SL Clinical And Medical Oncology

Special Issue Article "Gynecologic Oncology" Research Article

# Clinical Relevance of Co-Expression of HLA-DRα, CD74/II and NFkB in Epithelial Ovarian Cancer: Identification of Prognostic and Therapeutical Biomarker Profile

**Daltoé RD[1], Madeira KP[1], de Araújo KL[3], Lyra PCM Jr[1], Tessarollo NG[1], de Souza MLM[4], dos Santos DZ[4], de Carvalho AA[6], Rotstein C[7], Lima R[7], Farias P[7], Silva IV[2,5] and Rangel LBA[1,2*]**

[1]Laboratory of Cellular and Molecular Biology of Human Cancer, Federal University of Espirito Santo, Brazil

[2]Department of Pharmaceutical Sciences, Federal University of Espirito Santo, Brazil

[3]Laboratory of Cellular Biology of Aging, Federal University of Espirito Santo, Brazil

[4]Laboratory of Cellular and Molecular Biology of Human Cancer, Federal University of Espirito Santo, Brazil

[5]Department of Morphology, Federal University of Espirito Santo, Brazil

[6]Department of Pathology, Federal University of Espirito Santo, Brazil

[7]National Institute of Cancer, Brazil

**ARTICLE INFO**

Received Date: November 08, 2018
Accepted Date: March 18, 2020
Published Date: March 20, 2020

**KEYWORDS**

Ovarian cancer; HLA-DRα; HLA-DRβ CD74/Ii; NFkB; Biomarker



**Citation for this article:** Daltoé RD, Madeira KP, de Araújo KL, Lyra PCM Jr, Tessarollo NG, de Souza MLM, dos Santos DZ, de Carvalho AA, Rotstein C, Lima R, Farias P, Silva IV and Rangel LBA. Clinical Relevance of Co-Expression of HLA-DRα, CD74/II and NFkB in Epithelial Ovarian Cancer: Identification of Prognostic and Therapeutical Biomarker Profile. SL Clinical And Medical Oncology. 2020; 2(1):115

**Corresponding author:**
Leticia B. A. Rangel,
Laboratory of Cellular and Molecular Biology of Human Cancer, Federal University of Espirito Santo, Av. Marechal Campos, Vitoria, ES, Brazil,
Email: lbarangel@yahoo.com

## ABSTRACT

**Purpose:** Ovarian Cancer (OVCA) is the gynecologic malignancy of highest lethality rate. We've demonstrated that HLA-DRA, HLA-DRB and CD74/Ii, which code HLA-DRa, HLA-DRb and invariant chain of HLA-DR complex are over expressed in OVCA and other cancers. Nevertheless, HLA-DRb is suppressed in OVCA. Furthermore, CD74/Ii might interact with NFkB, a molecule related to cellular proliferation, angiogenesis, metastasis and chemoresistance.

**Experimental design:** Tissue array platforms were generated containing samples of neoplasic ovarian issue that were assayed by immunohistochemistry applying primary antibodies anti-HLA-DRα, anti-HLA-DRβ, anti-CD74/Ii and anti-NFkB.

**Results:** MEMBRANE (m) expression of HLA-DRα and CD74/Ii were restricted to OVCA. Tumors with the HLA-DRα, CD74/Ii (c,m); HLA-DRβ -/c; NFkB (c,n), (c=cytoplasm and n=nuclear) proteomic profile represented 42% of the OVCA cases and have demonstrated a more aggressive pattern than tumors with distinct protein expression profiles. Tumors with profile HLA-DRα,CD74/Ii (c,m); HLA-DRβ -/c; NFkB (c,n) have a 6-fold increased risk to relapse than tumors with other profiles. Additionally, they tend to be less sensitive to platin-based therapy in initial stages (I/II) of the disease. Survival rate over 15 months in patients with OVCA diagnosed in stages I/II was lower among the tumors presenting the more aggressive profile pattern. We had also observed that HLA-DRa expression has been related to CD74/Ii expression.

**Conclusion:** Even though more studies are needed, the more aggressive behavior of tumors with the HLA-DRα,CD74/Ii (c,m); HLA-DRβ -/c; NFkB (c,n) profile observed in these OVCA, suggest that the referred profile could be introduced in the OVCA clinic routine as prognostic and therapeutic biomarker.

## INTRODUCTION

Ovarian Cancer (OVCA) representing the *mortis* cause of highest incidence among all gynecological malignancies and is the 5th cause of cancer deaths among women [1].

Part of these statistics is due to the fact that OVCA diagnosis is usually done in advanced stages tumors [2], which are often less responsive - or refractory - to the first-choice platin-based antineoplastic treatment regimens. The epidemiological profile of OVCA reflects, then, the inefficiency of the available diagnostic strategies, the lack of sensitivity and specificity of diagnostic biomarkers, such as CA125 and the limited therapeutic arsenal against the disease [3,4] and the need of therapeutic biomarkers and/or predictors of response to chemotherapy and the discovery of alternative treatment strategies.

The number of immunocompetent women who succumb to the disease each year suggests that OVCA cells with increased proliferative capacity are probably selected from a heterogeneous cluster of tumor cells under selective pressure, favoring immune evasion and tumor growth. Aiming to investigate the OVCA gene expression pattern, Huang and collaborators [5] performed an elegant SAGE study using fresh OVCA and cellular lineages. Intriguingly, the highest super expressed gene in OVCA was HLA-DRA (human leukocyte antigen). OVCA cells also expressed HLA-DRB and CD74/Ii transcripts, however in much lower levels. HLA-DRA, HLA-DRB and CD74/Ii encode, respectively, the a chain of the HLA-DR complex (HLA-DRa), the β chain of the HLA-DR complex (HLA-DRb) and invariant chain (CD74/II) of the major histocompatibility complex class II (MHC II).

MHC class II molecules are encoded by genes in HLA-D region which contains at least three sub-regions: DP, DQ and DR. It is known that the initiation, propagation and regulation of adaptive immune responses depend on the precise regulation of MHC class II complex [6,7]. In contrast to MHC class I molecules, which are widely distributed, the expression of MHC class II is restricted, at least in normal conditions, to antigen-presenting cells such as macrophages, dendritic cells and B cells and it mediates the adaptive immune responses by interacting with the CD4 molecules on the surface of these cells. However, its expression can be induced by cytokines, such as IFN-g (interferon gamma), in other cell types as normal epithelial cells and cancerous cells. On the other hand, maturation of MHC class II molecules depends on the dimerization of α and β chains, encoded by distinct genes in a chromosomal region of MHC class II [8,9], yet in a coordinate fashion, under the regency of the transcriptional co-activator of Class II Transactivator (CIITA) [10].

From this, there is great interest in evaluating whether the expression of antigens by HLA class II correlates with the clinical course of the disease, but there is conflicting information about the expression of HLA class II antigens by tumor cells and the clinical significance of that [11]. In some malignancies, HLA class II antigen expression is associated with poor prognosis, while it is associated with favorable prognosis in others cancers [12] such as in colorectal carcinoma [13].

As another example, we mention the B-cell lymphoma. In this case HLA-DR antigen loss is associated with a more aggressive course of the disease and in acute promyelocytic leukemia, characterized as a subtype acute leukemia, and the abscence of HLA-DR cannot be used for diagnostic [14]. However, the expression of the same antigen was already reported to be associated with the progression of colon cancer [15] and plays an important role in the progression of metastatic breast carcinoma [16]. In malignant melanoma, there are controversies about the association of HLA class II antigen expression with poor prognosis [17,18].

*In silico* analysis of HLA-DR expression profile in high quality SAGE libraries of normal and cancerous tissues [19], as well as the protein expression investigated by immunohistochemistry in a tissue microarray platform containing several cancer types samples (TARP2; Tissue Array Research Program, NCI, NIH, USA) revealed that while many types of cancer express the transcripts HLA-DRA and HLA-DRB, they do not synthesize mature HLA-DR molecules (HLA-DRm). This is probably due to the suppression of HLA-DRb by post-transcriptional or post-translational mechanisms. Furthermore, tumor cells maintain cytoplasmic expression of HLA-DR a and CD74/Ii [19]. HLA-DRα, HLA-DRβ, CD74/Ii and CIITA expression in ovarian tissues was further studied in and ovarian cancer tissue array platform (Ovarray) generated by Rangel and collaborators [20] and Sherman-Baust and collaborators [21]. The authors' data have demonstrated that most primary OVCA overexpress HLA-DRα, but suppress HLA-DRβ, so that they are unable to synthesize HLA-DRm. The expression of HLA-DRβ, when observed, was confined to the cytoplasm of OVCA cells [19]. Surprisingly, OVCA cells not only maintain the cytoplasmic expression of



Clinical Relevance of Co-Expression of HLA-DRα, CD74/II and NFkB in Epithelial Ovarian Cancer: Identification of Prognostic and Therapeutical Biomarker Profile. SL Clinical And Medical Oncology. 2020; 2(1):115.

HLA-DRα and CD74/Ii but also sustain marked expression of these proteins in their membranes.

Based on the concept that cancer cells direct their energetic metabolism towards the expression of molecules that favor their proliferative, metastatic and cellular viability potentials, our group proposes that in addition to a unique immune modulation mode, OVCA development might depend upon a HLA-DR a/CD74/Ii-coupled signaling pathway. Furthermore, it has been demonstrated that the intracellular domain of CD74/Ii can interact with the Nuclear Factor Kappa B (NFkB) [22], an anti-apoptotic molecule important for the neoplastic transformation of various tissues, including ovarian, and for the acquisition of the cisplatin-resistant phenotype often observed in OVCA [23]. We hypothesize that women suffering from OVCA that express HLA-DRα and CD74/Ii in the membrane of the cancer cells associated with cytoplasmic and nuclear NFkB synthesis might present poor prognosis disease, possibly associated to the platin-resistance phenotype. Finally, we believe to be presenting an OVCA biomarker profile (HLA-DRα, CD74/Ii and NFkB) that represents novel avenues to better evaluate OVCA prognosis, as well as might configure a tool to envisage tumor platin responsiveness, decreasing the high obit number of women in which platin-resistant OVCA relapse.

## MATERIALS AND METHODS

### Study patients and OVCA database

We had evaluated 101 formalin-fixed-paraffin-embedded specimens of epithelial ovarian tumors: 91 primary ovarian cancer, 5 *borderline* tumors and 5 benign tumors (adenoma). Specimens were obtained from patients submitted to debulking surgery at Cassiano Antonio de Moraes Hospital, Santa Rita de Cassia Hospital and The National Institute of Cancer Hospital (Brazil) between 1997 and 2007. All live patients gave write informed consent before enrollment in the study. We had generated a complete OVCA database containing patients' clinical follow up information's (such as age at diagnosis, tumor histological type, tumor FIGO staging, tumor grade, tumor response to chemotherapy and patients survival).

### Tissue array and immunohistochemistry

Hematoxylin–eosin stained sections from each ovarian tissue histological specimen were reviewed by pathologists to confirm ovarian histological diagnosis and to select one representative 2 mm area of the tumor paraffin block for immunohistochemical analysis. Each 2 mm sample was further transferred to a transient tissue array platform, generating customized ovarian cancer tissue array blocks [24].

For immunohistochemistry, we had followed a protocol optimized by our group in 5 µm sample sections of the generated ovarian cancer tissue array platform [19]. Briefly, after deparaffinization, sections were immersed in preheated antigen-retrieval solution (citrate buffer 10 mM; pH 6,0) and incubated at 95° - 99° C for 30 minutes; then allowed to cool to room temperature for 30 minutes. Following, sections were incubated for 3 hours with the primary antibodies of interest: monoclonal mouse anti-human HLA-DRa, clone TAL. 1B5 (DakoCytomation, Carpinteria CA) dilution 1:20; monoclonal mouse anti-human MHC class II b chain, clone TDR 31.1 (Ancell, Bayport, MN) dilution 1:50; mouse monoclonal to CD74, clone LN-2 (Abcam Inc., Cambridge, MA) dilution 1:100; rabbit monoclonal to NFkB p65, clone E379 (Abcam Inc., Cambridge, MA) dilution 1:100. Sections were incubated with biotinilated universal secondary antibody (Dako Cytomation LSAB+ System-HRP, Dako Cytomation, Carpinteria CA) for 30 minutes. Endogenous peroxidase activity was blocked by 5 minutes incubation in 3% hydrogen peroxide. Antigen-antibody complexes were detected by the avidin-biotin-peroxidase method, using 3,3-Diaminobenzidine (DAB) as chromogenic substrate. The sections were incubated with streptavidin conjugated to peroxidase (LSAB+ System-HRP, Dako Cytomation, Carpinteria CA) for 30 minutes and then with DAB (Liquid DAB+ Substrat Chromogen System, Dako Cytomation, Dako Cytomation, Carpinteria CA) for 5 minutes. Slides were counterstained with hematoxylin and immersed in 5% ammonium hydroxide. Positive controls were included in the ovarian cancer tissue array platform in opposite positions of the chip in order to guarantee an accurate analysis of protein expression in ovarian tissue, as cited: amygdale tissue for HLA-DRa, HLA-DRb, CD74/Ii and mammary tissue for NFkB. Negative control experiments, conducted in the absence of the primary antibodies listed above, were conducted in parallel of all assays. Cases with less than 5% staining in tumor cells were considered negative for the expression of the protein of interest. Subcellular localization was also noted.

Each 2 mm tumor section was evaluated for protein expression by using 40× objective lens (Olympus AX70 microscope) with the guidance of a pathologist. The immunohistochemically stained sections were evaluated without previous knowledge of the clinical outcome of patient in a blinded-study fashion. After scoring protein staining intensity, immunohistochemistry results were correlated to each clinical parameter of our ovarian cancer database.

## STATISTICAL ANALYSIS

Statistical analysis was performed using Fisher's exact test to compare experimental data to clinical information. A p value of 0.05 or less was considered statistically significant. The risk to develop ovarian cancer associated with the proteomic profile herein studied was calculated using Odds Ratio (OR) test with Confidence Interval (CI) of 95%. Statistics were performed using the Graph Pad Prism 5 for Windows (version 5.00.288).

## RESULTS

In order to analyze the clinical impact of HLA-DRα, CD74/Ii and NFkB co-expression on OVCA, we had, initially, generated an ovarian database including cases of OVCA and *borderline* tumors, all of epithelial origin. The main features of the cases studied are compiled in (Table 1).

Table 1: Percentage of cases that express HLA-DRα, HLA-DRβ, CD74/Ii and NFkB.

| Samples | N | HLA-DRα (c,m) | HLA-DRα (c) | HLA-DRβ (c,m) | HLA-DRβ (c) | CD74/Ii (c,m) | CD74/Ii (c) | NFkB (c,n) | NFkB (c) |
|---|---|---|---|---|---|---|---|---|---|
| Adenomas | 5 | 0 | 40 | 0 | 0 | 0 | 20 | 0 | 0 |
| Borderline Tumors | 5 | 0 | 20 | 0 | 0 | 0 | 40 | 40 | 20 |
| Serous Adenocarcinoma | 43 | 60 | 5 | 0 | 14 | 54 | 33 | 93 | 7 |
| Endometrioid Adenocarcinoma | 16 | 56 | 6 | 0 | 31 | 69 | 19 | 88 | 6 |
| Mucinous Adenocarcinoma | 11 | 27 | 9 | 0 | 0 | 27 | 64 | 73 | 18 |
| Clearcell Adenocarcinoma | 9 | 44 | 0 | 0 | 22 | 44 | 11 | 89 | 0 |
| Adenocarcinoma Not Otherwise Specified | 12 | 42 | 9 | 0 | 0 | 50 | 18 | 92 | 0 |
| Total | 101 | | | | | | | | |

Table 2: Percentage of histological type by profile.

| Samples | Profile 1 | Profile 2 |
|---|---|---|
| Borderline Tumors | 4 | 4 |
| Serous Adenocarcinoma | 48 | 36 |
| Endometrioid Adenocarcinoma | 18 | 12 |
| Mucinous Adenocarcinoma | 11 | 16 |
| Clearcell Adenocarcinoma | 4 | 24 |
| Adenocarcinome Not Otherwise Specified | 15 | 8 |
| Total | 27 | 25 |



Clinical Relevance of Co-Expression of HLA-DRα, CD74/II and NFkB in Epithelial Ovarian Cancer: Identification of Prognostic and Therapeutical Biomarker Profile. SL Clinical And Medical Oncology. 2020; 2(1):115.

As observed, the majority of OVCA studied was diagnosed at late stages associated with very aggressive disease. Consequently, in the cases investigated, 23% of the women died before receiving any kind of treatment. These cases were excluded from the expression studies. Furthermore, among OVCA patients who received chemotherapy, all were initially treated with the first line chemotherapy for OVCA, that is platinum-based (cisplatin or carboplatin), mostly combined with other chemotherapeutics such as paclitaxel or cyclophosphamide, which increases response of the tumors to the treatment [25]. Of these patients, 51% (n = 32) were sensitive to platinum, showing no relapse of the disease for a period of at least 6 months after the end of cycles of chemotherapy; while 49% (n = 31) were refractory/resistant to platinum, describing no response to chemotherapy or relapsing the disease within the first 6 months of the beginning of the first line chemotherapy treatment. Combining all cases, the sensitive and the resistant to the platinum-based regimen, we had observed 78% of the OVCA cases relapsing after treatment. Additionally, most of the tumors initially sensitive to platinum acquired the platin-resistant phenotype (47%, n = 15).

The cases were then studied by immunohistochemistry, to determine the expression of HLA-DRα, HLA-DRβ, CD74/Ii and NFkB. In order to correlate the patients' clinical data registered in the OVCA database generated by our laboratory with the experimental data, we had nominated two tumor protein expression profiles: i) PROFILE 1: tumors expressing HLA-DRα and CD74/Ii in the cytoplasm (c) and cellular membrane (m), considered an OVCA specific feature to our knowledge [19], combined with the absence or strictly cytoplasmic expression of HLA-DRβ , and cytoplasmic and nuclear (n) expression of NFkB (the nuclear expression of NFkB denotes the activation of the transcription factor), ii) PROFILE 2: includes all tumors presenting a different profile as compared to profile 1. Of the all cases studied, 53% of OVCA presented the profile 1 protein expression pattern (n = 27) and 47% Profile 2 (n = 25), as described in (Table 2).

The stage of OVCA at diagnosis was independent of the proteomic profile, as well as the degree of cell differentiation of the tumors investigated. However, tumors describing the profile 1 protein expression pattern presented more than two-fold incidence of poorly differentiated cells (grade 3) (n = 20) than moderately differentiated cells (grade 2) (n = 10); while the referred ratio has not been observed in profile 2 tumors (poorly differentiated: n = 19; moderately differentiated: n = 14). Relapses, in turn, were more frequent in profile 1 tumors (90%, n = 19) than in tumors expressing the profile 2 protein pattern (59%, n = 16) ($p < 0.05$). Interestingly, profile 1 tumors describe a 6.5-fold greater risk to recidivate when compared to profile 2 tumors (Odds ratio = 6,531, 95% confidence interval), suggesting higher aggressiveness of profile 1 when compared to profile 2 OVCA (Figure 1).

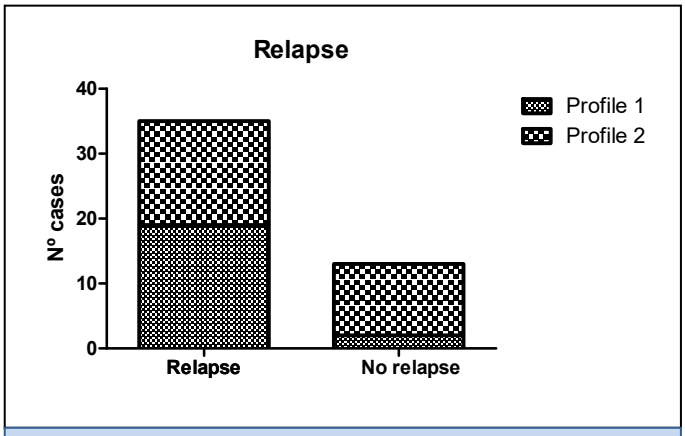


Figure 1: Occurrence or not of relapse, according to proteomic profile. Relapse were more frequent in profile 1 tumors than in tumors expressing the profile 2 protein pattern($p<0,05$, Fisher Exact Test).

In conformity with the profile 1 OVCA prevalent relapse rate, as compared to profile 2 tumors, proteomic profile was related to the tumor sensitivity to platinum-based chemotherapy. Surprisingly, we had observed that tumors diagnosed at stages I/II, corresponding to OVCA restricted to the ovaries and to best prognostic disease and which also expressed profile 1, tended to be less sensitive to platinum than tumors with profile 2. Indeed, our observations showed 33% (n = 1) of the tumors sensitive to platin with the profile 1 versus 71% (n = 5) of the tumors sensitive to platin with profile 2. On the other hand, when we analyzed protein expression profile in tumors diagnosed at stage III/IV, corresponding to metastatic and poor prognosis disease, the proportion of tumors sensitive to or resistant to platinum was similar in both profiles. In this matter, we had noted 47% (n = 8) of tumors sensitive to platin

expressing profile 1 and 54% (n = 17) of tumors sensitive to platin with profile 2 (Figure 2).

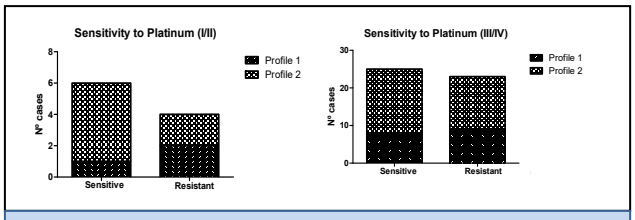


Figure 2: Sensitivity to platinum x proteomic profile. The graph on the left, which includes stage I and II tumors, shows that, of the tumors sensitive to platinum, 33% are profile 1 and 71% are profile 2. In the right panel, which includes stage III and IV at diagnosis, 47% of the tumors sensitive to platinum have profile 1 and 54% profile 2.

As expected, the compromised sensitivity to platinum observed in profile 1 OVCA affected adversely the survival of patients with tumors in stage I and II, so that 67% of these women (n = 4) survived less than 15 months. In the group with profile 2, considering only the cases diagnosed in stages I and II, only 12% (n = 1) had survived less than 15 months (Figure 3).

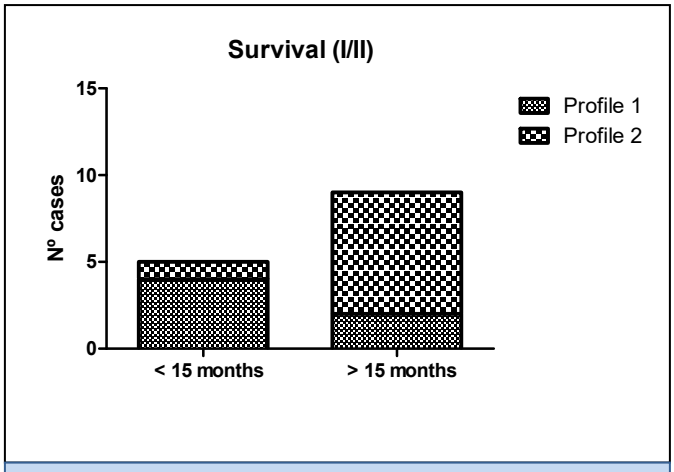


Figure 3: Survival x proteomic profile. In the figure on the left, which includes stage I and II tumors at diagnosis, is noted that 67% of the cases of tumors with profile 1 died in less than 15 months, compared to 12% of tumors with profile 2 (p<0,05).

Besides the clinical correlation, we had also analyzed the statistical significance of HLA-DRα and CD74/Ii co-overexpression, believing that OVCA tumorigenesis may depend, at least partially, on a pathway coupled to the expression of these molecules. Our immunohistochemistry data revealed that HLA-DRα and CD74/Ii membrane co-expression was positively correlated as 61% of the OVCA cases expressed both molecules whereas 15% of the cancer cases have not synthesize any of them (p <0.0001). Moreover, the expression of HLA-DRα in the membrane of OVCA seems to be required for the expression of membrane CD74/Ii, so that 81% of the cases that expressed CD74/Ii on the membrane of OVCA cells also expressed HLA-DRα (p <0.0001). However, when we analyzed the co-expression of HLA-DR and CD74/Ii for each profile, we observed that 100% of Profile 1 showed co-expression of both molecules while Profile 2 showed only 20% of simultaneous expression of antigens (Figure 4,5).

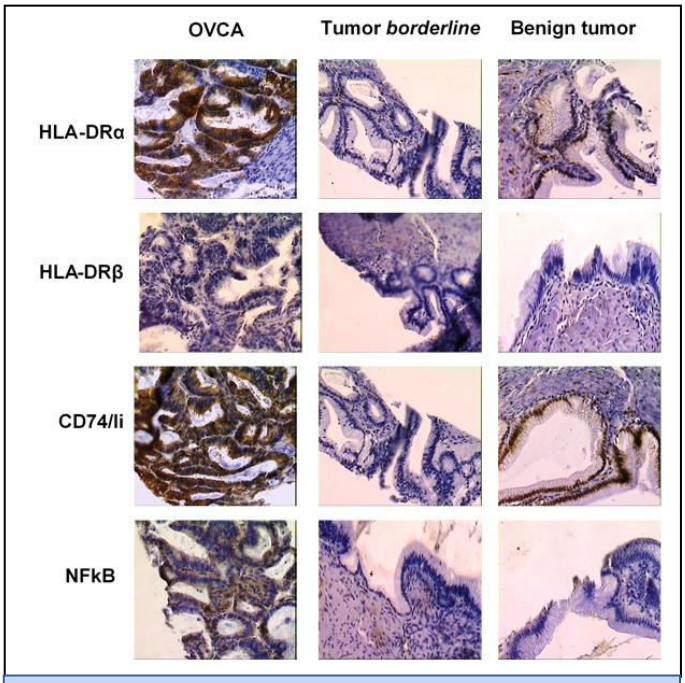


Figure 4: HLA-DRα, HLA-DRβ, CD74/Ii and NFkB expression in ovarian tissues. Protein expression were assessed using immunohistochemistry in a tissue array platform developed in our laboratory. Representative examples are shown for each tissue type. Expression is shown by brown staining and the slides counterstained with hematoxylin-eosin as described in materials and methods. (40× objective lens).

## DISCUSSION

Data showed in the present work, as the worldwide panorama, confirm the poor outcome of OVCA, mainly due to the low effective methods of diagnosing the disease at early stages, as well as novel and valuable therapeutic strategies to control such an aggressive cancer.

Herein, we had demonstrated that most of the tumors initially sensitive to platin acquired the chemoresistant phenotype during the disease progression (47%, n = 15). This is one of the

major limitations to the use of platin-based combinations in chemotherapy of cancer [26]. Undeniably, the high incidence of refractory OVCA or tumors with acquired resistance to platin, the first line anticancer treatment against OVCA, impacts Public Health globally as platin-resistant OVCA relapses are extremely aggressive and often culminate with the death of the patient. Indeed, significant fraction of the initial rate of satisfactory response to cisplatin from 40% to 80%, modestly increased by the combined treatment with platinum and paclitaxel [27,28] relapse as resistant disease. Consequently, only 25% of advanced OVCA treated with cisplatin remained for at least 4 years without recurrence [29]. In this study, in accordance with previous reports, combination of late diagnosis, poor cellular differentiation, high percentage of tumors resistant/refractory to platinum-based chemotherapy and high number of relapse resulted in 81% of deaths caused by OVCA.

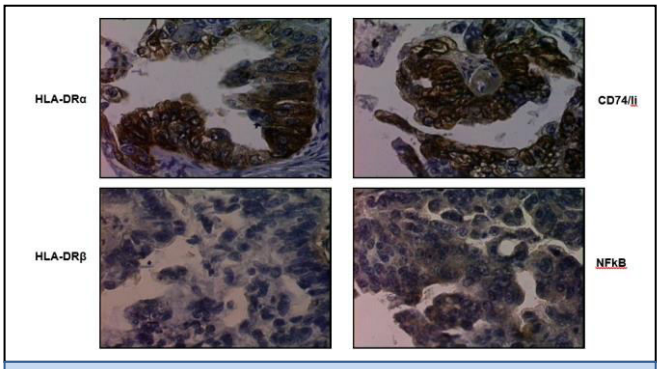


Figure 5: OVCA with profile 1. Study of immunohistochemical revealing representative OVCA case with profile HLA-DRα, CD74/Ii (c, m); HLA-DRβ -/c; NFkB (c, n): there is a marked cytoplasmic and membrane expression of HLA-DRα and CD74/Ii in neoplastic cells, and cytoplasmic and nuclear expression of NFkB and the absence of HLA-DRβ. Expression is shown by brown staining and the slides are counterstained with hematoxylin-eosin as described in materials and methods. c: citoplasmic, m: membrane, n: nuclear. (40× objective lens).

The large number of immunocompetent women who die of OVCA and other cancers supports the hypothesis of cancer immunoediting. According to this hypothesis, the immune system that initially protects the host against tumor progression, might then promote the development of the disease through the selection of cell clones within the heterogeneous tumor mass. Selected cell clones carry a reduced immunogenic phenotype, which, finally, escape the immune control and progress as an aggressive cancer [30,31]. To escape immune surveillance, some tumors develop adaptive strategies such as suppression or abnormal expression of molecules of the MHC class I or II [32]. Modulation of MHC class II molecules expression was found in an anomalous expression fashion or not in OVCA, liver, renal, gastric and colorectal cancer, among others [6,19,33-36].

In fact, in this work, we had confirmed the membrane expression of HLA-DRα and CD74/Ii in OVCA cells included in the study. In contrast with Tamiolakis and collaborators [27] who found only 25% of serous OVCA expressing HLA-DRα, our results showed, on average, 73% of OVCA expressing HLA-DRα (in 60% of cases membrane and cytoplasmic expression and in 5% of cases only cytoplasmic expression). Our findings are in agreement with those previously published [19] where we observed expression of HLA-DRα in 73% of serous OVCA. Analyzing the other histologic subtypes, all cases of SOE (ovarian surface epithelium) and 88% of endometrioid analyzed showed expression of HLA-DR (m/c). However, the antigen is only expressed in 29% of cases of clear cell carcinomas.

The membrane co-expression of HLA-DRα and CD74/Ii seems to be associated with increased aggressiveness of OVCA. Moreover, it has been shown by others that CD74/Ii can activate NFkB, which is associated with tumorigenesis, angiogenesis, metastasis and resistance to cisplatin and paclitaxel [5,23,37,38]. Interestingly, the profile 1 OVCA (HLA-DRα, CD74/Ii (c, m), HLA-DRβ (-/c); NFkB (c, n)) was correlated to some features of higher aggressiveness in studied OVCA, as will be further discussed.

In other studies, modulation of MHC II molecules expression by cancer cells has been correlated with survival and clinical outcome of the disease. In B-cell lymphoma, as well as in colorectal tumor, decreased expression of MHC II was associated with decreased survival. In colorectal cancer, a 10-year survival rate was observed in 67% of the cases expressing HLA-DR molecules and only in 34% of which have not express the complex [13]. However, the significant differences in patients' survival rate observed by Matsushita and collaborators [13] was only in tumors already in advanced

stages, while in the early tumor stages there was no significant difference in survival between tumors that express and those who have not express HLA-DR. Furthermore, the expression of HLA-DR in rectal tumors was associated with lower recurrence of the disease [36]. In hepatocellular carcinoma, low expression of MHC II was correlated to the occurrence of early relapse [39]. Although these data appear to be discordant from data obtained in our study, since the presence of MHC II predicts better clinical outcome and increased survival of patients with cancer in other studies, we emphasize that OVCA express HLA-DR molecules in a non-coordinated, anomalous mode [19] therefore ovarian cancer lacks mature and immunocompetent HLA-DR molecules which could present tumor antigens to CD4+ T cells and, therefore, trigger an effective antitumor response.

Besides the lack of immunocompetent HLA-DR complex, we had observed the overexpression of CD74/Ii in OVCA. Corroborating our data, the presence of CD74/Ii or CLIP in gastric cancers (class II invariant chain-associated peptide) has been related to worse prognosis disease [34]. Chamuleau and collaborators [40], in a study with acute myeloid leukemia, compared the survival of patients in which tumor cells maintained CLIP or CD74/Ii with patients in which CLIP has been properly removed. The investigators reported that the survival of the first was lower than the latter, suggesting that the anomalous expression to MHC II molecules may be associated with increased tumor aggressiveness. This is in concordance with data found in the current study.

Supported by the previous discussion, observing and analyzing the clinical information obtained in this study, we believe that the profile 1 OVCA (HLA-DRα, CD74/Ii (c, m), HLA-DRβ (-/c), NFkB (c, n)) is associated with worse prognosis of OVCA. Moreover, observing our data of responsiveness to chemotherapy, survival and mortality, stratified by stage (I/II vs. III/IV), for the different profiles 1 (HLA-DRα, CD74/Ii (c, m), HLA-DRβ (-/c); NFkB (c, n)) and 2 (all protein expression patterns distinct from profile 1), we had noticed that in stages I/II the impact of profile 1 is higher than in tumors of stage III/IV.

One explanation for this observation may be the fact that in later stages of OVCA, the prognosis of the disease is poor enough so that the importance of profile 1 (HLA-DRα, CD74/Ii (c, m), HLA-DRβ (-/c); NFkB (c, n)) phenotype is hidden within disseminated metastases destining patients with OVCA in these stages to low survival rate and high mortality rate, regardless of the proteomics profile expressed. Therefore, we believe that the presence of the proteomic profile studied in ovarian tumors may be related to increased aggressiveness of these, but may have more impact on tumors in early stages when chances for cure are better.

Our data suggest that the profile 1, HLA-DRα, CD74/Ii (c, m); HLA-DRβ -/c; NFkB (c, n), may be a new therapeutic and prognosis biomarker for OVCA. Nevertheless, clinical applicability is thought to have a higher impact on OVCA diagnosed at stages I/II when the actual clinical value of profile 1 expression on prognosis evaluation of the disease, as well as in the definition or the prediction of tumor responsiveness to platin-based chemotherapy, is more impressive.

Besides the clinical implications of the presence of the molecules studied, discussed above, the presence of HLA-DRα and CD74/Ii in the membrane of OVCA cells seems to be associated with ovarian tumorigenesis. We had noted that the presence of these two molecules appears to be linked, such that the majority of the cases co-expressed both molecules (70.3%, $p < 0.0001$) or just did not express both molecules. Moreover, the expression of membrane CD74/Ii seems to depend on the expression of HLA-DRα (81% of cells that expressed membrane CD74/Ii also expressed HLA-DRα) ($p < 0.0001$). As the activation of NFkB can be triggered by intramembranous cleavage of CD74/Ii (MATZA et al, 2002), the presence of CD74/Ii, probably stabilized in OVCA cell membrane by HLA-DRα, may be important for ovarian tumorigenesis and the metastatic and aggressive potential of OVCA, and for acquisition of resistant phenotypes to platin-based chemotherapy mediated by NFkB.

Also, in agreement with our current findings, our previous data clearly show that HLA-DRβ, although transcribed, is deleted during protein translation [19], strengthening the hypothesis of a tumorigenic pathway in which the anomalous expression of HLA-DRα and CD74/Ii, suppression of HLA-DRβ and activation of NFkB may contribute to the development and progression of OVCA reducing their cells immunogenicity and increasing their

proliferative potential, resulting in a disease with such dramatic epidemiology trends.

As a laboratory analysis of relative low cost, we believe that the assessment of the profile HLA-DRα, CD74/Ii (c, m); HLA-DRβ -/c; NFkB (c, n) during OVCA diagnosis can be introduced in the disease clinical routine intending to allow a more accurate evaluation of the disease, enabling better therapeutic planning and rationalizing the chemotherapeutical treatment of OVCA.

## CONCLUSION

The correlation between the expression profile of HLA-DRα, HLA-DRβ, CD74/Ii and NFkB in OVCA samples, *borderline* tumors and ovarian adenomas and their clinical information suggest that the evaluated expression profile may be associated with the worst prognosis and less responsiveness to chemotherapy of first choice of OVCA based on platinum derivatives. Given this scenario, we propose that the expression profile of HLA-DRα and CD74/Ii in membrane and cytoplasm, associated with the expression of NFkB nuclear and cytoplasmic or absent expression of HLA-DRβ be commonly evaluated in the OVCA clinical routine as a new biomarker diagnostic, therapeutic and/or predictive of responsiveness to chemotherapy in this disease. We believe that the introduction of the referred analysis in routine diagnostic of OVCA can optimize and rationalize the treatment of this malignancy, providing longer survival and better quality of life for patients affected by this neoplasm.

## ACKNOWLEDGEMENT

CAPES, FAPES and CNPq.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## REFERENCES

1. American Cancer Society. (2018). Key Statistics for Ovarian Cancer.
2. Jammal MP, Martins-Filho A, Silveira TP, Murta EFC, Nomelini RS. (2016). Cytokines and prognostic factors in epithelial ovarian cancer. Clinical Medicine Insights: Oncology. 10: S38333.
3. Jiang W, Huang R, Duan C, Fu L, Xi Y, et al. (2013). Identification of Five Serum Protein Markers for Detection of Ovarian Cancer by Antibody Arrays. Plos One. 8: e76795.
4. Zheng H, Zhang L, Zhao Y, Yang D, Song F, Wen Y, et al. (2013). Plasma miRNAs as Diagnostic and Prognostic Biomarkers for Ovarian Cancer. Plos One. 8: e77853.
5. Huang S, DeGuzman A, Bucana CD, Fidler IJ. (2000). Nuclear Factor-kB Activity Correlates with Growth, Angiogenesis, and Metastasis of Human Melanoma Cells in Nude Mice. Clin Cancer Res. 6: 2573-2578.
6. Cresswell P. (1994). Assembly, Transport, And Function Of Mhc Class Ii Molecules. Annu Rev Immunol. 12: 259-293.
7. Viret C, Janeway CA Jr. (1999). MHC and T cell development. Rev Immunogenet. 1: 91- 104.
8. Beck S, Trowsdale J. (1999). Sequence organisation of the class II region of the human MHC. Immunol Rev. 167: 201-210.
9. Handunnetthi L, Ramagopalan SV, Ebers GC, Knight JC. (2010). Regulation of MHC class II gene expression, genetic variation and disease. Genes and immunity. 11: 99-112.
10. Mach B. (1999). Perspectives: Immunology. Regulating The Regulator. Science. 285: 1402-1405.
11. Seliger B, Kloor M, Ferrone S. (2017). HLA class II antigen-processing pathway in tumors: Molecular defects and clinical relevance. Oncoimmunology. 6: e1171447.
12. Sconocchia G, Eppenberger-Castori S, Zlobec I, Karamitopoulou E, Arriga R, et al. (2014). HLA Class II Antigen Expression in Colorectal Carcinoma Tumors as a Favorable Prognostic Marker. Neoplasia. 16: 31-42.
13. Matsushita K, Takenouchi T, Shimada H, Tomonaga T, Hayashi H, et al. (2006). Strong HLA-DR antigen expression on cancer cells relates to better prognosis of colorectal cancer patients: Possible involvement of c-myc suppression by interferon-gamma in situ. Cancer Sci. 97: 57-63.
14. M Wetzler, McElwain BK, Stewart C, Blumenson L, Mortazavi A, et al. (2003). HLA-DR antigen-negative acute myeloid leukemia. Leukemia. 17: 707-715.
15. Norazmi M, Hohmann AW, Skinner JM, Bradley J. (1989). Expression of MHC class I and class II antigens in colonic carcinomas. Pathology. 21: 248-253.

16. Redondo M, García J, Villar E, Rodrigo I, Perea-Milla E, et al. (2003). Major Histocompatibility Complex Status in Breast Carcinogenesis and Relationship to Apoptosis. Human Pathology. 34: 1283-1289.
17. Konstadoulakis MM, Vezeridis M, Hatziyianni E, Karakousis CP, Cole B, et al. (1998). Molecular oncogene markers and their significance in cutaneous malignant melanoma. Ann Surg Oncol. 5: 253-260.
18. Ostmeier H, Fuchs B, Otto F, Mawick R, Lippold A, et al. (1999). Can immunohistochemical markers and mitotic rate improve prognostic precision in patients with primary melanoma? Cancer. 85: 2391-2399.
19. Rangel LB, Agarwal R, Sherman-Baust CA, Mello-Coelho Vd, Pizer ES, et al. (2004). Anomalous Expression of the HLA-DR α and β Chains in Ovarian and Other Cancer. Cancer Biol Ther. 3: 1021-1027.
20. Rangel LB, Agarwal R, D'Souza T, Pizer ES, Alò PL, et al. (2003). Tight junction proteins claudin-3 and claudin-4 are frequently overexpressed in ovarian cancer but not in ovarian cystadenomas. Clin Cancer Res. 9: 2567-2575.
21. Sherman-Baust CA, Weeraratna AT, Rangel LB, Pizer ES, Cho KR, et al. (2003). Remodeling of the extracellular matrix through overexpression of collagen VI contributes to cisplatin resistance in ovarian cancer cells. Cancer Cell. 3: 377-386.
22. Matza D, Kerem A, Medvedovsky H, Lantner F, Shachar I. (2002). Invariant chain-induced B cell differentiation requires intramembrane proteolytic release of the cytosolic domain. Immunity. 17: 549-560.
23. Mabuchi S, Ohmichi M, Nishio Y, Hayasaka T, Kimura A, et al. (2004). Inhibition of NFkB Increases the Efficacy of Cisplatin in *in Vitro* and *in Vivo* Ovarian Cancer Models. J Biol Chem. 279: 23477-23485.
24. Rosen DG, Huang X, Deavers MT, Malpica A, Silva EG, et al. Validation of tissue microarray technology in ovarian carcinoma. Modern Pathology. 17: 790-797.
25. Thigpen JT, Blessing JA, Vance RB, Lambuth BW. (1989). Chemotherapy in ovarian carcinoma: present role and future prospects. Semin Oncol. 6: 58-65.
26. Solomon LA, Ali S, Banerjee S, Munkarah AR, Morris RT, et al. (2008). Sensitization of ovarian cancer cells to cisplatin by genistein: the role of NF-kappa B. J Ovarian Res. 1: 9.
27. Omura G, Blessing JA, Ehrlich CE, Miller A, Yordan E, et al. (1986). A randomized trial of cyclophosphamide and doxorubicin with or without cisplatin in advanced ovarian cancer. A gynecologic oncology group study. Cancer. 57: 1725-1730.
28. McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Partridge EE, et al. (1996). Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N Engl J Med. 334: 1-6.
29. Kartalou M, Essigmann JM. (2001). Recognition of cisplatin adducts by cellular proteins. Mutat Res. 478: 1-21.
30. Dunn GP1, Bruce AT, Ikeda H, Old LJ, Schreiber RD..(2002). Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 3: 991-998.
31. Schreiber RD. (2005). Cancer vaccines 2004 opening address: the molecular and cellular basis of cancer immunosurveillance and immunoediting. Cancer Immun. 6: S1-8.
32. Seliger B, Maeurer MJ, Ferrone S. (2002). Antigen-processing machinery breakdown and tumor growth. Immunol . 21: 455-464.
33. Hippo Y, Yashiro M, Ishii M, Taniguchi H, Tsutsumi S, et al. (2001). Differential gene expression profiles of scirrhous gastric cancer cells with high metastatic potential to peritoneum or lymph nodes. Cancer Res. 61: 889-895.
34. Tamori Y, Tan X, Nakagawa K, Takai E, Akagi J, et al. (2005). Clinical significance of MHC class II-associated invariant chain expression in human gastric carcinoma. Oncol Rep. 14: 873-877.
35. Dengjel J, Nastke MD, Gouttefangeas C, Gitsioudis G, Schoor O, et al. (2006). Unexpected abundance of HLA class II presented peptides in primary renal cell carcinomas. Clin Cancer Res. 12: 4163-4170.
36. de Bruin EC, van de Velde CJ, van Krieken JH, Marijnen CA, Medema JP. (2008). Epithelial human leukocyte antigen-DR expression predicts reduced recurrence rates and prolonged survival in rectal cancer patients. Clin Cancer Res. 14: 1073-1079.
37. Karashima T, Sweeney P, Kamat A, Huang S, Kim SJ, et al. (2003). Nuclear Factor-kB Mediates Angiogenesis and

Metastasis of Human Bladder Cancer through the Regulation of Interleukin-8. Clin Cancer Res. 9: 2786-2797.

38. Mabuchi S, Ohmichi M, Nishio Y, Hayasaka T, Kimura A, et al. (2004). Inhibition of Inhibitor of Nuclear Factor-kB Phosphorylation Increases the Efficacy of Paclitaxel in *in vitro* and vivo Ovarian Cancer Models. Clin Cancer Res. 10: 7645-7654.
39. Matoba K, Iizuka N, Gondo T, Ishihara T, Yamada-Okabe H, et al. (2005). Tumor HLA-DR expression linked to early intrahepatic recurrence of hepatocellular carcinoma. Int J Cancer. 115: 231-240.
40. Chamuleau ME, Souwer Y, Van Ham SM, Zevenbergen A, Westers TM, et al. (2004). Class II-associated invariant chain peptide expression on myeloid leukemic blasts predicts poor clinical outcome. Cancer Res. 64: 46-50.