Published in final edited form as:

Histopathology. 2016 January ; 68(2): 262–271. doi:10.1111/his.12735.

Resolving quandaries: basaloid adenoid cystic carcinoma or breast cylindroma? The role of massively parallel sequencing

Nicola Fusco^{1,2}, Pierre-Emmanuel Colombo³, Luciano G. Martelotto¹, Maria R. De Filippo¹, Salvatore Piscuoglio¹, Charlotte K.Y. Ng¹, Raymond S. Lim¹, William Jacot³, Anne Vincent-Salomon⁴, Jorge S. Reis-Filho¹, and Britta Weigelt¹

¹Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

²School of Pathology, University of Milan, Milan, Italy

³Department of Surgical Oncology, Montpellier Cancer Institute (ICM), Montpellier, France

⁴Department of Tumor Biology, Institut Curie, Paris, France

Abstract

Aims—The aims of this study were to perform a whole-exome sequencing analysis of a breast cylindroma and to investigate the role of molecular analyses in the differentiation between breast cylindroma, a benign tumor that displays MYB expression and *CYLD* gene mutations, and its main differential diagnosis, the breast solid-basaloid adenoid cystic carcinoma, a malignant tumor that is characterized by the presence of the *MYB-NFIB* fusion gene and MYB overexpression.

Methods and Results—A 66-year old female underwent quadrantectomy after an irregular dense shadow was discovered in the right breast at the screening mammogram. Histologically, the tumor displayed features suggestive of a solid-basaloid variant of adenoid cystic carcinoma with a differential diagnosis of cylindroma. Fluorescence *in situ* hybridization, reverse transcription PCR, immunohistochemistry and whole-exome sequencing revealed absence of the *MYB-NFIB* fusion gene, low levels of MYB protein expression and a clonal somatic *CYLD* splice site mutation associated with loss of heterozygosity of the wild-type allele.

Conclusions—The results of the histologic, immunohistochemical and molecular analyses were consistent with a diagnosis of breast cylindroma, providing a proof-of-principle that the integration of histopathologic and molecular approaches can help differentiate between a low-malignant potential and a benign breast tumor of triple-negative phenotype.

Correspondence to: Britta Weigelt, PhD, Department of Pathology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. Phone: +1-212-639-2332, Fax: +1-212-639-2502, weigeltb@mskcc.org. **Jorge S. Reis-Filho, MD PhD FRCPath**, Department of Pathology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. Phone: +1-212-639-8054, Fax: +1-212-639-2502, reisfilj@mskcc.org.

Conflicts of interest: The authors have no conflict of interest to declare.

BW and JSR-F conceived the study. P-EC and WJ provided samples. NF, AV-S and JSR-F performed the pathologic review. LGM and SP carried out experiments. MRDF, CKYN and RSL performed the bioinformatics analysis. NF, BW and JSR-F wrote the first draft of the manuscript. All authors interpreted the data, and reviewed and approved the final version of the manuscript.

Keywords

breast cancer; cylindroma; adenoid cystic carcinoma; massively parallel sequencing; fluorescence *in situ* hybridization

INTRODUCTION

Salivary gland-like tumors and adnexal tumors of the breast comprise a spectrum of lesions that have histologic features similar to those of tumors that more often affect the salivary glands and the skin adnexae, respectively.¹ Owing to their rarity, salivary gland-like tumors and adnexal tumors of the breast not uncommonly pose diagnostic challenges. Their correct diagnosis, however, is not a mere academic exercise, as these lesions often display a triple-negative phenotype (i.e. they lack estrogen receptor (ER), progesterone receptor (PR) and HER2 expression), but have an indolent clinical behavior, at variance with the aggressive clinical behavior of triple-negative high-grade invasive ductal carcinomas of no special type.²

Despite its relative rarity, adenoid cystic carcinoma (AdCC) is one of the most frequent types of salivary gland-like tumor of the breast.^{3–6} AdCCs account for <1% of all invasive breast cancers and histologically resemble their salivary gland counterpart.^{3–6} When diagnosed in the breast, AdCCs often display the typical tubular or cribriform morphology, however a solid variant of AdCC with basaloid features was described by Shin and Rosen in 2002,⁷ which is characterized by mitotically-active basaloid looking cells organized in solid nests.^{3,7} Recent molecular analyses of breast AdCCs have revealed that both the classic forms of AdCC and the basaloid variant harbor a recurrent t(6;9) chromosomal translocation that results in the formation of the *MYB-NFIB* fusion gene, which is present in 23%–100% of breast AdCCs.^{3–6,8,9} This fusion gene has been shown to result in overexpression of the proto-oncogene *MYB*,^{5,9} possibly due to the loss of 3' miRNA binding sites that regulate *MYB* gene expression.⁹

One of the differential diagnoses of the basaloid variant of AdCC of the breast is the even rarer breast cylindroma, which is a benign, adnexal-type tumor.^{1,10} Only eleven cases of breast cylindromas have been reported in the English literature (Table 1).^{11–15} Albeit more frequently diagnosed in elderly women, cylindromas can affect younger women and may be part of hereditary syndromes such as Brooke-Spiegler syndrome and familial cylindromatosis.^{1,11,12} Cylindromas are invariably composed of a double population of cells (small basal cells at the periphery of the lobules and larger epithelial cells with vesicular nuclei in the center), organized in multiple lobules of various shapes and sizes,^{1,16} which may bear resemblance with the histologic appearance of the solid-basaloid variant of AdCCs.⁷ The lobules are typically surrounded by a rim of periodic acid-Schiff stain (PAS)-positive basement membrane material. Although germline mutations in the tumor suppressor gene *CYLD*, located in the 16q12.1 locus, have been shown to be causative of the Brooke-Spiegler and familial cylindromatosis syndromes¹⁷ and loss of heterozygosity (LOH) of the *CYLD* gene locus has been reported in up to 75% of sporadic cylindromas,^{13,18} limited knowledge about the somatic genetic features of cylindromas is presently available.

Importantly, however, examples of sporadic cylindromas harboring somatic mutations of the *CYLD* gene are on record.¹⁷ On the other hand, akin to AdCCs, up to 60% of dermal cylindromas have been found to harbor the recurrent *MYB-NFIB* fusion gene.¹⁹

While reviewing a consecutive series of breast AdCCs, we encountered a tumor composed of basaloid cells, initially diagnosed as a solid-variant of breast AdCC with basaloid features, which displayed a histologic appearance reminiscent of that of a cylindroma. Given the ambiguous histologic features displayed by this case, we sought to define whether a combination of reverse transcription PCR (RT-PCR), fluorescence *in situ* hybridization (FISH) and genomic analysis would assist in the differentiation between these two lesions and define the most accurate classification for this case, and to provide the first whole-exome sequencing analysis of a breast cylindroma.

MATERIAL AND METHODS

Case report

A 66-year old asymptomatic female patient underwent mammography screening, which revealed an irregular dense shadow involving a wide area of the upper outer quadrant of the right breast in the background of increased parenchymal density (Figure 1). Further ultrasonographic evaluation revealed a suspicious hypoechoic lesion measuring 0.9 cm, >6cm away from the nipple (Figure 1). No other relevant clinical conditions were present. A core biopsy was performed at the Montpellier Cancer Institute and a diagnosis of low-grade ER-negative, PR-negative and HER2-negative breast cancer consistent with an AdCC was rendered. Immunohistochemical analysis revealed expression of smooth muscle actin in the cells in the periphery of the cell nests, lack of vimentin expression in the neoplastic cells and strong AE1/AE3 cytokeratin expression in the larger cells located in the central aspects of the cell nests. The patient underwent a quadrantectomy with sentinel lymph node excision and a diagnosis of AdCC with clear margins and negative sentinel lymph node was made. No adjuvant therapy was performed. After 37 months of follow-up the patient is alive and free of disease (Table 1).

Samples, histopathologic and immunohistochemical analysis

All diagnostic blocks and slides were reviewed centrally by three pathologists (NF, AV-S & JSR-F). After obtaining approval from the Institutional Review Boards of the author's institution and obtaining written consent from the patient, 4µm-thick sections from a representative formalin-fixed paraffin-embedded (FFPE) tissue block of the case were subjected to periodic acid-Schiff (PAS) staining and to immunohistochemical analysis using antibodies against ER, PR, Ki67, HER2, cytokeratin (CK) 7, p63, c-KIT and MYB as previously described (Supplementary Table 1).^{5,19,20} Positive and negative controls were included in each slide run. ER, PR, and HER2 status were assessed following the American Society of Clinical Oncology/College of American Pathologists guidelines.^{21,22} The Ki67 index was assessed according to the recommendations of the International Ki67 in Breast Cancer working group.²³ All sections subjected to immunohistochemical analyses were independently analyzed by two of the authors (NF, JSR-F); discordant results were resolved on a multi-headed microscope.

Fluorescence *in situ* hybridization (FISH)

FISH was performed using a three-color probe mix consisting of BACs for 5' *MYB* (RP11-614H6, RP11-104D9; green), 3' *MYB* (RP11-323N12, RP11-1060C14; orange) and 3' *NFIB* (RP11-413D24, RP11-589C16; red) using validated protocols established at the Memorial Sloan Kettering Cancer Center Molecular Cytogenetics Core as previously described.²⁴ At least 50 non-overlapping, interphase nuclei of morphologically unequivocal neoplastic cells were analyzed as previously described,²⁵ and a case was considered positive if >15% of cells displayed at least one 5' *MYB*-3' *NFIB* fusion signal.²⁴

DNA and RNA extraction

Primary tumor and matched normal breast tissue samples were snap-frozen into liquid nitrogen immediately after surgical resection and stored at -80°C. Sections stained with hematoxylin and eosin were prepared, and reviewed by a dedicated breast pathologist who estimated 75% or greater tumor purity. DNA was extracted separately from the tumor and matched normal tissue using the DNeasy Blood & Tissue Kit (Qiagen) and concentration was measured using the Qubit Fluorometer assay (Life Technologies). RNA was extracted from representative tumor blocks using TRIzol as previously described.⁵

Reverse transcription PCR (RT-PCR)

The presence of the *MYB-NFIB* fusion transcript was investigated by RT-PCR as previously described (Supplementary Methods, Supplementary Table 2).^{5,19}

Whole-exome sequencing

Matched DNA from tumor and normal tissues were subjected to whole-exome capture (SureSelect Human All Exon, Agilent Technologies) and sequencing as previously described.²⁶ Sixty million 74-bp paired-end reads were generated on an Illumina HiSeq 2000 instrument for both tumor and germline DNA, achieving mean target coverage of 48x and 46x, respectively. Whole-exome sequencing data analysis for the detection of single nucleotide variants and insertions and deletions was performed as previously described²⁶ and detailed in the Supplementary Methods. To determine the presence of LOH of each mutated locus, the results obtained with the R package exomeCNV were employed and further manually curated, and copy number analysis was performed using Control-FREEC.^{27,28} To infer the tumor purity and ploidy, and the clonal heterogeneity of somatic mutations, we employed ABSOLUTE.²⁹ Whole-exome sequencing data have been deposited in the Sequence Read Archive (SRA) under accession number SRP052995. The *CYLD* mutation identified was subsequently validated by Sanger sequencing (Supplementary Methods).²⁶

RESULTS

Histopathology and immunohistochemistry

Histopathologic review of the core biopsy and the excision specimen revealed a non-encapsulated but relatively well demarcated lesion with irregular borders composed of nests and trabeculae of tumor cells arranged in a jigsaw puzzle pattern (Figure 2). The tumor nests

and trabeculae were surrounded by thick eosinophilic PAS-positive hyaline bands, and composed of two cell populations, basaloid cells with scanty cytoplasm and hyperchromatic nuclei, found preferentially at the periphery of nests and in trabecular areas, and larger cells with pale cytoplasm and more vesicular nuclei with finely granular chromatin, which were more frequent in the central areas of nests (Figures 2 and 3). Nuclear atypia was not observed. Duct-like structures filled up with eosinophilic material were found within nests, sometimes conferring a cribriform-like appearance (Figure 3). Mitotic figures were scarce (1/ 10 high power fields) and cytological atypia was unremarkable. Immunohistochemically, the lesion displayed a triple-negative phenotype (ER-, PR-, and HER2-negative) with a Ki67 labeling index of 3% (Figure 3). Analysis of CK7, vimentin and p63 expression confirmed the presence of the double population of neoplastic luminal/epithelial and basal/myoepithelial elements, respectively. c-KIT (CD117), which is often diffusely positive in AdCCs,³⁰ was expressed in <1% of tumor cells, whereas MYB expression was restricted to 5% of the cells (Figure 3).

Molecular characterization

Consistent with the limited MYB protein expression in tumor cells, FISH with *MYB-NFIB* probes and RT-PCR revealed the absence of the *MYB-NFIB* fusion gene (Figure 4). Whole-exome sequencing revealed a remarkably simple genome, with 17 somatic single nucleotide variants (SNVs) and no somatic small insertions and deletions (Figure 5, Supplementary Table 3). Using a combination of mutation function predictors with a high negative predictive value, 10 of those SNVs were considered to be passengers. Of the potential driver genetic alterations, five were clonal (i.e. present in all tumor cells); of these mutations, only one likely pathogenic splice-site mutation affected a *bona fide* cancer gene, *CYLD*, which was confirmed by Sanger sequencing (Supplementary Figure 1). This mutation involved intron 11 (c.1890+2 T>C) with a clonal frequency of 100% of the neoplastic cells sequenced. Allelic specific copy number analysis revealed LOH of the wild-type allele of *CYLD* in the form of a large deletion of 16q encompassing the *CYLD* locus (Figure 5, Supplementary Table 3).

Clonality analysis performed using ABSOLUTE²⁹ suggested that this tumor was composed of genetically distinct clones. The modal clone was characterized by the splice-site mutation affecting *CYLD*, and four additional potentially pathogenic somatic mutations, affecting *SLIT1*, *ZBTB4*, *LRP2* and *XDH* (Figure 5, Supplementary Table 3), none of which were found to be recurrently mutated in salivary gland AdCCs.^{24,31} Of the subclonal mutations identified, only the mutations affecting *SERPINE2* and *CDC27* were considered as potentially pathogenic, and only the latter affected a known cancer gene.

Based on the histologic features, absence of the *MYB-NFIB* fusion gene, lack of diffuse c-KIT expression, which is characteristic of breast AdCCs,³⁰ and presence of a somatic pathogenic mutation affecting *CYLD* coupled with LOH of the wild-type allele, a diagnosis of breast cylindroma was rendered.

DISCUSSION

Here we report on the potential use of an integrative approach, combining histopathology, immunohistochemistry, RT-PCR, FISH and sequencing methods to assist in the diagnosis of salivary gland-like and adnexal lesions of the breast. The information provided by the immunohistochemical analysis (i.e. lack of MYB and c-KIT expression), RT-PCR and FISH (i.e. lack of the *MYB-NFIB* fusion gene) and the presence of a *CYLD* splice-site mutation identified by whole-exome sequencing resulted in a diagnostic change from solid-basaloid variant of AdCC into cylindroma of the breast. This distinction is not a mere academic exercise, as the former is a low-malignant potential tumor associated with lymph node metastasis and rare distant relapses³, whereas the latter is a benign lesion with an excellent prognosis.¹ It should be noted, however, that the lack of diffuse c-KIT expression should favor a diagnosis of breast cylindroma in a lesion with the histologic features observed in this case. Given the challenging nature of the differential diagnosis between a solid-basaloid AdCC and a breast cylindroma in particular in core biopsies, ancillary studies, including c-KIT immunohistochemistry and potentially *CYLD* sequencing, are of potential diagnostic value.

Cylindromas are slow-growing benign tumors that only very rarely originate in the breast. Only 11 cases have been previously reported in the English literature.^{11–15} (Table 1) Although this study is the first comprehensive description of the landscape of somatic mutations in a cylindroma, previous studies have demonstrated that these tumors harbor germline and/or somatic mutations of *CYLD*.^{13,17,18} The *CYLD* gene encodes a tumor suppressor protein that plays several roles in cell signaling, through inhibition of the NF-κB and the JNK signaling pathways.³² Pathogenic germline *CYLD* mutations cause the Brooke-Spiegler and familial cylindromatosis syndromes,^{17,33} whereas the *CYLD* gene has also been implicated in the pathogenesis of sporadic cylindromas, based on the observations that somatic mutations and/or somatic LOH occur at the *CYLD* locus in both sporadic and familial dermal cylindromas.^{13,17,18} Importantly, *CYLD* somatic mutations have been shown to be absent in AdCCs in two independent studies (n=34³⁴ and n=60²⁴). In one out of 24 AdCCs reported by Stephens et al., however, a *CYLD* frameshift mutation was found;³¹ this case lacked the *MYB-NFIB* fusion gene,³¹ raising the possibility that that case might actually be a cylindroma. In fact, the reported low frequency of the *MYB-NFIB* fusion gene in solid-basaloid breast AdCCs³ may reflect the inclusion of lesions that would be best classified as breast cylindromas. Furthermore, the four additional genes affected by clonal and potentially pathogenic mutations in the present case, namely *LRP2*, *SLIT1*, *XDH* and *ZBTB4*, are either not mutated (i.e. *SLIT1*, *XDH* and *ZBTB4*) in AdCCs or mutated in a single case (i.e. *LRP2*) out of 84 AdCCs whose whole exomes have been reported to date.^{24,31}

Here we provide the first complete whole-exome sequencing analysis of a breast cylindroma, which revealed a rather simple pattern of somatic gene copy number alterations and a limited number of potentially pathogenic mutations, one of which affected a known cancer gene (i.e. *CYLD*). This pathogenic splice-site *CYLD* somatic mutation was found in conjunction with LOH of the *CYLD* wild-type allele in the form of a large 16q loss, and likely constituted a founder genetic event of this tumor, given that ABSOLUTE²⁹ analysis demonstrated that this somatic mutation was clonal (i.e. present in all tumor cells analyzed).

ABSOLUTE²⁹ also revealed the surprising observation that this breast cylindroma displayed intra-tumor genetic heterogeneity, with two potentially pathogenic mutations in *CDC27* and *SERPINE2* being present in minor subclones of the lesion. Further studies to characterize the repertoire of somatic mutations in cylindromas and to define their driver genetic alterations in addition to *CYLD* mutations are warranted.

This study illustrates the impact of combining pathology and genetics, where a rare form of triple-negative breast cancer could be differentiated from a benign adnexal-type tumor. Although careful histologic and immunohistochemical analysis can resolve the differential diagnosis between solid AdCC and breast cylindroma, with the availability of comprehensive analyses of the genomic landscapes of human cancers, genetics will undoubtedly play a role in helping resolve the diagnosis of lesions with ambiguous diagnostic features. This will only be possible if optimal annotation of the samples being subjected to genomics analysis is available, emphasizing the importance of a multi-disciplinary approach in this era of precision medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

SP is funded by a Susan G Komen Postdoctoral Fellowship Grant (PDF14298348). Research reported in this publication was supported in part by the Cancer Center Support Grant of the National Institutes of Health/National Cancer Institute under award number P30CA008748. The content of this study is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

REFERENCES

1. Lakhani, SR.; Ellis, IO.; Schnitt, SJ.; Tan, PH.; van de Vijver, MJ. Who classification of tumours of the breast. 4th ed.. Lyon: IARC press; 2012.
2. Foschini MP, Eusebi V. Rare (new) entities of the breast and medullary carcinoma. Pathology. 2009; 41:48–56. [PubMed: 19089740]
3. D'Alfonso TM, Mosquera JM, MacDonald TY, et al. Myb-nfib gene fusion in adenoid cystic carcinoma of the breast with special focus paid to the solid variant with basaloid features. Hum Pathol. 2014; 45:2270–2280. [PubMed: 25217885]
4. Marchio C, Weigelt B, Reis-Filho JS. Adenoid cystic carcinomas of the breast and salivary glands (or 'the strange case of dr jekyll and mr hyde' of exocrine gland carcinomas). J Clin Pathol. 2010; 63:220–228. [PubMed: 20203221]
5. Wetterskog D, Lopez-Garcia MA, Lambros MB, et al. Adenoid cystic carcinomas constitute a genomically distinct subgroup of triple-negative and basal-like breast cancers. J Pathol. 2012; 226:84–96. [PubMed: 22015727]
6. Wetterskog D, Wilkerson PM, Rodrigues DN, et al. Mutation profiling of adenoid cystic carcinomas from multiple anatomical sites identifies mutations in the ras pathway, but no kit mutations. Histopathology. 2013; 62:543–550. [PubMed: 23398044]
7. Shin SJ, Rosen PP. Solid variant of mammary adenoid cystic carcinoma with basaloid features: A study of nine cases. Am J Surg Pathol. 2002; 26:413–420. [PubMed: 11914618]
8. Brill LB 2nd, Kanner WA, Fehr A, et al. Analysis of myb expression and myb-nfib gene fusions in adenoid cystic carcinoma and other salivary neoplasms. Mod Pathol. 2011; 24:1169–1176. [PubMed: 21572406]

9. Persson M, Andren Y, Mark J, Horlings HM, Persson F, Stenman G. Recurrent fusion of myb and nfib transcription factor genes in carcinomas of the breast and head and neck. *Proc Natl Acad Sci U S A*. 2009; 106:18740–18744. [PubMed: 19841262]
10. Gokaslan ST, Carlile B, Dudak M, Albores-Saavedra J. Solitary cylindroma (dermal analog tumor) of the breast: A previously undescribed neoplasm at this site. *Am J Surg Pathol*. 2001; 25:823–826. [PubMed: 11395563]
11. Nonaka D, Rosai J, Spagnolo D, Fiaccavento S, Bisceglia M. Cylindroma of the breast of skin adnexal type: A study of 4 cases. *Am J Surg Pathol*. 2004; 28:1070–1075. [PubMed: 15252315]
12. Wang N, Leeming R, Abdul-Karim FW. Fine needle aspiration cytology of breast cylindroma in a woman with familial cylindromatosis: A case report. *Acta Cytol*. 2004; 48:853–858. [PubMed: 15581173]
13. Albores-Saavedra J, Heard SC, McLaren B, Kamino H, Witkiewicz AK. Cylindroma (dermal analog tumor) of the breast: A comparison with cylindroma of the skin and adenoid cystic carcinoma of the breast. *Am J Clin Pathol*. 2005; 123:866–873. [PubMed: 15899777]
14. Mahmoud A, Hill DH, O'Sullivan MJ, Bennett MW. Cylindroma of the breast: A case report and review of the literature. *Diagn Pathol*. 2009; 4:30. [PubMed: 19725978]
15. Aggarwal R, Gupta O, Yadav YK, Dogra S. Cylindroma of the breast: A rare case report. *Int J Trichology*. 2013; 5:83–85. [PubMed: 24403771]
16. LeBoit, PE.; Burg, G.; Weedon, D.; Sarasain, A. World health organization classification of tumours. Pathology and genetics of skin tumours. Lyon: IARC Press; 2006.
17. Bignell GR, Warren W, Seal S, et al. Identification of the familial cylindromatosis tumour-suppressor gene. *Nat Genet*. 2000; 25:160–165. [PubMed: 10835629]
18. Leonard N, Chaggar R, Jones C, Takahashi M, Nikitopoulou A, Lakhani SR. Loss of heterozygosity at cylindromatosis gene locus, cyld, in sporadic skin adnexal tumours. *J Clin Pathol*. 2001; 54:689–692. [PubMed: 11533075]
19. Fehr A, Kovacs A, Loning T, Frierson H Jr, van den Oord J, Stenman G. The myb-nfib gene fusion-a novel genetic link between adenoid cystic carcinoma and dermal cylindroma. *J Pathol*. 2011; 224:322–327. [PubMed: 21618541]
20. Weigelt B, Horlings HM, Kreike B, et al. Refinement of breast cancer classification by molecular characterization of histological special types. *J Pathol*. 2008; 216:141–150. [PubMed: 18720457]
21. Hammond ME, Hayes DF, Dowsett M, et al. American society of clinical oncology/college of american pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol*. 2010; 28:2784–2795. [PubMed: 20404251]
22. Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American society of clinical oncology/college of american pathologists clinical practice guideline update. *J Clin Oncol*. 2013; 31:3997–4013. [PubMed: 24101045]
23. Dowsett M, Nielsen TO, A'Hern R, et al. Assessment of ki67 in breast cancer: Recommendations from the international ki67 in breast cancer working group. *J Natl Cancer Inst*. 2011; 103:1656–1664. [PubMed: 21960707]
24. Ho AS, Kannan K, Roy DM, et al. The mutational landscape of adenoid cystic carcinoma. *Nat Genet*. 2013; 45:791–798. [PubMed: 23685749]
25. Lambros MB, Tan DS, Jones RL, et al. Genomic profile of a secretory breast cancer with an etv6-ntrk3 duplication. *J Clin Pathol*. 2009; 62:604–612. [PubMed: 19561229]
26. Weinreb I, Piscuoglio S, Martelotto LG, et al. Hotspot activating prkd1 somatic mutations in polymorphous low-grade adenocarcinomas of the salivary glands. *Nat Genet*. 2014; 46:1166–1169. [PubMed: 25240283]
27. Sathirapongsasuti JF, Lee H, Horst BA, et al. Exome sequencing-based copy-number variation and loss of heterozygosity detection: Exomecnv. *Bioinformatics*. 2011; 27:2648–2654. [PubMed: 21828086]
28. Boeva V, Popova T, Bleakley K, et al. Control-freec: A tool for assessing copy number and allelic content using next-generation sequencing data. *Bioinformatics*. 2012; 28:423–425. [PubMed: 22155870]

29. Carter SL, Cibulskis K, Helman E, et al. Absolute quantification of somatic DNA alterations in human cancer. *Nat Biotechnol.* 2012; 30:413–421. [PubMed: 22544022]
30. Crisi GM, Marconi SA, Makari-Judson G, Goulart RA. Expression of c-kit in adenoid cystic carcinoma of the breast. *Am J Clin Pathol.* 2005; 124:733–739. [PubMed: 16203286]
31. Stephens PJ, Davies HR, Mitani Y, et al. Whole exome sequencing of adenoid cystic carcinoma. *J Clin Invest.* 2013; 123:2965–2968. [PubMed: 23778141]
32. Massoumi R, Paus R. Cyldromatosis and the cyld gene: New lessons on the molecular principles of epithelial growth control. *Bioessays.* 2007; 29:1203–1214. [PubMed: 18008375]
33. van den Ouweland AM, Elfferich P, Lamping R, et al. Identification of a large rearrangement in cyld as a cause of familial cylindromatosis. *Fam Cancer.* 2011; 10:127–132. [PubMed: 20972631]
34. Daa T, Nakamura I, Yada N, et al. Plag1 and cyld do not play a role in the tumorigenesis of adenoid cystic carcinoma. *Mol Med Rep.* 2013; 7:1086–1090. [PubMed: 23404581]
35. Futreal PA, Coin L, Marshall M, et al. A census of human cancer genes. *Nat Rev Cancer.* 2004; 4:177–183. [PubMed: 14993899]
36. Kandoth C, McLellan MD, Vandin F, et al. Mutational landscape and significance across 12 major cancer types. *Nature.* 2013; 502:333–339. [PubMed: 24132290]
37. Lawrence MS, Stojanov P, Mermel CH, et al. Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature.* 2014; 505:495–501. [PubMed: 24390350]

Figure 1. Imaging findings

A, Mammogram images displays an irregular dense shadow in the upper outer quadrant with increased parenchymal density. **B**, Corresponding ultrasound image of the hypoechoic mass, measuring 0.9 cm.

Figure 2. Histologic features of the breast cylindroma

Scanning magnification (**A**) revealed a relatively well demarcated lesion in the breast tissue, with lobulated, irregular borders, composed of epithelial lobules and nests of various shapes and sizes arranged in a 'jigsaw puzzle' pattern (**B**, **C**). Note the presence of non-affected breast terminal duct-lobular units in the lower left corner of **B**. The epithelial nests were surrounded by a rim of hyaline eosinophilic material, and composed of a dual population of cells: small basaloid cells with limited cytoplasm and hyperchromatic nuclei, and larger cells with pale cytoplasm and vesicular nuclei (**D**, **E** and **F**). Note the lack of nuclear atypia, and presence of thick hyaline bands surrounding the cell nests (**E**, **F**) and the occasional hyaline globules within the neoplastic cell nests (**E**, **F**). Scale bar: **A**, 4 mm; **B**, 1mm; **C**, 600 μ m; **D**, 200 μ m; **E** and **F**, 100 μ m.

Figure 3. Histochemical and immunohistochemical features of the breast cylindroma

A, The tumor nests and trabeculae were surrounded by thick eosinophilic periodic acid-Schiff (PAS)-positive hyaline bands. The PAS-positive hyaline eosinophilic material was found inside some nests forming pseudo-glandular spaces. **B**, Weak-to-moderate expression of MYB was observed in 5% of the cells. **C**, The large neoplastic cells in the central aspects of the tumor nests and lobules expressed cyokeratin 7. **D**, p63 was expressed in the small, basaloid cells and to a lesser extent in the large cells in the central areas of the neoplastic nests and lobules. **E**, c-KIT was expressed in the membranes of <1% of the neoplastic cells,

and its expression was restricted to the large cells in the central aspects of the nests and lobules. **F**, A low Ki67 labeling index (3%) was observed. Original magnification x40 **A-F**.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 4. Fluorescence *in situ* hybridization (FISH) and reverse transcription PCR (RT-PCR) analysis of the *MYB-NFIB* fusion gene

A, FISH analysis using a three-color *MYB-NFIB* probes, with 5' *MYB* (green), 3' *MYB* (orange) and 3' *NFIB* (red). Inset: A previously tested adenoid cystic carcinoma of the breast was used as positive control. **B**, RT-PCR analysis. A previously tested adenoid cystic carcinoma of the breast was used as positive control. Primer set 1, *MYB* exon 14 – *NFIB* exon 8c; primer set 2, *MYB* exon 14 - *NFIB* exon 9. The *MYB-NFIB* fusion gene was not detected in the breast cylindroma.

Figure 5. Mutational and gene copy number profiles of the breast cylindroma

A, List of somatic single nucleotide variants identified. The mutation clonal frequency was determined using ABSOLUTE. Mutations considered by both MutationTaster and CHASM (breast) as non-deleterious/passengers were considered as passenger genetic alterations. Genes included in the Cancer Gene Census,³⁵ Kandoth et al. (127 significantly mutated genes),³⁶ and Lawrence et al. (Cancer5000-S set)³⁷ were considered as ‘cancer genes’. **B**, Analysis of the gene copy number alterations found in the cylindroma demonstrated the presence of a large heterozygous deletion involving most of 16q, encompassing the *CYLD* gene locus. In this genome plot, the copy number states are plotted on the y-axis according to the genomic positions on the x-axis. Red denotes regions of copy number loss. **B inset**, Chromosome 16 plot, where the copy number states are plotted on the y-axis according to the genomic positions on the x-axis. Note that the *CYLD* gene locus is encompassed by the loss of 16q (arrow).

Table 1

Cylindromas of the breast reported in the English literature.

Reference	N	Age (years)	Gender	Tumor size (cm)	Synchronous carcinoma	Clinic	Follow-up (months)	Distant metastasis	Familial cylindromatosis
Aggarwal et al., Int J Trichol 2013 ¹⁵	1	61	F	2	No	Palpable nodule	12	No	No
Mahmoud et al., Diagn Pathol 2009 ¹⁴	1	62	F	1.6	No	Incidental finding	6	No	No
Wang et al., Acta Cytol 2004 ¹²	1	59	F	NA	No	Incidental finding	NA	NA	No
Nonaka et al., Am J Surg Pathol 2004 ¹¹	1	79	F	1.2	No	Palpable nodule	30	No	No
Nonaka et al., Am J Surg Pathol 2004 ¹¹	1	62	F	1.1	No	Incidental finding	6	No	No
Nonaka et al., Am J Surg Pathol 2004 ¹¹	1	85	F	1.3	Yes (IDC, DCIS)	Incidental finding	NA	NA	No
Nonaka et al., Am J Surg Pathol 2004 ¹¹	1	37	F	0.7	No	Palpable nodule	192	No	Yes
Gokaslan et al., Am J Surg Pathol - 2001 ¹⁰ , Albores-Saavedra et al. - Am J Clin Pathol - 2005 ¹³	4	63-78	F	0.8-1.3	Yes (ILC LN+)	Incidental finding	NA	No	No
Present case	1	66	F	1.1	No	Incidental finding	37	No	No

DCIS, ductal carcinoma *in situ*; F, female; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; LN+, lymph node positive; M, male; N, number of patients; NA, not available.