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Original contribution

Gene expression microarray-based assay to determine tumor site of origin in a series of metastatic tumors to the ovary and peritoneal carcinomatosis of suspected gynecologic origin☆

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Summary The origin of the primary tumor is sometimes difficult to determine in peritoneal and ovarian metastases. A series of 25 metastatic tumors to the ovary and 7 cases of peritoneal carcinomatosis of suspected gynecologic origin were collected. Total RNA was extracted from frozen tumor tissue and studied by the Tissue of Origin-Frozen test, a microarray-based gene expression test from Pathwork Diagnostics (Redwood City, CA). Independently, formalin-fixed, paraffin-embedded tumor tissue was subjected to pathologic analysis. Immunohistochemical stains included keratins 7 and 20, estrogen and progesterone receptors, CDX2, villin, CEA, WT-1, TTF-1, mammoglobin, GCDF-15, and CD31. Clinical data were considered as gold standard, and after clinicopathologic evaluation, the tissue of origin was found in 29 cases. The Tissue of Origin-Frozen test correctly identified the ovary as site of origin in 7 of 7 peritoneal carcinomatosis cases, whereas immunohistochemical stains only allowed appropriate recognition in 5. In addition, the Tissue of Origin-Frozen test identified correctly the site of origin in 18 of the 22 metastatic tumors to the ovary with known origin. In the remaining 4 tumors, the correct origin was the second option in 2 cases and was not determined in the other 2. Immunohistochemistry correctly identified the site of origin in 17 of these 22 ovarian metastases. A combination of Tissue of Origin-Frozen and immunohistochemistry correctly identified the site of origin in 19 of 22 ovarian metastases of known origin. Although conventional pathologic examination and immunohistochemistry are commonly used for assessing the tumor site of origin, Tissue of Origin testing can be useful in difficult cases.

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1. Introduction

The ovary is a common site of metastasis [1-3]. In some cases, there is a known previous history of a primary tumor, and the diagnosis may be quite easy taking that information together with microscopic appearance. However, in other cases, the patients present with an ovarian mass with no known history of a previous neoplasm. Immunohistochemistry has been used to help in this differential diagnosis [4,5]. Cytokeratin staining (CK7/CK20) is frequently used in this differential diagnosis. Moreover, specific antibodies, such as steroid hormone receptors, mammoglobin, GCDF15, CDX2, villin, TTF1, and WT1, have been proven to be helpful [8-13].

Although recently recognized as having the origin in the tubal epithelium, high-grade serous ovarian carcinomas usually present as an adnexal mass and frequently show prominent peritoneal spread. Patients with high-grade ovarian serous carcinomas occasionally show peritoneal carcinomatosis, without a known history of an adnexal mass. Although the microscopic appearance of the tumor in the peritoneal implants is quite typical in a significant number of cases, the presence of a predominant solid pattern of growth may pose problems in differential diagnosis, particularly when the patient also has a previous history of a neoplasm outside the gynecologic tract. A panel of CK7, WT-1 and steroid receptors has been suggested as helpful in this differential diagnosis.

The Pathwork Tissue of Origin assay (Pathwork Diagnostics, Redwood City, CA) is one of the most studied and clinically validated microarray-based platforms for determining the site of origin of carcinomas of unknown primaries [14-18]. It was initially developed for use with frozen tissue samples (TOO frozen, TOO-FZN) and was cleared by the US Food and Drug Administration for clinical use in 2008. Recently, the Pathwork Tissue of Origin test has also been validated for use with formalin-fixed, paraffin-embedded tissue [19] and was cleared by the US Food and Drug Administration in 2010. TOO-FZN is a microarray-based gene expression diagnostic test for determining the similarity of a tumor specimen to 15 known tumor tissue types. The test assesses the expression of 1550 genes in each specimen by applying normalization and classification algorithms to gene expression data from a microarray.

In this study, we assessed the value of the TOO-FZN test and immunohistochemistry (IHC) in a series of 25 tumors metastatic to the ovary and 7 peritoneal metastases of suspected gynecologic origin.

2. Material and methods

2.1. Patients and samples

Archived fresh-frozen tumor tissue material was obtained retrospectively from the pathology departments of Hospital Universitari Arnau de Vilanova de Lleida, Spain, and Hospital de Sant Pau, Barcelona, Spain. Samples were obtained from 1992 to 2010. Informed consent was obtained from each patient, and the study was approved by the local ethics committee. Samples were frozen in the pathology departments, immediately after surgical removal in the operating room. Twenty-five patients were subjected to resection of adnexal masses with the preoperative diagnosis of a suspected primary ovarian tumor. Seven patients were subjected to laparoscopic surgery, with the preoperative diagnosis of a suspected gynecologic neoplasm. Matched tumor samples were fixed in formalin and embedded in paraffin for conventional pathologic evaluation. After surgery, patients were subjected to clinical follow-up for confirmation of the primary site of origin, which was detectable in 29 of the 32 patients.

2.2. Tissue microarrays and IHC

One tissue microarray (TMA) was constructed from the 32 paraffin-embedded samples of tumor tissue. A tissue arrayer device (Beecher Instrument, Sun Prairie, WI) was used to construct the TMA. Briefly, all the samples were histologically reviewed, and representative areas were marked in the corresponding paraffin blocks. Two selected cylinders (0.6 mm in larger diameter) from 2 different areas were included in each case. Control normal tissues from the same specimens were also included. TMA blocks were sectioned at a thickness of 3 μm, dried for 1 hour at 65°C before being dewaxed in xylene and rehydrated through a graded ethanol series, and washed with phosphate-buffered saline. Antigen retrieval was achieved by heat treatment in the Pre-Treatment Module, PT-LINK (Dako, Glostrup ,Denmark) at 95°C for 20 minutes in 50× Tris/EDTA buffer, pH 9. Before staining the sections, endogenous peroxidase was blocked. The antibodies used are listed in Table 1. After incubation, the reaction was visualized with the EnVision Detection Kit (Dako) using diaminobenzidine chromogen as a substrate. Sections were counterstained with hematoxylin. Immunohistochemical results were

Table 1 Antibodies used in this study

Protein	Clone	Source	Dilution
Cytokeratin 7	OV-TL 12/30	Dako	Ready to use
Cytokeratin 20	KS20.8	Dako	Ready to use
Estrogen receptor α	1D5	Dako	Ready to use
Progesterone receptor	PgR 636	Dako	Ready to use
CDX2	DAK-CDX-2	Dako	Ready to use
Villin	1D2 C3	Dako	Ready to use
CEA	II-7	Dako	Ready to use
WT-1	6F-H2	Dako	Ready to use
TTF-1	8G7G3/1	Dako	Ready to use
Mamoglobin	304-1A5	Dako	Ready to use
GCDF-15	23A3	Dako	Ready to use
CD-31	JC70A	Dako	Ready to use