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CHANGES OF PROTEIN EXPRESSION OF PLATINUM RESISTANCE INDICATORS UNDER THE INFLUENCE OF HYPERTHERMIA DURING THE HIPEC PROCEDURE IN RECURRENT OVARIAN CANCER

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Introduction. Ovarian cancer relapse and death are usually caused by acquired drug resistance. The mechanisms of platinum resistance are multifactorial. Excision repair cross-complementation group 1 (ERCC1) is a protein critical in removing platinum-induced DNA lesions. Microsatellite Instability (MSI) is present in a substantial proportion of ovarian cancers but knowledge about its clinical value is limited. Hyperthermia, one more promising treatment agent, delays the repair of DNA damage. Hyperthermic intraperitoneal chemoperfusion (HIPEC), which has been actively studied in recent years as a possible addition to therapy for advanced stages of epithelial ovarian cancer.

Material and methods. The study was retrospective, it included a total of 16 patients with stage IIIC epithelial ovarian cancer. For various reasons, these patients underwent suboptimal cytoreductive surgery with HIPEC + Second-look surgery with complete / optimal cytoreduction (6 patients) or relaparotomy with biopsy of residual disease due to surgical complications in 2–4 weeks interval. Immunohistochemical investigation of ERCC1 and MLH-1 expression were performed for the histological samples obtained from pre- and post HIPEC metastatic tumor tissue on the first and second surgical interventions.

Conclusions. DNA repair pathways are one of the most important factors of platinum drug resistance formation. Hyperthermia during HIPEC procedure leads to decrease in the efficiency of DNA repair pathways by reducing the expression of ERCC1 and MMR proteins. These changes may determine the proven effectiveness of HIPEC procedure with cytoreduction after NACT (which may lead to secondary platinum drug resistance formation) by overcoming platinum resistance.

Keywords: recurrent ovarian cancer, platinum resistance, HIPEC, ERCC1, MLH-1.

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ЗМІНИ ЕКСПРЕСІЇ БІЛКОВИХ МАРКЕРІВ РЕЗИСТЕНТНОСТІ ДО ПРЕПАРАТІВ ПЛАТИНИ ПІД ВПЛИВОМ ГІПЕРТЕРМІЇ ПІД ЧАС ПРОЦЕДУРИ HIPEC ПРИ РЕЦИДИВНОМУ РАКУ ЯЄЧНИКІВ

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Рецидив раку яєчників і подальша смерть зазвичай спричинені набутою медикаментозною резистентністю. Механізми резистентності до платини багатофакторні. Шляхи репарації ДНК є одним із найважливіших факторів формування стійкості до препаратів платини. Гіпертермія під час процедури HIPEC призводить до зниження ефективності репарації ДНК шляхом зменшення експресії ERCC1 (з 57,56% клітин до 5% клітин – $p < 0,05$) та білків MLH-1 (з 9,11% клітин до 0% клітин – $p < 0,05$). Цей механізм подолання вторинної резистентності до препаратів платини обґрунтовує ефективність процедури HIPEC з циторедукцією після неоад'ювантної хіміотерапії.

Ключові слова: рецидивний рак яєчників, резистентність до препаратів платини, HIPEC, ERCC1, MLH-1.

Introduction

Ovarian cancer is one of the most common malignant tumors of the reproductive organs and has the highest mortality rate among all gynecological malignancies. At diagnosis, approximately 3/4 of patients present with advanced disease resulting in a low five-year survival rate. The initial response to platinum-based chemotherapy is as high as 80%, but in most advanced patients, final relapse and death are caused by acquired drug resistance. The mechanisms of platinum resistance are multifactorial and comprise genetic and epigenetic alterations as well as immune and environmental factors frequently involving more than one mechanism of resistance [1].

Deoxyribonucleic acid (DNA) is the main target of platinum-based anticancer drugs, and the cell's ability to recognize and repair drug-induced DNA damage can influence its sensitivity or resistance to platinum chemotherapy. The primary mechanism through which platinum chemotherapy exerts its cytotoxic effects is the formation of DNA monoadducts that evolve through covalent binding to DNA crosslinks that can occur either on the same DNA strand or on the opposite strands, generating interstrand crosslinks that block DNA synthesis and

transcription if they are not repaired. DNA damage response consists of several signaling pathways responsible for enforcing cell-cycle arrest and, depending on the severity of DNA damage, either DNA repair or the activation of apoptosis for cells presenting with unrepairable DNA lesions. Six major DNA repair pathways have been described: mismatch repair (MMR), base excision repair, nucleotide excision repair (NER), homologous recombination, nonhomologous end joining, and Fanconi anemia [2].

Excision repair cross-complementation group 1 (ERCC1) is a protein critical in a nucleotide excision repair pathway. The key role of NER in removing platinum-induced DNA lesions has been suggested by the extreme sensitivity of cells lacking functional ERCC1. It was shown that neoadjuvant chemotherapy (NACT) treated epithelial ovarian cancer (EOC) tissues showed a two-fold increase in ERCC1 expression compared to chemo-native epithelial ovarian cancer tissues. This is why ERCC1 has been the most investigated potential biomarker of therapeutic response at the genomic level (analysis of single-nucleotide polymorphisms), at the transcriptional level (reverse transcriptase PCR) and at the protein level (immunohistochemistry – IHC) in different tumor types, in retrospective and prospective studies. However, the results obtained by some researchers have been contradictory [3–5].

Microsatellite Instability (MSI) is present in a substantial proportion of endometrioid ovarian cancers but can also occur in other tumor subtypes. MMR deficiency/MSI typically involves the entire tumor mass, suggesting that MMR inactivation occurs early in tumorigenesis in a subset of ovarian cancers [6]. Investigating for mismatch repair protein deficiency, microsatellite instability, and Lynch syndrome is widely accepted in endometrial cancer, but knowledge is limited on its value in epithelial ovarian cancer [7]. In ovarian cancer, data on intratumoral heterogeneity of MMR deficiency/MSI are lacking. From 7% to 16% of OCs are MMRd by IHC or MSI, respectively, although studies where both techniques are used do not suggest that one technique is superior. This is clinically significant as these cancers would potentially be amenable to immunotherapy; a treatment that has been shown be highly effective in solid cancers with MMRd [8].

Interval debulking surgery (IDS) is an option for treating patients with advanced ovarian carcinoma. Randomized trials have shown similar survival rates for primary debulking surgery (PDS) and IDS. NACT followed by IDS could improve the optimal debulking rate and decrease the postoperative adverse reactions. The question of whether overall survival and progression-free survival are improved compared with PDS followed by chemotherapy in patients with FIGO stages IIIc and IV ovarian carcinoma requires further research. One of the concerns with IDS is the potentially higher risk of inducing platinum resistance when treating patients with greater disease volume [9–11].

Hyperthermia, one more promising treatment agent, delays the repair of DNA damage caused by cisplatin or doxorubicin, acting upstream of different repair pathways to block histone polyADP-ribosylation, a known effect of chemotherapy [12]. Furthermore, hyperthermia blocks this histone modification as efficiently as pharmacologic inhibitors of PARP (PARPi), producing comparable delay in DNA repair, induction of double-strand breaks, and cell cytotoxicity after chemotherapy. Mild hyperthermia (41°C–42.5°C) induces degradation of BRCA2 and inhibits homologous recombination. It is demonstrated that hyperthermia can be used to sensitize innately homologous recombination-proficient tumor cells to PARP-1 inhibitors and that this effect can be enhanced by heat shock protein inhibition [13, 14].

Hyperthermic intraperitoneal chemoperfusion (HIPEC), which has been actively studied in recent years as a possible addition to therapy for advanced stages of epithelial ovarian cancer locally spread by the peritoneal cavity. We need to focus on the M06OVH-OVHIPEC phase 3 trial, which examines the combination of interval cytoreduction and HIPEC. This is the only randomized and controlled study to date that has reliably proven the effectiveness of this method [15].

Aim of the study. To investigate changes in immunohistochemical markers of platinum resistance ERCC1 and MLH-1 under the influence of hyperthermia during the HIPEC procedure in epithelial ovarian cancer.

Materials and Methods

The study was retrospective, including a total of 16 patients with stage IIIC epithelial ovarian cancer who were treated in 2016–2018 at the Center for Reconstructive and Renovative Medicine (University Clinic) of Odesa National Medical University. All patients in the neoadjuvant regimen received 3 courses of chemotherapy according to the scheme Carboplatin (AUC 5-6) and Paclitaxel 175 mg/m^2 in a three-week regimen. They have obtained CC 1-2 (suboptimal) cytoreductive surgery with HIPEC + Second-look surgery with CC 0-1 (complete – optimal) cytoreduction (6 patients) or relaparotomy with biopsy of residual disease due to surgical complications (anastomosis leakage in 2 patients, early adhesive intestinal obstruction – 6 patients, eventeration – 1 patient, Bowel perforation (acute ulcer) – 1 patient) at an interval of 2–4 weeks. The HIPEC procedure was performed on the Rand Performer HT device (Italy) using cisplatin 50 mg/m^2 of body surface area and doxorubicin 15 mg/m^2 of body surface area. Immunohistochemical studies were performed on the basis of the pathomorphological laboratory of the University Clinic of Odesa National Medical University and the laboratory "CSDHealthCare" (Kyiv). IHC-study of ERCC1 expression using monoclonal antibodies against human ERCC1 clone 4F9 (DAKO, Denmark) was performed for histological samples obtained from metastatic tumor tissue before and after HIPEC during the first and second surgical interventions. IHC-study of MLH-1 obtained using monoclonal antibodies MLH-1 (DAKO Clone ES05) was performed for histological samples obtained from metastatic tumor tissue before and after HIPEC during the first and second surgical interventions. Morphometric counting of the percentage of positive tumor cells was performed using the JMicroVision 1.2.7 computer software. Student's t-test for matched samples was used to compare the results. All patients provided written informed voluntary consent for medical care, as well as for participation in the research and educational process. The study was conducted in compliance with the principles of the World Medical

Association Code of Ethics for Research (Declaration of Helsinki "Ethical principles of medical research involving humans as research subjects" Adopted by the 18th General Assembly of the World Medical Association, Helsinki, Finland, June 1964, and revised by the 59th General Assembly of the World Medical Association, Seoul, October 2008 protocol N990_005) – Meeting protocol of the Bioethics Commission of Odesa National Medical University No. 06 dated October 14, 2022.

Research results and their discussion

The mean age of patients was 54.4 ± 10.2 years. All 16 patients demonstrated high baseline ERCC1 expression (> 50% of tumor cells. We then selected 9 of these patients with Pre-HIPEC IHC expression of MLH-1 > 5% of cells and compare it with the Post-HIPEC results.

Median Pre-Hipec expression of ERCC1 was 57,56% of cells. Median Post-Hipec expression of ERCC1 was 5% of cells (Fig. 1). Student's t-test for matched samples was used to compare the results. Empirical t-value was 22.3, critical for $p \leq 0.05 – 2.13$, for $p \leq 0.01 – 2.95$. The obtained reduction of ERCC1 expression in cells of metastatic nodes of epithelial ovarian cancer is statistically reliable ($p < 0.05$).

Median Pre-Hipec expression of MLH-1 was 9,11% of cells. Median Post-Hipec expression of MLH-1 was 0% of cells (Fig. 2). Student's t-test for matched samples was used to compare the results. Empirical t-value was 5.3, critical for $p \leq 0.05 – 2.31$, for $p \leq 0.01 – 2.36$. The obtained reduction of MLH-1 expression in cells of metastatic nodes of epithelial ovarian cancer is statistically reliable ($p < 0.05$).

Surgical interventions in the study patients were performed before there were available clinical trials and treatment protocols that demonstrated benefits in overall and disease-free survival for patients who underwent HIPEC with complete cytoreduction only. Nevertheless, this retrospective analysis provides valuable observations. Currently, there are only a few studies on biomarkers of chemotherapy resistance and their impact on the effectiveness of HIPEC or, conversely, the impact of the HIPEC procedure on biomarkers of chemotherapy resistance in the treatment of ovarian cancer. But there are some studies on this topic regarding colorectal cancer which we can compare with.

For example the obtained results are similar to a systematic review by Emma C. Hulshof et al. investigating the association between genetic biomarkers related to DNA repair and treatment outcome in patients with colorectal cancer undergoing systemic chemotherapy, because only two studies could be retrieved that investigated the association of biomarkers related to DNA repair and intraperitoneally administered mitomycin C or oxaliplatin. The most promising genetic biomarkers were *ERCC1* rs11615, *XPC* rs1043953, *XPD* rs13181, *XPG* rs17655, *MNAT* rs3783819/rs973063/rs4151330, MMR status, ATM protein expression, *HIC1* tandem repeat D17S5 and *PIN1* rs2233678. Combination studies of two DNA repair genes have also been studied and showed significant associations with treatment outcome [16].

The similar data was reported by M. Tonello et al. They have concluded that for patients affected by primary metastatic colorectal cancer who are eligible for cytoreductive surgery, clinical and pathologic criteria need to be integrated with molecular features (KRAS/BRAF mutation). Micro-satellite status should be strongly considered because MSI confers a survival advantage over microsatellite stable, even for mutated patients [17].

Ahmed B. Hamed at al. [18] and D. Massalou [19] also have reported that patients with primary metastatic

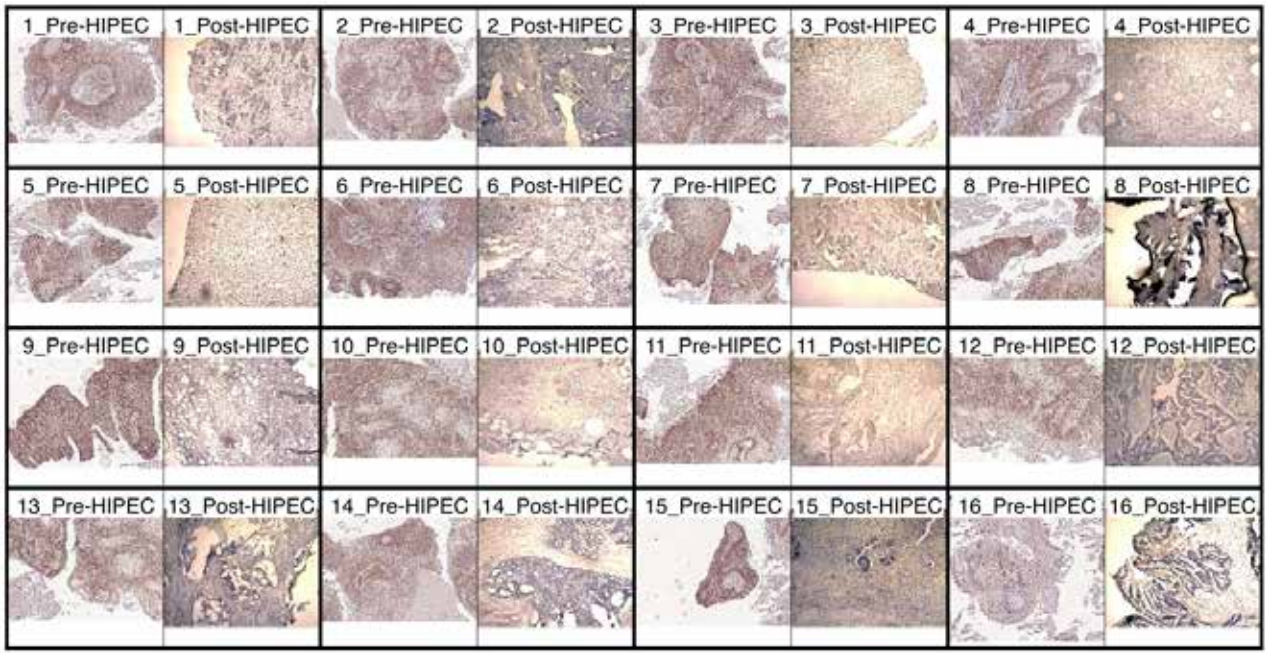

Fig. 1. Paired immunohistochemical micrographs of ERCC1 expression in tumors of 16 patients during the first surgery with HIPEC and after repeated surgery, x10 magnification, obtained using monoclonal Anti-Human ERCC1 Clone 4F9 antibody

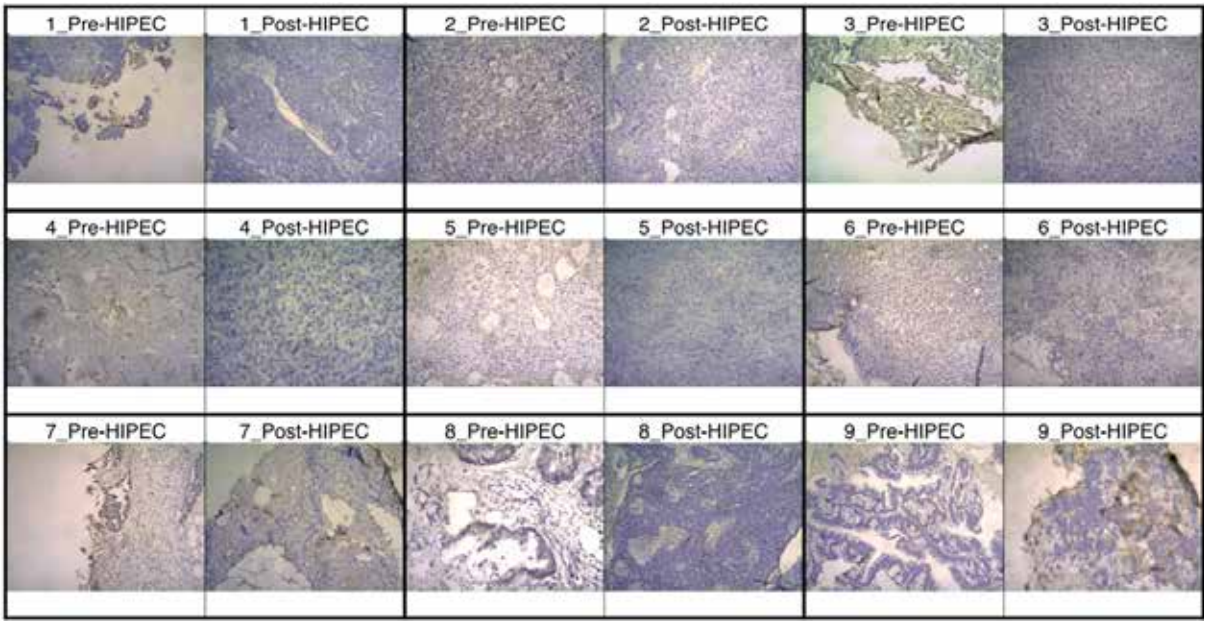

Fig. 2. Paired immunohistochemical micrographs of MLH-1 expression in tumors of 9 patients during the first surgery with HIPEC and after repeated surgery, x10 magnification, obtained using monoclonal MLH-1 Antibody Clone ES05

colorectal cancer and dMMR/MSI-H status have superior survival due to benefits of immune checkpoint-inhibitors in this subgroup.

Primary metastatic colorectal cancer with cytoreduction and HIPEC patients has a surprisingly high proportion of mutBRAF (24.7%) according to S.G. Larse et al. research. Survival was similar when comparing mutBRAF, mutKRAS and double wild-type cases, whereas a small subgroup with mutBRAF and MSI had better survival. Patients with mutBRAF tumours and limited peritoneal metastases should be considered for CRS-HIPEC [20].

Comparing these studies with those obtained by us on ovarian cancer patients, we can assume that the DNA repair biomarkers have the necessary role in its treatment result prediction in FIGO IIIC and IV stages EOC patients. The decreased IHC expression of ERCC1 and MMR proteins after the HIPEC procedure may lead to treatment benefits from platinum-based chemotherapy. Lower MMR proteins expression (dMMR) status after HIPEC procedure may also confer a therapeutic advantage from immune checkpoint inhibitors treatment in future researches on cytoreduction + HIPEC + chemotherapy + immunotherapy in EOC patients.

The limitation of this study is a small number of patients treated in a single institution and retrospective research.

A positive aspect of the study is the unique data that was obtained from the small group of patients with suboptimal cytoreduction and HIPEC in EOC which can be found in other institution's repositories from 2000–2015 years but can't be studied prospectively now due to proofed major overall survival and progression free survival benefits from complete cytoreduction (PDS or IDS).

Conclusions

Hyperthermia during HIPEC procedure leads to a decrease in the efficiency of DNA repair by reducing the expression of ERCC1 (from 57.56% of cells to 5% of cells – $p < 0.05$) and MLH-1 proteins (from 9.11% of cells to 0% of cells – $p < 0.05$). This mechanism of overcoming secondary platinum resistance supports the efficacy of the HIPEC procedure with cytoreduction after neoadjuvant chemotherapy.

Conflict of interest. The authors declare that there is no conflict of interest regarding the publication of this article.

The sponsor has not been specifically involved in the research.

All authors read and approved the final version of the manuscript.

The authors have nothing to disclose.

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