MOLECULAR ONCOLOGY XXX (2015) 1–10

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Gene expression-based classifications of fibroadenomas and phyllodes tumours of the breast

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ARTICLE INFO

Article history:
Received 14 October 2014
Received in revised form
6 January 2015
Accepted 8 January 2015
Available online ■

Keywords:
Fibroepithelial
Fibroadenoma
Juvenile fibroadenoma
Phyllodes tumours
Gene expression
Intrinsic subtypes and claudin-low subtype

ABSTRACT

Fibroepithelial tumors (FTs) of the breast are a heterogeneous group of lesions ranging from fibroadenomas (FAD) to phyllodes tumors (PT) (benign, borderline, malignant). Further understanding of their molecular features and classification might be of clinical value. In this study, we analysed the expression of 105 breast cancer-related genes, including the 50 genes of the PAM50 intrinsic subtype predictor and 12 genes of the Claudin-low subtype predictor, in a panel of 75 FTs (34 FADs, 5 juvenile FADs, 20 benign PTs, 5 borderline PTs and 11 malignant PTs) with clinical follow-up. In addition, we compared the expression profiles of FTs with those of 14 normal breast tissues and 49 primary invasive ductal carcinomas (IDCs). Our results revealed that the levels of expression of all breast cancer-related genes can discriminate the various groups of FTs, together with normal breast tissues and IDCs (False Discovery Rate $< 5\%$). Among FTs, the levels expression of proliferation-related genes (e.g. CCNB1 and MKI67) and mesenchymal/epithelial-related (e.g. CLDN3 and EPCAM) genes were found to be most discriminative. As expected, FADs showed the highest and lowest expression of epithelial- and proliferation-related genes, respectively, whereas malignant PTs showed the opposite expression pattern. Interestingly, the overall profile of benign PTs was found more similar to FADs and normal breast tissues than the rest of tumours, including juvenile FADs. Within the dataset of IDCs and normal breast tissues, the vast majority of FADs, juvenile FADs, benign PTs and borderline PTs were identified as Normal-like by intrinsic breast cancer subtyping, whereas 7 (63.6%) and 3 (27.3%) malignant PTs were identified as Claudin-low and Basal-like, respectively. Finally, we observed that the previously described PAM50 risk of relapse prognostic score better predicted outcome in FTs than the morphological classification, even within PTs-only. Our results suggest that classification of FTs using gene expression-based data is feasible and might provide clinically useful biological and prognostic information.

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http://dx.doi.org/10.1016/j.molonc.2015.01.003

1. Introduction

Fibroepithelial tumours (FT) of the breast represent a heterogeneous group of biphasic neoplasms, composed of both epithelial and stromal components, that account for about 0.5–1 % of all breast tumours (Fattaneh, 2003; Reinfuss et al., 1996). To date, 3 main groups of FTs of the breast have been identified based on morphology: fibroadenoma (FAD), juvenile FAD and phyllodes tumour (PT). PTs are further subclassified into benign, borderline or malignant categories on the basis of a series of histological features such as stromal cellularity, nuclear atypia and mitotic activity (Contarini et al., 1982). However, reliable classification of FTs based on morphology remains challenging (Contarini et al., 1982; Hart et al., 1988; Niezabitowski et al., 2001; Yonemori et al., 2006).

From a clinical perspective, FADs may be safely followed without further investigation or treated with simple enucleation, whereas PTs are usually treated with mastectomy or wide excision with adequate margins. Although surgical resection is sufficient to cure the vast majority of PTs, PTs can recur locally and/or undergo metastatic spread. Indeed, local recurrence rate of PTs is 10%–18% with negative and positive resection margins, respectively, and 9–27% of malignant PTs metastasize to distant organs (Barrio et al., 2007; Kracht et al., 1998; Lester and Stout, 1954; Lindquist et al., 1982). However, reports of benign and borderline PTs metastasizing also exist (Kracht et al., 1998; Lester and Stout, 1954; Lindquist et al., 1982). Thus, there is a need for an accurate diagnosis and management of FTs of the breast (Jones et al., 2008a; Tan and Ellis, 2013).

Similar to FTs, invasive breast carcinoma is a heterogeneous disease with respect to molecular alterations, cellular compositions and clinical outcomes. Over the last decade, studies based on gene expression analysis have identified and extensively studied 5 major classes of breast cancer (Luminal A, Luminal B, HER2-enriched, Basal-like and Claudin-low) (Perou et al., 2000; Prat and Perou, 2011; TCGA, 2012a). Known as the “intrinsic subtypes of breast cancer”, these groups of tumours have revealed critical differences in incidence, survival, dissemination sites and response to treatment (Parker et al., 2009; Prat et al., 2014a; TCGA, 2012b). To date, it is unknown how the FTs are classified according to the biology of the intrinsic breast cancer subtypes.

Among the different intrinsic subtypes, the Claudin-low shows a stromal-like phenotype characterized by the low expression of many tight junction-related genes such as claudins −3, −4 and −7 and E-cadherin and high expression of mesenchymal-related genes such as vimentin or ZEB1 (Prat et al., 2013c, 2010). Clinically, these tumours are usually aggressive and have a poor outcome. Interestingly, metaplastic breast cancer, which resembles many phenotypic features of malignant PTs, usually belongs to the Claudin-low and Basal-like intrinsic subtype (Prat et al., 2010). Both Claudin-low and metaplastic breast cancer have previously show high enrichment for cancer stem cell-related biological processes (Hennessy et al., 2009).

In this study, we analysed the expression of 105 breast cancer-related genes, including the genes that define the intrinsic subtypes of breast cancer, in a panel of FTs with clinical follow-up. In addition, we compared the expression profiles of FTs with those of normal breast tissues and invasive breast carcinomas.

2. Materials and methods

2.1. Patient samples

This is a retrospective and exploratory study. From 1998 to 2013, we identified all consecutive patients (n = 41) diagnosed of juvenile FAD, benign PTs, borderline PTs or malignant PTs who had undergone local treatment at the breast surgery unit of the Vall d’Hebron University Hospital. In addition, we randomly selected 34 surgically resected FADs from our records from the same period of time for a total of 75 FTs. All FADs were enucleated and PTs were resected with free margins. All FTs were classified by V.P. according to the 2012 World Health Organization (WHO) guidelines (Lakhani et al., 2012). Clinical reports and follow-up data were available for 64 patients. Moreover, we included an in-house FFPE-based dataset of 49 primary invasive ductal carcinomas and 14 normal breast tissue obtained from reduction mammoplasties. The project was approved by the ethics committee of our institution.

2.2. Immunohistochemistry

Immunohistochemical Ki-67 staining of 5 representative FTs was performed in sections from paraffin-embedded tissue blocks with the avidin-biotin-peroxidase technique. Five micrometer-thick sections were cut from the tissue specimens and placed on poly-L-lysine-coated glass slides. Sections were deparaffinized with xylene and rehydrated in graded alcohol. Endogenous peroxidase was blocked by immersing the sections in 0.1% hydrogen peroxidase in absolute methanol for 20 min. For antigen retrieval, tissue sections were heated in a pressure cooker in 10 mM citric acid monohydrate, pH 6.0 for 5 min, and then incubated with the primary antibody at room temperature. The primary antibody used was CONFIRM anti-Ki-67 (30–9) (Ventana Medical Systems, Tucson, AZ). Immunohistochemistry was performed with the Ventana BenchMark XT slide processing system and the iView detection kit (Ventana Medical Systems, Tucson, AZ). All slides were counterstained with hematoxylin, dehydrated, and mounted. Negative controls were performed by omitting the primary antibody.

2.3. Gene expression analysis

A section of the formalin-fixed paraffin-embedded (FFPE) breast tissue was first examined with a hematoxylin and eosin staining to confirm the diagnosis and determine the tumour area. For RNA purification (Roche® High Pure FFPET RNA isolation kit), 3 10 μm FFPE slides were cut for each tumour, and macrodissection was performed, when needed, to avoid normal breast contamination. A minimum of ~100 ng of total

Please cite this article in press as: Vidal, M., et al., Gene expression-based classifications of fibroadenomas and phyllodes tumours of the breast, Molecular Oncology (2015), http://dx.doi.org/10.1016/j.molonc.2015.01.003