Apocrine carcinoma of the breast: A brief update on the molecular features and targetable biomarkers

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ABSTRACT

Apocrine carcinoma of the breast is a rare, primary breast cancer characterized by the apocrine morphology, estrogen receptor-negative and androgen receptor-positive profile with a frequent overexpression of Her-2/neu protein (~30%). Apart from the Her-2/neu target, advanced and/or metastatic apocrine carcinomas have limited treatment options. In this review, we briefly describe and discuss the molecular features and new theranostic biomarkers for this rare mammary malignancy. The importance of comprehensive profiling is highlighted due to synergistic and potentially antagonistic molecular events in the individual patients.

KEY WORDS: Breast cancer; special types; apocrine carcinoma; androgen receptor; biomarkers; targeted therapy
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INTRODUCTION

Invasive apocrine carcinoma of the breast is a rare primary breast cancer characterized by the apocrine cells with abundant eosinophilic and granular cytoplasm, centrally to eccentrically located nuclei with prominent nucleoli and distinctive cell borders [1] (Figure 1A). Mammary apocrine epithelium has a characteristic steroid receptor profile that is negative for full length estrogen receptor-alpha (ER) and progesterone receptor (PR) and is androgen receptor (AR) positive [2,3]. The presence of apocrine cells in more than 90% of the tumor population defines invasive apocrine carcinoma, but it is necessary to have the characteristic immunohistochemical (IHC) profile of steroid receptors (ER-/PR-/AR+) to qualify as "pure" apocrine carcinoma (PAC) [3-5] (Figures 1B-D). Such strictly defined PAC constitutes < 1% of all breast cancers [6].

At the molecular level, PAC is characterized by detectable mRNA transcript of estrogen receptor, but only the alternatively spliced variant ER-36alpha (ER-36α) can be detected at the protein level (IHC) [7,8]. Progression of neoplastic disease from apocrine hyperplasia, apocrine ductal carcinoma *in situ* (DCIS) to invasive apocrine carcinoma had been proposed and investigated for genetic changes using a microdissection approach [9]. Array comparative genomic hybridization (aCGH) and next-generation sequencing (NGS) revealed accumulation of chromosomal losses and gains (particularly along the chromosome 17q) as well as tumor suppressor/oncogene mutations during the phases of progression [5,9].

Activation of the AR signaling pathway is a prominent feature present in apocrine lesions of the breast including apocrine DCIS and invasive apocrine carcinoma [2]. AR upregulation is mediated through forkhead box A1 (FoxA1) and human epidermal growth factor receptor 2 (HER2) activities [10]. Prolactin-induced protein (or GCDFP-15) is also actively regulated by the AR/extracellular signal-regulated kinase (ERK) feedback loop in apocrine cells [11,12]. Based on these data, targeting of the androgen receptor with androgen-targeting therapies, and potential combination strategies based on additional genomic alterations have been under investigation in clinical trials for triple-negative breast carcinomas, including PAC.

"Molecular apocrine" carcinomas exhibit prognostically poor gene signature (using validated qRT-PCR signature) with a high-risk recurrence score and a poor prognosis [13]. Comprehensive cancer profiling of PAC reveals numerous, yet individualized therapeutic options. *PIK3CA/PTEN/AKT* and *TP53* alterations are most common in apocrine carcinoma [5,9,13,14]. Deregulation of the mitogen-activated protein kinase (MAPK) pathway components (*KRAS*, *NRAS*,

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BRAF) is less frequent [5,9] (Table 1). AR expression presents an opportunity for the treatment with androgen targeting therapies seemingly in all cases [6,15-17]. The presence of *PIK3CA* mutations and androgen overexpression may confer sensitivity to the combination of phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) and AR inhibitors [18]. *HER2* amplification and over-expression are seen in ~30% of tumors, while *EGFR* gene is amplified in a small (6%) percentage of cases despite the common epidermal growth factor receptor (EGFR) protein expression [3,5,6,13]. These two receptors may be targeted with monoclonal antibodies; however the high frequency of *PIK3CA* mutation may contribute to attenuated responses [19], and a comprehensive sample profiling (e.g. IHC for receptors and sequencing for mutations) is needed to provide optimal personalized therapy.

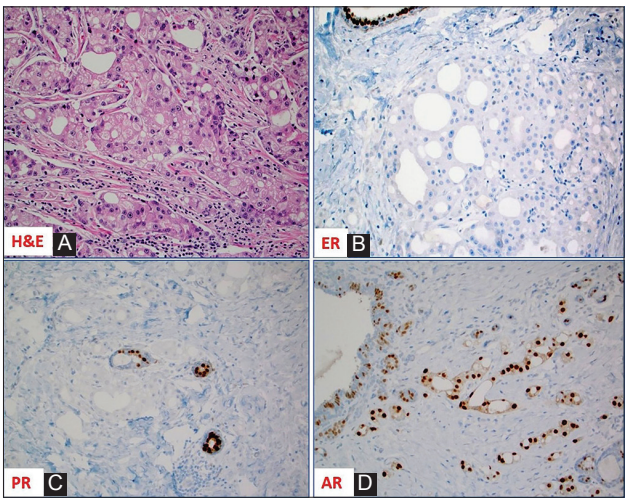

FIGURE 1. (A) Hematoxylin and Eosin slide of a case of invasive mammary carcinoma with apocrine morphology; The apocrine cells are negative for estrogen receptor [ER] (B) and progesterone receptor [PR] (C) but positive for androgen receptor [AR] (D); note the presence of ER and PR expression in adjacent normal ducts.

Biomarkers of classic chemotherapy that are variably expressed in individual cases of PAC (and the agents used to target them) include topoisomerase 2 alpha (TOPO2A) [anthracyclines], excision repair cross-complementation group 1 protein (ERCC1) [platinum drugs], ribonucleotide reductase catalytic subunit M1 (RRM1) [gemcitabine], transducin like enhancer of split 3 (TLE3) [taxanes], and thymidylate synthase (TS) [antifolates] (Table 1). Recently described immune therapies based on immune checkpoint inhibitors have not been described specifically for apocrine carcinomas, which in our limited experience do not express programmed death-ligand 1 (PD-L1) (0/8; using antibody clone SP142), in contrast

TABLE 1. A summary of the biomarker profiling using a multiplatform approach (immunohistochemistry, *in situ* hybridization and next-generation sequencing) [data from the Caris Life Science commercial laboratory, September 2016]

	Biomarker (n)	% Positive (↑) or % Negative (↓) Ratio	Therapy
NCI-Compendium Therapies	↑HER2 (n=23)	22	Anti-HER2 therapy (trastuzumab)
	↑TLE3 (n=16)	38	Taxanes (paclitaxel, docetaxel, nab-paclitaxel)
	↓TUBB3 (n=8)	100	
	↓PGP (n=12)	88	
	↑SPARC (n=15)	47	
	↓ERCC1 (n=14)	50	Platinum compounds (cisplatin)
	↑TOP2A (n=13)	38	Anthracyclines (doxorubicin)
	↑TOPO1 (n=20)	45	Topoisomerase inhibitors (irinotecan)
	↓TS (n=22)	68	Pyrimidine antagonists (capecitabine)
	↓ RRM1 (21)	81	Antimetabolites (gemcitabine)
Targets for Clinical Trials	↑AR (n=23)	91	Androgen-blockade (abiraterone)
	↑PD-1 TILs (n=4)	50	Immune checkpoint therapies
	↑PD-L1 (n=7)	0	
	↓PTEN (n=23)	65	Attenuator of anti-HER2 therapy/PI3K-beta inhibitors (clinical trials: GSK2636771)
	↑cMET (n=8)	25	cMET inhibitors
	↑EGFR_IHC (n=5)	100	Anti-HER therapy (trials)
	*EGFR*_ISH (n=3)	33	
NGS	*AKT* (n=6)	17	PIK3CA pathway inhibitors (trials)
	PIK3CA (n=12)	50	Attenuators of anti-HER2 therapy/ PIK3CA pathway inhibitors (trials)
	PTEN (n=8)	25	
	HRAS (n=7)	14	MEK inhibitors (trials)
	TP53 (n=8)	63	Not targetable

ISH=*In-situ* hybridization; IHC=Immunohistochemistry; NGS=Next-generation sequencing, NCI=National Cancer Institute. ↑ Indicates upregulation/high expression; ↓ indicates downregulation/low expression; AR=Androgen receptor; TOPO1 & 2=Topoisomerases 1 and 2; TS=Thymidylate synthase; TLE3=Transducin-like enhancer of split 3; RRM1=Ribonucleotide reductase M1; SPARC=Osteonectin; TUBB3=Tubulin beta-3 chain; PGP=P-glycoprotein; ERCC1=Excision repair cross-complementation group 1; SPARC=Secreted protein acidic and cysteine rich; EGFR(HER1) = Epidermal growth factor receptor; HER2=Human epidermal growth factor receptor 2; cMET=Hepatocyte growth factor receptor; PD-1=Programmed cell death protein 1; PD-L1=Programmed death-ligand 1; AKT=AKT Serine/Threonine kinase 1; PTEN=Phosphatase and tensin homolog; PIK3CA=Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; MEK=Mitogen-activated protein kinase kinase, MAPK/ERK pathway; HRAS: Transforming protein p21; TP53: Tumor suppressor p53; TILs=tumor-infiltrating lymphocytes

to classic triple-negative breast cancers, which are more frequently PD-L1 positive (9-59%) [20,21] (Table 1).

In summary, apocrine carcinoma is a rare, distinct subtype of breast cancer with consistent AR overexpression/full length ER-α loss, frequent deregulation of the PIK3CA/phosphatase and tensin homolog (PTEN) pathway and HER2 activation, which along with the biomarkers of conventional chemotherapy may tailor optimal treatment modalities for the patients with advanced and/or metastatic apocrine carcinoma. Targeted therapy based on immune check point inhibitors needs additional investigations as preliminary results show lack of PD-L1 expression in this cancer type.

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DECLARATION OF INTERESTS

Zoran Gatalica is an employee of Caris Life Sciences; Semir Vranic and Rebecca Feldman declare no conflict of interest.

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