Honjo, Nangia-Makker, Inohara, & Raz, 2001).

2.3.3. Ability of SrTPO on induction of apoptosis

2.3.3.1. DAPI (4′, 6-diaminidino-2-phenylindole) staining and apoptotic evaluation. AGS cells were seeded into 60 mm-dishes (2×10^5 cells/dish) overnight and treated with SrTPO and doxorubicin (10, 20 and 30 μg/mL) and incubated for 48 h. After harvesting the cells with trypsin treatment, the cells were washed with PBS