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Nestin and CD146 expression in metaplastic breast cancer: stem-cell therapy in need? Lessons reported from a male patient

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Abstract. – OBJECTIVE: Metaplastic breast carcinomas represent a rare subtype of breast cancer exhibiting aggressive clinical features. They appear as highly chemoresistant tumors, therefore showing poor outcome and high rates of local recurrence or distant metastasis.

CASE REPORT: A 37-year-old greek man was referred to our hospital for evaluation of a locally advanced, ulcerated, fixed, irregular and hard in consistency mass covering his left breast and chest wall. Further work out with CT and biopsy of the tumor revealed a triple negative metaplastic breast cancer classified as cT4cN3cM1. The patient received first line chemotherapy and afterward a palliative resection of the tumor. The histology revealed the presence of a combined triple negative adenocarcinoma with a predominant metaplastic squamous carcinoma and a spindle cell (sarcomatoid) carcinoma of the breast. In the tissue sample stem cell markers, nestin and CD146 (MCAM) were expressed, enhancing the theory that cancer cells of this tumor could possibly harbor stem cell properties. The patient received several chemotherapy regimens but died 6 months after the initiation of treatment.

CONCLUSIONS: Metaplastic breast cancer consists of cells with stem cell properties. New targeted therapies are warranted in the view of the tumor's high resistance to conventional chemotherapy. Targeting nestin and CD146 might be a promising therapy as they seem to be implicated in the EMT pathway.

Key Words
Nestin, CD146, Metaplastic breast cancer, Cancer stem cells, Resistance to chemotherapy, Poor survival.

Abbreviations

MBCs: Metaplastic breast carcinomas, RECIST: Response Evaluation Criteria in Solid Tumors, CD146: Cluster of Differentiation 146, MCAM: Melanoma cell-adhesion molecule, PI3K: phosphoinositide 3-kinase, EMT: epithelial to mesenchymal transition, VEGF: Vascular endothelial growth factor.

Introduction

Metaplastic breast carcinomas (MBCs) represent a rare subtype of breast cancer exhibiting aggressive clinical features. They appear as highly chemoresistant tumors showing poor outcome and a high risk of local recurrence or distant metastasis[1].

Case Presentation

A 37-year-old greek man was referred to our hospital for evaluation of a locally advanced, ulcerated, fixed, irregular and hard in consistency mass covering his left breast and chest wall (Figure 1). The mass was growing in size since it was first noticed, 11 months ago. The patient had no comorbidities and referred to a smoking history of 50 pack-years. Furthermore, he had no history of undertaking estrogen related drugs or having surgery in the past and denied any previous neoplasia or a family history of cancers. Socially, the patient was a farmer but had no history of significant radiation or chemical exposures.

Clinical examination revealed a big ulcerating mass in his left breast with palpable, enlarged axillary lymph nodes. The patient was not obese and had no signs of cachexia. CT scan of the chest and abdomen revealed an 8 × 7.5 × 4.5-mm enhancing lesion arising from the outer quadrants of his left breast infiltrating the left *pectoralis*

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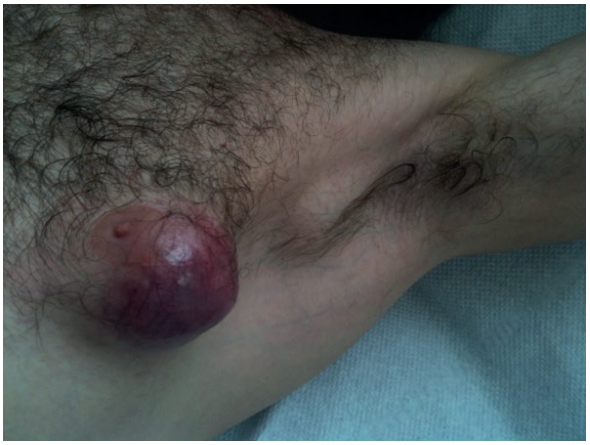

Figure 1. Locally advanced, ulcerated, fixed, irregular and hard in consistency mass covering the patient's left breast and the chest wall with macroscopically enlarged lymph nodes.

major muscle. Moreover, pathologically enlarged axillar, mediastinal, and supraclavicular lymph nodes were present as well as a solid lesion in the lower lobe of the left lung measuring less than 1 cm. A core needle biopsy of the large mass was performed, and revealed a primary triple negative breast cancer with the Ki67 cell proliferation marker reaching up to 50%. The patient was classified at a clinical stage of cT4cN3cM1 (although there was no biopsy of the lung, the solid lung lesion was growing further and new lung lesions appeared in the follow up).

The patient received first line chemotherapy with Adriamycin 50 mg/m^2 and Cyclophosphamide 750 mg/m^2 (1st cycle doses). Due to chemotherapy-induced thrombocytopenia along with grade IV neutropenia, the patient continued with further three cycles of small-sized liposomal Adriamycin at a dose of 40 mg/m^2 every 3 weeks. A 30% partial response according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria was observed and the patient received a palliative lumpectomy. Histological diagnosis revealed a combined triple negative adenocarcinoma with a predominant metaplastic squamous carcinoma and a spindle cell (sarcomatoid) carcinoma of the breast. The imnunohistochemical profile of the tumor is presented in Table I. For research reasons the tissue was immunohistochemically stained with two stem cell markers (nestin and CD146 [MCAM] or otherwise known as melanoma cell adhesion molecule) and presented an intense expression of both markers. Chemotherapy was further administered in 4 cycles containing liposomal Adriamycin at a dose of 40 mg/m^2 and Paclitaxel at a dose of 135 mg/m^2 every 3 weeks. Follow up with CT scan demonstrated disease progression with osteolytic lesions of the spine for which the patient was palliatively treated with zoledronic acid at a dose of 4 mg/kg every 28 days. Chemotherapy plan was then changed to 4 cycles with Bevacizumab 8 mg/kg, Gemcitabine 1200 mg/m^2 and Vinorelbine 30 mg/m^2 on day 1 and 8 every 4 weeks. Further progression was observed with skeletal, lung and new liver metastases. The patient finally received Capecitabine 1250 mg/m^2 combined with Fulvestrant BID on days 1-14 every 3 weeks and died 6 months after receiving initial treatment.

Discussion

Metaplastic breast carcinoma represents a rare subtype of breast cancer with glandular and alternatively non glandular epithelial cell types. From a biologic point of view, they most commonly exhibit the triple negative molecular subtype. They often present activation of the phosphoinositide 3-kinase (PI3K) and therefore they strongly correlate with a cancer-stem cell derived genomic profile based on this activity. Also, they harbor cancer stem-cell properties and it has been suggested that they frequently relate to epithelial to mesenchymal transition (EMT) mechanisms. Finally, they exhibit high levels of neovascularization as well as high levels of vascular endothelial growth factor (VEGF) expression. These aspects may account for their lack of response to conventional chemotherapy as they show a worse prognosis even when compared to basal-like cancers[1]. This hypothesis was confirmed by our patient considering that a metastatic disease occurred

Table I. Immunohistochemical profile of the tumor.

Carcinoma IMC	CK7	CK18	CK14	CK5/6	34bE12	p63	Vim	SMA	EGFR
Adenocarcinoma	+	+	-	-	+	-	-	-	+
Squamous cell	Focal+	Focal+	Focal+	+	+	+	-	-	+
Spindle cell	Focal+	-	-	-	Focal+	Focal+	Strong+	-	+

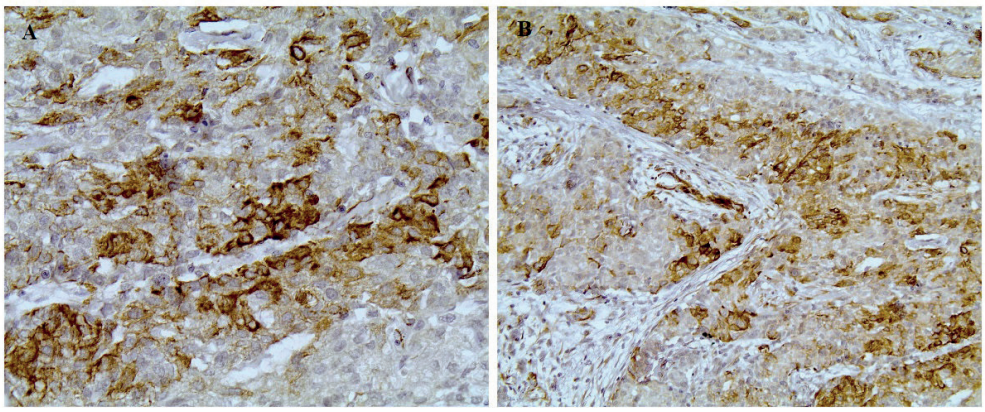

Figure 2. A, Least intense nestin cytoplasmic expression in the neoplastic cells of the patient (x40). **B**, Intense CD146 cytoplasmic expression in the tumor cells of the patient (x40).

11 months after the first sign of disease and, 6 months after treatment initiation with aggressive chemotherapy, the patient died.

The patient's tumor cells expressed nestin (Figure 2). In a previous study[2], we showed that nestin is expressed in a variety of tumors carrying cells with stem cell properties. More specifically, nestin might be implicated to neovascularization through cytoskeleton changes promoted from the interaction between cancer cells exhibiting stem cell properties and the endothelial cells of the tumor blood vessels. Notably, Zhao et al[3] showed that nestin-expression in triple negative cancers has been correlated with poor survival as it enhanced tumorigenicity in breast cancer stem cells. Moreover, it has been demonstrated [3] that nestin positively regulates the Wnt/β-catenin pathway, which is required for the activation of the EMT pathway, providing these changes. Therefore, the question raised here is whether nestin could be a potential target for drug therapy in patients exhibiting aggressive clinical features like the MBC patients, which might be induced through pluripotency and resistance to chemotherapy of the cancer stem/stem-like cells.

Of note, the patient of the present report harbored a strong expression of the CD146 (Figure 2) stem cell marker. Imbert et al[4] demonstrated *in vitro* working with breast cancer cell lines that the expression of CD146 is correlated with a specific EMT phenotype. The same results *in vitro* were confirmed from Zeng et al[5] as they showed that CD146 silencing induced an up-regulation of the epithelial marker E-cadherin, while its expression induced the expression of mesenchymal markers vimentin and fibronectin. Therefore, it could be concluded that activation of the EMT pathway as a basic mechanism of metastasis might explain partially the quick disease progression of the patient, who died only six months after the initiation of his treatment.

Conclusions

The metaplastic breast cancer consists of cells with stem cell properties. New targeted therapies are warranted in the view of the tumor's resistance to conventional chemotherapy. Targeting nestin and CD146 might be a promising therapy, as they seem to be implicated in the EMT pathway.

Availability of data and materials

All data analyzed are presented through the list of figures and tables in the manuscript.

Authors' Contributions

AT helped to analyze the clinical and research data and draft the manuscript. ECT collected the clinical and research data, helped to analyze the data and draft the manuscript, DT determined the patients therapy, helped to collect the clinical data and reviewed the manuscript, AN performed and assessed the immunohistochemistry, KK helped to collect the clinical data of the patient, EP assessed the immunohistochemistry, MK performed the surgery and GK was involved in the critical revision of the manuscript before submission. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests

Consent for publication

Written informed consent for data publication was acquired from the patient.

Ethics approval and consent to participate

All research carried out is in compliance with the Helsinki Declaration and was approved by the Ethics Committee of Laiko General Hospital, Faculty of Medicine, National and Kapodistrian University of Athens.

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