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Original Article

Integrins and haptoglobin: Molecules overexpressed in ovarian cancer

Julio César Villegas-Pineda[a], Olga Lilia Garibay-Cerdenares[a,1], Verónica Ivonne Hernández-Ramírez[a], Dolores Gallardo-Rincón[b], David Cantú de León[b], María Delia Pérez-Montiel-Gómez[b], Patricia Talamás-Rohana[a,*]

[a] Departamento de Infectómica y Patogénesis Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, Delegación Gustavo A. Madero, México, DF 07360, Mexico
[b] Instituto Nacional de Cancerología, SSA, Av. San Fernando 22, Sección 16, Tlalpan, México, DF 14080, Mexico

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ABSTRACT

Integrins are adhesion molecules whose expression is upregulated during different cellular processes such as adhesion, growth, proliferation, migration, survival and differentiation, all of which are involved in neoplastic development. Several reports have linked the overexpression of integrins with epithelial ovarian cancer (EOC). Furthermore, fucosylated haptoglobin (Hp) isoforms with antioxidant activity and synthesized primarily in the liver have also been associated with various types of cancer, including ovarian cancer. Here, we determined the level of expression of three integrin heterodimers (α5β1, α6β4, and αVβ3) and fucosyltated Hp in two different settings: cell cultures and biopsies from ovarian cancer patients. On the one hand, integrin heterodimers were analyzed in the ovarian cancer cell line (SKOV-3), two primary cultures (INCan017 and INCan019) and a tumor derived from INCan017 (T-017) by Western blot. Statistical analysis was performed using one-way ANOVA. The SKOV-3 cell line, INCan017 and INCan019 primary cultures, and the T-017 tumor showed increased expression patterns of the α5, αV, β1, β3, and β4 integrin subunits when compared with healthy ovary tissue. We then analyzed the expression pattern of the integrin subunits as well as the fucosylated Hp in biopsies from patients with different histotypes of EOC by immunofluorescence. α5β1 and α6β4 integrins were expressed by 90% of the samples, whereas 80% expressed the αVβ3 integrin. Furthermore, Hp, fucosylated or not, was present at high levels in most biopsies. In fact, there was a statistical correlation between the expression of integrins or Hp and the presence of the disease given that α5β1, α6β4, and αVβ3 integrins, Hp, fucosylated Hp and additional fucosylation state of proteins were highly expressed in biopsies of EOC histotypes when compared with healthy ovarian tissue. However, the statistical analysis showed no association of the presence of integrins, Hp or fucosylation with clinical or pathological characteristics of EOC patients. These results suggest that increased expression of these molecules and of the fucosylation modification are characteristics of the malignant process itself. Therefore, these molecules may be promising therapeutic targets in patients with this type of neoplasia.

1. Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecologic neoplasma, mainly due to its asymptomatic presentation in early stages and its typical diagnosis in advanced disease stages when it is no longer curable [1]. EOC is a heterogeneous disease and can be categorized into one of four common subtypes based on histopathological examination: serous, endometrioid, clear cell, and mucinous [2]. This type of neoplasia has its origin in the superficial epithelial layer of the ovary, a monolayer that covers these organs. Once the epithelial cells undergo transformation, they separate from the subjacent matrix and then propagate, most frequently as cell clumps, toward the adjacent organs and the peritoneum [3].

The metastatic transformation of the cells requires the synthesis of proteins important for tissue invasion. When the cells arrive at new tissues, they require proteins such as integrins to adhere to the new tissue and generate new tumors in different anatomical sites either adjacent to or distant from the primary

* Corresponding author.
E-mail addresses: jcvillegas@cinvestav.mx (J.C. Villegas-Pineda), olgaribay@hotmail.com (O.L. Garibay-Cerdenares), arturomvi@hotmail.com (V.I. Hernández-Ramírez), dolores.gallardo@prodigy.net.mx (D. Gallardo-Rincón), dcantude@gmail.com (D. Cantú de León), madeliapmg@yahoo.com.mx (M.D. Pérez-Montiel-Gómez), ptr@cinvestav.mx (P. Talamás-Rohana).
[1] Current address: Unidad Académica de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero (Cátedra CONACYT), Mexico.

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tumor site [3]. Moreover, efficient cell migration requires constant endocytosis and recycling of integrins rather than their degradation [4], establishing the recycling of integrins as an important determinant of tumor progression.

Integrins are heterodimeric receptors present on the cell surface, and they are composed of two non-covalently bound subunits, one α-subunit and one β-subunit; to date, 18 α-subunits and 8 β-subunits have been identified, and they can combine to produce different heterodimers with different specificities for extracellular components, such as collagen, fibronectin, laminin, vitronectin, etc. Integrins are able to induce intracellular signaling to trigger the expression of a diverse set of genes; moreover, these proteins actively participate in cellular adhesion processes in the extracellular environment [5].

Cell adhesion processes are involved in many essential cellular functions both during normal physiological as well as disease conditions. They participate in cell survival, growth, differentiation and cell migration; moreover, adhesion is also crucial during inflammation, platelet aggregation and tissue repair. In fact, some integrins are overexpressed during cancer development [6].

The α5β1 integrin actively participates in the migration of transformed cells; moreover, it has been shown that this integrin favors tumor growth, angiogenesis and metastasis in several animal models [7,8]. Recently, Hu et al. analyzed the patterns of integrin expression together with the Lewis antigen "y". They found an association between elevated expression of the α5β1 integrin and the Lewis "y" antigen, along with increased resistance to chemotherapy, in EOC biopsies [9].

In neoplastic cells, overexpressed α6β4 cooperates with several growth factor receptors to potentiate the signals that promote cellular events related to tumor progression [10]. In a cell migration assay with breast cancer cells treated with the inhibitor curcumin, tumor mobility and invasion were reduced due to the direct actions of this molecule on the α6β4 integrin [11].

The αVβ3 integrin was the first αV characterized, and it has been associated with regulation of angiogenesis [12,13]. This integrin is able to bind to multiple extracellular matrix components (EMC) including vitronectin, fibronectin, fibrinogen and osteopontin. The expression of this integrin together with the Lewis "y" antigen was also evaluated in the previously cited study performed in Chinese women; a positive correlation between the expression of these two markers and disease progression was observed [14]. This study concluded that the αVβ3 integrin is overexpressed in malignant ovarian tissue in comparison with its expression level in borderline or benign tumors, whereas it is not expressed at all in healthy ovaries.

On the other hand, haptoglobin (Hp) is a protein synthesized in the liver, and its antioxidant activity is well known; furthermore, Hp acts as an acute phase protein, but has recently been associated with disease, specifically cancer [15–17]. Moreover, in its fucosylated state, Hp has been suggested as a biomarker for different cancers [18–20].

In a previous study, we reported the identification of fucosylated Hp isoforms in ascitic fluid and in biopsies [20], reinforcing the idea that this fucosylated protein may be related to EOC disease stage.

In this study, we determined the expressions levels of α5β1, α6β4, and αVβ3 integrins in cells isolated from ascitic fluid samples as well as in biopsies of Mexican patients with EOC by western blot and immunofluorescence assays, respectively; also, the levels of Hp and fucosylation were determined in the same biopsies by immunofluorescence assays. A correlation analysis between the expression of integrins or Hp and the presence of the disease or EOC histotypes was also performed by the statistical Spearman correlation test.

2. Material and methods

2.1. SKOV-3 cell line

The SKOV-3 cell line (ATCC, Cat. No. HTB-77) was originally established from malignant ascites of a patient with advanced ovarian carcinoma at Memorial Sloan-Kettering Cancer Center. SKOV-3 cells are capable of generating tumors in the peritoneal cavity of nude mice but are unable to form cell aggregates. This cell line was used as a reference.

2.2. Purification of transformed cells from ascitic fluid

Recently, our research group characterized two primary cultures, INCan017 and INCan019, obtained from ascitic fluid of two EOC diagnosed Mexican patients admitted to the Department of Medical Oncology of the Instituto Nacional de Cancerología de México; these primary cultures are from the endometrioid and serous histotypes, respectively. Both primary cultures have the ability to induce tumors in an animal model with characteristics that are very similar to those presented by tumors that develop in patients (data not shown). Patients were admitted for a first time diagnosis; histopathology and tumor grade were assigned by a pathologist according to the International Federation of Gynecology and Obstetrics (FIGO) criteria [21]. The study was approved by the Institutional Scientific and Bioethics Committees (protocol numbers INCAN/CC/134/09 and CB/549/09), and written consent was obtained from patients prior to sample collection.

Cell selection was performed from clear malignant ascitic fluid that was free of hemolysis (colorless) and bacteria. Ascitic fluid was placed in culture flasks under sterile conditions and incubated at 37 °C with 5% CO_2 for at least 3 weeks without any change in the conditions. Over time, non-tumor cells died (leukocytes, fibroblasts, and erythrocytes) while cancer cells remained adherent and proliferative. After three weeks, 10% McCoy's 5A (ATCC, 30-2007) medium supplemented with 10% fetal bovine serum (FBS) (PAA, Cat. No. A15-701) was added. At six weeks, the culture medium was removed without disturbing the attached cells. The adherent cells were gently washed with sterile PBS to remove cellular debris, and McCoy 5A medium supplemented with 10% FBS was added. The culture flasks were further incubated at 37 °C with 5% CO_2. Periodically, cell viability (confluence, cell refringence and the number of adherent cells) was assessed. All of the cultures were established in McCoy's 5A medium with 10% FBS and 1% penicillin/streptomycin (PAA, Cat. No. P11-010). Trypsin (Cat. No. T4674)/EDTA (Cat. No. E-4748), both from Sigma, were used for culture propagation. Recovered primary cultures were designated INCan017 and INCan019.

2.3. nu/nu mice and tumor production in the in vivo model

Nude mice, strain Crl:NU-Foxn1nu, were obtained from the Production Unit of Experimental Laboratory Animals (UPEAL) with the approval of the Internal Committee for the Care and Use of Laboratory Animals of CINVESTAV-IPN and managed under controlled conditions according to the Mexican Official Standard for Technical Specifications for the Production, Care and Use of Laboratory Animals (NOM-062-ZOO-1999). INCan017 cells (1×10^6) were inoculated in 1 ml of sterile PBS intraperitoneally to generate tumors in nu/nu mice. Animals were sacrificed by cervical dislocation at 9 weeks post-inoculation. Tumors were dissected and sonicated to prepare total tissue lysates that were used for western blot assays.