

Altered Expression of miR-146a-5p and Bcl-2 in Oncocytic Carcinoma of the Breast: A Case Report

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Case Report

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Abstract

Background: Oncocytic carcinoma of the breast is extremely rare, and its molecular profile is poorly understood. The distinctive feature of oncocytic carcinoma of the breast is that the granular eosinophilic cytoplasm contains numerous mitochondria. Recently, microRNA-146a-5p has been identified as a contributor to carcinogenesis and as a mitochondria-related microRNA, which regulates the mitochondrial function affecting Bcl-2. Bcl-2 plays a role in mitochondrial apoptosis.

Case presentation: We report the clinical features, histopathological features, and immunohistochemical and molecular findings of oncocytic carcinoma of the breast in a 76-year-old woman. Immunohistochemistry studies revealed that the tumor cells were positive for antimitochondrial antibody but negative for gross cystic disease fluid protein 15, which confirmed the diagnosis. For molecular profiling, expression of microRNA-146a-5p and Bcl-2 messenger RNA (mRNA), which are mitochondria-related small molecules, were evaluated using real-time reverse transcription polymerase chain reaction. We found that the expression of microRNA-146a-5p was significantly lower ($p < 0.01$) and that of Bcl-2 mRNA was significantly higher ($p < 0.01$) compared to the control group (with no specific type of breast cancer).

Conclusions: The significant changes in the expression of microRNA-146a-5p and Bcl-2 are specific to oncocytic carcinoma of the breast. Therefore, we suggest that the use of microRNA-146a-5p to target Bcl-2 has a potential therapeutic effect on oncocytic carcinoma of the breast.

Background

Oncocytic carcinomas (OC) are rare malignant tumors composed of oncocytes, which are epithelial cells characterized by a granular eosinophilic cytoplasm containing numerous mitochondria [1]. OC of the breast is uncommon, accounting for less than 1% of all breast cancers [2]. Hence, little is known about its pathologic molecular features.

MicroRNAs (miRNAs) are small, non-coding RNAs that play an important role in post-transcriptional gene regulation [3, 4]. They interact with their target messenger RNAs (mRNAs) to control translation through mRNA degradation or translational repression [5, 6]. Research has revealed that miRNAs are involved in various biological processes, including cancer [7, 8]. The miRNA expression profiles differ between diseases, suggesting that miRNAs have an important role in the diagnosis and treatment of cancers [9–11].

One of the miRNAs that has recently received much attention is miR-146a-5p because of its function as a tumor suppressor and its deregulation observed in various cancers [12]. It has also been identified as a mitochondrial miRNA (mito-miR) that targets the mitochondria and regulates mitochondrial protein expression and function [13, 14]. A previous study suggested that miR-146a-5p affects mitochondrial function by targeting Bcl-2, which plays an apoptogenic role in mitochondria [15].

In view of the tumor-specific expression of miRNAs and characteristic features of OC, we hypothesized that the expression of mitochondria-related miRNAs could be altered in OC. To test our hypothesis, we compared the expression of miR-146a-5p in tissues from specimens of OC with that in tissues from specimens of a more typical type of invasive ductal carcinoma (IDC) of the breast. We also assessed the mRNA and expression levels of Bcl-2, which is the target of miR-146a-5p. In this report, we describe a case of OC of the breast that exhibited alterations in the expression of miR-146a-5p and Bcl-2, in comparison with IDC. To the best of our knowledge, these alterations have not been previously reported.

Case Presentation

A 76-year-old woman was referred to our hospital with a left breast tumor. She had noticed a gradual enlargement of the mass over the past 3 years. Physical examination revealed a 65-mm, hard, non-mobile mass with a focal cystic appearance in the upper inner quadrant of the left breast (Fig. 1A). No axillary lymph node was palpable. Ultrasonography revealed a solid mass with a marked cystic component, which could have represented hemorrhage and swelling of the left axillary lymph nodes (Fig. 1B). Magnetic resonance imaging revealed a solid mass with a cystic component and apparent involvement of the skin and pectoralis major muscle (Fig. 1C). Positron emission tomography revealed abnormal uptake in the mass and axillary lymph nodes (maximum standardized uptake values: 10.2 and 8.0, respectively) but no evidence of systemic metastases (Figs. 1D and E). A core needle biopsy was performed, and the mass was diagnosed as an invasive carcinoma. Cytologic examination of a specimen obtained with fine-needle aspiration of the lymph node revealed the presence of malignant cells. The patient underwent mastectomy and axillary lymph node dissection.

The tumor measured 45 × 12 mm, and four involved lymph nodes were found (Figs. 2A and B). Hematoxylin and eosin staining revealed a granular cytoplasm, ranging from eosinophilic to clear, in ovoid cancer cells (Fig. 2C). Strongly positive immunostaining for an antimitochondrial antibody revealed that the tumor cells were of oncocytic origin (Fig. 2D). Furthermore, immunohistochemical staining showed that the tumor cells were positive for estrogen and progesterone receptors but negative for human epidermal growth factor receptor 2 (HER2) and gross cystic disease fluid protein 15 (GCDFP-15; Fig. 2E). The Ki-67 labeling index was 15%. Based on these results, the diagnosis of OC of the left breast was made. The patient received postmastectomy radiation therapy of 50 Gy in 25 fractions to the chest wall and supraclavicular lymph nodes, sequentially followed by adjuvant endocrine therapy with an aromatase inhibitor for the next 10 years. In the 21 months after surgery, the cancer had not recurred.

For molecular profiling, we collected fresh tissue samples from the surgical specimens and stored them at 80°C until use. For comparison, we also collected fresh tissue samples of IDC from a 72-year-old patient, who had the same phenotype (positive estrogen and progesterone receptors and negative HER2). Reverse transcription polymerase chain reaction (RT-PCR) was used to evaluate the expression levels of miR-146a-5p and Bcl-2 mRNA. miRNA was extracted using an miRNeasy Mini Kit (QIAGEN, Crawley, West Sussex, UK), in accordance with the manufacturer's protocol. RT-PCR was performed as previously described [16]. U6 small nuclear RNA and glyceraldehyde 3-phosphate dehydrogenase were

used for normalization in RT-PCR with miRNA and mRNA, respectively. All experiments were conducted in triplicate. For RT-PCR, the results were calculated as the mean±standard deviation of triplicate data, and the expression level was illustrated as a fold difference of 1 for IDC. We used JMP® 12 software (SAS Institute Inc., Cary, NC, USA) to perform statistical analysis and compared the data of the two groups using the t-test. The significance threshold was set at $P<0.05$. In addition, we determined the Bcl-2 protein level by immunohistochemical staining using formalin-fixed, paraffin-embedded samples.

Interestingly, the expression level of miR-146a-5p was 0.18 times less in OC than in IDC (Fig. 3A), which was a significant difference. In addition, mRNA expression of Bcl-2 was 2.26-fold higher in OC than in IDC, which was also significant and could further explain the data from immunohistochemical staining, which showed a higher intensity of Bcl-2 protein (Figs. 3B and C).

Discussion And Conclusions

OC is an extremely rare type of breast cancer, characterized by mitochondrial accumulation. In our patient's tumor, we demonstrated significant changes in the expression of miR-146a-5p and Bcl-2 in OC compared to that of IDC, which suggests that miR-146a-5p, by targeting Bcl-2, has a potential therapeutic effect on OC.

The World Health Organization classification defines OC of the breast as a tumor in which more than 70% of all cells have oncocytic features [2]. The distinctive feature of oncocytes is the enlargement of cells containing abundant eosinophilic granules in their cytoplasm due to an increase in altered mitochondria [17]. Morphologically, OC resembles apocrine carcinoma, which also has an abundant eosinophilic granular cytoplasm [18]. On hematoxylin and eosin staining, it is difficult to distinguish OC from apocrine carcinoma based on morphological features. Thus, accurate diagnosis of OC necessitates immunohistochemical staining for several markers, including mitochondrial markers. In addition, GCDFP-15, a marker for apocrine differentiation, must be absent for the diagnosis of OC. In our patient, the morphologic features and use of immunostaining for antimitochondrial markers and GCDFP-15 were consistent with OC.

The mechanism of oncocytic tumor formation remains to be fully elucidated. Mitochondrial DNA mutations or mitochondrial genome alterations have been found in OC, which lead to alterations in mitochondrial metabolism and tumorigenesis [19, 20]. However, these observations have not been investigated in OC of the breast, and the unique miRNA expression in OC of the breast is poorly described because of its rarity. In other organs in which OC has been reported, such as the thyroid, kidneys, and salivary glands, miR-146a-5p expression has not been found. In relation to miR-146a and oncocytes, Fridman et al. demonstrated a specific downregulation of miR-146a in renal oncocytoma in comparison with other histological types of renal tumors [21]. Other investigators have detected miRNAs in multiple subcellular compartments, including the mitochondria [22–24]. Regarding the accumulation of mitochondria as the hallmark of OC, we hypothesized that the expression of mito-miR would be altered in OC, in contrast to IDC. Indeed, our data showed that the expression of miR-146a-5p, a mito-miR, was

significantly decreased in OC compared to that in IDC. We found no evidence that the altered expression of miR-146a-5p is associated with carcinogenesis of oncocytes; however, our results raise the possibility of a connection between miR-146a-5p dysregulation and mitochondrial abnormality in OC of the breast.

In breast cancer, miR-146a-5p has been shown to be upregulated, promoting proliferation and invasion and inducing epithelial–mesenchymal transition [25, 26]. Furthermore, upregulation of miR-146a-5p plays an important role in regulating Bcl-2 proteins via the mitochondrial pathway of apoptosis in aging-related diseases, including cancer [15]. Bcl-2 proteins exist in the outer membrane of mitochondria and their overexpression in breast cancer has been shown to increase invasion and migration [27, 28]. The higher expression of Bcl-2 and lower expression of miR-146a-5p in OC tissues compared to IDC tissues in our study indicate that miR-146a-5p may contribute to altered expression of Bcl-2 through mitochondria.

Our study has several limitations. First, we investigated only one sample of OC; we could not obtain multiple OC samples because of its rarity. We acknowledge that our results would be insufficient to generalize to all cases of OC; however, providing descriptions of molecular profiling in a fresh sample is important and beneficial for patients with very rare tumors, from both clinical and translational standpoints [29]. In addition, although investigations of both tumors and noncancerous samples would be more desirable, we could not obtain noncancerous fresh samples for experimentation. Finally, we could not identify the mechanisms underlying the association between miR-146a-5p and Bcl-2 in OC in this study. Further studies are needed to confirm these findings.

OC is a rare type of breast cancer. To the best of our knowledge, significant changes in the expression of miR-146a-5p and Bcl-2 in OC, in comparison with IDC, have not been previously reported. Therefore, our findings could help improve the understanding of this rare tumor.

Abbreviations

mRNA: messenger RNA

OC: oncocytic carcinoma

miRNA: microRNA

IDC: invasive ductal carcinoma

HER2: human epidermal growth factor receptor 2

GCDFP-15: gross cystic disease fluid protein 15

RT-PCR: reverse transcription polymerase chain reaction

mito-miR: mitochondrial miRNA

Declarations

Ethics approval and consent to participate: This study was approved by the Institutional Review Board of Hiroshima University Hospital. Written informed consent was obtained from the patients before enrollment.

Consent for publication: Written informed consent for the publication of the clinical data including photos and images was obtained from the patients.

Availability of data and materials: All analyzed data is included in this published article.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: RT provided the clinical data included in the text; YK wrote the manuscript; TS and KK acquired and interpreted clinical data; YD performed the pathological examination; YY, SF and HT performed and analyzed the experimental examination; MO revised the manuscript. All authors issued final approval for the version to be submitted.

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Figures

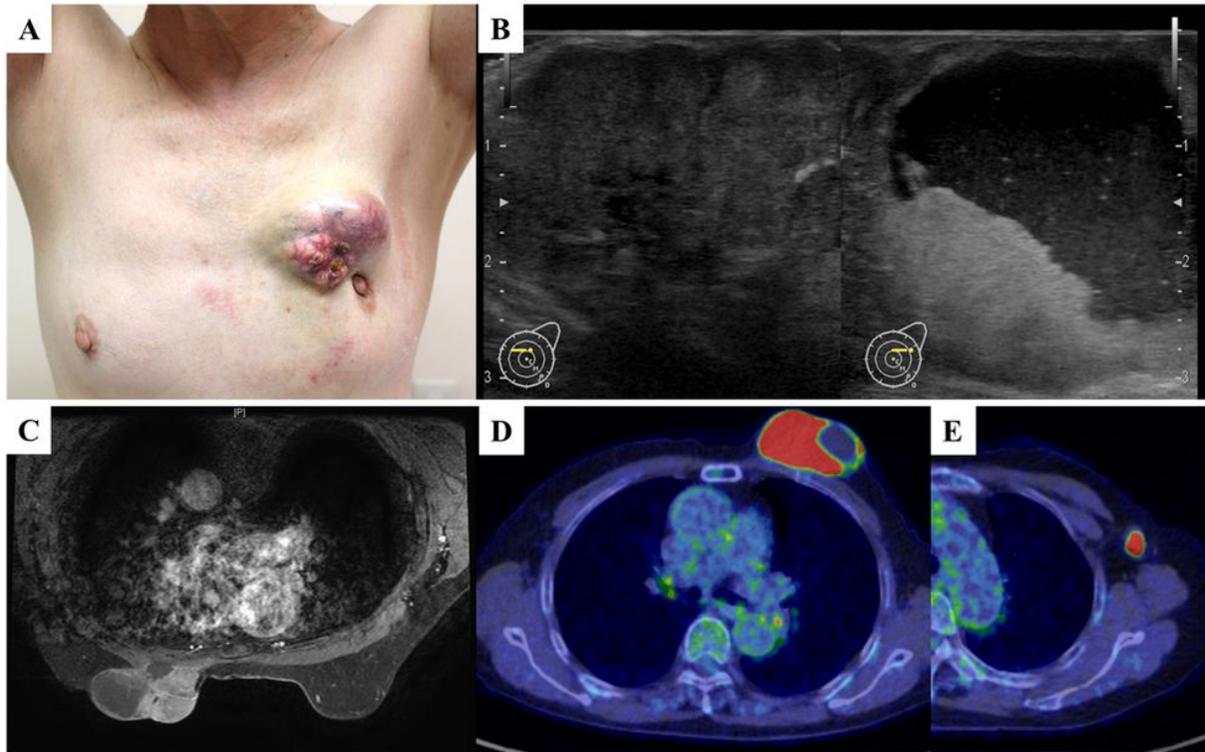


Figure 1.

Figure 1

Preoperative imaging studies A, Gross appearance of the left breast. B, Ultrasonographic view of a mass with uncircumscribed margin, showing a complex cystic and solid echo pattern. C, Magnetic resonance image of a partially enhanced, heterogeneous mass indicating invasion into the skin and pectoralis major muscle. D and E, Views on positron emission tomography–computed tomography, showing 18-F fluorodeoxyglucose uptake values of 10.2 for the breast tumor (D) and 8.0 for the axial lymph node (E).

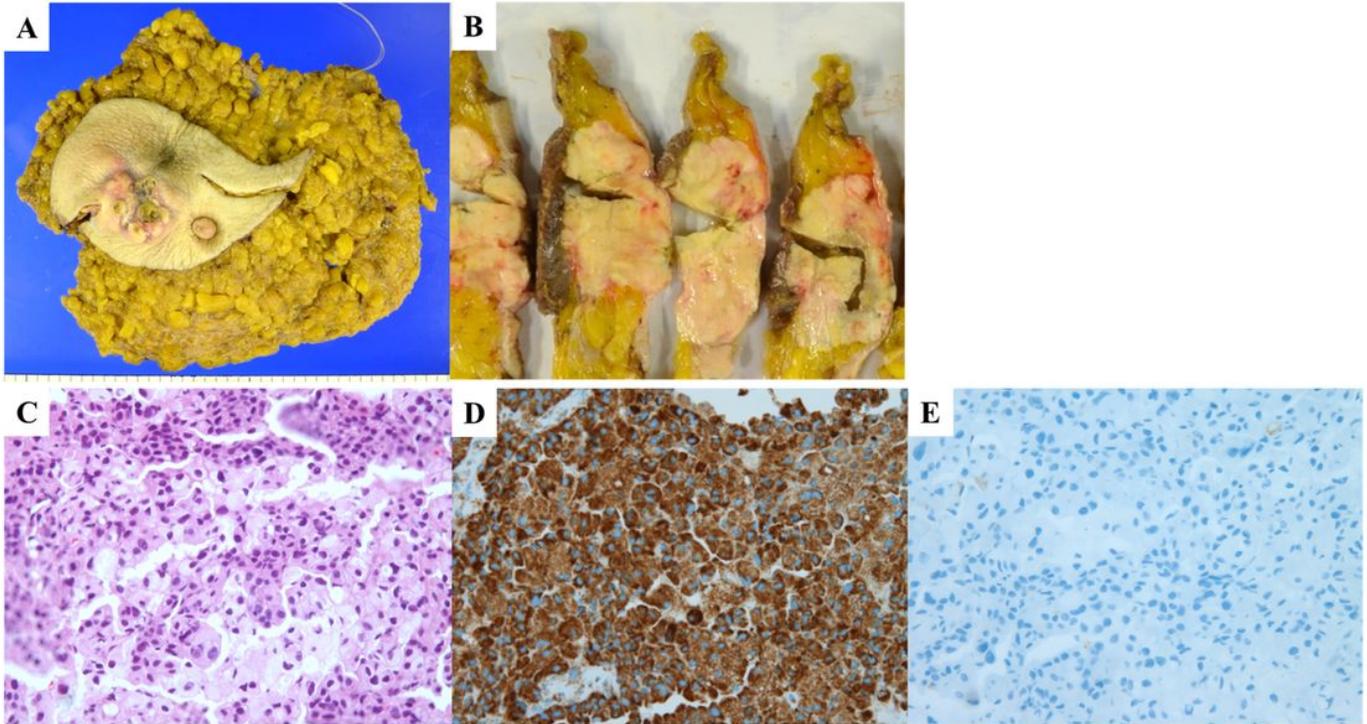


Figure 2.

Figure 2

Pathological findings in the surgical specimen from the left breast A, Macroscopic appearance. B, A slice of the specimen showing involvement of the skin and pectoralis major muscle. C, Hematoxylin and eosin staining showing tumor cells with abundant acidophilic granular cytoplasm (400×). D and E, Immunohistochemical staining showing positive antimitochondrial antibody and negative gross cystic disease fluid protein 15 (400×).

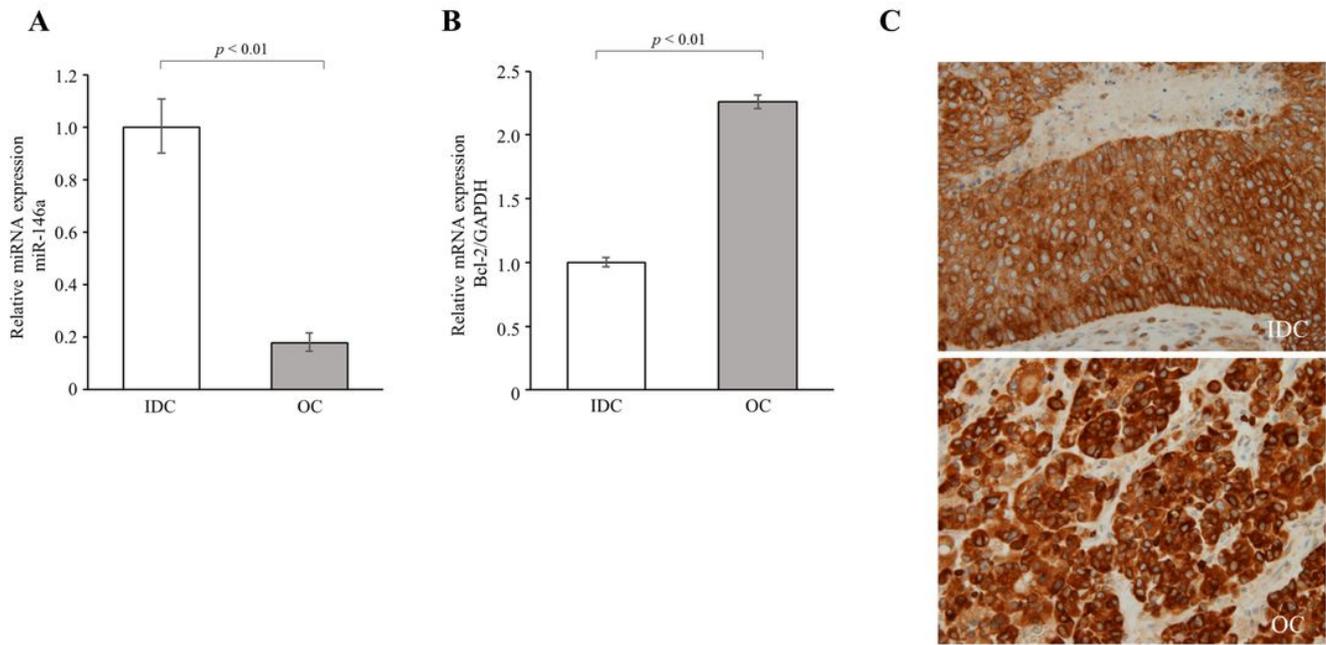


Figure 3.

Figure 3

Differential expression of miR-146a-5p and Bcl-2 in oncocytic carcinoma (OC) and invasive ductal carcinoma (IDC) A and B, The relative expression level of miR-146a-5p (A) and Bcl-2 messenger RNA (B) in IDC and OC, which was examined with reverse transcription polymerase chain reaction ($p < 0.01$). C, immunohistochemical staining of Bcl-2 in IDC (upper) and OC (lower). The intensity of staining was stronger in OC than in IDC (400 \times). GAPDH, glyceraldehyde 3-phosphate dehydrogenase; mRNA, messenger RNA; miRNA, microRNA.

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