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Consistency in biomarkers expression between matched tissue microarray cores from primary gallblader and ovarian cancers

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ABSTRACT

Introduction. Tissue microarray (TMA) technique has been widely used, especially in immunohistochemical assays of new prognostic and predictive markers. The main objections raised by its opponents are the small amount of sampled material and the associated risk of inadequate assessment of analysed expression, resulting from the potential heterogeneity of tumour tissue.

Material and methods. This study evaluated the compatibility of biomarker expression in two independent tissue cores, 1.5 mm in diameter, obtained by TMA technique from patients with gallbladder cancer (ERβ, cytoPgR, HER2, CTGF) and ovarian cancer (PTEN, BCL2, PIK3CA, IGF1R). Comparison of the expression of individual biomarkers between cores was performed using the intraclass correlation coefficient (ICC), assuming a kappa < 0.4 as a weak, ≥ 0.4 as sufficient, ≥ 0.6 as good, and ≥ 0.75 as optimal correlation, and Kendall's tau test — ICC package.

Results. Evaluation of biomarker expression in the primary tumour was performed in 60 patients with gallbladder cancer and in 64 patients with high-grade serous ovarian cancer. Additionally, in patients with follicular cancer, the expression of the tested markers was assessed in the epithelium free from neoplastic malignancy. In both tumours, a good or sufficient level of homogeneity was observed in the expression of the analysed biomarkers between tissue cores. The correlation coefficient for the expression of individual markers in gallbladder cancer and adhering healthy tissue was: 0.68 (95% CI: 0.53–0.79)/0.62 (95% CI: 0.39–0.78) for ERβ, 0.44 (95% CI: 0.23–0.61)/0.77 (95% CI: 0.61–0.87) for cytoPgR, 0.77 (95% CI: 0.65–0.85)/0.66 (95% CI: 0.44–0.80) for HER2, and 0.68 (95% CI: 0.53–0.79)/0.62 (95% CI: 0.39–0.78) for CTGF. In patients with ovarian cancer, the correlation coefficient within the primary tumour was 0.82 (95% CI: 0.71–0.89) for PTEN, 0.84 (95% CI: 0.75–0.90) for BCL2, 0.71 (95% CI: 0.56–0.81) for PIK3CA, and 0.77 (95% CI: 0.65–0.85) for IGF1R.

Conclusions. Tissue microarray technique allows reliable assessment of the expression of tissue biomarkers within the primary tumour of gallbladder cancer and ovarian cancer.

Key words: tissue microarrays, biomarkers, gallbladder cancer, ovarian cancer

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Introduction

The technique of tissue microarray (TMA) was first described in the 1980s [1]. In the following years, a modified method has been widely used, especially in immunohistochemical studies on new prognostic and predictive markers [2, 3]. It enables tissue material from tens or even hundreds of patients to be placed on a single microscope slide. In the first stage, the pathologist makes a microscopic evaluation of the whole specimen stained with haematoxylin and eosin to determine the most representative necrosis-free tumour area for further analysis. In the second stage, from a paraffin tissue block (the so-called "donor") containing a formalin-fixed fragment of the tumour, a small, cylindrical core with a diameter of 0.6 to 2 mm is collected using a special needle. This core is then placed in a pre-prepared hole located in another paraffin block called the "recipient". To increase the representativeness of the material being tested and to reduce the risk of tissue loss in the staining process, at least two cores are usually taken for each case. In addition, a map is created containing information about the location of the material, which allows it to be quickly identified in the block. After completion of the material collection process, sections are obtained for examination using the microtome; one microscopic slide usually contains of 50 to 150 cases [4]. The main objection raised by the opponents of this method is the small amount of material tested and the associated risk of inadequate assessment of analysed biomarker expression resulting from the potential heterogeneity of tumour tissue. Data on the reliability of TMA in gallbladder and ovarian cancer are scarce. This study evaluated the compatibility of biomarker expression between two tissue cores obtained by TMA in both tumours.

Material and methods

Characteristics of the assessed biomarkers (proteins)

The analysis included patients in whom the expression of a panel of tissue biomarkers was examined as part of two retrospective clinical studies. Proteins for immunohistochemical analysis were selected on the basis of available literature, taking into account the availability of antibodies and technical feasibility of assessment on archived formalin-fixed paraffin-embedded (FFPE) tissue. In the project concerning gallbladder cancer, the expressions of following receptors were analysed: steroid hormones receptors: estrogen α (ERα) and β (ERβ), progesterone (PgR), human epidermal growth factor 2 (HER2), and connective tissue growth factor (CTGF). In turn, in ovarian cancer, the expression of the following proteins was determined: human protein encoded by the *PTEN* suppressor gene (phosphatase and tensin homolog deleted on chromosome 10) on the long arm of chromosome 10, proteins belonging to the BCL2 family (B-cell CLL/lymphoma 2), protein of the catalytic subunit α phosphatidyl inositol 3-kinase (PI3K-CA), and insulin-like growth factor-1 receptor (IGF1R).

Preparation of tissue microarrays

In the analysed group, sections stained with haematoxylin and eosin were subjected to histopathological reassessment, which allowed verification of the diagnosis and determination of the most representative fragments of cancer and healthy tissues. Selected samples together with the corresponding paraffin blocks were used to determine the tumour areas from which the sections for tissue microarray were taken using a 1.5 mm diameter needle. Biopsy specimens of tumour-containing fragments were placed in previously prepared, tissue-free paraffin blocks — "recipients". Tissue microarrays were performed using a Manual Tissue Arrayer I by Beecher Instruments (MTAI, K7 BioSystems). Two fragments (biopsies) of primary tumours were collected in both groups, and in the gallbladder cancer project, additionally, excisions from adjacent healthy tissues. Immunohistochemistry was performed on tissue sections of microarrays with a thickness of 4 μm. Table 1 presents a list of the antibodies used in the study along with the methodology of performing immunohistochemical staining.

Statistical analysis

Statistical analysis was performed using the statistical environment R, version 3.4.3 [5] on the basis of data contained in a specially prepared database. A comparison of the expression of individual biomarkers between the "tissue cores" was performed using the intraclass correlation coefficient (ICC), assuming kappa < 0.4 as weak, ≥ 0.4 as sufficient, ≥ 0.6 as good and ≥ 0.75 as optimal correlation, and Kendall tau test — ICC package [6].

Results

In the gallbladder cancer project, biomarker expression was evaluated in tissue material from cholecystectomy in 60 patients treated between 2004 and 2016 in four oncology centres in Poland: The Military Institute of Medicine in Warsaw, the University Clinical Centre of the Medical University of Gdansk in Gdansk, Professor Tadeusz Koszarowski Opole Oncology Centre in Opole, and Janusz Korczak Provincial Specialist Hospital in Slupsk. In the ovarian cancer project, the analysis was carried out in the primary tumour, in the postoperative material in 64 patients diagnosed with high-grade serous

Table 1. Antibodies tested and immunohistochemical methods

Antibody	Manufacturer Catalogue No.	Concentration	Epitope recovering	Exposure time	Control tissue	Assessment method
ERα	DAKO; anti-human; rabbit clone EP1	RU	HIER; DAKO PT-link, high pH	20′	Breast cancer	Semiquantitative
ERβ	Abcam; anti-human; rabbit clone EPR3778; ab133467	1:70	HIER; DAKO PT-link, high pH	Night incubation	Breast cancer	Semiquantitative
PgR	DAKO; anti-human; mouse clone 636	RU	HIER; DAKO PT-link, high pH	20′+linker mouse 15′	Breast cancer	Semiquantitative
HER2	Ventana; rabbit clone 4B5	RU	Epitope recovering in the machine	20′	Breast cancer	Semiquantitative
CTGR	Santa Cruz, California; goat sc-14939	1:100	HIER, DAKO PT-link, high pH	60′	Smooth muscles	Semiquantitative
PTEN	DAKO; clone 6H2.1	1:50	HIER, DAKO PT-link, high pH	30′	Placenta	Semiquantitative
BCL2	DAKO monoclonal mouse clone 124	RU	HIER, DAKO PT-link, high pH	20′	Lymph node	Semiquantitative
PIK3CA	Cell signalling Rabbit monoclonal	1:50	HIER, DAKO PT-link, low pH	60′	Breast cancer	Semiquantitative
IGF1R	Roche Rabbit Monoclonal (G11)	RU	Epitope recovering in the machine	30′	Placenta	Semiquantitative

cancer, treated surgically between 2010 and 2016 at the Military Institute of Medicine in Warsaw.

In both tumours, a good or sufficient level of homogeneity was observed in the expression of the analysed biomarkers between tissue cores. ERα expression was not demonstrated in gallbladder and healthy tissue. The correlation coefficient for the expression of other biomarkers in gallbladder carcinoma and adhering healthy tissue was: 0.68 (95% CI: 0.53–0.79)/0.62 (95% CI: 0.39–0.78) for ERβ, 0.44 (95% CI: 0.23–0.61) 0.77 (95% CI: 0.61–0.87) for cytoplasmic PgR, 0.77 (95% CI: 0,65–0.85)/0.66 (95% CI: 0.44–0.80) for HER2, and 0.68 (95% CI: 0.53–0.79)/0.62 (95% CI: 0.39–0.78) for CTGF. In patients with ovarian cancer, the correlation coefficient within the primary tumour was 0.82 (95% CI: 0.71–0.89) for PTEN, 0.84 (95% CI: 0.75–0.90) for BCL2, 0.71 (95% CI: 0.56–0.81) for PIK3CA, and 0.77 (95% CI: 0.65–0.85) for IGF1R (Table 2 and 3).

Table 2. Compatibility analysis for ERβ, cytoPgR, HER2, and CTGF expression between tissue cores in gallbladder cancer and adherent healthy tissue (intraclass correlation coefficient [ICC], assuming kappa: < 0.4 as weak, ≥ 0.4 as sufficient, ≥ 0.6 as good, and ≥ 0.75 as optimal correlation, and Kendall tau test — ICC package)

HER2	
In total	0.74 (95% CI: 0.64–0.82)
Gallbladder cancer	0.77 (95% CI: 0.65–0.85)
Healthy tissue	0.66 (95% CI: 0.44–0.80)
cytoPgR	
In total	0.80 (95% CI: 0.73–0.86)
Gallbladder cancer	0.44 (95% CI: 0.23–0.61)
Healthy tissue	0.77 (95% CI: 0.61–0.87)
CTGF	
In total	0.66 (95% CI: 0.55–0.76)
Gallbladder cancer	0.68 (95% CI: 0.53–0.79)
Healthy tissue	0.62 (95% CI: 0.39–0.78)
ERβ	
In total	0.66 (95% CI: 0.55–0.76)
Gallbladder cancer	0.68 (95% CI: 0.53–0.79)
Healthy tissue	0.62 (95% CI: 0.39–0.78)

Discussion

Neoplasms are heterogeneous in nature, which means that there may be significant genotype differences in the primary tumour or its distant lesions, resulting from the selection of cell clones [7–9]. Therefore, the heterogeneity of tumours is spatial and temporal. In turn, in diagnostics and qualifications for treatment, especially molecularly targeted, there is a need to determine reliable prognostic and predictive factors — biomarkers. Undoubtedly, intra-tumour heterogeneity in

Table 3. Compatibility analysis for *PTEN*, BCL2, PIK3CA, and IGF1R expression between tissue cores in ovarian cancer (intraclass correlation coefficient [ICC], assuming kappa: < 0.4 as weak, ≥ 0.4 as sufficient, ≥ 0.6 as good, and ≥ 0.75 as optimal correlation, and Kendall tau test — ICC package)

PTEN	
In total	0.82 (95% CI: 0.71–0.89)
BCL2	
In total	0.84 (95% CI: 0.75–0.90)
PIK3CA	
In total	0.71 (95% CI: 0.56–0.81)
IGF1R	
In total	0.77 (95% CI: 0.65–0.85)

neoplastic disease can lead to erroneous conclusions and hinder the development of personalised medicine [7–9]. For this reason, validation of diagnostic methods used in scientific research is very important. The technique of tissue microarray, due to the gathering of material from different patients on one slide, significantly shortens the time of staining and evaluation, saves tissue material and the amount of reagents used, and allows testing in uniform conditions and with the same dilutions of the antibodies used. On the other hand, the evaluation of such small fragments of tissue raises doubts as to their representativeness in relation to the whole tumour. Previous studies on this issue, carried out in various cancers, indicate high consistency of results evaluated in microarrays and in full tumour sections [10–18]. In individual studies, the discrepancy in the number of cores needed to obtain an acceptable sample representation could be due to the heterogeneity of the expression of antigens in tumours [14, 16, 17, 19]. In a breast cancer study it was found that one or two TMA cores in each case yielded results that were 95% similar to those obtained using tumour sections [10]. However, most validation studies have shown that analysis of two to three cores with a diameter of 0.6 mm gives higher compliance rates than using one core [10, 14–16]. Therefore, two cores, 1.5 mm in diameter, were used in this work. High homogeneity in the expression of the analysed biomarkers with the use of tissue microarray technology in tumours has been demonstrated, which until now have not been the subject of a similar assessment. The reliability and usefulness of this method in the diagnosis of other cancers requires similar research.

Conclusions

In immunohistochemical studies on new prognostic and predictive biomarkers in gallbladder and ovarian cancer, the tissue microarray technique is a reliable diagnostic method.

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