Immunophenotypes of Glycogen Rich Clear Cell Carcinoma

Sung Eun Kim,* Ja Seung Koo, and Woo-Hee Jung

Department of Pathology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea.

Received: November 10, 2011
Revised: January 17, 2012
Accepted: January 18, 2012

Corresponding author: Dr. Woo-Hee Jung,
Department of Pathology,
Gangnam Severance Hospital,
Yonsei University College of Medicine,
211 Eonju-ro, Gangnam-gu,
Seoul 135-720, Korea.
Tel: 82-2-2019-3541, Fax: 82-2-3463-2103
E-mail: Jungwh96@yuhs.ac

*Current address: Department of Pathology,
CHA Gangnam Medical Center, CHA
University, Seoul, Korea.

- The authors have no financial conflicts of interest.

Purpose: Glycogen rich clear cell carcinoma (GRCC) of the breast is a rare subtype of invasive ductal carcinoma and involves a poor prognosis. In the literature, less than 150 cases have been reported. Many researchers have attempted to characterize GRCC according to electron microscope, flow cytometry, or clinical data. However, an organized study of the immunophenotype of GRCC has yet to be reported. Materials and Methods: Here, we present three cases of GRCC and their immunohistochemical profiles. Results: Histologically, all three cases contained periodic acid stain (PAS) positive and d-PAS labile granules in their clear cytoplasm. Case I showed positivity for only estrogen receptor (ER) and c-erbB2. Case II exhibited positivity for progesterone receptor and negativity for ER and c-erbB2. Case III presented with triple negative invasive carcinoma. The expression pattern of E-cadherin was concordant with epidermal growth factor receptor and c-kit, but discordant with ki-67. Among these three cases, p53-positive cases exhibited a low proliferative index (ki-67: 15%), while p53-negative cases showed a high proliferative index (ki-67: 50-60%). Conclusion: In conclusion, the immunophenotype of GRCC is not uniform, but is similar to that of conventional ductal carcinoma.

Key Words: Breast, carcinoma, clear cell, glycogen, immunohistochemistry

INTRODUCTION

Glycogen rich clear cell carcinoma (GRCC) of the breast is known as a rare subtype of invasive ductal carcinoma, and involves a poor prognosis. It was first described by Hull, et al.1 in 1981. Since then, fewer than 150 cases have been reported. Many researchers have attempted to characterize GRCC, according to, electron microscope (EM),1,2 flow cytometry,3 or clinical data,4-6 the results of which have been summarized in Table 1.

Hull, et al.1 confirmed intracytoplasmic glycogen of GRCC by EM and stated that the H&E and EM features of GRCC were very similar to the morphology of 13-week old fetal developing mammary gland. In diagnosing GRCC, tumor cells that contain abundant fine glycogen granules in their clear cytoplasm, are stained by periodic acid stain (PAS), but not stained by diastase-periodic acid stain (d-PAS). The incidence of GRCC is estimated to be 1 to 3% of breast carcinoma. Opinions differ with among researchers concerning the prognosis of GRCC, ranging from favorable to unfavorable.2-6 Immunohistochemical (IHC) staining for estrogen receptor (ER) is usually positive. However, IHC for progesterone receptor...
(PR) and c-erbB2 has been shown to vary.\textsuperscript{5-8} Other than hormone receptors, no organized study concerning the immunophenotype of GRCC has been published. Here, we present three cases of GRCC and their immunohistochemical profiles.

## MATERIALS AND METHODS

### Clinical data

Table 2 summarizes the clinical data. There were only three cases found during the last 10 years. One case was lost to follow up after diagnosis by biopsy. The ages at diagnosis were 49 to 69 years old and follow up data were 14 months and 18 months. They were currently disease-free states. They were treated with total and partial mastectomy followed by chemotherapy and radiation therapy.

### Immunohistochemical (IHC) staining

The antibodies used to conduct this study are listed in Table 3. Paraffin blocks were sectioned to a thickness of 4 \( \mu \text{m} \) and tissues were attached on silane coated slides. The slides

## Table 1. GRCC in Previous Publications

|                        | Fisher, et al.\textsuperscript{4} | Hull, et al.\textsuperscript{1,2} | Toikkanen and Joensuu\textsuperscript{5} | Hayes, et al.\textsuperscript{6} | Kuroda, et al.\textsuperscript{7} | Varga, et al.\textsuperscript{8} | Akbulut, et al.\textsuperscript{9} |
|------------------------|-----------------------------------|-----------------------------------|------------------------------------------|---------------------------------|---------------------------------|---------------------------------|----------------------------------|
| Numbers of cases       | 45                                | 10                                | 6                                        | 21                              | N/A                             | 28                              | 12                               |
| Incidence (%)          | 2.9                               | 1.0                               | 1.4                                      | N/A                             | 2.8                             | N/A                             | 12                               |
| Age (mean)             | N/A                               | 59.4                              | 58.3                                      | 54                              | N/A                             | N/A                             | N/A                              |
| Mass size (cm)         | N/A                               | 2.6-5                             | 4-8                                      | 1.8                             | 1.6-5                            | 3.7                             | N/A                              |
| TNM stage              | N/A                               | T1N0-T3N1                         | T2N0-4N1                                 | T1N0-T3N1                       | N/A                             | N/A                             | N/A                              |
| F/U months             | N/A                               | 1-175                             | 7-75                                     | 2-84                            | 16-72                            | N/A                             | N/A                              |
| Operation name (%)     | N/A                               | RM (60), SM (10), MRM (20), Ex (10) | RM (66), MRM (33)                       | MRM (77), Ex (23)               | RM (65)                         | N/A                             | N/A                              |
| Post-op treatment (%)  | N/A                               | N/A                               | RT (83)                                  | RT (23), Chx (8)                | RT (25)                         | N/A                             | N/A                              |
| Prognosis              | Poor                              | Poor                              | Poor                                     | Same                            | Same                            | Same                            | Same                             |
| Flow cytometry (%)     | DNA index                         | N/A                               | 100 (>1.3) ND                           | N/A                             | N/A                             | N/A                             | N/A                              |
|                        | S-phase fr.                       | N/A                               | 19.20                                    | N/A                             | N/A                             | N/A                             | N/A                              |
|                        | ER                                | N/A                               | 67% (6/9)                                | 35% (7/20)                      | 75% (9/12)                      | 62% (16/37)                     |
|                        | PR                                | N/A                               | 0% (0/3)                                 | 30% (6/20)                      | 33% (4/12)                      | 43% (16/37)                     |
|                        | C-erbB2                           | N/A                               | 20% (4/20)                               | 33% (4/12)                      | 44% (16/36)                     |
|                        | Her2-Fish amplification           | N/A                               | 25% (3/12)                               | N/A                             |
|                        | P53                               | N/A                               | 58% (21/36)                              | N/A                             |
|                        | Ki-67 index                       | N/A                               | 20%                                      | N/A                             |

N/A, not available; F/U, follow up; RM, radical mastectomy; SM, simple mastectomy; MRM, modified radical mastectomy; Ex, excision; RT, radiation therapy; Chx, chemotherapy; fr, fraction; ND, non-diploid; ER, estrogen receptor; PR, progesterone receptor; GRCC, glycogen rich clear cell carcinoma.

## Table 2. Clinical Findings

|                        | Case 1 | Case 2 | Case 3 |
|------------------------|--------|--------|--------|
| Age                    | 57     | 49     | 69     |
| Ass. Lesion            | FCD    | FCD    | N/A    |
| Mass size (mm)         | 23     | 12     | N/A    |
| pTNM                   | T2N0M0 | T1N0M0 | N/A    |
| Location               | RUL    | LUM    | N/A    |
| Last F/U               | 2011-11-06 | 2011-12-16 | N/A    |
| F/U mos                | 18     | 14     | N/A    |
| Op name                | TM/c SLNB | PM/c SLNB | N/A    |
| Post-op Tx             | 6th AC | 4th AC+post RT | N/A    |

FCD, fibrocystic change; RUL, right upper lateral; LUM, left upper medial; F/U, follow up; TM/c SLNB, total mastectomy with sentinel node biopsy; PM/c SLNB, partial mastectomy with sentinel node biopsy; AC, cyclophosphamide+adriamycin; RT, radiation therapy; N/A, not available.
receptor (AR), p53, p63, and ki-67; cytoplasmic positivity for gross cystic disease fluid protein 15 (GCDFP-15); nuclear and cytoplasmic positivity for c-kit and galectin3; and cytoplasmic membrane positivity for c-erbB2, e-cadherin, and epidermal growth factor receptor (EGFR). Additional-ly, a proportional index was evaluated to describe the percentage of positivity for a few of the antibodies, including hormone receptors (ER, PR, and AR) and p53.

**RESULTS**

Three patients were diagnosed with invasive glycogen rich clear cell carcinoma from 2000 to 2011 at our institution. On gross examination, GRCCs appeared as well-circumscribed, irregular and solid masses. No difference was found in comparison to conventional invasive ductal carcinoma. Microscopically, GRCCs consisted of solid nests of clear cells with fine meshwork vasculatures. The individual cancer cells showed clear cytoplasm with fine granules and relatively low nuclear and cytoplasmic ratio. Nuclei were

---

**Table 3. Antibodies Used in This Study**

| Antibody  | Clone     | Dilution | Source          |
|-----------|-----------|----------|-----------------|
| ER        | 6F11/16   | 1:500    | Novocastra, UK  |
| PR        | 16        | 1:1000   | Novocastra, UK  |
| HER-2/neu | 4B5       | Prediluted | Ventana, USA |
| p53       | DO-7      | 1:2000   | Novocastra, UK  |
| p63       | 4A4       | 1:1000   | DAKO, Denmark   |
| Ki-67     | MIB-1     | 1:800    | DAKO, Denmark   |
| E-cadherin| 36B5      | 1:50     | Novocastra, UK  |
| AR        | 4R441     | 1:100    | Lab vision Corp.|
| EGFR      | 3C6       | 1:15     | Ventana, USA    |
| GCDFP-15  | 23A3      | 1:100    | Neomarkers      |
| C-kit     | Polyclonal| 1:100    | DAKO, Denmark   |
| Galectin3 | 9C4       | 1:400    | Novocastra, UK  |

ER, estrogen receptor; PR, progesterone receptor; AR, androgen receptor; EGFR, epidermal growth factor receptor; GCDFP-15, gross cystic disease fluid protein 15.

---

Fig. 1. Histologic features of GRCC. (A) Tumor shows solid nests of clear cells with fine meshwork vasculatures (×100). (B) The individual cancer cells have clear cytoplasm with fine granules, low N-C ratio, wrinkled nuclei, and prominent nucleoli (×400). (C) The granules in tumor cells are stained with PAS (×400 and ×1000 in box). (D) The granules are not stained with d-PAS (×400 and ×1000 in box). GRCC, glycogen rich clear cell carcinoma; PAS, periodic acid stain.

IHC slides were evaluated by light microscopy. The scoring intensity of IHC staining was based on a three-grade system; 1 positive (weak), 2 positive (intermediate) and 3 positive (strong). The grade intensity of positive staining was assessed as follows; nuclear positivity for ER, PR, androgen receptor (AR), p53, p63, and ki-67; cytoplasmic positivity for gross cystic disease fluid protein 15 (GCDFP-15); nuclear and cytoplasmic positivity for c-kit and galectin3; and cytoplasmic membrane positivity for c-erbB2, e-cadherin, and epidermal growth factor receptor (EGFR). Additionally, a proportional index was evaluated to describe the percentage of positivity for a few of the antibodies, including hormone receptors (ER, PR, and AR) and p53.

**Interpretation of immunohistochemical staining**

IHC slides were evaluated by light microscopy. The scoring intensity of IHC staining was based on a three-grade system; 1 positive (weak), 2 positive (intermediate) and 3 positive (strong). The grade intensity of positive staining was assessed as follows; nuclear positivity for ER, PR, androgen receptor (AR), p53, p63, and ki-67; cytoplasmic positivity for gross cystic disease fluid protein 15 (GCDFP-15); nuclear and cytoplasmic positivity for c-kit and galectin3; and cytoplasmic membrane positivity for c-erbB2, e-cadherin, and epidermal growth factor receptor (EGFR). Additionally, a proportional index was evaluated to describe the percentage of positivity for a few of the antibodies, including hormone receptors (ER, PR, and AR) and p53.
somewhat wrinkled. Nuclear membranes were irregularly thickened and their nucleoli were prominent. All of them contained PAS positive and d-PAS labile granules in the cytoplasm of more than 90% of tumor cells (Fig. 1). Mucin was not detected upon alcian blue and mucicarmin staining. Tests for GCDFP-15 were negative. The tumors were, therefore, considered to involve no apocrine differentiation.

The three cases revealed three different immuno-phenotypes (Table 4). Case 1 showed positivity for ER and negativity for PR. Case 2 was positive for PR, but negative for ER. Case 3 presented with triple negative invasive carcinoma. All three cases were negative for c-erbB2. Androgen receptor was positive in the ER positive case 1. EGFR, E-cadherin, and CD117 (c-kit) showed positivity in two cases (cases 2 and 3). Among these three cases, p53-positive case exhibited a low proliferative index (ki-67: 15%), while p53-negative cases showed high proliferative index (ki-67: 50-60%).

Table 4. Immunophenotypes of GRCC

|         | Case 1 | Case 2 | Case 3 |
|---------|--------|--------|--------|
| ER      | 100%/2 pos | Neg  | Neg   |
| PR      | Neg    | 60%/2 pos | Neg   |
| C-erbB2 | 1 pos  | Neg    | Neg   |
| P53     | 80%/2 pos | Neg  | Neg   |
| P63     | Neg    | Neg    | 5%    |
| Ki-67   | 15%    | 40%    | 60%   |
| E-Cad   | No loss | Gain (3 pos) | Gain (3 pos) |
| AR      | 90%/2 pos | Neg  | Neg   |
| EGFR    | Neg    | 3 pos  | 2 pos |
| GCDFP-15| Neg    | Neg    | Neg   |
| C-kit   | Neg    | 2 pos  | 1 pos |
| Galectin3 | 2 pos | 1 pos  | Neg   |

ER, estrogen receptor; PR, progesterone receptor; E-Cad, E-cadherin; AR, androgen receptor; EGFR, epidermal growth factor receptor; GCDFP-15, gross cystic disease fluid protein 15; pos, positive; neg, negative.
nuclei, resulting in immuno-positivity of p53 protein. Mutated p53 protein cannot suppress genetic errors leading to cancer development. Nevertheless, p53 over-expression has been considered a poor prognostic marker and accompanied by a high mitotic index in invasive ductal carcinoma. Our three cases demonstrate just the opposite. Case 1 showed strong positivity for p53 with low mitotic index, while cases 2 and 3 showed negativity for p53 with high mitotic index. These findings support the possibility of an association of cell proliferation with the signal transduction of surface molecules (EGFR, c-kit and E-cadherin), not with mutation of p53 protein, in GRCC.

Case 3 presented as a triple negative carcinoma and also expressed p63 protein. Case 3 was a basal-like breast cancer and was predicted to involve a poor prognosis. Increased proliferative activity as determined by ki-67 (60%) and over-expression of EGFR supported the possibility of a poor prognosis. The diagnosis of this case was confirmed only 3 months prior to the preparation of this manuscript; therefore, follow up data are currently not available. Based on IHC results, this case will require continued attention for future treatment. Presently, researchers agree that if GRCC is comparable to the conventional ductal carcinoma of the same TNM stage, the prognosis of the two types of carcinomas would be the same. As only three cases were available for study, the drawing of any conclusions would be limited. However, these cases did reveal different IHC characteristics for GRCC. We believe that the cases presented herein substantiate a need to re-examine the general features of GRCC. While the immunophenotypes of GRCC were not uniform, they appeared to be very similar to conventional ductal carcinoma.

REFERENCES

1. Hull MT, Priest JB, Broadie TA, Ransburg RC, McCarthy LJ. Glycogen-rich clear cell carcinoma of the breast: a light and electron microscopic study. Cancer 1981;48:2003-9.
2. Hull MT, Warfel KA. Glycogen-rich clear cell carcinomas of the breast. A clinicopathologic and ultrastructural study. Am J Surg Pathol 1986;10:553-9.
3. Toikkanen S, Joensuu H. Glycogen-rich clear-cell carcinoma of the breast: a clinicopathologic and flow cytometric study. Hum Pathol 1991;22:81-3.
4. Fisher ER, Tavares J, Bulatao IS, Sass R, Fisher B. Glycogen-rich, clear cell breast cancer: with comments concerning other clear cell variants. Hum Pathol 1985;16:1085-90.
5. Hayes MM, Seidman JD, Ashton MA. Glycogen-rich clear cell carcinoma of the breast. A clinicopathologic study of 21 cases. Am J Surg Pathol 1995;19:904-11.
6. Kuroda H, Sakamoto G, Ohnisi K, Itoyama S. Clinical and pathological features of glycogen-rich clear cell carcinoma of the breast. Breast Cancer 2005;12:189-95.
7. Varga Z, Zhao J, Ohlschlegel C, Odermatt B, Heitz PU. Preferential HER-2/neu overexpression and/or amplification in aggressive histological subtypes of invasive breast cancer. Histopathology 2004;44:332-8.