Adipocyte Derived Hormones Gene Expression, Resistin and Visfatin, in AGS Gastric Cancer Cell Line

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Abstract

Background: Adipose tissue has characteristics of an endocrine organ which releases a number of adipocyte-specific factors, known as adipocytokines. It has recently suggested that adipocytokines might play a role in pathogenesis and progression of certain cancers, especially in gastric cancer. This study has managed to investigate endogenous and/or exogenous expression of Visfatin and Resistin in gastric cancer cell line.

Methods: Cell culture and semi-quantitative reverse transcription polymerase chain reaction has performed to measure mRNA and protein expression of Resistin and Visfatin in gastric cancer cell lines. ELISA test has performed for cell lysate and supernatant of cell culture to measure Resistin and Visfatin protein expression and secretion.

Results: Human gastric cancer cell line (AGS cell line) has found to express Visfatin mRNA and protein but Resistin mRNA and protein has not expressed.

Conclusion: Visfatin has expressed endogenously in AGS human gastric cancer cells. Conversely Resistin has no expression. The results of this study has suggested that expression of adipocytokine proteins in real samples, could be a biomarker for gastric cancer.

Keywords: Visfatin; Nampt; Resistin; Gastric cancer; AGS cell line

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Introduction

Gastric adenocarcinoma (GC) is the fourth most common cancer worldwide (7.8% of cancers) then the second leading cause of cancer death (9.7% of cancer deaths) [1]. Several epidemiological studies have evaluated the effects of increasing body weight as well as, Metabolic Syndrome (MS), Body Mass Index (BMI) and other anthropometric indexes, all above on the risk of gastric cancer [2-4]. Since it has identified that adipose tissue displays characteristics of an active endocrine organ, adipocytotokines have become subjects of extensive research [5, 6] particulary in the previous two decades, many researchers have tried to discover the possible role of adipocytokines in the regulation of angiogenesis and tumor growth [7]. The previous studies in gastric cancer has shown that Resistin and Visfatin increased more than other adipocytokines and might be appropriate gastric cancer biomarkers [8].

Resistin is a member of family of cysteine-rich proteins, is called "Resistin-Like Molecules" (RELMs). Its gene, has referred as Retn mapping to the p13.3 band of chromosome [9], has encoded a 114-amino acid polypeptide [10] which is secreted as a disulphide linked homodimer [11] and circulates in two distinct assembly states: an abundant high-molecular weight hexamer and a less abundant, but more bioactive, trimer [12]. A few studies, in human, has evaluated the association of Resistin and RELMs with gastric cancer. In previous study that has performed by Zheng et al., a higher expression of RELMb has recorded in intestinal-type in comparison to diffuse-type gastric carcinomas. In addition, RELMb has correlated positively with tumor differentiation and inversely with tumor infiltration,

lymph node metastasis and heparanase expression. Recently study has performed by Nakajima et al. About Resistin role in gastric cancer, has found significantly correlation with stage progression, perhaps encouraging its use as a biomarker for gastric cancer progression.

Visfatin is also known as Pre-B Cell Enhancing Factor (PBEF), a growth factor for early B cell proliferation [13], and it is the secretory form of Nicotinamide phosphoribosyl-transferase (Nampt) [14], the rate-limiting enzyme of mammalian Nicotinamide Adenine Dinucleotide (NAD) biosynthesis. Nicotinamide Adenine Dinucleotide (NAD+) is central to many cellular processes and cancer cells have a high rate of NAD+ turnover compared to normal cells [15]. Several studies have shown the role of Visfatin in different cancers [16-19].

In this present study, we have tried to investigate whether high serum level of Visfatin and Resistin in gastric cancer patients are good biomarkers, then could be used as a potential diagnostic and prognostic tools. For this purpose, at first step we should investigate if Visfatin and Resistin express endogenously in gastric cancer cell line. In this study we have used only one human gastric cancer cell line and have focused on Resistin and Visfatin mRNA and protein expression.

Materials and Methods

Cell Lines and Culture Conditions

Human gastric adenocarcinoma consists of mucus-secreting epithelial cells and have obtained or purchased from the institute pastor cell bank in Iran. The cells have incubated in Ham's F-12 culture medium containing 100 ng/ml of penicillin, 100 ng/ml of streptomycin, 15 mM Hepes, 1.2 g/1 sodium bicarbonate, and 10% fetal bovine serum. The cell monolayer in a 25-cm 2 flask has subcultured at 1:5 ratios every 3 days by treatment with 0.1% trypsin and 0.03% EDTA. The flask has maintained in incubator at 37°C in a humidified atmosphere with 5% CO2.

Total RNA Extraction

In T25 flasks have seeded 7×10^5 cells, after 24 incubation at 37°C, AGS cells we have cut out by harvesting medium for each flask. Adherent cells have washed twice with PBS and trypsini zed; the cell pellets have been collected by centrifugation in 1000 g for 10 min at 4°C. Total RNA has extracted from each cell culture flask using the guanidine isothiocyanate based RNX-plus solution (Sinna Gen INC, IRI) according to the manufacturer protocol. The amount of extracted RNA has quantified by measuring the absorbance at 260 nm. The purity of the RNA has checked by measuring the ratio of the absorbance at 260 and 280 nm. The absence of degradation of the RNA has confirmed by RNA electrophoresis on a 1.5% agarose gel containing ethidium bromide.

Reverse Transcription Polymerase Chain Reaction

First-strand cDNA has generated using the High-Capacity cDNA Archive kit (Fermentas. K1621), with random hexamers, according to the manufacturer's protocol. An aliquot of the RT reaction has amplified under conditions (1 min at 94°C, 45 sec at94°C, 1 min at 60°C, and 2 min at 72°C) in a total volume of 25 µl. In parallel, GAPDH, as a housekeeping gene, PCR has performed to control for the RNA input in the RT-PCR. The following primers have used: human Visfatin forward sequence 5′-TGCCTTCGGTTCTGGTGGAGGTT-3′; human Visfatin reverse sequence 5′-ACAAAATTCCCTGCTGGCGTCCT-3′; its PCR product size is 189 bp. human Resistin forward sequence 5′-TGGAAGAAGCCATCAATGAGAGG-3′, human Resistin reverse sequence 5′-CGCACTGGCAGTGACATGTG-3′, its PCR product size is 210 bp. Housekeeping gene GAPDH the following primers have used: forward sequence 5′-CAAGGTCATCCATGACAACTTTG-3′, GAPDH reverse sequence 5′-GTCCACCACCCTGTTGCTGTAG-3′, its PCR product size is 496 bp. Reaction products e separated on polyacrylamide gel.

Measurement of Resistin and Visfatin

Visfatin expression and secretion has detected by a human Visfatin ELISA, using a competitive enzyme immunoassay technique (RayBiotech. Human/Mouse/Rat Visfatin. EIA-VIS-1) respectively, from the cell lysate and cell supernatant (after 24 and 48 hours without serum) according to the manufacturer's instructions. Also Resistin expression and secretion has detected by a human Resistin ELISA using quantitative sandwich enzyme immunoassay technique (KOMA BIOTHECH. INK, Human Resistin ELISA Kit.K0331199. 2) respectively, from cell lysate and cell supernatant (after 24 and 48 hours without serum) according to the manufacturer's protocol. Briefly, cell lysate has harvested from culture flask of AGS cell also supernatants have harvested from serum free culture flask of AGS after 24 and 48 hours. Standards, controls (positive control was available in kit and used H2O as negative control),

and samples have assayed at a wavelength of 450 nm for wavelength correction. A range of Visfatin and Resistin dilutions has used to generate a standard curve to determine Visfatin and Resistin concentration in the sample supernatant.

Statistical Analysis

Results e analyzed using SPSS 15. Student's t-test has used for comparisons between two groups. *P* value of less than 0.05 has considered to be statistically significant.

Results

Visfatin Expression and Secretion

To determine the expression of Visfatin in gastric cancer AGS cell line in mRNA level, reverse transcription polymerase chain reaction has performed that have positively detected in cell line (Figure 1). For confirming the results we have performed ELISA based on competitive enzyme immunoassay in cell lysate sample and cell free serum supernatant after 24 and 48 hours. Results have shown Visfatin positively express and secrete in all samples. In cell lysate, Visfatin concentration was higher than cell supernatants but this differentiation was not significant. Also in 48 hours Visfatin concentration was higher than 24 hours but differentiation was not significant (Figure 2).

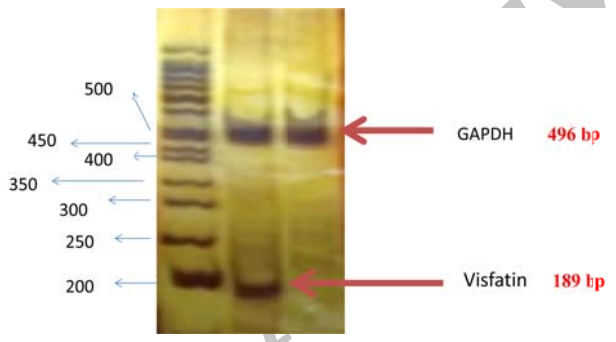

Figure 1. It shows RT-PCR detection of human Visfatin and GAPDH, internal control gene expression in human gastric AGS cell line. Resistin has not expressed.

Risfatin Expression and Secretion

To determine the expression of Resistin in gastric cancer AGS cell line in mRNA level has performed reverse transcription polymerase chain reaction and have not detected in cell line. To confirm of this result we have performed ELISA based on sandwich immunoassay in cell lysate sample and cell free serum supernatant after 24 and 48 hours. Results have shown Resistin could not express and secrete in all samples.

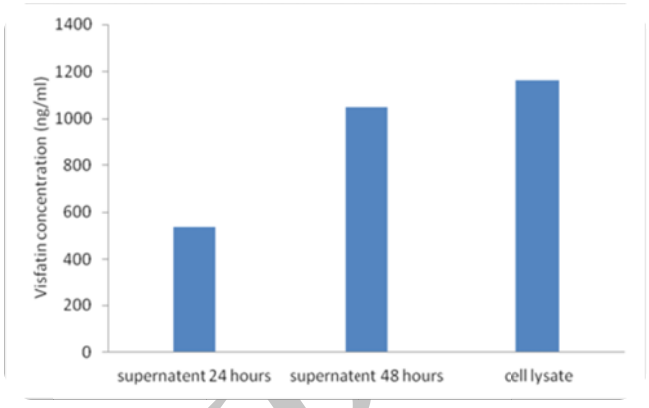

Figure 2. Protein levels of Visfatin in human gastric cancer AGS cell line is shown.

Discussion

Based on previous analyses of the adipokines alterations, we have realized that in underlying gastric cancer, increases of Visfatin and Resistin have measured higher than other adipokines, then we have hypothesized in addition expression adipose tissue and other sources, there would be endogenous expression of Visfatin and Resistin in gastric cells.

Based on our study, there was no express Resistin in gastric cancer cells, although the previous studies in gastric cancers has shown that Resistin serum level has increased more than other adipocytokines and might be good biomarkers of gastric cancer [8]. Otherwise the underlying mechanisms of relation between increased levels of serum Resistin and lack of its expression in gastric cancer cells are not clear, but there are some suggestions about the reasons of higher serum Resistin levels in gastric cancer patients. This increase might be due to leukocytes and macrophages and molecules of the RELM family that have found into the inflamed tissues. Resistin has abundantly expressed in bone marrow cells and, in particular, in leukocytes and macrophages, and molecules of the RELM family that have found in inflamed tissues [20]. In fact Resistin is a molecule accumulating in time of inflammation and supports the inflammatory process by triggering cytokine production and NF-κB activation while simultaneously up-regulating its own expression. Pro inflammatory cytokines (IL-1, IL-6, and TNF-α) increases the expression of Resistin in the human Peripheral Blood Mononuclear Cells (PBMC) (seem to be major source of Resistin) [21].

Also our study has demonstrated that Nampt express endogenous in gastric cancer cell line AGS and increased level in cancer cells as well as serum concentration of Visfatin in patients. In fact, in mammals, Nampt has two different forms: intracellular and extracellular Nampt (iNampt and eNampt, respectively). eNampt, has also been named Visfatin, as it has been found releasing from adipocytes mostly in form of serum Nampt, and acting as a cytokine in circulation [22]. iNampt has participated in the salvage pathway of NAD synthesis — NAD has played a vital role in energy metabolism, serving as a cofactor of histone deacetylasesirtuins, regulated cell death through poly (ADP-ribose) polymerase 1 (PARP-1) [23], thus linking iNampt to these important cellular processes [24]. Increased iNampt expression has been reported in primary colorectal cancer [15, 16]. Additionally, iNampt has involved in angiogenesis by activating the extracellular signal-regulated kinase 1/2 pathway and inducing vascular endothelial growth factor and MMP2/9 [25]. iNampt also has induced proliferation and capillary-like tube formation in human umbilical vein endothelial cells in a dose- and time dependent manner [26]. These findings have indicated that iNampt could enlarge the growth of some types of tumors.

In conclusion, our study has determined that increased Resistin serum level in gastric cancer patient is due to other cells infiltrate in to the cancer tissues, according to the previous study Resistin is a molecule which accumulates in inflammation sites, in order to support the inflammatory process by triggering cytokine production and NF-KB activation while simultaneously up-regulating its own expression from inflammation cells [27]. Therefore Resistin couldn't be a good biomarker. But Visfatin expresses endogenous in gastric cancer AGS cell line. According to the recent study that has performed by Nakajima et al., increased Visfatin serum level, has correlated significantly with stage progression. Therefore it is suggested that expression of Visfatin in real samples could be biomarker for gastric cancer , then could be used as a potential diagnostic and prognostic tool.

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Conflict of Interest

The authors have no conflict of interests in this article.

Authors' Contribution

Masumeh Gorgian Mohammadi has designed the study, analyzed the data and written the paper. Mehdi Hedayati and Nosratollah Zarghami has contributed the study design and analysis and approved the final manuscript. Sara Ghaemmaghami has contributed the data entry. Mojtaba Mohaddes has contributed the study design.

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