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Expression of genes encoding for enzymes associated with O-GlcNAcylation in endometrial carcinomas: clinicopathologic correlations

Ekspresja genów kodujących enzymy związane z O-GlcNAcylacją w rakach błony śluzowej trzonu macicy: korelacja z parametrami kliniczno-patologicznymi

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Abstract

Objectives: *O-GlcNAcylation is an abundant modification of cellular proteins which consist of single N-acetylglucosamine residues attached by O-linkage to serine or threonine residues. Abnormal O-GlcNAcylation seems to be a feature of malignant cancer cells. The aim of the present study was to determine the relationship between the expression of genes encoding O-GlcNAc cycling enzymes (OGT and MGEA5) and clinicopathological parameters of endometrial carcinomas.*

Materials and methods: *The mRNA expression levels of O-GlcNAc cycling enzymes in series of 76 samples of endometrial carcinoma were studied by real time RT-PCR method.*

Results: The OGT and MGEA5 mRNA expression was significantly higher in tumors of higher histological grade than in well-differentiated tumors. Statistically significant association was found between OGT and MGEA5 mRNA expression and depth of myometrial invasion. Both OGT and MGEA5 expression profiles showed no significant association with the clinical stage of endometrial cancer.

Conclusion: *O-GlcNAcylation may be an important regulatory modification involved in endometrial cancer pathogenesis but the actual significance of this modification for endometrial cancer progression needs to be investigated further.*

Key words: **O-GlcNAcylation** / **OGT** / **MGEA5** / **endometrial carcinoma** /

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Streszczenie

Cel pracy: *O-GlcNAcylacja jest powszechną modyfikacją białek komórkowych polegającą na przyłączeniu wiązaniem O-glikozydowym pojedynczych reszt N-acetyloglukozoaminy do reszt seryny i treoniny. Zaburzenia O-GlcNAcylacji wydają się być istotną cechą związaną z agresywnością komórek nowotworowych. Celem prezentowanej pracy było określenie zależności pomiędzy ekspresją genów kodujących enzymy związane z O-GlcNAcylacją białek a kliniczno-patologicznymi parametrami raka błony śluzowej trzonu macicy.*

Materiał i metody: *Poziom ekspresji mRNA enzymów analizowano techniką real time RT-PCR w 76 preparatach raków błony śluzowej trzonu macicy.*

Wyniki: *Nowotwory o wyższym stopniu złośliwości histologicznej wykazywały wyższą ekspresję mRNA dla OGT i MGEA5 w porównaniu z rakami dobrze zróżnicowanymi. Stwierdzono również istotną statystycznie zależność pomiędzy ekspresją badanych genów a głębokością naciekania mięśniówki macicy. Nie stwierdzono natomiast zależności pomiędzy ekspresją mRNA OGT i MGEA5 a stopniem klinicznego zaawansowania nowotworu.*

Wniosek: *Wydaje się, że O-GlcNAcylacja może być ważną regulatorową modyfikacją włączoną w patogenezę raka błony śluzowej trzonu macicy ale dokładne określenie jej roli w progresji tego nowotworu wymaga dalszych badań.*

Słowa kluczowe: **O-GlcNAcylacja** / **OGT** / **MGEA5** /
/ **rak błony śluzowej trzonu macicy** /

Introduction

O-GlcNAcylation is an abundant post-translational modification of nuclear and cytoplasmic proteins. *O*-GlcNAc transferase (OGT) catalyses covalent attachment of β-D-N-acetylglucosamine (GlcNAc) sugars to serine or threonine residues and *O*-GlcNAcase (OGA) catalyses the removal of *O*-GlcNAc residues. OGT is encoded by a single gene on the X chromosome. The cloned sequence of *O*-GlcNAcase was found to be identical to that of MGEA5 (meningioma-expressed antigen 5) which was identified genetically in human meningiomas. *MGEA5* localizes to chromosome 10q24.1-q23.4 region [1, 2].

There is growing evidence that *O*-GlcNAcylation plays a role in cancer-relevant processes, such as cell signaling, transcription, cell division, metabolism and cytoskeletal regulation [3]. Several malignancies have been shown to have abnormal *O*-GlcNAcylation level compared to normal tissues [4-8]. Elevated *OGT* mRNA expression was found in breast cancer cell lines and breast invasive ductal carcinoma compared with normal breast cells and normal breast tissue [4, 9]. It has been shown that poorly differentiated breast tumors (grade II and III) have higher OGT expression than grade I tumors and lymph node metastasis is significantly associated with decreased *MGEA5* mRNA expression [9]. It is suggested that *O*-GlcNAcylation influences the malignant properties of breast cancer cells and promotes metastasis [5]. Elevated *O*-GlcNAcylation in colon and lung cancers is correlated with increased expression of OGT [6]. The intracellular *O*-GlcNAcylation has been found to be associated with the pathogenesis of chronic lymphatic leukemia –CLL [7] and this modification plays a role in tumor recurrence of hepatocellular carcinoma following liver transplantation [8].

Endometrial cancer is the most common malignant tumor of the female genital tract. In the endometrium different subtypes of cancer can develop. Endometrioid endometrial carcinoma (EEC) or Type 1 cancer is the most prevalent subtype and accounts for over 70% of cases. EECs are estrogen-dependent and often develop in the background of atypical complex hyperplasia. EECs tend to present as low grade, early stage tumors with good outcomes [10-12]. However, a subset of patients have a biologically aggressive disease characterized by lymphovascular invasion, high histological grade, and myometrial invasion. Patients with these characteristics are at an increased risk of recurrence following hysterectomy. For early-stage disease, surgery alone or in combination with local therapy is generally curative. For patients with stage III or stage IV of the disease and for those with recurrent endometrial cancer, the prognosis remains poor. The identification of differentially expressed genes between late vs. early stage endometrioid endometrial carcinoma might be important for predicting tumor progression and tumor outcome. The new markers could help to decide the need for adjuvant treatment and to identify new treatment strategies.

In this study the authors analyzed the mRNA expression of genes encoding *O*-GlcNAc transferase and *O*-GlcNAcase (*OGT* and *MGEA5*, respectively) in endometrial cancers and the relationship between their expression and clinicopathological parameters.

Materials and Methods

The study included 76 patients with endometrial carcinoma who had undergone surgery at the Department of Gynecological Oncology Copernicus Memorial Hospital, Łódź, Poland.

All studied endometrial carcinoma specimens were hysterectomy specimens. Information regarding the clinical and pathological characteristics of the patient populations was obtained from the medical records. The endometrial patients characteristics are presented in Table I. Endometrial carcinomas were classified according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). Histological typing and grading were done according to the WHO classification.

RNA extraction and cDNA synthesis

Total RNA was isolated using Trizol® Reagent (Sigma Aldrich, USA) according to manufacturer's protocol and quantified spectrophotometrically. First-strand cDNAs were obtained by reverse transcription of 1 µg of total RNA using RevertAid™ First strand cDNA synthesis kit (Fermentas International, Lithuania) following the manufacturer's protocol.

Quantitative real-time PCR

Real-time gene expression analysis of target genes (*OGT and MGEA5*) was performed using TaqMan® Gene Expression Assays (Applied Biosystems, USA) according to manufacturer's instructions. The *GAPDH* gene was used as internal control. The assay numbers for these genes were as follows: Hs00201970, Hs00269228 and Hs99999905.

Each PCR reaction was performed in a 10 µl volume that included 5 µl of 2x TaqMan Universal PCR MasterMix (Applied Biosystems, USA), 4.5 µl of water diluted cDNA template (50 ng) and 0.5 µl of TaqMan® Gene Expression Assay consisted of a pair of unlabeled PCR primers and TaqMan probe with a FAM™. The RT-qPCR reaction was carried out using the Mastercycler ep realplex (Eppendorf, Germany) under the following conditions: denaturation for 10 min at 95ºC followed by 50 cycles of 15 sec at 95ºC, 1 min annealing and extension at 60ºC.

Relative RNA quantification was performed using the ΔCt method. ΔCt (Ctgene – CtGAPDH) values were recalculated into relative copy number values (number of *OGT* or *MGEA5* mRNA copies per 1000 copies of *GAPDH* mRNA).

Statistical analysis

The statistical analyses were performed using the STATISTICA version 9.0 (StatSoft, Poland). Since levels of expression in endometrial cancer specimens did not show normal distribution (Kolmogorov-Smirnow test), the non-parametrical statistical tests (Mann-Whitney U test, Kruskal-Wallis test) were applied. The chi square test was used to identify the relationship between expression of *OGT* or *MGEA5* and clinicopathological parameters. A p-value <0.05 was considered statistically significant.

Results

OGT and *MGEA5* mRNA expression was found in all 76 samples of the studied endometrial cancers. The comparison of the q-RT-PCR data for *OGT* and *MGEA5* in EECs with different pathological parameters is shown in Figure 1 and 2.

The *OGT* and *MGEA5* mRNA expression was significantly higher in tumors of grade II and III than in well-differentiated tumors of grade I (Fig.1). The mean *OGT* and *MGEA5* expression levels in grade II were 7 and 4.6 –fold higher than in grade I, respectively. Statistically significant association was found between *OGT* and *MGEA5* mRNA expression and depth of myometrial invasion. However, there were no significant differences (p>0.05) in *OGT* and *MGEA5* mRNA expression between tumors with different lymph node metastasis status (Figure1) and different stages of development according to the FIGO classification (Figure 2).

Table I. Clinical characteristics of the endometrial carcinoma.

Median age (range)	62.5 (31 – 85)
FIGO stage	
I	35
II	31
III	9
IV	1
Histological grade	
GI	14
G II	49
G III	13
Depth of myometrial invasion	
<1/2	38
>1/2	35
Lymph node metastasis	
No	60
Yes	16

Discussion

The role of *O*-GlcNAcylation in tumorigenesis and cancer progression has not been fully elucidated. However, many oncogenic proteins and tumor suppressor proteins are regulated by *O*-GlcNAcylation. The protooncogene c-Myc which regulates transcription of genes involved in cell proliferation, apoptosis and metabolism is modified by *O*-GlcNAc at Thr58 and this potentially stabilizes the protein [13]. The important tumor suppressor p53, which is mutant or dysregulated in many cancers including some endometrial cancers, bears *O*-GlcNAc at Ser149. Increased *O*-GlcNAcylation of p53 at Ser149 results in decreased p53 ubiquitination and stabilizes the p53 protein [14]. It was found by immunohistochemistry that high expression of p53 was correlated with an advanced stage, poor differentiation, lymph node metastasis and deep myometrial invasion of endometrial cancers [15, 16]. It is possible that high expression of p53 protein in endometrial cancer, at least partially, may be due to increased stability of this protein caused by *O*-GlcNAcylation.

Over 25% of the *O*-GlcNAc modified proteins are involved in transcriptional regulation. *O*-GlcNAc modification of transcription factors is important in regulation of gene expression in various tissues [17]. One of the best studied *O*-GlcNAcylated transcription factors is Sp1 which targets genes encoding for factors involved in cell cycle progression, both pro- and anti-angiogenic factors involved in invasion and metastasis, pro- and anti-apoptotic factors involved in genomic stability, proto-oncogene and tumor suppressors stimulating cell proliferation and oncogenesis [18]. *O*-GlcNAcylation of Sp1 has been shown to regulate Sp1 nuclear localization, transactivation capability, protein stability, and its interaction with other transcriptional regulators [19-22]. Dynamic changes of *O*-GlcNAcylation of Sp1 as well as many other transcription factors can modulate the malignant properties of cancer cells.

Abnormal *O*-GlcNAcylation seems to be a feature of aggressive cancer cells. It was shown that high *O*-GlcNAcylation associated with high OGT expression might be a marker for progression of breast cancer [5,9]. Low expression of OGA was an independent prognostic factor for predicting tumor recurrence of hepatocellular cancer following liver transplantation [8].

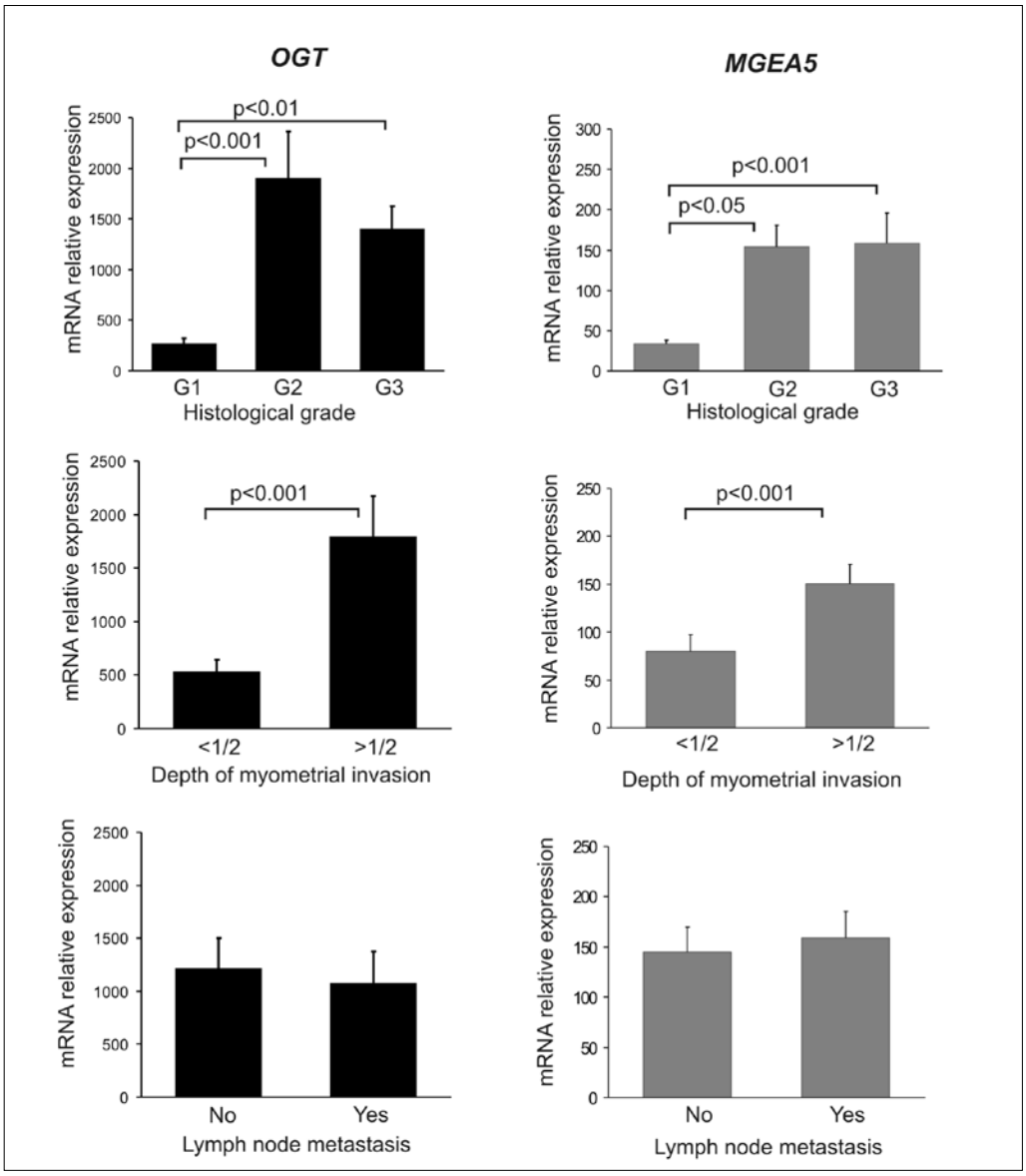

Figure 1. *OGT* and *MGEA5* mean mRNA expression level in endometrial carcinomas: a comparison between subgroups with different tumor grade, depth of myometrial invasion and lymph node metastasis status.

The aim of the present study was to determine whether the expression of *O*-GlcNAc cycling enzymes is correlated with endometrial cancer malignancy. Clinicopathological studies show that poor prognosis is related among others to histological grade, deep myometrial invasion of malignant cells and lymph node metastasis. The results have shown that expression of both genes is increased in endometrial cancers of higher histological grade what might suggest that less differentiated cancers have a high rate of *O*-GlcNAc cycling. Although increased expression of both genes was observed, the relative expression of *OGT* was much higher than *MGEA5*. The extent of myometrial invasion is one of important prognostic factors. Expression of *OGT* and *MGEA5* was higher in case of cancers with deep myometrial invasion. However, there was no association between expression of the studied genes and lymph node status or clinical stage. Although the results of our preliminary studies concerning expression of *O*-GlcNAc cycling enzymes suggest that *O*-GlcNAcylation may be an important regulatory modification involved in endometrial cancer pathogenesis, the actual significance of this modification for endometrial cancer progression needs to be further elucidated. Better insight into processes involved in endometrial cancer pathogenesis may lead to identification of novel biomarkers and targets for the development of diagnostic and therapeutic approaches for prevention and treatment of endometrial cancer.

Conclusions

The expression of genes encoding O-GlcNAc processing enzymes is significantly associated with aggressive features such as high-grade and deep myometrial invasion which suggest that *O*-GlcNAcylation may be involved in endometrial cancer progression. Additional investigations of *O*-GlcNAc cycling enzymes as a biomarker of malignant potential and as a novel target for therapeutics in endometrial carcinoma are necessary.

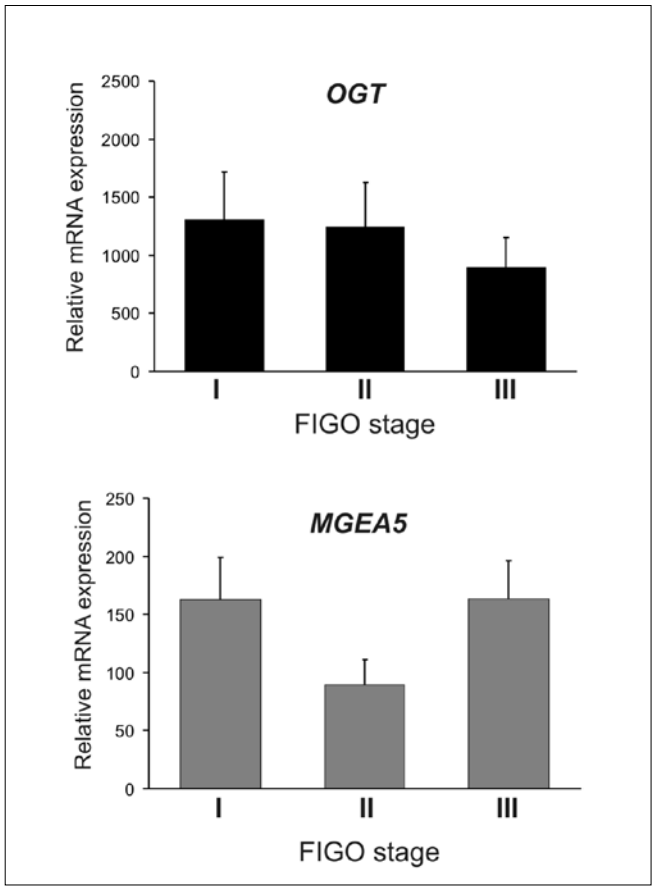

Figure 2. *OGT* and *MGEA5* mRNA expression level in endometrial carcinomas: a comparison between subgroups of different FIGO stage.

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