

The effects of growth inhibitory peptide on follicular thyroid cancer cell growth, migration, and invasion

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ABSTRACT

Aims and background. Thyroid cancer is the most common endocrine neoplasm worldwide. Although differentiated thyroid cancers are associated with a favorable survival, the prognosis worsens dramatically for patients with distant metastasis. Metastases from follicular thyroid carcinoma (FTC) occur earlier and are more aggressive than those from papillary thyroid carcinoma. For FTC that is resistant to radioactive iodine, new treatments are urgently needed. Human alpha-fetoprotein (HAFP) is a tumor-associated fetal protein that has been demonstrated to regulate tumorigenesis. Growth inhibitory peptide (GIP), a synthetic 34-mer peptide isolated from the third domain of HAFP, has been shown to have antitumor growth ability in various human cancers. However, the effects of GIP in FTC have not yet been studied. The aim of this study was to investigate the antitumor ability of GIP in FTC.

Methods and study design. Using both PBS and GIP control peptide as a negative control, the antiproliferative activity of GIP in the WRO human FTC cell line was determined using a tetrazolium-based colorimetric assay. In addition, cell migration and invasion assays were used to measure tumor metastasis inhibition effects *in vitro*.

Results. GIP did not inhibit WRO cell proliferation in a time- or dose-dependent manner. However, in WRO cells treated with GIP for 4 days, migration was significantly inhibited at concentrations of 50 and 100 μM (33.3% and 19.5%, respectively; both $P < 0.05$). Cell invasion was also significantly inhibited at 50 and 100 μM (67.1% and 39.0%, respectively; both $P < 0.05$).

Conclusions. Although GIP failed to suppress FTC cell growth, it effectively interrupted both FTC cell migration and invasion abilities *in vitro*. Further validation in an animal model and elucidation of the underlying mechanisms will be required. GIP may potentially serve as an anti-FTC metastasis agent aiding current chemotherapy regimens. Free full text available at www.tumorionline.it

Introduction

Thyroid cancer is the most common malignancy among endocrine-related cancers¹. Thyroid cancer includes the following tumors with different molecular and clinical characteristics: differentiated papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), poorly differentiated thyroid carcinoma (PDTC), and anaplastic thyroid carcinoma (ATC). Among thyroid cancers, ATC is the most lethal but also the rarest. Histologically, 94% of thyroid carcinomas are well-differentiated, including PTC and FTC¹. FTC accounts for 10-32% of the well-differentiated thyroid cancers^{2,3}. Differentiated thyroid cancers are associated with

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a favorable survival, but the prognosis worsens dramatically for patients with distant metastasis⁴. Although patients with FTC are ranked second in survival rates behind patients with PTC, patients with FTC have an increased risk of tumor metastases to lung and bone. Metastases of FTC occur earlier and are more aggressive than PTC metastases⁵. The current treatment strategies of metastatic FTC are surgical resection and radioactive iodine ablation therapy. All thyroid cancers are resistant to chemotherapy and some subsets of FTC have poor I-131 uptake, with a high mortality rate. The inadequacy of the currently available treatment modalities as a rescue for radioactive iodide suggests an urgent need for the development of novel treatment strategies.

Human alpha-fetoprotein (HAFP) is a tumor-associated fetal protein that has been demonstrated to enhance tumor growth⁶⁻⁹ and to have proangiogenic properties^{10,11}. However, a synthetic 34-mer peptide isolated from the third domain of HAFP, termed growth inhibitory peptide (GIP), possesses antitumor growth ability. GIP has been shown to suppress tumor growth in many kinds of human cancers *in vitro*, including colon cancer, ovarian cancer, breast cancer, prostate cancer, non-small cell lung carcinoma, melanoma, central nervous system cancer, renal carcinoma, and leukemia¹². GIP has also been found to inhibit the growth of various types of human breast cancer cells grown as xenografts in nude mice^{12,13}. The mechanism of GIP's antigrowth ability has not been established. GIP is proposed to be a decoy peptide ligand for an orphan G-protein-coupled receptor (GPR-30) which competitively binds GPR-30, displacing the natural ligand and uncoupling the G-protein signal transduction pathway of adenyl cyclase¹⁴.

This peptide has also been shown to inhibit tumor cell adhesion to ECM proteins and tumor cell migration, and to block platelet aggregation¹⁵. When the amino acid sequences of GIP were compared with protein sequences derived from the GenBank databases they were found to be similar to those of integrins, metalloproteinases, adhesion proteins, ECM proteins, and blood clotting factors¹⁵. GIP might be involved in cell-to-cell and cell-to-ECM interactions and it has been speculated that GIP also inhibits thyroid cancer cell adhesion, migration, and metastasis. Vivacqua *et al.*¹⁶ showed that the growth of WRO human FTC cells is induced by 17 β -estradiol via GPR-30. In contrast, GIP suppresses thyroid cancer cell growth by blocking the GRP-30-related growth signal transduction pathway.

GIP is a potential novel anticancer agent for tumor growth, adhesion, and metastasis. However, data on the effect of GIP on thyroid cancer cells have not been available so far. In the current study we investigate the hypothesis that GIP inhibits FTC tumor growth, invasion, cell migration, and metastasis.

Materials and methods

Cell culture

WRO cells were kindly provided by Dr JD Lin (Department of Endocrinology and Metabolism, Chang Gung Memorial Hospital, Taiwan)¹⁷. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS; Invitrogen Corp., Carlsbad, CA, USA) at 37 °C and in a 5% CO₂ humidified chamber. The amino acid sequences of GIP (P149) and GIP control peptide (P263) have been published previously¹⁵. GIP and GIP control peptide were synthesized by Genemed Synthesis, Inc. (San Antonio, TX, USA) and diluted in PBS.

Cell viability assay

Cells were seeded at 5×10^3 cells/well in 96-well plates. The cells were treated with GIP or GIP control peptide, and cell survival and proliferation were determined by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide (MTT)] purchased from Sigma-Aldrich. (St. Louis, MO, USA).

Cell migration assay

In the cell migration and invasion assay, WRO cells were pretreated with PBS, GIP, or GIP control peptide for 4 days. Cells were trypsinized and plated on 24-well Transwell plates (5×10^4 cells/well) with serum-free medium and cultured overnight. Transwell chambers were established to investigate the cell migration ability. Cell migration was evaluated by Milliwell assays (6.5-mm diameter, 8- μ m pore size polycarbonate membrane). Cells were placed in the upper chamber in 0.1 mL of serum-free medium, while 10% FBS medium was loaded in the lower chamber. After a 6-hour incubation, the cells migrating to the lower chamber were collected and counted. Three wells were examined for each condition and cell type, and the experiment was repeated 3 times.

Cell invasion assay

The cell invasion assay was conducted using 0.2 mL/well Matrigel-pre-coated 24-well invasion chambers, with ECM-coated filters on the upper surface. Three independent experiments in each condition were counted after incubation for 6 hours.

Results

No growth inhibition by GIP and GIP control peptide in FTC cell line

As compared with PBS, GIP and GIP control peptide did not inhibit the growth of thyroid cancer cells. Neither GIP nor GIP control peptide suppressed the growth of WRO cells in a dose- or time-dependent manner (Figure 1).

Migration inhibition of FTC cells treated by GIP

As compared with PBS, GIP at concentrations of 50 and 100 μM suppressed the migration ability of WRO cells but exhibited no dose dependency (Figure 2). There were no significant differences in the cell migration assay of WRO cells treated by GIP control peptide (data not shown).

Invasion inhibition of FTC cells treated by GIP

GIP at concentrations of 50 and 100 μM significantly inhibited cell invasion of WRO cells but showed no dose dependency (Figure 3). PBS and GIP control peptide (data not shown) produced no significant invasion inhibitory effect.

Discussion

Based on the results of the current study, we conclude that GIP fails to inhibit tumor cell growth of the FTC cell line, WRO; we did find, however, that GIP could

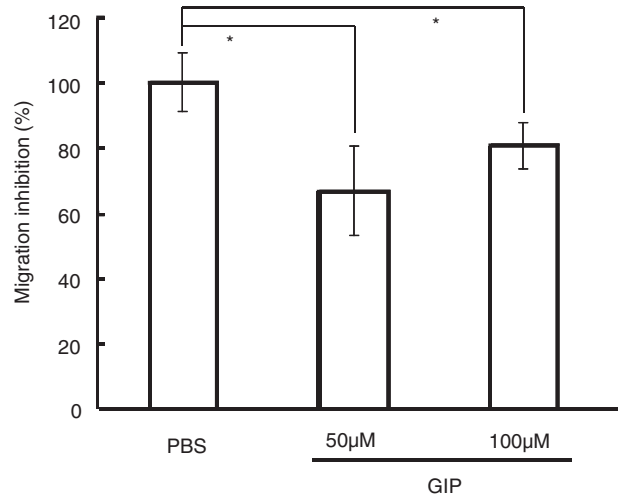


Figure 2 - Inhibition of migration of WRO cells treated with or without growth inhibitory peptide (GIP). WRO cells were treated with PBS or GIP (50 and 100 μM) for 4 days. Cell migration inhibition was determined using a Transwell migration model as described in the Methods section. Each data point represents the results of 3 repeated experiments; data are presented as mean \pm standard deviation. * $P < 0.05$.

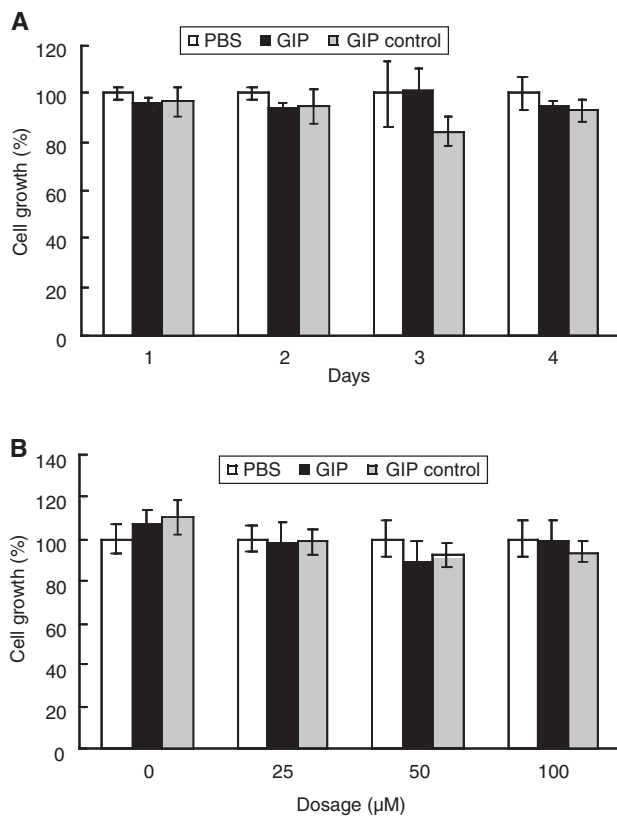


Figure 1 - Viability of WRO cells treated with or without growth inhibitory peptide (GIP). A) WRO cells were treated with GIP or GIP control peptide (100 μM) for various days. Cell viability was detected with an MTT assay kit. B) WRO cells were treated with various doses of GIP or GIP control peptide for 4 days. Cell viability was analyzed with an MTT assay kit. PBS was used as negative control. Each data point represents the results of 5 repeated experiments; data are presented as mean \pm standard deviation.

significantly suppress both the migratory and invasion abilities of tumor cells.

A summary of the screening of GIP by the National Cancer Institute Therapeutics Drug Screening Program (Bethesda, MD, USA) was presented using 60 different human cancer cell lines⁸. In a 6-day cell proliferation assay with sulforhodamine-B, GIP was shown to be cytostatic (non-cytotoxic) against 38 of 60 cancer cell lines. In subsequent reports, GIP was effective in various human breast cancer cell lines, both *in vitro* and *in vivo*^{13,18}. Compared with previous *in vitro* studies, GIP could not inhibit WRO cell growth in our 4-day cell proliferation assay with MTT. However, to fully elucidate the tumor suppression effect of GIP in WRO cell lines, a xenograft nude mouse model is needed. GIP's mechanism of tumor growth suppression is not fully known, although receptor blockade of G-protein-linked signal transduction has been suggested to play a role¹⁵. In breast cancer, an interaction with a G-coupled orphan receptor GPR-30 has been proposed. GIP serves as a decoy ligand to the receptor and blunts adenylyl cyclase downstream signal transduction¹⁰. FTC WRO cells have been shown to possess GPR-30 and proliferation can be induced by 17- β -estradiol, genistein, and 4-hydroxytamoxifen through GPR-30¹⁶. However, GIP failed to inhibit the growth of WRO cells in the current study. A possible explanation is that the GPR-30 pathway blockade is not the major growth-promoting pathway in FTC. We also cannot exclude that GIP may regulate GPR-30-related signaling pathways to control other physiological processes in FTC. However, GIP may suppress WRO cell proliferation after treatment with GPR-30-related growth-promoting agents

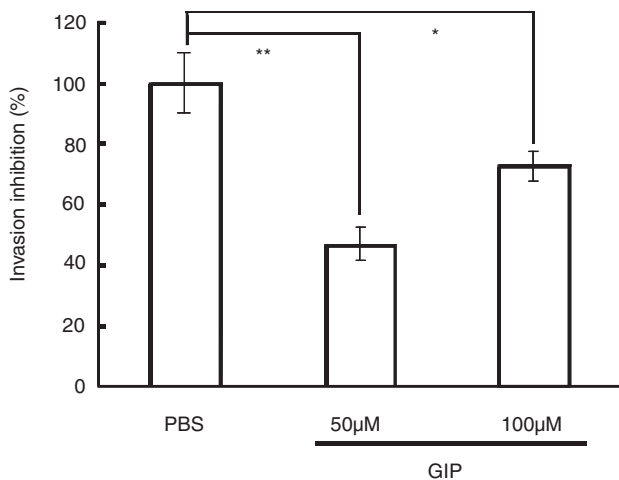


Figure 3 - Inhibition of invasion of WRO cells treated with or without growth inhibitory peptide (GIP). WRO cells were treated with PBS or GIP (50 and 100 M) for 4 days. Cell invasion was determined by an invasion assay model as described in the Methods section. Each data point represents the results of 3 repeated experiments; data are presented as mean \pm standard deviation. * P < 0.05, ** P < 0.01.

such as 17β -estradiol, genistein, or 4-hydroxytamoxifen.

The present study showed that GIP significantly suppressed migration of WRO cells. GIP has also been reported to inhibit MCF-7 breast cancer cell migration¹⁵. When compared with protein sequences from the GenBank databases, the amino acid sequences of GIP were found to match those of integrins, ECM proteins, metalloproteinases, clotting factors, and adhesion proteins. Matches were also found with integrin-associated ECM ligands such as collagen, laminin, fibronectin, and fibrinogen. Therefore, GIP is suspected to be involved in cell-to-cell and cell-to-ECM interactions^{15,18}. GIP is implicated in interfering with cell surface activities, such as invasion and migration and cell-to-ECM interactions related to integrin involvement.

Our study demonstrated that GIP can suppress the invasion ability of WRO cells, but the results need to be validated *in vivo*. The definite mechanisms of thyroid cancer invasion interrupted by GIP also need further investigation. In conclusion, although GIP failed to inhibit FTC tumor growth, it may serve as a potential agent to inhibit tumor metastasis. Combination treatment with effective cytotoxic chemotherapy may be further studied.

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