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Integrative genomic and transcriptomic characterization of papillary carcinomas of the breast

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ABSTRACT

Papillary carcinoma (PC) is a rare type of breast cancer, which comprises three histologic subtypes: encapsulated PC (EPC), solid PC (SPC) and invasive PC (IPC). Microarray-based gene expression and Affymetrix SNP 6.0 gene copy number profiling, and RNA-sequencing revealed that PCs are luminal breast cancers that display transcriptomic profiles distinct from those of grade- and estrogen receptor (ER)-matched invasive ductal carcinomas of no special type (IDC-NSTs), and that the papillary histologic pattern is unlikely to be underpinned by a highly recurrent expressed fusion gene or a highly recurrent expressed mutation. Despite displaying similar patterns of gene copy number alterations, significant differences in the transcriptomic profiles of EPCs, SPCs and IPCs were found, and may account for their different histologic features.

1. Introduction

Breast cancer is a heterogeneous disease comprising numerous distinct entities with different biological features and clinical behaviors (Reis-Filho and Pusztai, 2011; Weigelt and Reis-Filho, 2009). Invasive ductal carcinomas of no special type (IDC-NST), the commonest histologic type of breast cancer, have been shown to be heterogeneous at the

Abbreviations: PC, papillary carcinoma; EPC, encapsulated papillary carcinoma; SPC, solid papillary carcinoma; IPC, invasive papillary carcinoma.

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transcriptomic and genomic levels, and can be classified into several molecular subtypes (Cancer Genome Atlas, 2012; Reis-Filho and Pusztai, 2011). The 'intrinsic' gene classification is the most widely used molecular taxonomy for IDC-NSTs, and classifies breast cancers into luminal A, luminal B, HER2-enriched and basal-like subtypes (Parker et al., 2009; Perou et al., 2000). These subtypes have been shown not only to be associated with distinct clinical outcomes, but also with different risk factors, clinicopathologic features and repertoires of genetic aberrations (Cancer Genome Atlas, 2012; Parker et al., 2009; Reis-Filho and Pusztai, 2011).

The complexity and diversity of breast cancers have also been documented at the histologic level (Weigelt et al., 2010c; Weigelt and Reis-Filho, 2009), however this information has not been fully explored in molecular subtyping studies, which have primarily focused on IDC-NSTs. Based on the cytological and architectural features of invasive breast cancers, the World Health Organization (WHO) recognizes the existence of 21 special histologic subtypes in addition to IDC-NSTs (Lakhani et al., 2012). Genomic and transcriptomic analyses of special histologic types of breast cancer conducted by our group and others (Bertucci et al., 2008; Duprez et al., 2012; Geyer et al., 2010; Gruel et al., 2010; Horlings et al., 2013; Lacroix-Triki et al., 2010; Lopez-Garcia et al., 2010b; Marchio et al., 2009, 2008; Vincent-Salomon et al., 2007; Weigelt et al., 2009a, 2010b, 2008, 2009b; Wetterskog et al., 2012) have demonstrated that tumors from each of the special histologic types of breast cancer are more homogeneous amongst themselves than IDC-NSTs. In addition, some of the histologic special types have been shown to be driven by recurrent fusion genes resultant of chromosomal translocations. For example, secretory carcinomas of the breast are characterized by the t(12; 15) translocation, which results in the formation of the *ETV6-NTRK3* fusion gene (Tognon et al., 2002), and >90% of adenoid cystic carcinomas of the breast display the t(6; 9) translocation, which leads to the fusion of *MYB* with *NFIB* (Persson et al., 2009; Wetterskog et al., 2012).

Papillary carcinoma (PC) is a rare (<1%) special histologic type of breast cancer that often affects postmenopausal women and has an overall favorable outcome (Grabowski et al., 2008; Pal et al., 2010; Rakha et al., 2011; Solorzano et al., 2002; Weigelt et al., 2010a). PCs comprise a morphologically heterogeneous group of lesions, all of which share a growth pattern characterized by the presence of arborescent fibrovascular stalks lined by a layer of neoplastic epithelial cells devoid of an intervening myoepithelial cell layer, a feature that distinguishes them from benign intraductal papillomas and papillary carcinomas *in situ* (Collins and Schnitt, 2008; Hill and Yeh, 2005; Pal et al., 2010; Weigelt et al., 2010a). Papillary neoplasms of the breast include three histologic subtypes, namely encapsulated papillary carcinoma (EPC), solid papillary carcinoma (SPC) and invasive papillary carcinoma (IPC). EPC is a well-circumscribed lesion where the involved duct is surrounded by a thick fibrous capsule; in EPCs, the neoplastic cells are arranged in papillary fronds in the majority of cases, however areas with cribriform and/or solid patterns are not uncommonly found (Lakhani et al., 2012; Wynveen et al., 2011). SPC is also a well-circumscribed lesion that is densely cellular and composed of expansile nodules of epithelial cells, and IPC comprises papillary lesion with neoplastic cells arranged in finger-like projections with clear invasion into adjacent stroma. Although the classification of EPCs and SPCs as invasive or *in situ* disease still remains a matter of controversy, these tumors have the potential to disseminate to axillary lymph nodes and, albeit rarely, distant metastatic deposits of PCs have been documented (Rakha et al., 2011; Wynveen et al., 2011). Based on these observations, it has been proposed that EPCs and SPCs should be considered forms of invasive breast cancer with excellent outcome (Lakhani et al., 2012; Rakha et al., 2011; Wynveen et al., 2011).

Our group has previously demonstrated that PCs are preferentially estrogen receptor (ER) and progesterone receptor (PR) positive, lack *HER2* gene amplification, and display relatively simple genomes in terms of their repertoires of gene copy number aberrations (Duprez et al., 2012). In addition, the similarities in the gene copy number profiles of PCs and grade- and ER-matched IDC-NSTs have led us to suggest that PCs may be best positioned as part of the spectrum of ER-positive IDC-NSTs, rather than a distinct entity (Duprez et al., 2012). On the other hand, the transcriptomic characteristics of PCs and whether these tumors would differ from IDC-NSTs at the gene expression level remain to be determined. Therefore, the primary aims of this study were i) to investigate whether PCs would constitute a molecular entity distinct from grade- and ER-matched IDC-NSTs at the transcriptomic level, and ii) to define whether PCs would be driven by recurrent fusion genes or pathognomonic mutations. In addition, we carried out an exploratory, hypothesis-generating analysis to investigate whether EPCs, SPCs and IPCs would display distinct transcriptomic and genomic profiles.

2. Materials and methods

2.1. Samples

Nineteen PCs of the breast were retrieved from Institut Curie, Paris, France (from 1995 to 2009). In this study, we included PCs from patients diagnosed and managed in the above institution, whose tumors were <5 cm and who had no clinical and/or radiological evidence of distant metastases. Exclusion criteria were (a) patients with multiple tumors, either ipsi- or contra-lateral, (b) patients who received neoadjuvant chemotherapy, (c) patients for whom all histologic slides and blocks were not available for review, (d) tumors not consistent with the final diagnosis of EPC, SPC or IPC, and (e) tumors whose frozen samples contained <50% of tumor cell content. For sixteen cases, both DNA and RNA of sufficient quality and quantity were available for microarray-based gene expression and copy number profiling; for three cases only RNA could be extracted due to limited frozen tissue availability (Supplementary Table S1). Samples were anonymized prior to analysis and the study was approved by local research ethics committees of the authors' institutions. All cases were independently reviewed by two pathologists (AV-S and JSR-F), who subtyped the tumors into EPC, SPC and IPC following the WHO criteria (Lakhani et al., 2012), and graded the tumors according to the Nottingham grading system (Elston and Ellis, 1991). Histologic grade- and ER-matched IDC-NSTs, whose

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