Human Pathology (2012) 43, 2041–2046

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Hyalinized stroma in clear cell carcinoma of the ovary: how is it formed?

Noriko Kato MD[a,*], Junko Takeda MSc[a], Masayuki Fukase MD[b], Teiichi Motoyama MD[a]

[a]Department of Pathology, Yamagata University School of Medicine, 2-2-2 Iida-nishi, Yamagata, 990-9585, Japan
[b]Department of Pathology, Tsuruoka Municipal Shonai Hospital, Tsuruoka, Japan

Received 17 January 2012; revised 9 February 2012; accepted 15 February 2012

Keywords:
Clear cell carcinoma;
Ovary;
Hyalinized stroma;
Type IV collagen;
In vitro

Summary Ovarian clear cell carcinoma often shows stromal hyalinization. The main constituents of hyalinization are basement membrane materials, including laminin and type IV collagen. Although it is known that clear cell carcinoma cells produce these materials, it remains unclear whether they can form hyalinized stroma by themselves or if cooperation with stromal cells is required. In the present study, we first reviewed 35 surgical specimens for the pattern of early hyalinization. It occurred either in a globule-like pattern or in a circumferential pattern. In the former, compact hyaline globules abruptly appeared within tumor cell aggregates. In the latter, hyalinized materials appeared around the preceding spherule-like mucoid spaces among tumor cells. In either pattern, hyalinization is most likely to begin in the intercellular spaces among tumor cells, where stromal cells rarely intervene. To verify this, 2 ovarian clear cell carcinoma cell lines (JHOC-5 and HAC-2) were analyzed in vitro. Each cell line was monocultured in suspension: if any deposition occurred in floating multicellular aggregates, it should be in the intercellular spaces. Deposition of type IV collagen occurred in a globule-like pattern (JHOC-5) or a circumferential pattern (HAC-2) within multicellular aggregates, and it developed into a structure comparable with the hyalinized stroma in surgical specimens. Intercellular deposition of type IV collagen was reproduced by culture in 3-dimensional type I collagen gels. All of these findings showed that clear cell carcinoma cells themselves form hyalinized stroma by depositing self-made basement membrane materials in the intercellular spaces.

1. Introduction

Hyalinized stroma is one of the characteristics of clear cell carcinoma of the ovary [1,2]. It is acellular and avascular stroma composed of amorphous eosinophilic materials and is more frequently found in areas showing papillary architecture. The main constituents of the amorphous material are basement membrane materials, including laminin and type IV collagen [3]. Immunohistochemical and ultrastructural studies showed that tumor cells themselves can produce basement membrane materials and subsequently form basement membrane-like structures [3-5]. However, it remains unclear whether tumor cells can form hyalinized stroma by themselves or if cooperation with stromal cells is required.

In the present study, we first reviewed 35 surgical specimens of ovarian clear cell carcinoma for the pattern of early hyalinization. Two ovarian clear cell carcinoma cell lines were analyzed in vitro to clarify how hyalinized stroma is formed.

* Corresponding author.
E-mail address: nkato@med.id.yamagata-u.ac.jp (N. Kato).

0046-8177/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humpath.2012.02.012

2. Material and methods

2.1. Surgical specimens

We examined 35 ovarian clear cell carcinomas that had been surgically resected from patients. All of them had distinct areas showing stromal hyalinization, which was confirmed by immunohistochemistry for type IV collagen. All of the specimens were fixed in formalin and embedded in paraffin. A total of 2 to 4 slides were examined for each case.

2.2. Cell lines

Two ovarian clear cell carcinoma cell lines, JHOC-5 [6] and HAC-2 [7], were used. JHOC-5 was established from tumor tissues, whereas HAC-2 was established from tumor cell aggregates in ascites. JHOC-5 was obtained from Riken Cell Bank (Tsukuba, Japan), and HAC-2 was a generous gift from Dr M. Nishida (Kasumigaura Medical Center, Tsuchiura, Japan). Each cell line was cultured in RPMI1640 supplemented with 10% fetal bovine serum in a humidified 5% CO_2 atmosphere at 37°C.

2.3. Suspension culture

Cells were dispersed using trypsin, suspended in RPMI1640 and cultured in 24-well ultra low cluster plates (Costar, Corning, NY). Each cell line was cultured in a humidified 5% CO_2 atmosphere at 37°C. After 14 and 28 days, cells were collected, centrifuged, and prepared for smears or cell blocks. Smears were fixed in 95% ethanol and stained with Papanicolaou. Four-micrometer-thick paraffin sections of cell blocks were stained with hematoxylin-eosin, immunostained with anti–type IV collagen antibody, and reacted with hyaluronan-binding protein.

2.4. Culture in 3-dimensional collagen gels

Type I collagen (Nitta Gelatin Inc, Osaka, Japan), 10× concentrated Eagle minimal essential medium (MEM), 2% $NaHCO_3$, and 200 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were mixed on ice to make collagen solution. Half of the collagen solution was poured into the 24-well dishes and placed in a 37°C incubator to make base layers. 2×10^5 single JHOC-5 or HAC-2 cells were suspended in the other half of the collagen solution, poured onto the base layers, and gelled immediately by placing at 37°C. After the cell layers were formed, they were overlayered with RPMI1640 and cultured in a humidified 5% CO_2 atmosphere at 37°C. After 14 days, collagen gels were fixed in 10% formalin and embedded in paraffin to make cell blocks. Four-micrometer-thick paraffin sections were stained with hematoxylin-eosin and immunostained with anti–type IV collagen antibody.

2.5. Immunocytochemistry and immunohistochemistry for type IV collagen

Immunocytochemistry/immunohistochemistry was performed on paraffin sections. For antigen retrieval, the sections were digested with Difco Trypsin 250 (Becton, Dickinson and Company, Sparks, MD) at 37°C for 30 minutes. The slides were exposed to 3% skim milk in phosphate-buffered saline for 20 minutes to block nonspecific binding of the antibody. Then, they were incubated with anti–type IV collagen antibody (dilution 1/100; Dai-ichi Chemical, Tokyo, Japan) at 4°C overnight. The slides were visualized using Histofine SAB-PO kit (Nichirei, Tokyo, Japan).

2.6. Measurement of hyaluronan and cytochemistry of hyaluronan

After each cell line was cultured to confluence in plastic dishes, the medium was removed, then the cells and subcellular matrix remaining on the dish surface were collected by scraping in 1 mL of phosphate-buffered saline per dish. The cell suspension of each dish was centrifuged at 2000 rpm for 3 minutes, and the supernatant was obtained for measurement of hyaluronan by latex agglutination immunoturbidimetry (SRL, Tokyo, Japan). Each cell line was set up in triplicate.

Paraffin sections of cell blocks were deparaffinized, exposed to 1% hydrogen peroxide for 5 minutes to inactivate endogenous peroxidase, then incubated in 10 μg/mL biotinylated hyaluronan-binding protein (Hokudo, Sapporo, Japan) overnight at 4°C. The slides were visualized using Histofine SAB-PO kit.

3. Results

3.1. Patterns of hyalinization in surgical specimens

Two patterns of early hyalinization were recognized (Fig. 1). One of the patterns was a globule-like pattern. Compact hyaline globules were observed among tumor cells. The other pattern was a circumferential pattern. It was found around the spherule-like mucoid spaces that occupied the cores of small papillae. The shape or thickness of the hyaline materials was variable: linear, band-like, or doughnut-like. In either pattern, stromal cells or vessels were rarely identified. Of 35 cases, 28 (80%) were associated with both patterns within a tumor, either concomitantly or separately. Both patterns were usually found in the cores of small papillae, although the globule-like pattern was also found in the small foci of solid nests.

3.2. Suspension culture

Each cell line formed multicellular aggregates or spheroids in suspension. Spheroids of JHOC-5 were different