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Neural Signature Expressed by Cells from Ovarian Carcinoma (A Case Report)

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Abstract

Aim: To demonstrate the presence of differentiated cells in ovarian carcinomatosis nodules after chemotherapy.

Patient and method: A patient of 85 years, who presented a pelvic mass of 10 cm. The anatomo-pathological study was performed on the biopsies (before treatment) and post operational samples (after treatment by Carbotaxol). Histological samples were analyzed with ovarian cancer markers for diagnosis. The immune cells (CD3, CD4, CD8 and CD20) and neural markers such as anti: neurofilament (NF), neural cell adhesion molecules NCAM (CD56), chromogranin A, neuronal specific enolase (NSE), S100 protein and synaptophysine were used for demonstrating the neuronal differentiation tendencies of carcinomatosis cells. Proliferation activities were studied by using proliferative index and Ki67 antibody.

Results: The histological result of biopsies of bilateral ovarian carcinomatosis showed the poorly differentiated monomorphic cell serous carcinoma (cytokeratin$^+$, estrogen receptor$^+$, protein S100$^+$, anti-wilms tumor-1$^+$ with proliferative index (31%) and high Ki67 marker (45%). Semi-quantitative histological evaluations of post operational samples presented two cellular quotas. One was composed of monomorphic cells with high proliferative index (19%) and ki67 marker (30%). In another quota, large size polymorphic cells with no proliferative index and Ki67 marker were distinguished. Before treatment, all neuronal markers except NSE and S100 protein were found negative in primary tumors. In the proliferative zone of post-operative samples, NSE and S100 protein markers persist with any other neuronal markers. These zones were highly infiltrated by CD3, CD4 and CD20 immune cells. In contrast, in degenerative non-proliferative zone, all primary tumor markers except Ki67, all neuronal markers except synaptophysine, and dramatically decreased infiltrated immune cells.

Conclusion: These results are in favors of differentiation of poorly differentiated ovarian cancer cells with high proliferative index to other tissue with no proliferation potential. Targeting of differentiation of cancer cells by differencing inductors may be a new way for cancer therapy.

Keywords: Anatomo-pathological study; Ovarian carcinomatosis; Neuronal markers; Immune cells; Cancer cell differentiation; NCAM (CD56); Chromogranin A; Neuronal specific enolase

Introduction

Ovarian carcinoma (OC) is the sixth most common malignancy in woman and the leading cause of death from gynecological cancer in the world [1]. Over 90% of malignant tumors are epithelial. It has been hypothesized that tumor can arise either from single layer of cells covering the ovary or from the epithelial lining of the fimbrial end of fallopian tube [2]. OC has a predisposition to metastatic involvement of the peritoneal cavity and form ovarian carcinomatosis nodules [3,4]. Late stage OC is characterized by widespread peritoneal dissemination, ascites and a high mortality rate with an overall survival ranging from 20 to 30% at 5 years after surgery [5].

Platinum associated to taxans chemotherapy is a standard treatment for ovarian cancer and has achieved a high response rate [6]. The development of drug-resistant cancer cells exhibiting the multidrug resistance phenotype is one of the major limitations of efficacy [5,7]. The mechanisms underlying chemoresistance in cancer are not clear. A growing amount of studies are inspiring the role of the cancer stem cells (CSC) in OC development [8]. Resistance to platinum-based chemotherapy is also associated with epithelial to mesenchymal transition in epithelial ovarian cancer [9,10]. A link between CSC and EMT has been suggested [11] and cancer cell can acquired differentiated phenotype after cytotoxic chemotherapy [12].

Previously, we reported that an ovarian cancer cell line OVCAR-3 NIH express both CD133 and CD117 stem cell markers and secret cytokines implicated in tumor growth and cell differentiation [13]. On the basis of pluripotency of cancer stem cell, the aim of this study is to demonstrate, co localization of ovarian carcinomatosis cells with high proliferative tendency and cells that expressed neural proteins without proliferative markers in peritoneal ovarian carcinoma.

Materials and Methods

Patient

We report the case of a female multipara patient of 85 years, who presented a pelvic mass of 10 cm wide painless axis associated with abdominal distension, nausea and ascites. The PET CT (positron

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emission tomography) and pelvic MRI (**magnetic resonance imaging**) confirmed the presence of a mixed primary ovarian tissue mass of about 10 cm and a large abdominal ascites with pleural effusion on right side and an important peritoneal carcinomatosis. Patient was evaluated as unoperatic in laparoscopy. Peritoneal biopsies were in favor of a poorly differentiated carcinoma, high-grade malignancy and evocative of a serosa nature.

After chemotherapy "Carbotaxol for a total of 7 treatments", the marker CA 125 dropped from 3200 to 174 ng / ml and a regression of peritoneal carcinoma was observed. The patient was operated on for a cytoreduction associated with a posterior pelvectomy, protected omentectomy, appendectomy histero-ovarectomy, and lymph node dissection. Tumors samples were obtained at diagnostic by biopsies and after chemotherapy, by radical surgeries of ovarian cancer patient. Stage of tumor categorized according International Federation of Gynecology and Obstetrics (FIGO) as malignant stage 4 serous adenocarcinoma.

Antibodies

16 antibodies (Table 1) as histopathological markers were used; cytokeratin (Ck AE1/AE3), neurofilament (NF), synaptophysine (SYP), chromogranin-A (CHR-A), neuronal specific enolase (NSE), proliferation marker (Ki67), neuronal protein S100 (S100), neural cell adhesion molecule (NCAM-CD56), wilms tumor-1 (WT-1), estrogen receptor (ER), progesterone receptor (PR), stem cell growth factor receptor CD117 C-kit and β-catenin). Immune cells were identified by anti:-CD3 (pan T cells), CD4 (T helper cells), CD8 (T suppressor cells) and CD20 (B cells).

Immunohistochemistry

Five nodules were extracted from patient and used for this study. For anatomo-pathological analysis, the samples were dissected, fixed in PAF (4%) and embedded in paraffin. The slides (4 micron) were produced and colored by hematein-eosin-safran according to classical methods in the anatomo-pathogical laboratory. In parallel several slides were stained by antibodies coupled peroxidase using Benchmark Ultra apparatus (ROCHE VENTANA, Tucson, Arizona, USA) according their manufacturers. Proliferative index was evaluated according the sum of all cells in phase as metaphase, anaphase and telophase, counted in ten slid digital image on 40X (0.705 mm^2) for each tissue slide.

All antibodies and their origin and degree of dilution presented in Table 1. As controls, isotypic antibodies were used and the nuclei were labeled by hematoxylin bleuing (Ventana, Tuscan, USA). The pictures were taken by Leitz (Diaplan) microscopy with Nikon Cool pix 995 apparatus (Japan).

Results

Anatomo-pathological studies

Figure 1A and 1B presents anatomo-pathologic aspect of tumor nodule before chemotherapy (biopsy). The tumor with high vascularization is poorly differentiated (1A). As presented in Figure 1B, only one quota made up of tumor cells of average size with a monomorphic cell distribution and high proliferative index (31%) without psammoma bodies.

Surgical biopsies of peritoneal nodules presented in Figure 2. The tumor nodule was well limited (2A). In all samples (n=5), the tumor nodule in addition of necrotic zone, is composed of two cellular quotas (2B and 2C). A quota made up of tumor cells of average size with a monomorphic (2D) with high proliferative index (19%). Another quota of tumor cells was observed as a large size polymorphic cells (2E) with almost no one proliferative index (<1%). All proliferative, non-differentiated zones are shown with black star and non-proliferative, degenerative with white star.

Immunohistochemistry analysis

Tissues markers: Immunohistochemistry analysis of all tissues, before and after cancer treatment using tissues markers presented in Table 2. Before treatment, all monomorphic proliferative cells express only CK (3A), Ki67 (3B), ER (3C) and WT-1 (3D) protein markers (Figure 3).

As presented in Figure 4, these cells express some cancer stem cell markers such as β-catenin (Figure 4A) and stem cell growth factor receptor CD117 (4B). Except S100 protein (4C) and NSE (4D), no pro-neuronal marker were detected such as NCAM (CD56) (Figure 5A), NF (5B), CHR-A (5C), and SYP (5D).

After treatment as presented in Figure 6, all ovarian cancer cell markers such as ER (6A and 6B), Ki67 (6C) and WT-1 (6D) were

Antibodies	Origin	City	Country	Clone	Dilution
Ck AE1/AE3	Dako	Carpinteria -Californie	USA	AE1/AE3	1/50
NF	Dako	Glostrup	Denmark	2F11	1/100
SYP	Dako	Glostrup	Denmark	DAK-SYNAP	1/500
CHR-A	Dako	Glostrup	Denmark	DAK-A3	1/100
NSE	Dako	Glostrup	Denmark	BBS/NC/VI-H14	1/100
Ki67	Dako	Glostrup	Denmark	MIB-1	1/50
S100	Dako	Glostrup	Denmark	No clone	1/1000
CD56	Leica Biosystems	Benton	UK	1B6	1/50
WT-1	Dako	Carpinteria -Californie	USA	6F-H2	1/150
ER	Ventana	Tucson - Arizona	USA	Sp1	Not diluted
PR	Ventana	Tucson - Arizona	USA	1E2	Not diluted
CD117 C-kit	Dako	Carpinteria -Californie	USA	No clone	1/50
β -catenin	BD-biosciences	Erembodegem	Belgium	14/β -catenin	1/50
CD3	Fisher scientific	Fremont	USA	Sp7	1/100
CD4	Ventana	Tucson - Arizona	USA	Sp35	Not diluted
CD8	Dako	Glostrup	USA	C8/144B	1/50
CD20	Dako	Glostrup	USA	CD20	1/50

Table 1: The name, origin, clone name and dilution for each antibody used in this study.

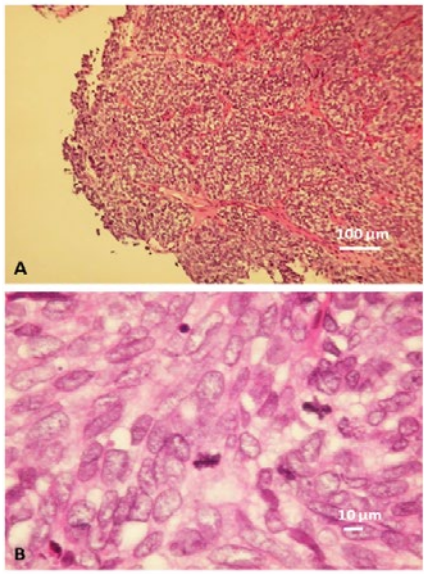

Figure 1: Anatomo-pathologic aspect of tumor nodule before chemotherapy (biopsy). The tumor is highly vascularized (A) and is poorly differentiated with a monomorphic cell distribution and high proliferative index (B).

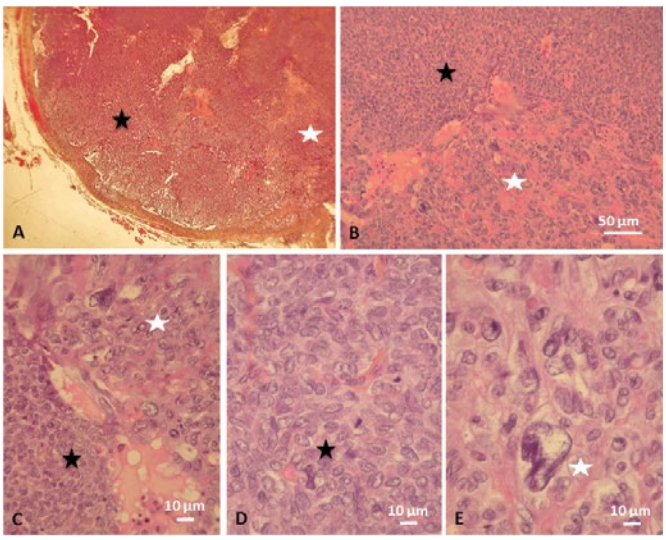

Figure 2: Anatomo-pathologic aspect of surgical biopsies of peritoneal nodules. The tumor nodule was well limited (2A) and composed of two cellular quotas (2B and 2C). A quota with proliferative monomorphic cells (2D) indicated with black star. Another quota with degenerative polymorphic cells (2E) indicated with white star.

detected in proliferative zone. CK, NSE, S100 and ER immunoreactivity was presented in both proliferative and non-proliferative regions (Figure 7 A, B and C). PR immunoreactivity was absent in all, pre and after chemotherapy (7D).

In contrast, in non-proliferative-degenerative zone, all neural markers studied in this study were present. In addition of NSE and S100 proteins, several pro neuronal markers as presented in Figure 8 such as NCAM (CD56) (8A), CHR-A(8B), NF(8C), except SYP (8D) were stained. As presented in Figure 6D, proliferative marker (Ki67) was stained predominantly in monomorphic proliferative zone. Any Ki67 immunoreactivity was not observed in degenerative zone. NF and CHR-A, markers of neuroendocrine tumors were detected only in some cells and also in non-proliferative degenerative zone (8B and C). After chemotherapy, the cell markers β-catenin persisted in all cells but CD117 C-kit not expressed (Figure 9).

Immune cells markers: Tumor infiltrative immune cells such as T cells (CD3) and their derivate cells, T helper (CD4), T suppressor (CD8) and B cells (CD20) were identified in surgical biopsies of peritoneal nodules after treatment. Significant differences were observed according the proliferative ($Ki67^{+}$) and non-proliferative ($Ki67^{-}$) zones. As presented in Figure 10. All degenerative zones were private from effective immune cells.

Discussion

In this case report, we demonstrated the neuronal signature in the nodule of peritoneal ovarian Carcinomatosis. Before treatment, tumor composed of small monomorphic cells with high score of mitosis and hyper vascularization. Numerous immune and inflammatory cells were identified in hematoxylin and eosin sections. This observation was confirmed by using anti: immune and inflammatory antibodies in immunocytochemistry.

Antibodies	Before Treatment		After Treatment	
	Proliferative zone	degenerative zone	Proliferative zone	degenerative zone
Ki67 (mitotic index)	+ (45%)	Non	+ (30%)	(<1%)
NSE	+	Non	+	+
Ck AE1/AE3	+	Non	+	+
WT-1	+	Non	+	+
β-catenin	+	Non	+	+
ER	+	Non	+	+
S100	+	Non	-	+
$CD117^{low}$	+	Non	-	-
NCAM-CD56	-	Non	-	+
NF	-	Non	-	+
CHR-A	-	Non	-	+
SYP	-	Non	-	-
PR	-	Non	-	-

Table 2: Comparison of immunohistochemical analysis of different tissue markers before and after cancer treatment.

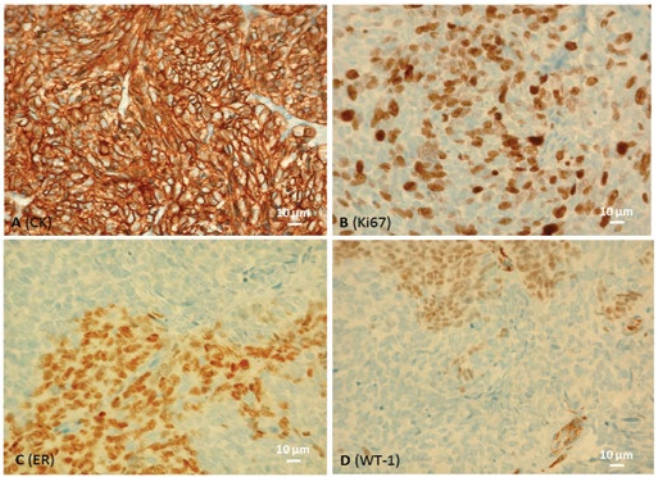

Figure 3: Immunohistochemistry analysis of ovarian cancer. Immunoreactivity with anti CK (A), Ki67 (B), ER (C) and WT-1 (D) was presented as protein markers in ovarian cancer (before treatment).

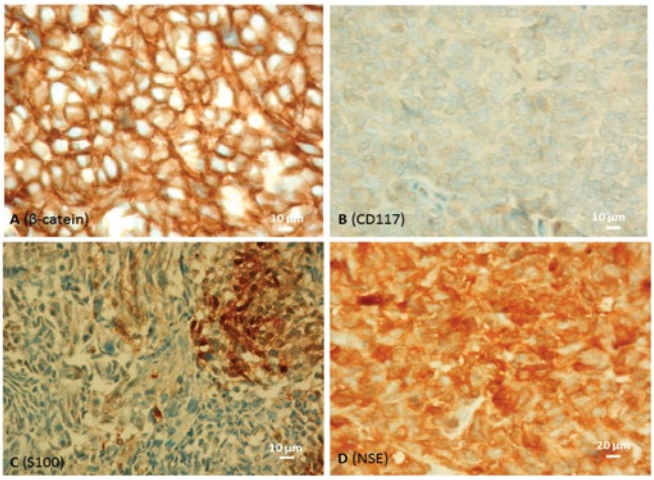

Figure 4: The expression of β-catenin (A), stem cell growth factor receptor CD117 (B), S100 protein (C) and NSE (D) were presented in ovarian cancer tissues (before treatment).

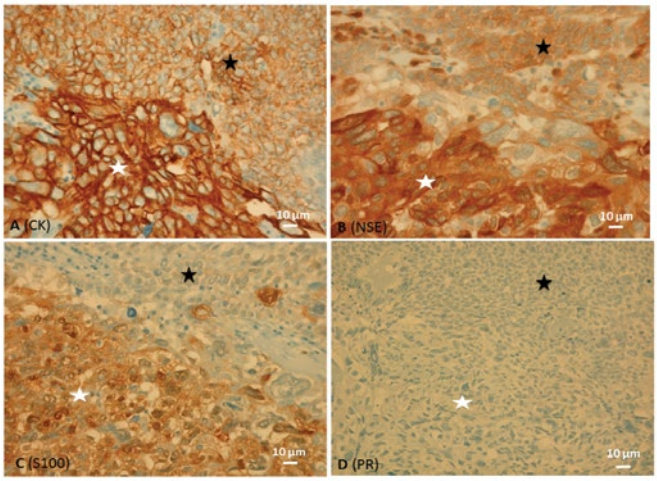

Figure 7: Presence of CK (A), NSE (B), S100 (C) in both proliferative and non-proliferative regions of ovarian tumor. Absence of PR Immunoreactivity (7D).

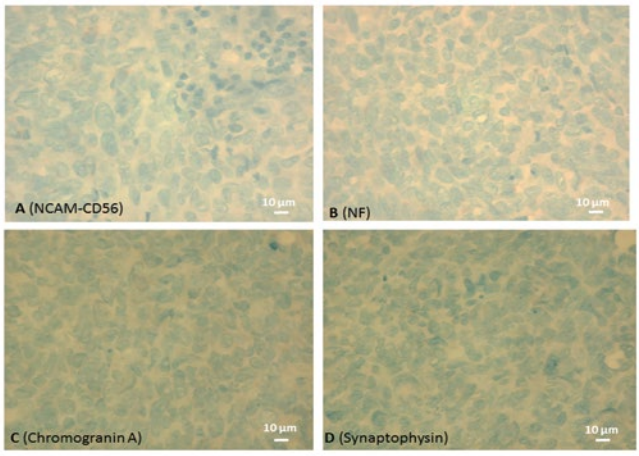

Figure 5: Absence of pro-neuronal marker, NCAM (CD56) (A), NF (B), CHR-A (C), and SYP (D) in ovarian cancer before treatment.

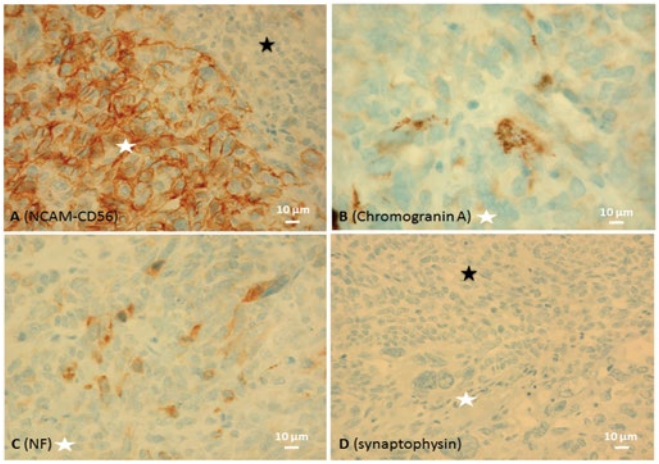

Figure 8: Presence of pro neuronal markers NCAM (CD56) (8A), CHR-A (8B), NF (8C), except SYP (8D) in degenerative zone of ovarian cancer after treatment.

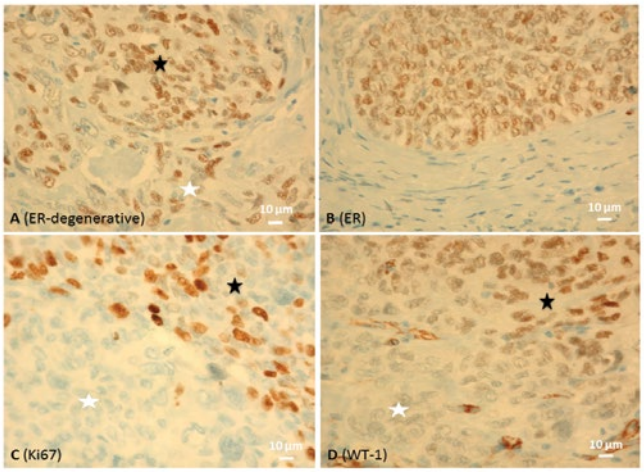

Figure 6: The presence of ovarian cancer cell markers ER in degenerative zone (A) and proliferative zone (B) and Ki67 (C) and WT-1 (D) in proliferative zone.

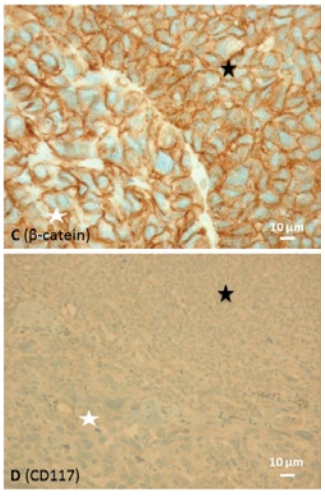

Figure 9: The presence of β-catenin (A) and absence of CD117 C-kit Immunoreactivity after treatment in both proliferative and degenerative zones.

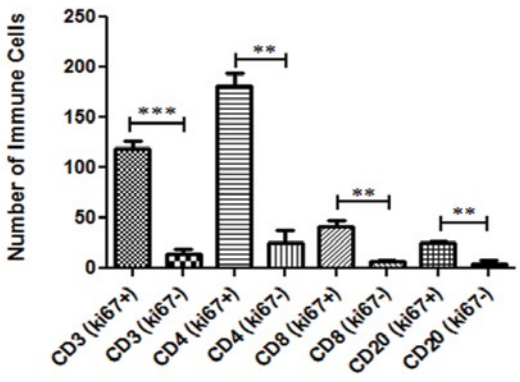

Figure 10: The distribution of immune cells CD3, CD4, CD8 and CD20 in both proliferative (Ki67$^+$) and degenerative (Ki67$^-$) zones.

After treatment, two zones were distinguished in all nodules; a zone with small monomorphic and proliferative cells and mitosis and the other non-proliferative zone without cell mitosis and absence of Ki67 marker. Curiously, all neural markers such as NSA, NCAM (CD56), S100 protein and CHR-A were found in non-proliferated zone. These results suggest that neuronal markers are associated with non-proliferative characteristic of ovarian cancer cells.

A consensus panel convened by the "American Association for Cancer Research" defined a cancer stem cell as "cell within a tumor that possesses the capacity to self–renew and to cause the heterogeneous lineage of cancer cells that comprise the tumor". Like normal stem cells, cancer stems cells were characterized by their radio and chemo resistance properties [14]. Differentiation of these stem cells can be benefic especially, when they differentiated into other cells. Induction of differentiation of human promyelocytic leukemia cell line (HL60) by retinoic acid was first demonstrated by Breitman et al. [15]. This finding suggests that the differencing agents can be used in therapeutic.

In this study we observed the expression of several neuronal markers in carcinomatosis nodules after the treatment of patients by chemotherapy. Belong to these; CD56 may be a crucial marker. CD56 antigen or NCAM is a glycoprotein expressed on the surface of neurons, glia and skeletal muscle. CD56 has been implicated in cell adhesion between them. Normal cells that stain positively for CD56 include NK cells, activated T cells, the brain and cerebellum, tissues and neuroendocrine. Tumors that are CD56-positive are myeloma, myeloid leukemia, neuroendocrine tumors, wilms tumor, neuroblastoma, lymphomas, NK / T cells, carcinoma of pancreatic acinar cells, pheochromocytoma, the paraganglioma, carcinoma small cell lung, and the family of Ewing sarcoma tumors. All this observation indicates ectopic localization of NCAM in different cells. Here, NCAM only expressed in non-proliferative quota of carcinomatosis nodule. In addition, some of these cells expressed NF and CHR-A. These results may suggest the differentiation of undifferentiated lineage cancer cells to other tissues with neuronal markers in carcinomatosis nodule after chemotherapy. Zueva et al. reported that 48.5% of the serous ovarian adenocarcinoma patient presents immunoreactivity with anti-NCAM antibodies [16].

NSE detected in ovarian cancer before and after treatment. The anti-NSE antibodies can be used to identify neuronal cell cells and with neuroendocrine differentiation. NSE is produced by pulmonary small carcinoma cells that are of neuroendocrine origin [11]. NSE is therefore a useful tumor marker for patients with lung cancer. In ovarian tumor, NSE as serum markers reported in ovarian immature teratoma and dysgerminoma. The presence of NSE in ovarian tumors may be in favor of neuronal differentiation tendencies.

Another neural signature is NF. It is a major corposant of neuronal cytoskeleton. Neurofilament light polypeptide as a novel hypermethylated gene associated with resistance to cisplatin-based chemotherapy [17]. In this study, the presence of NF positive cells in non-proliferative or dystrophic zone indicates that the ovarian carcinomatosis cells can be oriented to pre-neuronal cells. These results were confirmed by the presence of CHR-A in degenerative zone. CHR-A as a granin neuroendocrine secretory protein, is located in secretory vesicle of neuron and endocrine cells. CHR-A as well as NSE, NF and SYP were described in neuroendocrine tumors (carcinoid). In this study, in all nodules no cells were stained by SYP. Absence of mitosis and Ki67 index as well as lymph angiogenesis (tested by D2-40, Dako) (results not shown) in degenerative zone is in favor of non-carcinoid origin of the tumors.

Curiously, the amount of immune cells decreased dramatically in degenerative zone. These observations are in favors of lake of immunogenicity of cancer cells when they are differentiated to non-tumor cells. Over all, these results indicate that the poorly differentiated cancer cells can be switched in some stress condition such as chemotherapy to well differentiated cells. In this cell transition, proliferative cancer cells lake their mitotic activity and in consequence, all pathway signaling proteins implicated in this transition may be a target for cancer treatment. Future protocol using differencing inductors may be a new way for cancer therapies.

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