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Original article

Regulation of leptin receptor expression in human papillary thyroid cancer cells

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ABSTRACT

Epidemiological studies suggest an important link between obesity and thyroid cancer. The adipose tissue-derived polypeptide leptin acting via leptin receptor may modulate cell migration of thyroid cancer cells. Previously we have demonstrated that leptin receptor is overexpressed in papillary thyroid cancer and is associated with tumor aggressiveness. The present study was undertaken to explore the possible regulatory factors which would influence leptin receptor expression in papillary thyroid cancer cells. We found that DNA methyltransferase inhibitor (5-Aza-2′-deoxycytidine) and histone deacetylase inhibitor (trichostatin A) reduced leptin receptor expression. Conversely, insulin upregulated leptin receptor expression in a time- and dose-dependent manner. Hypoxia-mimicking agent (cobalt chloride) had no effect on leptin receptor expression. Taken together, our study provides evidence that epigenetic events and insulin stimulation take part in regulation of leptin receptor expression in papillary thyroid cancer cells.

1. Introduction

Thyroid cancer is the most common malignancy of endocrine organs [1]. The majority of thyroid tumors arise from thyroid follicular epithelial cells, and papillary carcinoma is the most common thyroid cancer type. Accumulating evidence indicates that obesity is a risk factor for thyroid cancer [2]. Interestingly, the link between obesity and cancer has also been observed in other cancer types [3]. Overweight-related changes of the adipose tissue lead to insulin resistance, chronic inflammation, and altered secretion of adipokines [4]. These mechanisms may synergistically contribute to cancer development and progression.

Polypeptide hormones derived from adipocytes are collectively termed adipokines. Leptin is a 16 kDa adipokine encoded by the obese (OB) gene [5]. The levels of leptin polypeptides in the circulation essentially reflect the amount and distribution of adipose tissue in the body. Leptin exerts its physiological and pathophysiological effects by binding to the leptin receptor [6]. Several isoforms of leptin receptor resulting from alternative splicing or proteolysis of membrane bound isoforms have been found, including trans-membrane receptors and a soluble receptor [7]. Only the long isoform with an extended intracellular domain has various motifs required for the interaction with other proteins and subsequent signaling pathway activation.

Leptin was originally discovered as a hormone regulating appetite and energy expenditure. Recent studies have shown that leptin has mitogenic and anti-apoptotic properties in some types of cancer cells [8–10]. Polymorphism in leptin receptor gene may associate with obesity and tumor progression [11]. In addition, leptin has been implicated in the pathogenesis of primary hyperparathyroidism [12]. Given the association between obesity and thyroid cancer, we have recently demonstrated that leptin and leptin receptor are overexpressed in papillary thyroid cancer [13]. Furthermore, the overexpression is associated with tumor aggressiveness. It is consistent with our experimental observations that leptin modulates cell migration of thyroid cancer cells [14,15]. However, the mechanisms responsible for the altered expression of leptin receptor in thyroid cancer have not been investigated. The aim of this study was to explore the possible regulatory factors, which would influence leptin receptor expression in papillary thyroid cancer cells.

2. Materials and methods

2.1. Reagents and antibodies

5-Aza-2′-deoxycytidine (5-Aza), trichostatin A (TSA), insulin, and cobalt chloride ($CoCl_2$) were purchased from Sigma

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Chemical Co. (St. Louis, MO). Goat polyclonal antibody against long-form specific leptin receptor (C-20) and rabbit polyclonal antibody against short and long forms of leptin receptor (H-300) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies for β-actin and α-tubulin were obtained from Sigma.

2.2. Cell culture

The human papillary thyroid carcinoma cell line K1 was purchased from the European Collection of Cell Cultures (ECACC, Salisbury, United Kingdom). It has been authenticated to be a unique thyroid cancer cell line [16]. K1 cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in Dulbecco's modified Eagle's medium (Invitrogen/Gibco, Carlsbad, CA) mixed with Ham's F12 (Gibco) and MCDB 105 (Sigma) medium in 2:1:1 proportions, supplemented with 10% fetal bovine serum and 2 mM L-glutamine.

2.3. Western blotting analysis

Whole cell lysate was prepared by resuspending cells in M-PER protein extraction reagent (Thermo Scientific/Pierce, Rockford, IL) according to manufacturer's instructions. Cell lysates were sonicated for 30 s on ice and centrifuged at 14,000 x g for 10 min at 4 °C. Protein concentration was measured using Bradford assay (Bio-Rad Laboratories, Hercules, CA). An aliquot of protein lysate (30 μg) was mixed with 10X Laemmli sample buffer (Bio-Rad) and separated in 10-12% sodium dodecyl sulfate polyacrylamide gels. Fractionated proteins were transferred to a nitrocellulose membrane, and transfer was controlled by reversible Ponceau staining. After transfer, the membrane was blocked with 5% skimmed milk or 5% bovine serum albumin for 30 min at room temperature. The proteins were probed with primary antibodies at 4 °C overnight. After three washes, the blots were incubated with horseradish peroxidase-conjugated secondary antibodies (Sigma) for 1.5 hours at room temperature. The blots were visualized with enhanced chemiluminescence kit (Pierce) and Amersham Hyperfilm ECL (GE Healthcare, Piscataway, NJ).

2.4. Statistical analysis

All experiments were independently performed at least three times. Bar graphs with error bars represent mean ± standard error of the mean (SEM). The differences between two experimental conditions were compared on a one-to-one basis using the two-tailed Student's t test. A difference of $P < 0.05$ between groups was considered statistically significant.

3. Results

3.1. DNA methyltransferase inhibitor, 5-Aza, reduces leptin receptor expression in thyroid cancer cells

DNA methylation represses transcription directly by inhibiting the binding of specific transcription factors, and indirectly by recruiting methyl-CpG-binding proteins [17]. The DNA methyltransferase inhibitor may induce DNA demethylation and re-expression of epigenetically silenced genes [18]. K1 cells were treated with DNA methyltransferase inhibitor 5-Aza for 72 hours. Leptin receptor expression evaluated by Western blotting clearly showed that long-form leptin receptor expression in K1 cells decreased with 5-Aza treatment in a dose-dependent fashion (Fig. 1). The expression of short-form leptin receptor decreased with increasing 5-Aza doses as well.

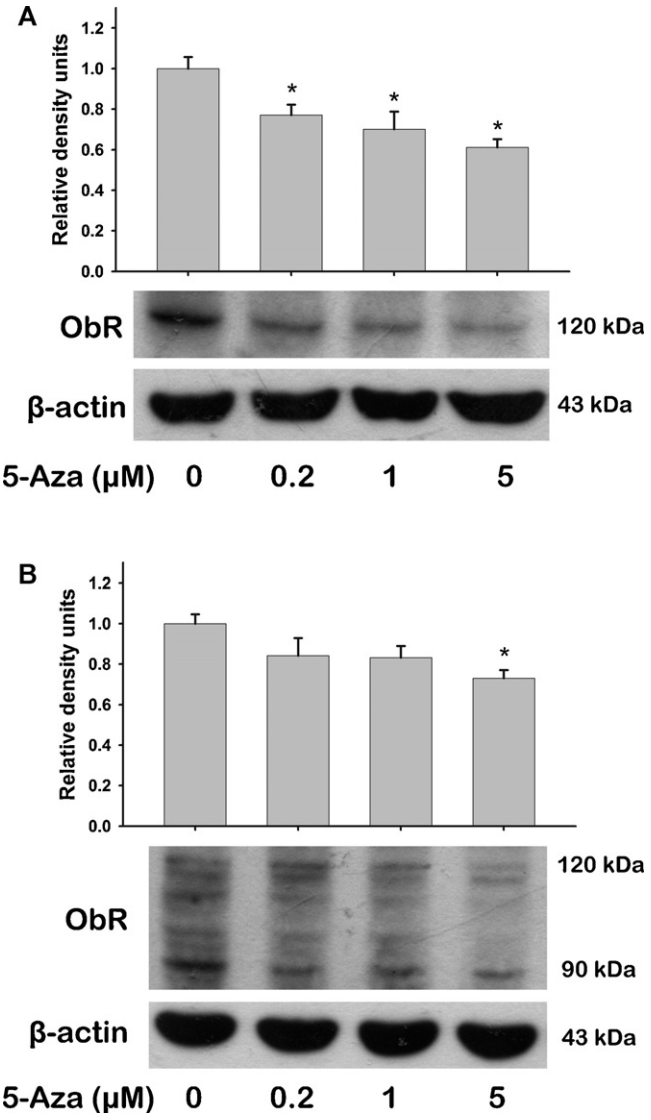

Fig. 1. Effects of DNA methyltransferase inhibitor, 5-aza-2′-deoxycytidine (5-Aza), on the expression of leptin receptors. K1 thyroid cancer cells were treated with various doses of 5-Aza for 72 hours. Protein expression was visualized by Western blotting with anti-leptin receptor antibodies C-20 (A) and H-300 (B). The blot signals were quantified by densitometry and normalized to β-actin. Columns, mean of independent experiments; bars, SEM. *$P < 0.05$, compared with vehicle controls.

3.2. Histone deacetylase inhibitor, TSA, reduces leptin receptor expression in thyroid cancer cells

Emerging data also implicate histone modification in the pathophysiology of cancer and other diseases [19]. Histone acetylation is mediated by histone acetyl transferases, which transfer acetyl groups to amino-terminal lysine residues in histones and result in chromatin expansion and increased accessibility of regulatory factors to DNA. On the contrary, histone deacetylases catalyze the removal of acetyl groups, leading to chromatin condensation and transcriptional repression [20]. TSA is an inhibitor of the histone deacetylase which relaxes the chromatin structure and upregulates transcription [21]. TSA treatment for 24 hours caused a distinct dose-dependent inhibition of long-form leptin receptor expression in K1 cells (Fig. 2). There was no significant change in the expression of short-form leptin receptor.