The role of nesfatin and selected molecular factors in various types of endometrial cancer

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ABSTRACT

Objectives: Endometrial cancers (ECs) are the most common gynaecological cancers in well developed countries. Diabetes and metabolic syndrome are among the biggest risk factors. Nesfatin-1, the adipokine derivative of NUCB2 (nucleobindin derivative 2) is linked to the clinical course of EC. Molecular factors, including mutations in MLH1 and MHS2 genes, c-MET and ARID1A are also related to prognosis in endometrial cancer.

Material and methods: Using sections of paraffin-embedded preparations and immunohistochemistry, the expression of NESF1, MLH1, MSH2,c-MET and ARID1A were examined.

Results: In this study on protein expression, EC tissues manifested (although insignificantly) an elevated expression of NESF-1 in type II EC. In type I EC, NESF-1 expression was significantly higher in G1 in comparison to G2 and G3 together. A significantly lower expression of MLH1 was demonstrated in type I EC.

Conclusions: The most pronounced expression involved c-MET in all EC I and EC II tissues (in over 80% of cases). A tendency was detected for a high expression of NESF-1 in patients with type II EC, who also exhibited a high expression of MSH2.

Key words: endometrial cancer; NESF-1; MLH1; MSH2; c-MET; ARID1A

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INTRODUCTION

Endometrial cancer (EC) is the most commonly occurring gynaecological malignancy in economically developed countries. In Europe, the incidence rate amounts to 13.6 per 100.000 women. In many European countries an increased incidence of this tumor has been noted since 2005 [1–3]. In over 90% of EC cases, the cancer develops after the age of 50, with a median age of 63; in 10–15% it is diagnosed before the age of 45 [2, 4].

Bokhman's hypothesis led to the identification of two types of EC differing in etiology, biology and clinical course [5].

Type I EC (*endometrioid adenocarcinoma*) involves 80% of all EC cases. In most cases it is sporadic and linked to unbalanced estrogen stimulation and metabolic syndrome. This EC type manifests slow clinical course and positive prognosis. This type of cancer contains mutations in the *PTEN, KRAS, CTNNB1, P1K3CA* genes and in mismatch repair *(MMR)* genes, typical for Lynch syndrome [2, 6–8].

Type II EC (*non-endometrioid adenocarcinoma*) histologically encompasses serous, clear cell, poorly differentiated cancers of aggressive biology and an unfavorable clinical course. They contain mutations in *TP53*, *HER2-neu* and *BRCA* [2, 9, 10–12].

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Type I EC with a high histological grade (G3) manifests a similarly poor prognosis to uterine serous carcinoma (USC), belonging to type II [6, 3, 13, 14], some well-differentiated, early diagnosed endometrioid EC gives recurrent disease, which suggests that the traditional Bokman's division may not be up to date.

Recent results have shown that more EC types exist. The heterogeneity of ECs has been proven by genomic analysis of endometrioid and serous cancers. The Cancer *Genome Atlas* (TCGA) developed in 2013, indicates there are 4 types of ECs [15]:

— *unique nucleotides spectrum* ultra-mutated tumors *(POLE)* — associated with favourable outcome,
— highly-mutated with microsatellite instability, mostly with MLH1 promoter methylation,
— a group of low frequency of mutations with low gene copy numbers,
— a group with high gene copy numbers and a low frequency of mutations, mainly serous cancers, connected to a poor prognosis.

It is suggested that the Genomic Atlas classification (TGGA) should be linked to the histological classification of ECs, particularly ECs of poor differentiation [16].

The acknowledged and recognized factors which affect the clinical course and type of oncological treatment of EC include the histological type, grading (G) and clinical stage of disease. However, these traditional prognostic factors do not allow for tailoring of treatment to individual patients which could spare many side effects for over-treated women and reduce the number of recurrences in under-treated patient groups.

Molecular studies also indicate the relationship between EC clinical course and several molecular variables, such as: condition of hormonal receptors (ER, PR), ARID1A and c-MET linked to poor prognosis, metastases and mutations in MMR genes detected in Lynch syndrome [17–21]. Recent published studies indicated a relationship between Nesfatin-1 (NESF-1) with its precursor, NUCB2-nucleobindin 2, and the clinical course of EC [22, 23]. NESF-1 is associated with obesity and diabetes type II (main risk factors for EC) and participates in the regulation of hunger and fat storage, it is associated with insulin resistance and glucose homeostasis.

The aim of this study was to estimate the expression of NESF-1, MLH1 and MLH2 belonging to MMR, c-MET and ARID1A in two types of endometrial cancer and determine relationships between the mentioned factors.

MATERIAL AND METHODS

This was a multi-centre, retrospective study. The investigated material involved archival, histological preparations, obtained from uterine endometrial cancer samples taken from patients primarily treated surgically between 2007–2014.

All 146 patients included in this retrospective study were monitored for the entire five to eight-year period from diagnosis of EC through the duration of their treatment and subsequent observation. In order to fulfil the selection criteria for the study, patients must have been diagnosed at one of the centres participating in the study. Only patients where information on FIGO grading and histology were complete, who underwent treatment for EC at the centre where they were originally diagnosed and then monitored for the stated study period by practitioners at that specific centre were included in this study.

Out of a total of 146 patients, 38 (26%) patients were diagnosed at stage IA according to FIGO, 36 patients (24.7%) at stage IB, 37 patients (25.4%) at stage II, 22 (15%) at stage III and 13 (8.9%) at stage IV.

In the studied group, 115 patients were diagnosed with type I EC (78.8%), in 31 type II EC (21.2%) was detected, this included 18 serous types (12.3%), 11 clear cell types and 2 mucinous types (1.4%).

In 38 patients (25%), endometrial cancer manifested a high histological maturity (G1), in 59 patients (38.8%) it displayed an intermediate histological differentiation (G2) while in 55 patients (36.2%) undifferentiated tumors were identified (G3) (Tab. 1).

Table 1. Clinicopathologic characteristics of studied patients with endometrial carcinoma	
Clinical Staging acc. to FIGO	**Number of patients**
Endometrioid adenocarcinoma (n = 115)	
IA	33
IB	32
II	27
III	14
IV	9
Serous adenocarcinoma G3 (n = 18)	
IA	4
IB	3
II	7
III	4
Clear cell *adenocarcinoma* G3 (n = 11)	
IA	1
IB	1
II	2
III	4
IV	3
Mucinous adenocarcinoma (n = 2)	
II	1
IV	1
Grading	**Number of patients**
Endometrioid adenocarcinoma (n = 115)	
G1	36
G2	56
G3	23

The mean age of the entire patient group studied was 65.3; in women with endometrioid cancer the mean age was 64.7 (34–83) and in women with non-endometrioid cancer it was 67.6 (40–83) (p > 0.05).

The tissue material was fixed in 10% buffered formalin, pH 7.4 and placed in a processor. The neoplastic tissue was embedded in paraffin at 60ºC using standard histopathological techniques. The paraffin blocks were sliced in a microtome to 4–5 um thick sections, placed on adhesive glass slides and left for one hour at a temperature of 60ºC. In the study, the immunohistochemical method — DAKO Envision™ Flex+ (Dako system, Dako, Santa Clara, USA) was applied. Antigens were detected in the paraffin sections using the Target Retrieval Solution, high pH, DAKO in the PT-link (Dako, Santa Clara, USA) at a temperature of 97ºC, for 20 min.

Nesfatin-1 was estimated using Nesfatin-1/Nucleobinding-2 Antibody (Novus Biologicals, Littleton, USA, NBP1-87383). In order to detect antigens present in the tissue material antibodies were used against ARID1A (Novus Biological, Littleton, USA, NBP1-88932), Met (Santa Cruz Biotechnology, Santa Cruz, USA, clone C-12, MLH1 (Leica NCL-L, Buffalo Grove, USA. Clone E-305), MSH2 (Invitrogen, Carlsbad, USA , clone FE11).

The intensity of NESF-1, ARID1A, c-MET, MLH1, MSH2 staining was estimated using a 4-degree scale:
no reaction
+ 1 to 50 immunopositive cells (cell nuclei or the cytoplasm)
++ 50 to 75 immunopositive cells
+++ 75 to 100 immunopositive cells
per 10 visual fields.

A positive reaction was accepted in the case of preparations manifesting ++ or +++ staining.

Statistical calculations were made using the Mann-Whitney, Kruskall-Wallis and Spearman's tests (STATISTICA StatSoft Inc, USA). Statistical significance was concluded when p was less than 0.05.

RESULTS

A high expression of NESF-1, MLH1, MSH2, c-MET and ARID1A was detected, respectively, in 53.1%, 57.7%, 47.3%, 88.7% and 48.2% patients with endometrial cancer.

The immunohistochemical reaction with antibodies against NESF-1 and c-MET was seen in the cytoplasm; MLH1, MSH2 and ARID1A manifested nuclear localization.

Histopathological type

In respect to histopathological diagnosis the patients formed two groups: endometrioid type (n = 115) and non-endometrioid type (n = 31), which included patients with serous, clear cell and mucinous cancers.

Positive expression of nesfatin was determined in 50.99% of patients with type EC I and in 61.3% of patients with type II EC. No significant difference was disclosed between the groups (p = 0.410). MLH1 expression was statistically lower in type EC I in comparison to type II EC (51.9% vs 77.4%, p = 0.013). No significant difference was disclosed in the expression of MSH2, c-MET and ARIDIA between the two subgroups (p < 0.05) (Fig.1).

Clinical stage according to FIGO

Patients with endometrial cancer were subdivided depending on clinical stage into early stage (IA) and later stage of the disease (IB–IV). In the entire group of patients with endometrial cancer, no relationship was detected between intensity of NESF-1 expression and the stage of clinical ad-
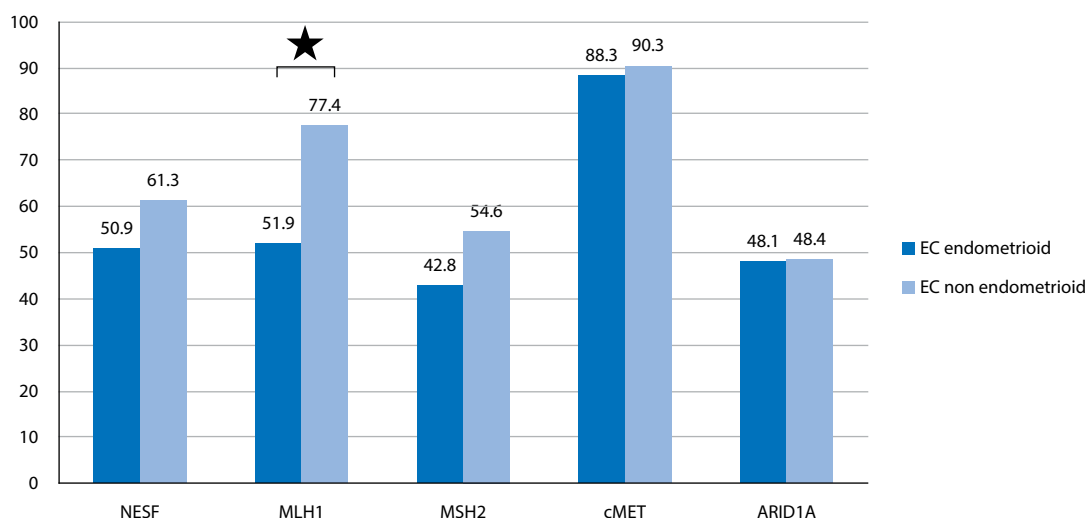

Figure 1. Percentage of endometrial cancer cases manifesting a high expression of a given protein in endometrioid and non-endometrioid cancer cells; ★ p < 0.05

vancement of the disease (p = 0.382). Also no such relationship was detected for MLH1, MSH2, c-MET and ARIDIA.

Grading

Among all the analyzed patients no relation was detected between the level of NESF-1 expression and histological grade of cancer differentiation (p = 0.3145). Furthermore, no such relationship was detected for MLH1, MSH2, c-MET and ARID1A (p > 0.05) (Fig. 2, 3). The group of patients with type I EC with grade 1 manifested a statistically higher expression of nesfatin than the patients with histologically less mature cancer (G1 68.97% vs G2 + G3 45.45%, p = 0.0487). The relationship between the level of studied protein expression and histological grading of non-endometrioid cancers was

not analyzed since the latter by definition are poorly differentiated cancers (G3).

Correlation between evaluated proteins

Among all the studied patients with ECs, a high expression of NESF-1 correlated with a high expression of the MLH1 protein (p = 0.039). No relationship was detected between the level of NESF-1 expression and MSH2, c-MET and ARID1A in the entire population of patients with endometrial cancer (p = 0.43, p = 0.24, p = 0.4158).

DISCUSSION

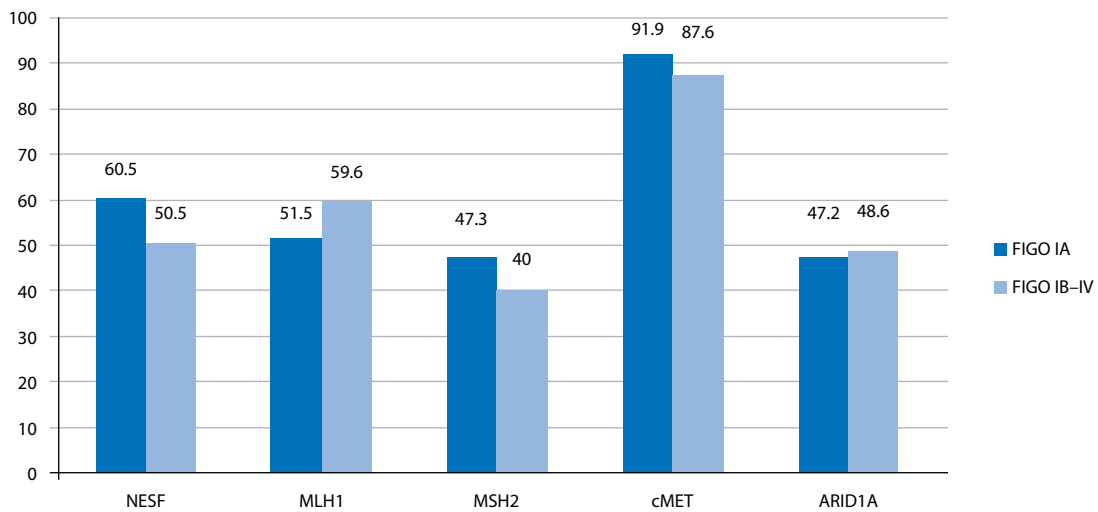NESF-1, the amino acid derivative of NUCB2 carries prognostic significance in type I EC; its immunoreactiv-

Figure 2. Percentage of endometrial cancer cases manifesting a high expression of a given protein in relation to the clinical stage
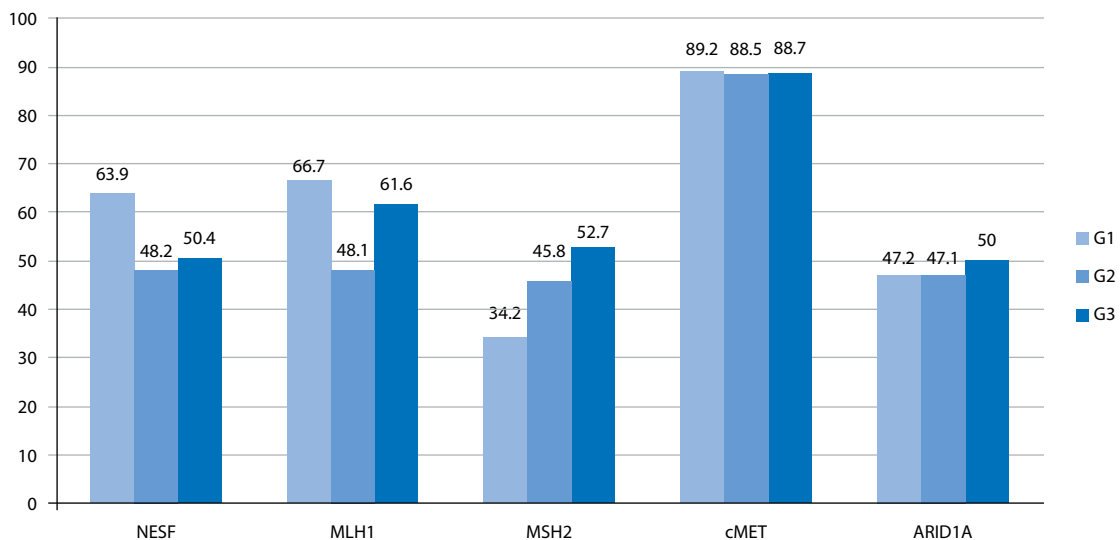

Figure 3. Percentage of endometrial cancer cases in the entire population of patients manifesting high expression of a given protein in relation to histopathological grading

ity correlated with an augmented risk of relapse and poor clinical course [23]. In the presented study, two groups of patients were analysed — women with endometrial cancer endometrioid type (EC I) and non-endometrioid (EC II). The expression of NESF-1 was more pronounced in type II EC (61.3%) with a poorer prognosis compared to type I EC (NESF-1 expression in 50.9% cells). However, no statistically significant values were determined (p = 0.410). The evaluation of expression in various grades of histological grading determined that patients with type I EC at G1 manifested a higher expression of NESF-1 than those with type I EC at both G2 and G3 (G1 — 68.97% compared to G2 and G3 — 50.4%, p = 0.0487). Similar results were observed in studies of Takagi et al. [23] in type I EC, though no statistical significance was identified (in G1 cancers the proportion of positive reaction was higher than in G2 and G3).

Mutations in MLH1 and MSH2 linked with Lynch syndrome carry a risk of developing type I EC. In the group of patients presented in this study, the expression of MLH1 protein was significantly higher in type II EC. Among all 146 patients, a high expression of NESF-1 correlated with a high expression of MLH1 (p = 0.039), which seems to be slightly controversial. In the literature, a high expression of NESF-1 was linked to poorer prognosis, while MLH1, associated with type I EC, was linked to better prognosis [2, 23, 24]. Dividing the patients according to the histological type of the tumor, a tendency was noted for a high expression of NESF-1 in patients with a high expression of MSH2 (p = 0.0596). This might indicate that despite the normal function of the MMR protein group, a high expression of NESF-1 is significant for prognosis in the patient group.

c-Met, also called tyrosine-protein kinase Met, is proto-oncogene tyrosine kinase, located on chromosome 7q21-31. Physiologically, it is essential for the disruption of cadherin-based cell–cell contacts and subsequent cell motility which takes place is embryonic development, organogenesis and wound healing [25]. It activates a wide range of different cellular signalling pathways, including those involved in proliferation, motility, migration and invasion. In many human primary tumours, amplification of the c-*MET* gene, with consequent protein overexpression and kinase activation, has been found. In our study, the expression of c-MET was most pronounced among the studied parameters: it was noted in over 80% of all tissues in both type I and type II ECs. No relationship was detected between the expression of c-MET and clinical stage or grading, although in the literature there is a correlation between its expression and metastatic disease and poor prognosis [26, 27].

ARID1A is located on chromosome 1p36.11 and encodes protein ARID1A, an important member of the SWI/SNF complex. This complex is responsible for chromatin remodelling, which regulates gene expression depending on changes in chromatin structures, and participates in replication, transcription and repair processes [28]. Dysfunction in the mechanism leads to carcinogenesis mainly via PI3K/AKT pathway. Mutations in *ARID1A* are found in many cancers and are common in gynaecological cancers such as clear cell and endometrioid ovarian cancer, but also in around 40% of endometrioid EC [29–31]. In our material, expression of ARID1A protein was detected in around 48% of all EC patients.

No differences in the expression of ARID1A protein were revealed in our study between clinical stages and histological grades. No relationship was also detected between ARID1A expression and the studied proteins (NESF-1, MLH1, MSH2, c-MET).

In both subgroups of patients, no other relationships were detected between the levels of expression of NESF, MLH1, MSH2, c-MET and AR1D1A.

CONCLUSIONS

In this study on protein expression, EC tissues manifested (although insignificantly) an elevated expression of NESF-1 in type II EC. In type I EC, NESF-1 expression was significantly higher in G1 in comparison to G2 and G3 combined. A significantly lower expression of MLH1 was demonstrated in type I EC. The most pronounced expression involved c-MET in all EC I and EC II tissues (in over 80% of cases). A tendency was detected for a high expression of NESF-1 in patients with type II EC, who also exhibited a high expression of MSH2.

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