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Case Report

Genetic Alterations in Invasive Breast Carcinoma with a Glycogen-Rich Clear Cell Pattern: A Case Report

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Glycogen-rich clear cell carcinoma · Breast cancer · Next-generation sequencing · *ARID1A* · *MAP2K4*

Abstract
Invasive carcinoma with a glycogen-rich clear cell pattern (IC-GRCCP) is a rare and understudied subtype of invasive breast carcinoma of no special type (IBC-NST). Here we report the molecular characteristics of a mammary IC-GRCCP diagnosed in a 69-year-old woman. Next-generation sequencing of the tumor revealed an inv(1)(p36.12,q32.1) leading to loss-of-function of *ARID1A* gene, a *MAP2K4* truncating mutation (p.E376), *MYC* amplification, a variant of uncertain significance of *PTPRB* gene (p.D1848N) and deep deletions of *NCKAP5*, *CCNT2*, *MAP3K19*, *LRP1B*, and *KMT2A*. The analysis of the involved pathways shows close resemblance to the ovarian clear cell carcinoma and indicates similarities in the molecular mechanisms of development of glycogen-rich clear cell carcinomas in different organs. Our findings and the literature review suggest new potential strategies for treatment of mammary IC-GRCCP, including epigenetic therapies, checkpoint inhibitors, radiation, or other double-strand DNA breaks-inducing agents. Nevertheless, larger studies are needed to substantiate those ideas.

Introduction

Invasive carcinoma with a glycogen-rich clear cell pattern (IC-GRCCP), formerly classified as glycogen-rich clear cell carcinoma, is a rare subtype of invasive breast carcinoma of no special type (IBC-NST) accounting for approximately 0.01% of all breast malignancies [1]. It is characterized by the presence of neoplastic cells with abundant clear cytoplasm that

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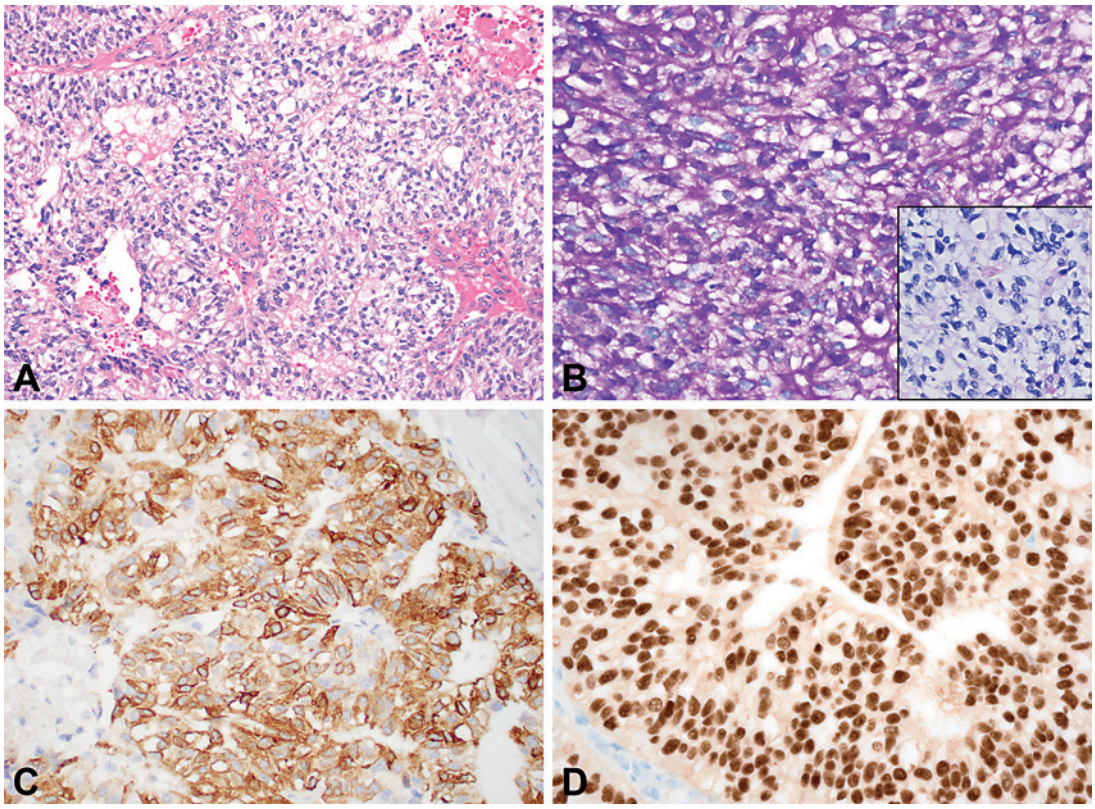

Fig. 1. Microphotographs of the breast tumor. **A** Hematoxylin and eosin stain. **B** Periodic acid-Schiff with (**inset**) and without digestion. **C** Immunohistochemical stain for mammaglobin. **D** Immunohistochemical stain for estrogen receptor. Original magnifications, ×200 for **A**, ×400 for **C–D**.

contains glycogen. Due to the low incidence of this tumor, the information about the specific molecular alterations, their prognostic significance, and potential therapeutic implications is quite limited.

Case Report/Case Presentation

A 69-year-old woman with no significant past medical history presented with a 1.8 cm right breast mass. The resection specimen revealed an invasive neoplasm with glandular and solid papillary growth patterns composed exclusively of polygonal cells with clear cell cytoplasm and distinct cell borders (shown in Fig. 1A). Tumor cells were strongly positive for periodic acid-Schiff (PAS) and PAS-diastase sensitive (shown in Fig. 1B). The tumor was also positive for mammaglobin and ER (shown in Fig. 1C–D), while negative for PR, HER2, and PAX-8. The morphologic features and histochemical staining results are consistent with an invasive carcinoma with a glycogen-rich clear cell pattern. The immunophenotype supports a breast primary site of origin.

Tumor-only sequencing using a hybrid-capture next-generation sequencing (NGS) assay was performed using formalin-fixed paraffin-embedded tissue. The NGS panel included the coding regions of 479 cancer-related genes, selected introns of 47 genes, and the *TERT* promoter. The NGS found alterations in the following genes: *ARID1A*, *KDM5B*, *MAP2K4*, *MYC*, *PTPRB*, *NCKAP5*, *CCNT2*, *MAP3K19*, *KMT2A*, and *LRP1B*. The details are shown in Table 1.

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Table 1. Genetic alterations in the patient's tumor

Gene	Name	Genetic alteration	Gene functions/pathways[+]
ARID1A	AT-Rich Interaction Domain 1A	inv(1)(p36.12,q32.1); large inversion between exon 20 of *ARID1A* and intron 2 of *KDM5B* leading to loss-of-function of ARID1A tumor suppressor gene	DNA and protein binding; transcription coactivator activity; negative regulation of transcription by RNA polymerase II
KDM5B	Lysine Demethylase 5B	inv(1)(p36.12,q32.1)	Transcription corepressor activity; chromatin organization and remodeling; regulation of transcription by RNA polymerase II
MAP2K4	Mitogen-Activated Protein Kinase Kinase 4	p.E376* truncating mutation with a variant allelic frequency of 87% suggestive of loss-of-heterozygosity	Protein serine/threonine kinase activity; protein tyrosine kinase activity; activation of MAPK activity; apoptotic process
PTPRB	Protein Tyrosine Phosphatase Receptor Type B	p.D1848N a variant of uncertain significance	Protein dephosphorylation; angiogenesis
MYC	MYC Proto-Oncogene, BHLH Transcription Factor	Amplification (5×)	DNA-binding transcription factor activity, RNA polymerase II-specific; negative regulation of transcription by RNA polymerase II; MAPK cascade; G1/S transition of mitotic cell cycle; re-entry into mitotic cell cycle
CKAP5	Cytoskeleton Associated Protein 5	Deep deletion	Microtubule bundle formation and depolymerization
CCNT2	Cyclin T2	Deep deletion	Transcription elongation from RNA polymerase II promoter
MAP3K19	Mitogen-Activated Protein Kinase Kinase Kinase 19	Deep deletion	Protein serine/threonine kinase activity; stress-activated protein kinase signaling cascade; regulation of mitotic cell cycle
KMT2A	Lysine Methyltransferase 2A	Deep deletion	Chromatin organization; regulation of transcription, DNA-templated; apoptotic process
LRP1B	LDL Receptor Related Protein 1B	Deep deletion	Protein and calcium ion binding; receptor-mediated endocytosis

[+] Selected GO, terms from GeneCards®: The Human Gene Database.

Discussion/Conclusion

Limited information exists about genetic changes in the IC-GRCCP of the breast. Genetic alterations have been reported in *PIK3R1*, *BRCA2*, *TP53*, *PTEN*, *CDKN2A*, *BCOR*, and *EGFR* genes [2, 3]. To our knowledge, there are no reports implicating other known cancer-associated genes, such as *ARID1A*, *MYC*, and *MAP2K4*, in the development of mammary IC-GRCCP. Other genetic alteration, such as the *LRP1B* or *KMT2A* deletions, may have also contributed to the development of the tumor, but further studies are required to determine their significance since limited information exists.

The AT-Rich Interaction Domain 1A (*ARID1A*) gene is located within chromosomal region 1p36. The encoded protein is a component of SWI/SNF chromatin remodeling complexes that is involved in the regulation of gene expression, proliferation, apoptosis, differentiation, and DNA repair [4]. ARID1A confers target specificity to the SNF/SWI complex by recruiting it to the specific sites of chromatin remodeling [4]. *ARID1A* acts as a tumor suppressor gene and

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is genetically altered or demonstrates loss of protein expression in a wide variety of tumor types [4]. In the TCGA PanCancer Atlas study (http://www.cbioportal.org) genetic alterations of *ARID1A* gene were found in approximately 5% of the breast cancers, while abnormal mRNA or protein expression was detected in 4–5% of the cases.

As a part of the SWI/SNF complex ARID1A participates in differentiation-associated repression of cell cycle genes some of which, such as *MYC*, *CDK1*, and *CCNB2*, are directly targeted at the time of repression [5]. This suggests synergism between the *ARID1A* mutation and *MYC* amplification, in our case leading to further enhancement of the effect of MYC on the cell cycle.

MAP2K4 is a part of mitogen-activated protein kinase (MAPK) pathways. In response to various stress stimuli MAP2K4 activates Jun N-terminal kinases (JNKs) and p38 MAPKs that control apoptosis, proliferation, differentiation, and cell migration. In our case, we found *MAP2K4* p.E376* truncating mutation that involves the DVD domain which contains a docking site critical for MAP2K4 activation by MAP3Ks. This is in agreement with the majority of the studies that suggest a tumor suppressor role of *MAP2K4* [6]. Experimental studies have also demonstrated that JNK pathway defects that result in loss of JNK signaling are "driver" mutations in mammary carcinogenesis [7].

Interestingly, the review of the literature shows genetic resemblance between our case and the glycogen-rich clear cell carcinomas from other organs, which indicates similarities in the molecular mechanisms of their development. For example, *ARID1A* genetic alterations and/or protein downregulation have been reported in up to 62% of ovarian clear cell carcinomas [8], 26% of endometrial clear cell carcinomas [9], and 67% of renal clear cell carcinomas [10]. Our finding of alterations in *ARID1A*, *MYC*, and *MAP2K4* genes is very similar to the results of Murakami et al. [8], who reported genetic damage involving the SWI/SNF complex, the MYC-CDK2/4-RB1 pathway, and the KRAS-PIK3CA-AKT1-PTEN pathway in 85, 79, and 82% of ovarian clear cell carcinomas, respectively. Although we did not detect alterations in the PIK3CA/PTEN pathway in contrast to Murakami et al. [8], the inactivating *MAP2K4* mutation in our case may have a similar effect since AKT phosphorylates and inactivates MAP2K4 [11] causing loss of JNK signaling upon PIK3CA/PTEN pathway activation. Co-existing genetic damage in two of those pathways (*PTEN* mutation/loss and a *CDKN2A* mutation) has been reported by Skenderi et al. [2] in one of their five cases of mammary IC-GRCCP.

Some of the genetic alterations in the tumor may have therapeutic implications. For example, experimental data suggest that *ARID1A* and SWI/SNF-subunit mutations result in epigenetic vulnerabilities in the tumor cells that can be targeted through inhibition of histone deacetylase (HDAC) and/or the catalytic subunit (EZH2) of the polycomb repressive complex 2 (PRC2) [12]. In addition, loss of *ARID1A* function causes DNA repair deficiency and may confer sensitivity to immune checkpoint inhibitors [13], radiation [14], or other double-strand DNA breaks-inducing treatments such as PARP and ATR inhibitors [14]. Mutations causing loss of MAP2K4 function sensitize tumors with RAS/RAF dysfunction to MEK inhibitors by inactivating JNK-JUN mediated feedback loop [15]. New anti-MYC therapies using inhibition of *MYC* transcription, partner protein dimerization, activating post-translational modifications, and turnover are in pre-clinical and clinical testing phases [16].

In summary, here we report new genetic alterations in mammary IC-GRCCP involving the chromatin remodeling machinery, MYC-CDK2/4-RB1 pathway and JNK pathway and demonstrate molecular similarities in the pathogenesis of clear cell carcinomas with high glycogen contents observed in different organs. More extensive molecular studies are needed to further elucidate the genetic mechanisms of mammary IC-GRCCP and the potential therapeutic opportunities for those patients.

Statement of Ethics

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. The study was approved by the Institutional Review Board at the University of California San Francisco (IRB#18-26671).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Carlo De la Sancha MD: took the lead in writing the manuscript. Roberto Ruiz-Cordero MD: analyzed NGS data and contributed to the final version of the manuscript. Nikolay Popnikolov MD, PhD: contributed to discussion and supervised the project.

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