An exceptional metaplastic lobular breast carcinoma diagnosed through exome sequencing

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ABSTRACT

Metaplastic breast carcinoma is a rare subtype of breast cancer. This subtype is mostly found in association with poorly differentiated ductal breast carcinomas and rarely with other breast carcinoma types. We report the case of a 69-year-old woman with an exceptional invasive lobular breast carcinoma associated with metaplastic squamous cell bone metastasis occurring 2 years after the initial breast cancer diagnosis. Whole-exome sequencing and subsequent immunohistochemistry of the lesions were used to link the squamous cell bone metastasis of unknown origin to the primary breast carcinoma initially diagnosed. Searching for primary carcinoma when metastatic lesions of unknown origin occur can be complex. Current molecular biology techniques may help pathologists in associating metastasis with the primary carcinoma by identifying shared specific gene mutations, even when different morphological and immunohistochemical profiles are observed between the tumours.

INTRODUCTION

Metaplastic breast carcinomas (MBC) are rare and account for approximately 0.2%–5% of all invasive breast cancers.[1] Pure primary breast squamous cell carcinomas (SCC) are very rare, accounting for <1% of all invasive mammary carcinomas. Although squamous metaplasia is one of the most common metaplastic alterations in breast carcinomas, its genesis is not well understood.[2] The majority of pure breast SCCs probably originate from benign squamous metaplasia, which can occur either in the glandular epithelium of benign epithelial proliferation or in benign breast neoplasms, such as papillomas or fibroadenomas.[3] However, the specific cell type leading to metaplastic carcinoma remains uncertain. Some authors suggest that metaplastic carcinomas arise directly from pluripotent mammary neoplastic stem cells.[3] Others suggest that they originate from misplaced pluripotent embryonic stem cells or myoepithelial cells with stem cell characteristics.[4] A metaplastic origin from luminal epithelial cells has also been suggested as a possible mechanism by other groups.[5 6] An interesting hypothesis is that metaplastic carcinomas might emerge from a process of dedifferentiation, secondary to 'epithelial to mesenchymal transition' (EMT) in non-MBC. Immunoreactivity for epithelial and mesenchymal markers in squamous metaplastic cells may support this idea. These specific phenotypic markers are the expression of genotypic characteristics resulting from a multistep and reversible process of dedifferentiation. Through this transition, epithelial cells acquire the morphological phenotype and functional characteristics of mesenchymal cells. EMT occurs physiologically during embryogenesis and tissue repair.[3] Immunohistochemical studies of MBC have revealed that >90% are triple-negative for oestrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor-2 (HER2). Moreover, these tumours are often resistant to adjuvant chemotherapy and have a worse prognosis than conventional triple-negative carcinomas.[5]

Invasive lobular carcinoma (ILC) represents 5%–15% of breast tumours.[5] The histological profile of ILC is well known to pathologists, as it is the second most frequent histological subtype after non-specific (invasive ductal) carcinoma. The classic ILC pattern is characterised by a proliferation of small cells lacking cohesion and appearing individually dispersed through fibrous connective tissue or organised in single-file linear cords invading the stroma. ILC features, that is, low or intermediate grade and expressing high levels of ER and PR, are associated with a good prognosis. HER2 amplification and overexpression are rare in this type of tumour, and the lack of E-cadherin expression is generally related to *CDH1* somatic mutation.[5]

This study reports a case of a 69-year-old woman with ILC treated by surgery, chemotherapy and radiotherapy, associated with squamous cell bone metastases of unknown primary origin. Exome sequencing and subsequent immunohistochemistry analysis of these different lesions established the link between the two neoplasms.

CASE REPORT

We report the case of a 69-year-old woman referred to the Georges-François Leclerc Cancer Center (Dijon, France) in March 2015 after detection of a palpable mass in her left breast with pathological axillary lymph nodes. No family history of breast cancer was reported. The mammogram showed architectural distortion, whereas ultrasound identified a tissue mass of 33 mm in diameter located above the left nipple. The contralateral breast was normal. Tissue mass ultrasound-guided core needle biopsy revealed an ILC of intermediate grade according to the Elston-Ellis grading system. Immunohistochemistry analysis showed that the tumour was negative for ER, PR and HER2 and

also for E-cadherin. Positron emission tomography did not show any distant spread of the disease, particularly in the lungs or bones. Left mastectomy with axillary lymph node dissection was performed. The final pathological examination confirmed the initial biopsy findings of an intermediate grade ILC. The tumour measured 4 cm in diameter. Early left nipple infiltration, without epidermal extension was also observed. The Ki67 (MIB1) index was approximately of 2%. The 14 nodes from axillary lymph node dissection were positive with capsular ruptures and neoplastic vascular emboli. The tumour was classified pT2N3 according to the TNM system (AJCC, Cancer Staging Manual, Eighth Edition (8th Edition)).

Two months later, a CT scan performed before adjuvant chemotherapy showed a nodule with a diameter of 25 mm, with spiculated margins in the apico-dorsal segment of the upper left pulmonary lobe. However, there was no associated bone localisation at the time. A CT-guided core needle biopsy was performed to determine the primary or secondary origin of this lung tumour. Pathological examination showed a well-differentiated adenocarcinoma with a predominant acinar pattern. The tumour was strongly and diffusely positive for TTF1, slightly and focally positive for P63, but negative for P40, confirming primary lung origin. No ALK or ROS1 rearrangement was detected, but molecular testing revealed a 6-base insertion in exon 20 of the epidermal growth factor receptor (*EGFR*) gene, conferring sensitivity to tyrosine kinase inhibitors. Surgery was the sole treatment, as no other distant spread of the lung disease was detected. Left superior lobectomy was performed with mediastinal lymph node dissection. The lung tumour was finally classified pT1N0M0 according to the TNM system (AJCC, 8th Edition). Chemotherapy and radiotherapy were then performed to treat breast cancer.

In April 2017, after almost 2 years of clinical and radiological remission, the patient presented bone pain localised in the fifth right rib and pelvis. Thoracoabdominopelvic CT scan showed two 22 mm diameter secondary nodular bone lesions, infiltrating the surrounding soft tissue. The lesions were located on the middle arc of the fifth right rib and on the right iliac wing. There was no evidence of lung or breast carcinoma progression. To determine the origin of the bone lesions, ultrasound-guided core needle biopsy of the right costal lesion was performed. Pathological analysis revealed a moderately differentiated SCC. Immunohistochemistry analysis showed that the tumour cells were diffusely positive for P40, attesting to their squamous origin. Consequently, CT scan, positron emission tomography, as well as a head-and-neck and gynaecological examination were performed to find a primitive SCC. All examinations were unremarkable as no tumour was found. Metastasis of the lung adenocarcinoma with squamous differentiation, not found in the primary tumour, was suspected. The patient was thus treated with a combination of carboplatin and gemcitabine chemotherapy, associated with radiation therapy for pain management.

Two months later, in August 2017, the patient complained of a rapidly changing telangiectatic erythematous cutaneous lesion, infiltrating the left chest wall. The biopsy showed a subcutaneous metastatic localisation of the lobular carcinoma of the breast diagnosed 2 years earlier. The secondary tumour was also triple-negative for ER, PR and HER2. The patient was enrolled in a clinical trial (the Exoma trial, NCT02840604), which required tumour whole-exome sequencing analysis. Consequently, the genetic profile of the bone lesion was compared with that of the lung, breast and subcutaneous tumours. Technical details of whole-exome sequencing are described in the online supplementary material 1. Whole-exome analysis of the primary breast carcinoma, bone and subcutaneous metastases revealed nine genetic alterations common to all three locations. Only one of these genetic alterations was of established pathological significance, namely *CDH1* gene loss of function (c.1051 C>T, p.Gln351Ter). Four genetic alterations were shared by the primary breast carcinoma and squamous cell metastasis; two of them had established pathological meaning: *ZNF717* (p.Ter915LeufsTer) and *ROBO2* (p.Gln36Ter) gene loss of function. Ten genetic alterations were common to the primary breast carcinoma and subcutaneous metastasis; none of which

Table 1 Genetic alterations and tumour immunohistochemical profiles

		Immunohistochemistry								
Tumour	**Morphology**	**E-cadherin**	**AR**	**ER**	**PR**	**HER2**	**P40**	**P63**	**CK5/6**	**Genetic alterations**
Primary breast tumour	Invasive lobular carcinoma	–	+	–	–	–	–	–	–	***CDH1***, TRIM6*, HOMEZ*, SPHKAP*, CX3CR1*, PFKM*, HAUS4*, SRR*, CBX2*, MAGED2†, RHOA†, **ZNF717†**, **ROBO2†**, HES4‡, NFKBIZ‡, ICAM5‡, FOXC1‡, PRUNE2‡, SIPA1L2‡, PHRF1‡, ZNF260‡, IQSEC2‡, CDRT15‡*
Bone metastasis	Squamous cell carcinoma	–	+	–	–	–	+	+	+	***CDH1***, TRIM6*, HOMEZ*, SPHKAP*, CX3CR1*, PFKM*, HAUS4*, SRR*, CBX2*, MAGED2†, RHOA†, **ZNF717†**, **ROBO2†**, TP53§, ADGRG4§, **HEYL§**, SPTA1§, **TNRC18§**, ANKRD44§, CSMD3§, **CTDSP2§**, **PIK3CB§**, **PKD1L2§**, REPS1§, **PTEN§**, GBP2§, CDK11A§.*
Subcutaneous metastasis	Invasive lobular carcinoma	–	+	–	–	–	–	–	–	***CDH1***, TRIM6*, HOMEZ*, SPHKAP*, CX3CR1*, PFKM*, HAUS4*, SRR*, CBX2*, HES4‡, NFKBIZ‡, ICAM5‡, FOXC1‡, PRUNE2‡, SIPA1L2‡, PHRF1‡, ZNF260‡, IQSEC2‡, CDRT15‡, TP53§, ADGRG4§, **HEYL§**, SPTA1§, **TNRC18§**, ANKRD44§, CSMD3§, **CTDSP2§**, **PIK3CB§**, **PKD1L2§**, REPS1§, **PTEN§**, GBP2§, CDK11A§.*
Primary lung tumour	Well-differentiated adenocarcinoma	+	+	±	–	–	–	±	–	***EGFR*** (No genetic alteration common with others tumours).

Genetic alterations with pathological significance are set in bold.
*Genetic alterations common to primary breast tumour, bone metastasis and subcutaneous metastasis.
†Genetic alterations common to primary breast tumour and bone metastasis.
‡Genetic alterations common to primary breast tumour and subcutaneous metastasis.
§Genetic alterations common to bone metastasis and subcutaneous metastasis.
AR, androgen receptor; CK5/6, cytokeratin 5/6; ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

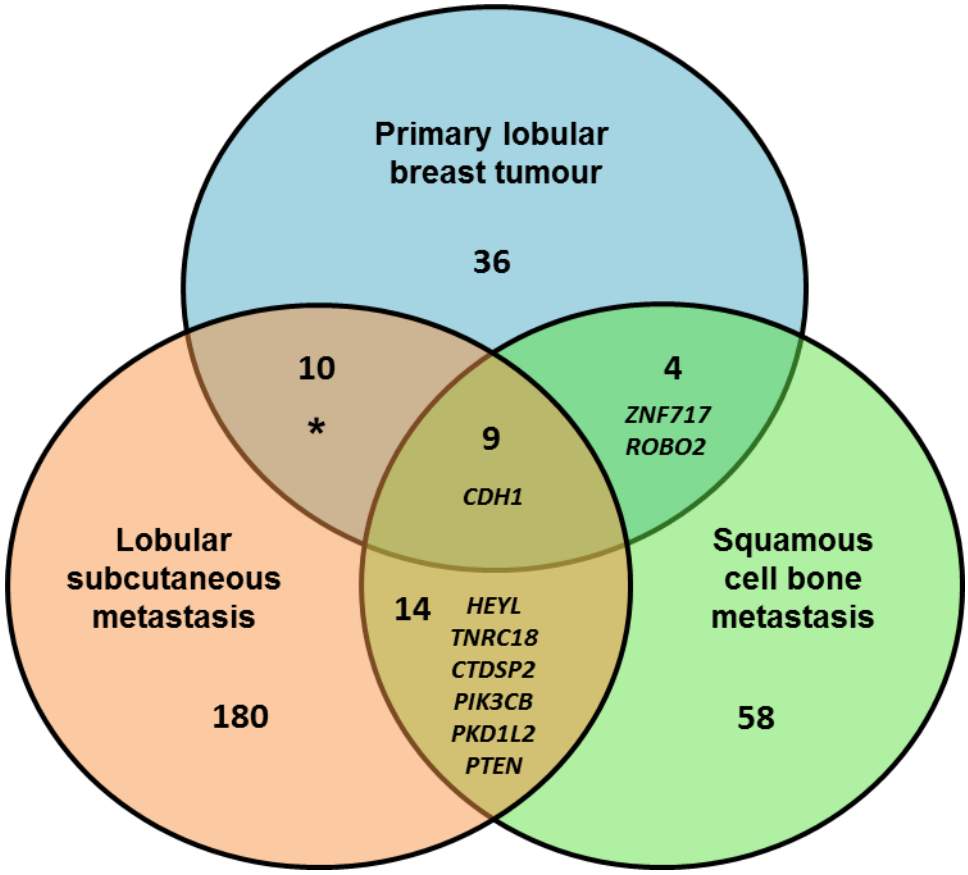

Figure 1 Venn diagram summarising the results of the comparative genomic study. The number of genetic alterations common to each tumour is presented and those with pathological meaning are in bold italics. *No other common genetic alteration with pathological meaning was found between the primary breast tumour and lobular subcutaneous metastasis.

were of established pathological significance. Fourteen genetic alterations were common to the squamous cell metastasis and subcutaneous metastasis. Six of them had well-known pathological meaning: *HEYL* (p.Ser300Ter), *TNRC18* (p.arg1692Ter), *CTDSP2* (p.Leu214HisfsTer24), *PKD1L2* (p.Thr2081SerfsTer), *PTEN* (p.Thr319LysfsTer24) gene loss of function and *PIK3CB* (p.Glu552Lys) gene activation. No *EGFR* gene mutation was found in the three tumours, and there was no other shared mutation with the primary lung adenocarcinoma. The results of the comparative genomic analysis are summarised in table 1 and illustrated in figure 1. Additionally, the complete set of mutations identified for each tumour is reported in the online supplementary table 1.

These data strongly suggested a mammary origin of the bone metastasis expressing squamous differentiation markers. These surprising results led to complementary immunohistochemical analysis in order to compare the phenotypic profiles of the primary and secondary tumours. This analysis showed that all tumours were triple-negative for ER, PR and HER2 and none expressed E-cadherin. In addition, all lesions were positive for androgen receptor. We thus concluded that all tumours had a similar phenotypic profile. The morphological and immunohistochemical findings are summarised in table 1 and illustrated in figure 2.

Based on these results, the carboplatin/gemcitabine combination initially prescribed was replaced by paclitaxel/bevacizumab chemotherapy, which is appropriate for triple-negative breast metastases. After multiple lines of chemotherapy, the patient died of her disease in November 2017.

DISCUSSION

This study focuses on the origin of SCC presented by a 69-year-old woman 2 years after the initial ILC diagnosis. Different hypotheses were envisaged: for example, the bone lesion could be a metastasis of the prior lung adenocarcinoma, or metastasis of a new SCC of unknown origin, or a distant metaplastic recurrence of ILC.

Case reports of invasive lobular metaplastic carcinomas associated with SCC are exceptional,[7 8] and therefore, breast cancer metastasis was not initially considered likely. Moreover, the bone lesion diffusely expressed squamous differentiation markers such as P40, P63 and cytokeratin 5/6, but did not express hormone receptors (ER and PR). Secondary analyses also revealed the expression of the androgen receptor, found in 80%–85% of primary, and 60%–75% of metastatic breast cancers.[9] As with the primary breast tumour, the bone lesion did not express E-cadherin, suggesting a common origin of both tumours. Indeed, E-cadherin expression may be decreased or absent in SCC, distinctive of a poorly differentiated tumour.[10] Subsequent subcutaneous localisation of the breast carcinoma further supported the hypothesis of a common origin. The whole-exome analysis revealed that a common somatic mutation in the *CDH1* gene was present in breast carcinoma, the SCC and the subcutaneous lobular metastases. Moreover, this mutation was not found in either the lung adenocarcinoma or the germline. These results suggested that, despite different morphologies, these tumours might have a shared genetic origin.

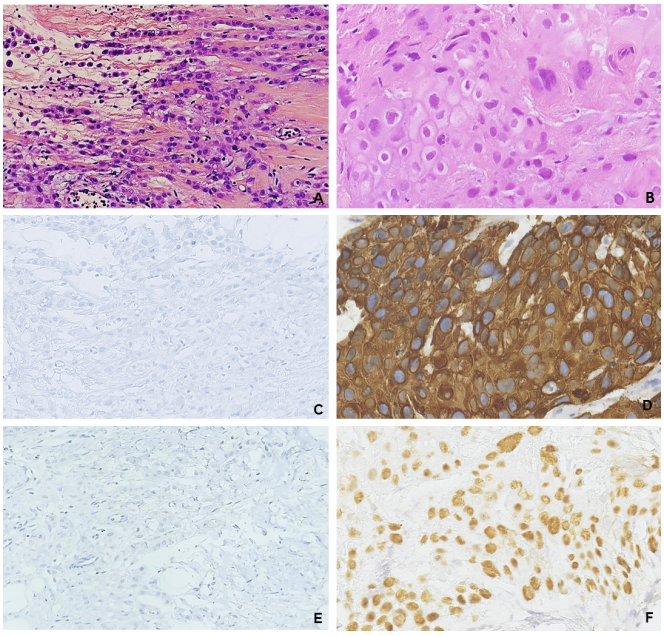

Figure 2 Morphological and immunohistochemical characterisation of primary breast tumour and squamous cell metastasis. Mild power view of primary breast invasive lobular carcinoma, microscopically characterised by small cells individually dispersed or arranged in single-file linear cords in a fibrous stroma (A) and high-power view of squamous cell metastasis forming sheets of large cohesive cells with intercellular bridges and keratinisation foci (B) (H&E-saffron). Invasive lobular carcinoma was negative for cytokeratin 5/6 (CK5/6) and P40 markers (C–E), whereas membranous CK5/6 and nuclear P40 immunostaining was found in squamous cell metastasis (D–F).

The *CDH1* gene is a tumour suppressor gene, located on the long arm of chromosome 16 (16q22.1) and encoding the epithelial-cadherin protein (E-cadherin),[11] part of the cadherin protein family, involved in cell adhesion and tissue formation.[12] Somatic mutations of the *CDH1* gene are acquired at an early stage of tumorogenesis.[1] These mutations are found in 30% to 80% of lobular carcinoma series, mainly in women with no family history of breast neoplasia.[13] Mutations of the *CDH1* gene have also been identified in other types of cancer, particularly diffuse gastric carcinoma. These cancers show infiltration with morphological loss of cell cohesion comparable with lobular breast carcinoma.[1 13] Loss of E-cadherin function leads to loss of cell adhesion and could promote carcinogenesis via cell division, proliferation and metastatic extension.[14] In SCC, a decrease in, or lack of E-cadherin expression is never due to *CDH1* mutation, but rather to hypermethylation of the *CDH1* promoter.[15]

MBC are rare tumours, often triple-negative for ER, PR and HER2. These tumours have a poor prognosis and are often not very sensitive to conventional chemotherapy.[5] No consensus or recommendations have yet been established regarding their management. MBC are genetically complex and heterogeneous.[16] Several studies have found somatic mutations in genes such as *TP53* (64% to 78%), *PIK3CA* (29%–61%) or more rarely *PTEN* (11%–25%), as with the metaplastic tumour described in this case, for which targeted therapies are available.[16–18] MBC are mainly associated with poorly differentiated ductal carcinomas and almost never with other breast carcinoma types.[3] Although metaplastic and classic invasive ductal components present very different morphologies, several studies have shown they are not separate cancers.[19] Indeed, the different histologies seem to be genetically related, sharing an almost identical set of somatic mutations. These results confirm the monoclonality of the paired metaplastic and ductal components when associated with a single primary breast tumour.[4 17]

In the case reported here, the *CDH1* gene mutation encountered in the primary breast ILC and also detected in the two metastases raised the possibility of a common genetic origin shared by the three neoplastic locations. The secondary acquisition of *TP53*, *PTEN* and *PIK3CB* gene mutations in the bone metastasis, 2 years after the initial diagnosis, could be responsible for the development of the metaplastic squamous cell component, as has been suggested in different studies.[4 17 19] The third subcutaneous location with a typical lobular phenotype expressed exactly the same mutations, without any foci of metaplastic squamous metaplasia. This phenomenon might be the consequence of epigenetic changes modulating gene expression involved in the tumour phenotype.[4 17 19] These molecular mechanisms leading to different tumour morphologies may have been favoured by the chemotherapy.[20]

Our study describes a triple-negative transformed recurrence of invasive lobular breast carcinoma with metaplastic squamous cell bone metastasis occurring 2 years after the initial diagnosis. It is important to highlight the utility of whole-exome analysis in this report. Despite the completely different morphologies, genetic analysis and additional immunohistochemical testing were key elements in linking the squamous cell bone metastasis of unknown origin to the primary breast ILC initially diagnosed. The genetic analysis had also a direct impact on the patient's management, as the metastatic origin determines the therapeutic decision. Therefore, the chemotherapy initially prescribed for squamous cell metastasis of unknown origin was replaced by a more appropriate therapy to treat a tumour of mammary origin.

CONCLUSIONS

Although metaplastic SCC is often associated with ductal breast carcinoma, its occurrence in association with ILC remains exceptional. Despite being an unusual pathology, this diagnosis should not be excluded in patients with a history of invasive lobular breast carcinoma and presenting metastatic lesions of unknown origin. Loss of E-cadherin expression and *CDH1* gene mutation might be crucial in establishing a molecular connection between the metastasis and the primary breast cancer.

In the presence of metastatic lesions of unknown origin, the search for the primary carcinoma can be complex. Wide gene panel or exome analysis can help establish a link between the metastasis and the primary carcinoma by identifying shared specific gene mutations between the tumours. Knowing the origin of metastasis is fundamental in providing specific and appropriate treatment. Our study highlights the importance of current molecular biology techniques, in addition to classic morphological and immunohistochemical analysis, especially in complex and unique diagnostic situations.

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