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ORIGINAL ARTICLE

Claudin-4 in ovarian cancer and its relation to platinum compounds resistance

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KEYWORDS
Claudin-4;
Ovarian cancer;
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Abstract
Background: Claudin-4, a component of the tight junction, plays an important role in tumorigenesis and metastasis of ovarian cancer, but its role in platinum resistance has not been elucidated.
Aim of the work: To determine the presence of claudin-4 in ovarian cancer tissues in relation to platinum compounds resistance.
Patients and methods: Patients with advanced ovarian malignancy (FIGO stages III and IV) that have undergone primary surgery for maximal cytoreduction, followed by first line chemotherapy with platinum compounds and paclitaxel, were followed up for 6 months to determine chemotherapeutic response. Claudin-4 expression in ovarian cancer tissue resected from the patients surgically was evaluated immunohistochemically.
Results: Claudin-4 is associated with more aggressive behavior of ovarian tumors and the advanced stage of the tumors. High expression of claudin-4 was found in high grade tumors, of the papillary serous subtype. High expression is linked to chemotherapeutic resistance, whereas low expression is associated with good response to first line chemotherapy.
Conclusion: High claudin-4 expression can predict poor chemotherapeutic response in advanced ovarian cancer.

PALABRAS CLAVE
Claudina-4;
Cáncer ovárico;
Compuestos de platino;
Resistencia

Claudina-4 en cáncer ovárico y su relación con la resistencia a compuestos de platino

Resumen
Antecedentes: La claudina-4, componente de la zona de oclusión, desempeña un papel importante en la oncogenia y metástasis del cáncer ovárico, pero su papel en la resistencia al platino no se ha aclarado todavía.

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Objetivo: Establecer la presencia de claudina-4 en los tejidos de cáncer ovárico con relación a la resistencia a compuestos de platino.
Pacientes y métodos: Pacientes con neoplasia maligna de ovario (clasificación FIGO fase III y IV) sometidos a una intervención citorreductora primaria seguida de quimioterapia de primera línea con compuestos de platino y paclitaxel; se realizó un seguimiento durante 6 meses para valorar la reacción a la quimioterapia. Se llevó a cabo un análisis inmunohistoquímico de la expresión de la claudina-4 en los tejidos de cáncer ovárico.
Resultados: La claudina-4 se asocia con un comportamiento más agresivo de los tumores ováricos y con una fase más avanzada de los mismos. Se encontró una alta manifestación de claudina-4 en tumores de grado alto, de subtipo papiloma seroso. Se relaciona la expresión alta con la resistencia quimioterápica, mientras que una expresión baja se asocia a una buena reacción a la quimioterapia de primera línea.
Conclusión: Una expresión alta de claudina-4 puede predecir una mala respuesta quimioterápica en el cáncer de ovario avanzado.

Introduction

Ovarian cancer is the most common fatal cancer of the female reproductive tract and is responsible for 5% of all cancer deaths in women. About 70% of ovarian cancers are diagnosed in Stages III/IV due to lack of specific signs and symptoms.[1] Patients undergo surgery aiming for maximal cytoreduction and this is followed by first line chemotherapy with platinum compounds and paclitaxel.[2] Approximately 80% of the patients show an initial response but unfortunately, the majority of the patients relapse.[3] The specific mechanisms for drug resistance have not been yet identified. Therefore, there is a pressing need for the identification of prognostic markers for first line chemotherapy.

Apical junctional complexes are responsible for holding epithelial cells together and they are formed of tight junctions (TJs), adherens junctions and desmosomes. Claudins are the main constituents of TJs and comprise 24 closely related transmembrane proteins.[4] Tight junctions are essential for the tight sealing of the cellular sheets, controlling para-cellular ion flux and therefore maintaining tissue homeostasis and cell polarity.[5] Also, because of the ability of tight junction proteins to recruit signaling proteins, tight junctions have been hypothesized to be involved in the regulation of proliferation, differentiation, and other cellular functions.[6] The expression of claudins is variable in different cancers.[7] Regarding cancer ovary, it was found that claudin-3 and claudin-4 are up-regulated,[8] and claudin-4 is over-expressed in ovarian cancer in comparison with ovarian cystadenomas.[9] This high expression suggests that claudins are responsible for increased invasion, motility and cell survival, the characteristics required for metastasis.[10] This can also suggest that high expression of claudins could be associated with chemoresistance. Therefore, this study was conducted to investigate the relationship between the expression of claudin-4 and the response to first line chemotherapy, aiming to identify a prognostic marker to therapy.

Patients and methods

This study was conducted on 25 patients with advanced ovarian malignancy (FIGO stages III and IV). Informed consent was taken from the patients and they were all subjected to history taking, clinical examination, radiological investigation with trans-vaginal ultrasound (TVUS) and CT, and laboratory detection of pre-operative serum CA 125 levels. All patients underwent maximal cytoreductive surgery in El Shatby University Hospital, aiming at leaving a residual tumor $<$1 cm. The operation comprised of ascetic fluid aspiration, total abdominal hysterectomy and bilateral salpingo-opherectomy, omentectomy and lymph node sampling (pelvic and para-aortic). The patients then received adjuvant chemotherapy of platinum compounds and paclitaxel. The patients were followed up for a period of six months to assess the response to chemotherapy. Resistance was identified by the detection of pelvic masses either clinically or radiologically (TVUS or CT), and by the level of CA 125, either as a failure to decrease, or as an increase after an initial decrease. The patients were divided into two groups according to chemotherapeutic response: the resistant group and the responder group.

Immunohistochemistry

Ovarian cancer tissues were fixed with 10% buffered formalin and embedded in paraffin. Only epithelial ovarian tumors were included in this study. Three-micrometer sections were deparaffinized and hydrated. They were incubated in the microwave for 10 min. Claudin-4 protein expression was detected by primary rabbit monoclonal claudin-4 antibody (Cat. No. RB-9266-R7, Thermo Fisher Scientific, Lab Vision Corporation, Fremont, CA, USA). The antibody was diluted at 1:300 in antibody diluents and incubated with the tissue specimens for two hours. Ten randomly selected areas of each slide were analyzed using high power fields objective ($\times$40) with 100 cells counted per field, and the positively stained cells were determined in respect to the total number of cells. For semiquantitative evaluation, tumorous and non-tumorous epithelia were considered negative if less than 5% of the cells reacted. The following further values were given: 1 (6—20% positivity), 2 (21—40% positivity), 3 (41—60% positivity), 4 (61—80% positivity), and 5 (81—100% positivity). Only membranous staining was classified as positive. Intensity scores were from 0 to 3; where 0 is negative, 1 is weakly