Pathology - Research and Practice xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original article

Prognostic value of GRIM-19, NF-κB and IKK2 in patients with high-grade serous ovarian cancer

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ARTICLE INFO

Keywords:
Immunohistochemistry
Serous cystadenocarcinoma
Ovarian neoplasms
Platinum
Recurrence
Prognosis

ABSTRACT

Aims: High grade serous carcinoma (HGSC) is an aggressive tumour, and most patients relapse after treatment, acquiring resistance to platinum-based chemotherapy. One of the resistance mechanisms proposed is apoptosis evasion triggered by drug-related cytotoxic effect in the cell. In this context, this study aims to evaluate the protein expression of GRIM-19, NF-κB and IKK2, their association with chemotherapy response and to determine their prognostic values in HGSC.

Methods: GRIM-19, NF-κB and IKK2 expression was evaluated by immunohistochemistry (IHC) in 71 patients with HGSC selected between 2003 and 2013, whose underwent primary debulking surgery with complete cytoreduction. Protein expression was analyzed in relation to platinum response groups, tumour progression, clinicopathological data and survival.

Results: Positive IKK2 expression was related to resistance ($p = 0.011$), shorter disease-free survival ($p = 0.001$) and overall survival ($p = 0.026$) and was also a risk factor for relapse ($p = 0.002$) and death ($p = 0.032$). The association between IKK2 and NF-κB positivity predicted a subgroup with shorter overall survival ($p = 0.004$), disease-free survival ($p = 0.003$) and resistance to platinum-based chemotherapy ($p = 0.036$). NF-κB positivity was associated with worse overall survival ($p = 0.005$) and disease-free survival ($p = 0.027$) and was a positive predictor for relapse ($p = 0.032$) and death ($p = 0.008$). Higher expression of GRIM-19 was associated with higher disease-free survival ($p = 0.039$) and was a negative predictor for relapse ($p = 0.046$).

Conclusions: GRIM-19 is a potential predictor of prognosis and disease recurrence in HGSC. IKK2 and NF-κB are related to poor prognosis and are potential predictors of response to platinum-based chemotherapy in HGSC. IHC analyses of GRIM19, IKK2 and NF-κB may be important in the attempt to provide prognostic values for relapse and response to treatment in patients with HGSC.

1. Introduction

Ovarian cancer has the highest mortality rate among malignant tumours in the female genital tract. This disease represents the fifth cause of death from cancer in women, with an estimative of 22,000 new cases and 14,000 deaths in the United States in 2016 [1]. High grade serous carcinoma (HGSC) is an aggressive tumour that comprises 70% of epithelial ovarian cancer cases [2] and is often diagnosed in advanced stages, hampering curative treatments [3,4].

Treatment of ovarian cancer is based on surgery followed by platinum-based chemotherapy [5]. Although patients have an initial good response to platinum, 70% of these patients will relapse after treatment, and eventually become resistant to platinum chemotherapy [6]. In this scenario, the comprehension of the molecular pathways involved in chemotherapy resistance is still obscure. Precise information on biomarkers is insufficient to determine during diagnosis which patients will become resistant or not to chemotherapy.

The anticancer effects of platinum compounds are DNA-damage induction and increase of cell oxidative stress, leading to the permeabilization of mitochondrial membranes [7] and activation of caspases,

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https://doi.org/10.1016/j.prp.2017.12.002
Received 27 September 2017; Received in revised form 21 November 2017; Accepted 4 December 2017

molecular executioners of apoptosis process, and cell death [8]. Studies have showed that an increase in expression of anti-apoptotic factors can be related with resistance to drugs which induce apoptosis [9,10]. Molecules able to control directly or indirectly the apoptotic process, such as Gene Associated with Retinoid-IFN-induced Mortality 19 (GRIM-19), Nuclear Factor-κB (NF-κB) and Inhibitor of Nuclear Factor-κB Kinase subunit beta (IKK2) [11–14], can be associated with response to chemotherapy.

GRIM-19 was identified for the first time in association with interferon-beta (IFN-β) and retinoid acid, two molecules that induce cell death by apoptosis, and therefore described as a new apoptotic factor and tumour suppressor [11,12]. The anticancer and apoptotic property of GRIM-19 protein is related to binding and inhibition of signal transducer and activator of transcription-3 (STAT3). STAT3 is constitutively activated in several tumours and considered as an inhibitor of apoptosis by promoting upregulation of anti-apoptotic Bcl-2 and Bcl-XL [15,16].

NF-κB is an important family of transcription factors that regulate several cellular processes, such as immune and inflammatory response, apoptosis, differentiation and proliferation [17]. In cancer, NF-κB pathway can be activated by growth factors and cytokines that are directed to NF-κB-related anti-apoptotic effect in the tumour cell. NF-κB activates the expression of anti-apoptotic genes during tumour progression, such as anti-apoptotic Bcl-2 family and inhibitors of apoptosis (IAPs), conferring resistance to drug-induced apoptosis [13]. The anti-apoptotic effect of NF-κB is due to IKK2 protein function, which activate NF-κB transcriptional function [14]. IKK2 activity primarily occurs in the cytoplasm, phosphorylating NF-κB inhibitor (IκB) to degradation by proteasome and releasing NF-κB from cytoplasm to nucleus [17].

To determine whether GRIM-19, NF-κB, and IKK2 are important in evaluating response to platinum-based chemotherapy and determine their prognostic value, we evaluated their expression in 71 cases of HGSC.

2. Materials and methods

2.1. Sample selection

Seventy-one cases of HGSC diagnosed in the Department of Gynecologic Oncology, AC Camargo Cancer Center, Brazil, were selected between 2003 and 2013. All patients underwent primary debulking surgery having achieved complete cytoreduction, later receiving 6–8 cycles of adjuvant platinum-based chemotherapy. Patients with missing clinical information or insufficient paraffin-embedded material were excluded. Original H&E slides were reviewed by gynecologic pathologist (LDB) to confirm the diagnosis and to select the most suitable paraffin-embedded tissue for immunohistochemical study. The samples were obtained from Anatomical Pathology Department at AC Camargo Cancer Center.

Patients were classified according to time of relapse after last chemotherapy infusion. Patients who relapsed prior to 6 months after the last platinum infusion were classified as platinum resistant. Patients who relapsed between 6 and 12 months after the last platinum infusion were classified as platinum partially sensitive. Those who had relapse after 12 months were classified as platinum sensitive. Relapse status was defined by CA-125 levels and RECIST criteria.

In order to evaluate protein expression according to tumour progression, we selected from the overall number of patients that relapsed a subcohort of 24 paired cases of primary tumours of HGSC and relapse tumours. This study was approved by the AC Camargo Cancer Center Research Ethics Committee under registry number 1863/14B.

2.2. Immunohistochemistry (IHC)

IHC was performed on tissue microarray (TMA) slides using Ventana Benchmarck XT stainer (Ventana Medical Systems, Tucson, AZ 85755 USA). The antibodies used were: anti-GRIM-19 (GeneTex®, Irvine, CA 92606 USA, clone EPR4471(2), diluted at 1:100), anti-NF-κB p65 (Abcam®, Cambridge, CB4 0FL, UK, clone E379, diluted at 1:250), anti-IKK2 (Biorbyt®, Cambridge, CB4 0WY, UK, clone orb86857, diluted at 1:750). All batches included positive controls, and omission of primary antibody was used as negative control.

2.3. Scoring of immunohistochemical expression

Blinded assessment of TMA slides was performed through optical microscope by two pathologists (LL and LDB). Scoring of protein expression of GRIM-19 took into account the cytoplasmic expression. It was established by the percentage of positive cells (PPC) and intensity of immunostaining (II) [HSCORE = (PPC x II), in which PPC varied from 0 to 100% and II varied from 0 (negative staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining)], with a score ranging between 0 and 300 [18]. Scoring > 120 was considered as higher expression and ≤120 was considered as lower expression, determined by statistic model from R software, version 3.2.4.

NF-κB and IKK2 expression were scored by percentage of positive nuclear cells, as it follows: 0 (absence of positive cells or until 10% of positive cells, negative), 1 (11%-49% of positive cells, irregular stain) and 2 (≥50% of positive cells, diffuse stain). The categories 1 and 2 were considered positive stains [19].

2.4. Clinicopathological parameters

The clinical data obtained from medical records were: age, FIGO staging, last platinum-based chemotherapy date, relapse date, death date and follow-up. Pathological data, such as involvement of ovarian surface and/or fallopian tube, bilateral disease, lymphatic and blood vascular invasion and lymph node involvement were evaluated from slides review.

2.5. Sample characteristics

Clinicopathological features of all 71 patients are described in Table 1. The mean age was 57 years, ranging from 33 to 77 years. The mean follow-up time was 49.2 months, ranging from 2 to 110 months. All of selected patients were diagnosed in advanced stages (FIGO stage III–IV) and 54 (76.1%) developed relapsed disease. Fourteen (19.7%) patients were resistant to platinum and 44 (62%) were classified as

Table 1
Clinicopathological data (N = 71).

Clinicopathological variables	Category	n/Total(%)
Age (years), mean (range)	57.17 (33–77)	–
SD	± 10.8	
Response groups	Resistant	14/71 (19.7)
	Partially sensitive	13/71 (18.3)
	Sensitive	44/71 (62)
	Partially sensitive + Sensitive	57/71 (80.3)
Follow-up (months), mean (range)	49.2 (2–110)	–
SD	± 24.4	
Death	Yes	26/71 (36.6)
	No	45/71 (63.4)
Relapse	Yes	54/71 (76.1)
	No	17/71 (23.9)

SD = Standard Deviation.