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Minireview

Molecular pathology of breast apocrine carcinomas: A protein expression signature specific for benign apocrine metaplasia

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Abstract **Breast cancer is a heterogeneous disease that encompasses a wide range of histopathological types including: invasive ductal carcinoma, lobular carcinoma, medullary carcinoma, mucinous carcinoma, tubular carcinoma, and apocrine carcinoma among others. Pure apocrine carcinomas represent about 0.5% of all invasive breast cancers according to the Danish Breast Cancer Cooperative Group Registry, and despite the fact that they are morphologically distinct from other breast lesions, there are at present no standard molecular criteria available for their diagnosis. In addition, the relationship between benign apocrine changes and breast carcinoma is unclear and has been a matter of discussion for many years. Recent proteome expression profiling studies of breast apocrine macrocysts, normal breast tissue, and breast tumours have identified specific apocrine biomarkers [15-hydroxyprostaglandin dehydrogenase (15-PGDH) and hydroxymethylglutaryl coenzyme A reductase (HMG-CoA reductase)] present in early and advanced apocrine lesions. These biomarkers in combination with proteins found to be characteristically upregulated in pure apocrine carcinomas (psoriasin, S100A9, and p53) provide a protein expression signature distinctive for benign apocrine metaplasias and apocrine cystic lesions. These studies have also presented compelling evidence for a direct link, through the expression of the prostaglandin degrading enzyme 15-PGDH, between early apocrine lesions and pure apocrine carcinomas. Moreover, specific antibodies against the components of the expression signature have identified precursor lesions in the linear histological progression to apocrine carcinoma. Finally, the identification of proteins that characterize the early stages of mammary apocrine differentiation such as 15-PGDH, HMG-CoA reductase, and cyclooxygenase 2 (COX-2) has opened a window of opportunity for pharmacological intervention, not only in a therapeutic manner but also in a chemopreventive setting. Here we review published and recent results in the context of the current state of research on breast apocrine cancer.**

Keywords: Apocrine metaplasia; Benign apocrine metaplasia signature; Pre-malignant apocrine lesions; Invasive apocrine carcinomas; Proteomics; Chemoprevention

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Abbreviations: IHC, immunohistochemistry; 2D PAGE, two-dimensional polyacrylamide gel electrophoresis

1. Introduction

Breast cancer is the leading cause of cancer deaths in women today and is the most common cancer among women in the Western world (22% of all cancers). According to the World Health Organization, more than 1.2 million people will be diagnosed with breast cancer this year worldwide and the Danish Health authorities estimate that in 2005, approximately 4200 women were diagnosed with the disease in Denmark. Moreover, there is a significant increase in the number of new cases diagnosed every year in Denmark as in the past 40 years the number of cases has tripled. Routine mammography screening has increased the number of breast lesions detected, but clearly, in spite of many efforts, new strategies for early detection, prevention and treatment are needed in order to control the disease and improve survival.

Breast cancer is a heterogeneous disease that encompasses a wide range of histopathological types including invasive ductal carcinoma (IDC), lobular carcinoma (ILC), medullary carcinoma, mucinous carcinoma, tubular carcinoma, and apocrine carcinoma among others [1]. Apocrine carcinoma is a rare lesion that represents about 0.5% of all invasive breast carcinomas according to the Danish Breast Cancer Cooperative Group (DBCG) Registry, and even though these lesions are morphologically distinct from other breast carcinomas, there are at present no available standard histopathological criteria for their diagnosis [2–4] and as a result, there is no precise information as to their prognosis. Molecular profiling of breast cancer tumours with cDNA microarrays have provided a new way to classify breast tumours in subgroups based on their expression patterns [5], as well as to derive signatures for prognosis [6–8] and response to treatment [9–11]. The original Stanford classification [5], subsequently refined by Sorlie and colleagues [12], describes five tumour sub-classes with characteristic molecular features and clinical outcomes. The five types defined as: luminal A, luminal B, basal, normal and ERBB2, represent distinct biological entities that with the exception of the ERBB2 class can be related to cell type and differentiation status. The Stanford classification, however, is by no means exhaustive as recently Farmer and colleagues [13] using principal component analysis and hierarchical clustering analysis of cDNA microarray data identified a novel subset of breast tumours with increased androgen signalling and

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apocrine expression profile that they termed "molecular apocrine", as these lesions do not exhibit all the histopathological features of classical apocrine carcinomas. Molecular apocrine tumours share some common expression characteristics with the ERBB2 class in the Stanford classification and exhibit some of the features of the basal group [13], underlining the heterogeneity of these lesions. In addition, Japaze and co-authors [14] have recently defined histopathological criteria for the diagnosis of pure invasive apocrine carcinoma (PIAC), an intermediate group of apocrine tumours representing a distinct clinicopathological entity. These lesions have a less aggressive behaviour than invasive ductal carcinomas otherwise not specified (IDC-NOS) [14]. In summary, given the lack of criteria, histological as well as molecular, to reproducibly categorize apocrine breast carcinoma and the continuing controversy over its definition, one cannot determine with certainty the true clinical significance of these lesions.

Our laboratories are part of a multidisciplinary and long-term research initiative aimed at the comprehensive analysis of breast cancer lesions using multiple experimental paradigms from proteomics and functional genomics to study fresh mammary tumours and matched benign tissues, along with the integration of the multiplatform "omic" data sets with clinical data from each patient [15–20]. In this minireview we focus on novel proteomic strategies employed by our laboratories that have (i) shed some light as to the origin and phenotype of apocrine carcinomas, (ii) aided in the identification of precancerous lesions in the linear histological model of breast cancer progression [21], and (iii) revealed specific targets for chemoprevention [20]. Published results as well as new data are reviewed here and set in the context of the current state of research on breast apocrine cancer.

2. Origin of apocrine tumours: relationship between benign apocrine metaplasia and apocrine carcinomas

Apocrine differentiation of breast epithelial cells has attracted a great deal of attention as up to 50% of breast invasive carcinomas exhibit areas with apocrine morphology features [22–28], and because available data have shown that women with breast apocrine cystic disease have an increased propensity for developing breast cancer later in their lives [29–33].

Presently, little is known about the molecular mechanisms underlying apocrine metaplasia, a process that entails the conversion of breast epithelial cells in the terminal duct lobular units (TDLUs) into sweat gland type cells [34]. Breast apocrine cells are cytologically identical to cells in apocrine glands in that they exhibit rather large vesicular nuclei with prominent nucleoli, as well as abundant eosinophilic cytoplasm that occasionally present apical snouts that are shed into the lumen of the ducts [29,35–37]. Apocrine lesions are usually diagnosed based on morphological features [27] and their relationship with invasive disease remains controversial as different authors have described these lesions as either a precursor in malignant apocrine transformation, or as benign lesions with no correlation with malignancy [24,29,35,37,39–44]. Up to one third of the women aged 30–50 bear large apocrine cysts in their breasts [29,36], and both their frequency and their proposed association with an increased risk of breast cancer have underpinned the importance of investigating these lesions at the molecular level in order to enlighten their putative relationship with invasive disease, in particular apocrine carcinoma [13,20,37,45].

The strategy we have used to unravel the relationship between benign apocrine metaplastic epithelium and invasive apocrine cancer was based on the assumption that invasive apocrine carcinomas evolve from epithelial cells in TDLU's in a stepwise manner, going through different sequential stages that involve metaplasia, hyperplasia, atypia, and carcinoma in situ (CIS) [21,37,46]. Firstly, we derived specific protein biomarkers for benign apocrine metaplasia by comparing the protein expression profiles of non-malignant breast epithelia and apocrine cells. Secondly, we generated specific antibody probes against these markers to use in immunohistochemistry (IHC) based analysis of tissue specimens to determine if these markers were indeed expressed by apocrine carcinomas [20].

2.1. *Apocrine differentiation of breast epithelial cells: identification of specific biomarkers of apocrine metaplasia*

Gross cystic disease is the most commonly observed benign disease of the breast occurring in about 7% of all adult women [47]. Cysts are divided into apocrine – or type I, flattened – or type II, and intermediate types based on the K^+/Na^+ concentration ratio [31,48–51]. Type I cysts (apocrine) have a higher K^+/Na^+ ratio ($\geqslant 1.5$) [48], and are lined by metaplastic apocrine secretory epithelium that exhibit tight cell–cell junctions. The conversion of breast epithelial cells to apocrine cells is a common phenomenon in the female breast, with microscopic apocrine changes appearing, typically, after the age of 30 and increasingly thereafter [29,36]. Apocrine microcysts are derived by either: (i) cystic transformation of glandular cells, followed by dilation of the ductules due to secretion, (ii) metaplasia of larger ducts, or (iii) by apocrine metaplasia of intraductal papillary lesions [20,45,52–54]. Microcysts can enlarge to become macrocysts reaching sizes of a couple of cm's in diameter, causing pain and discomfort due to the tension effects on the surrounding stroma. We have recently presented evidence indicating that apocrine macrocysts arise by coalescence of smaller microcysts [20]. Apocrine macrocysts are heterogeneous in their cytological characteristics and in some cases present transitions from cuboidal to flat apocrine epithelia, most likely representing differences in their stage of differentiation and/or metabolic activity.

Apocrine micro- and macrocysts do not express estrogen receptor alpha (ER-alpha) and progesterone receptor (PR) [55–57], but are positive for the androgen receptor (AR) and Her-2 Neu [13,20]. In addition, these lesions exhibit a low proliferative index as determined by staining with the Ki67 antibody [20,56].

To generate specific biomarkers of apocrine metaplasia we compared the protein expression profiles of fresh breast apocrine macrocysts with those of matched non-malignant tissues collected from the same patient [20] as these lesions are relatively large in size and provide abundant material for gel-based protein analysis. By combining an improved tissue sample preparation procedure that allows the proteomic analysis of only a few cryostat sections under a well-defined histological framework (Fig. 1) [19,20] with two-dimensional polyacrylamide gel electrophoresis (2D PAGE) and mass spectrometry, we have identified several proteins that are expressed preferentially, or solely by apocrine cells (Fig. 2A and B) [20]. These

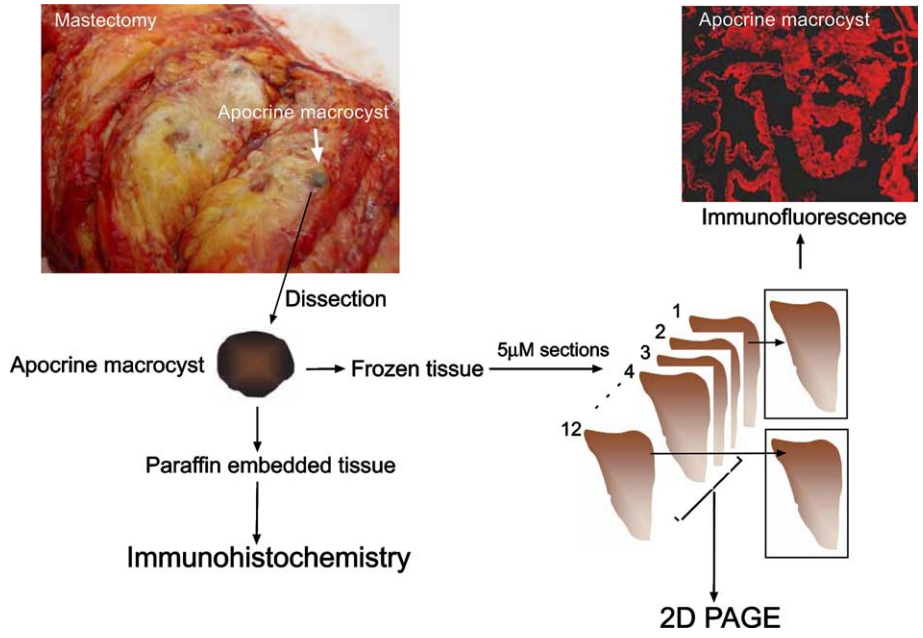

Fig. 1. Overview of the various steps involved in the analysis of apocrine macrocysts. A similar procedure was used to analyze non-malignant and tumour specimens.

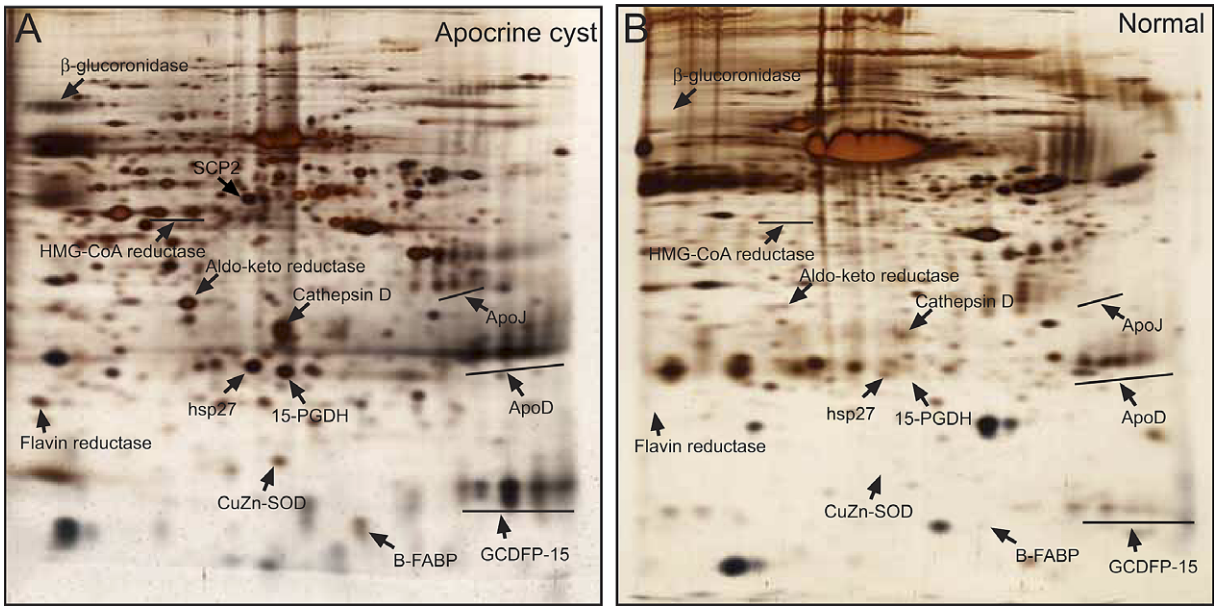

Fig. 2. IEF 2D PAGE of lysates of cryo sections from (A) apocrine macrocyst and (B) non-malignant epithelia from the same patient. Proteins that are preferentially expressed by the apocrine cyst are indicated with their respective names.

include 15-hydroxy prostaglandin dehydrogenase (15-PGDH), 3-hydroxymethylglutaryl CoA synthase (HMG-CoA synthase), gross cystic disease fluid protein-15 (GCDFP-15), apolipoprotein D (Apo D), apolipoprotein J (Apo J), beta-glucuronidase, steroid carrier-binding protein 2 (SCP2), flavin reductase, aldo–keto reductase family 1, CuZn–superoxide dismutase (CuZn–SOD), brain fatty acid binding protein (B-FABP), cathepsin D, and heat shock protein 27 (hsp 27). Most of the expression changes observed by 2D PAGE analysis were validated by IHC, with some examples being presented in Fig. 3A–N. In addition, we surmised two other biomarkers from our data, HMG-CoA reductase, a protein that is coordinately expressed with HMG-CoA synthetase [58,59] (Fig. 3P), and cyclooxygenase 2 (COX-2) [60], a 15-PGDH antagonist activity (results not shown), both of which were shown by IHC to be up-regulated in apocrine cells and present in micro and macrocysts [20]. An annotated 2D gel image of all the apocrine cyst proteins identified so far will be made available through our website (http://proteomics.cancer.dk).

Of all the up regulated proteins we identified, only 15-PGDH (Fig. 3B) and HMG-CoA reductase (Fig. 3P) are expressed specifically by apocrine cysts, and by apocrine metaplastic cells

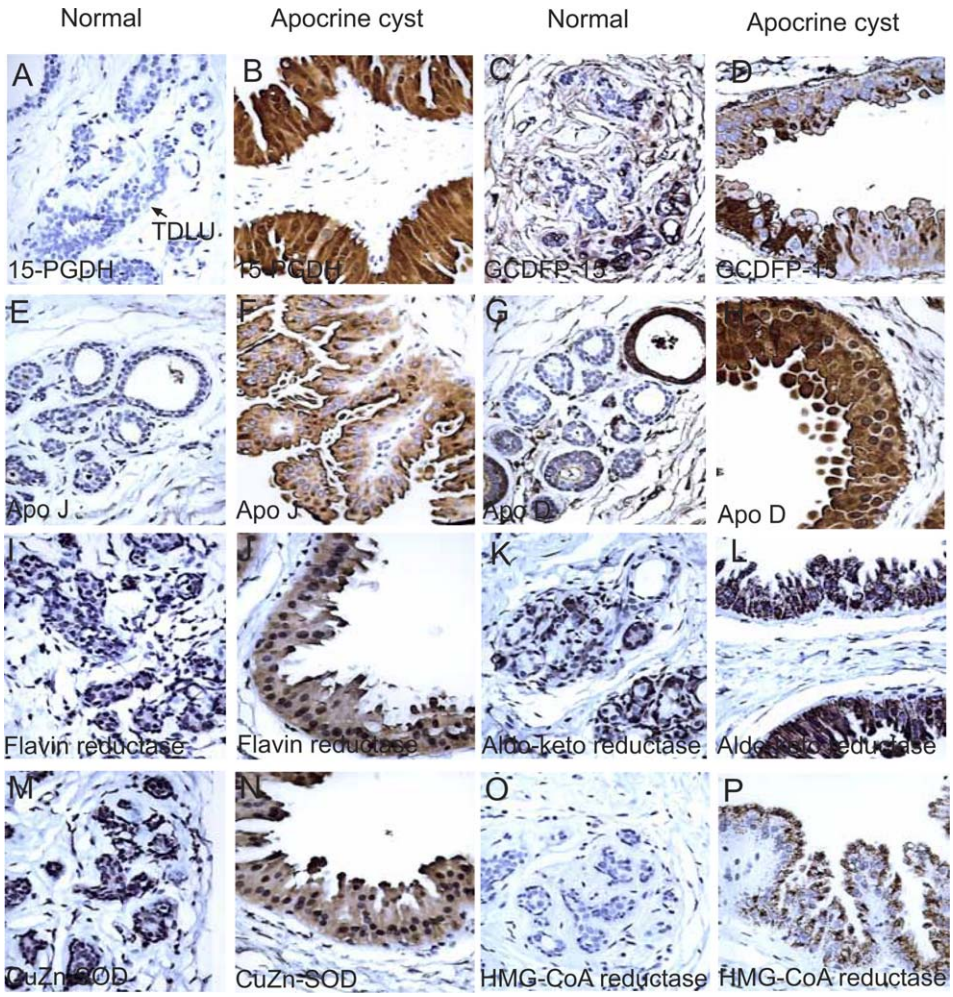

Fig. 3. IHC pictures of apocrine microcysts and non-malignant breast tissues reacted with antibodies against proteins expressed preferentially, or solely by apocrine cells. The peptide antibodies against GCDFP-15, Apo J, Apo D, flavin reductase, and CuZn-SOD were prepared by Eurogentec. The antibodies against aldo–keto reductase family 1 and the HMG-CoA reductase were a kind gift of R.J. Cenedella (Kirksville, Mo) and H. Aburatani (Tokyo), respectively. The antibodies against 15-PGDH were prepared in the laboratory and have been described before [20].

present in type I cysts, terminal ducts, and intraductal papillary lesions [20]. IHC analysis of hundreds of sections of non-malignant tissue biopsies collected from 93 patients with invasive breast cancer showed that these markers are not expressed by cells present in non-malignant terminal ductal lobular units (TDLUs) (Fig. 3A), type II flat microcysts, stroma (Fig. 3A), fat tissue, or any other cell type of mammary tissue examined [20], underscoring their value as specific markers for apocrine lesions. These biomarkers are far superior for discrimination of apocrine cells compared to GCDFP-15, a protein generally regarded as a specific functional marker of apocrine cells [61], which cannot be used alone to pinpoint apocrine lesions as it is expressed by some non-apocrine breast epithelial cells [20,37,62].

2.2. *Expression of specific apocrine metaplasia biomarkers by pure invasive apocrine carcinomas establish a direct link between these lesions*

An important question that has been debated for some years concerns the relationship between apocrine changes and breast carcinoma, in particular apocrine carcinoma [29,38,40,41,43,44]. So far, published studies have shown contradictory results as apocrine lesions have been considered as either a precursor in malignant apocrine transformation, or as benign lesions with no correlation with malignancy [24,29,35, 37,39–44].

Having demonstrated the strict specificity of our antibody probes recognizing 15-PGDH and HMG-CoA reductase antigens, we analyzed by IHC 93 prospective breast tumour samples for which we had collected proteomic data during a period of three years [16,17,19,20]. These studies revealed that only one of the lesions, classified by the attending pathologist as invasive pure apocrine carcinoma (RH-79), expressed 15-PGDH (Fig. 4A), although no HMG-CoA reductase could be detected. These results are in harmony with the fact that our set of tumours, consisting of 75 invasive ductal carcinomas, 15 invasive lobular carcinomas, one mucinous carcinoma, one tubular lesion, and one single apocrine carcinoma, reflecting the expected incidence of mammary tumour types prevalent in the general population [63,64].

Since the expression of 15-PGDH by an invasive apocrine carcinoma suggested a link between benign apocrine metaplasia and the tumour phenotype, we proceeded to analyze by IHC and 2D PAGE six retrospective pure apocrine carcinomas for which we had snap-frozen tissue and paraffin-embedded

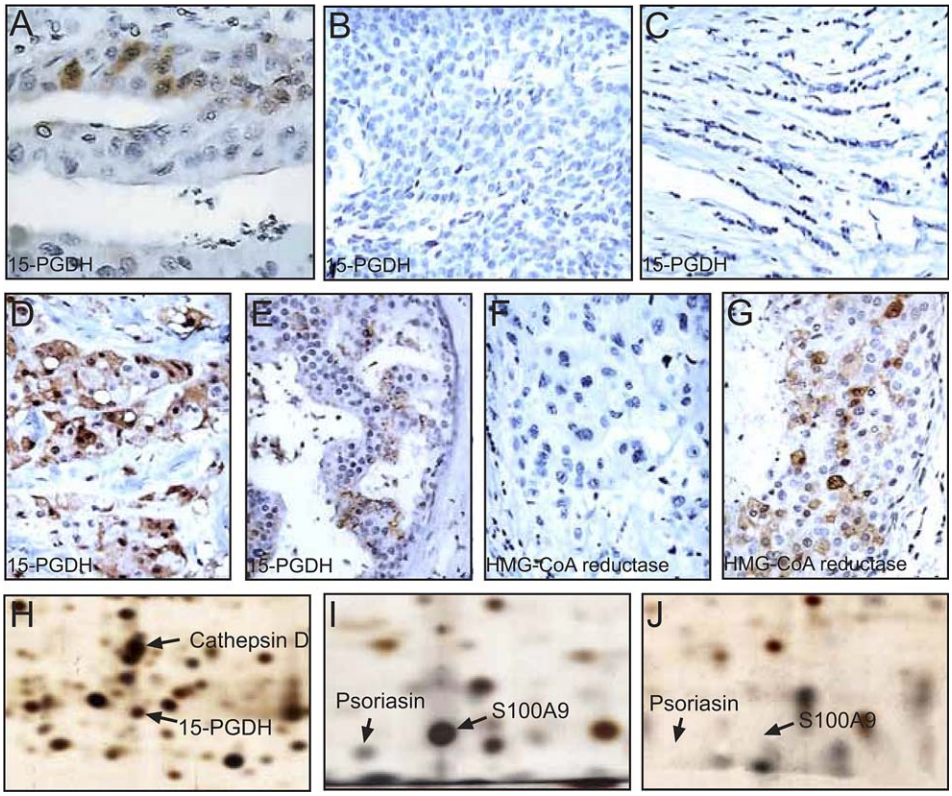

Fig. 4. IHC staining and 2D PAGE of invasive breast carcinomas. (A) Pure apocrine carcinoma RH-79 reacted with the 15-PGDH antibody. (B) and (C) Ductal and lobular invasive carcinomas reacted with the 15-PGDH antibody. (D) Invasive area of apocrine carcinoma RH-34697 reacted with the 15-PGDH antibody. (E) as (D), but depicting an area with apocrine CIS. (F) Invasive area of apocrine carcinoma RH-21990 reacted with the HMG-CoA reductase antibody. (G) as (F), but depicting an area with apocrine CIS. (H) Silver stained 2D gel of whole lysates from pure apocrine carcinoma RH-9777. (I) Silver stained 2D gel of whole lysates from pure apocrine carcinoma RH-21990. (J) Silver stained 2D gel of whole lysates from the apocrine cyst shown in Fig. 2A.

tissue blocks. Of these, five lesions stained positively with the 15-PGDH antibody, which decorated both invasive (Fig. 4D) and CIS apocrine cells (Fig. 4E; RH-34697). All five apocrine cancers were ER-alpha and PR negative, AR positive, while four were p53 positive [20]. Interestingly, only one of the 15-PGDH positive tumours (RH-34697) expressed HMG-CoA reductase both in the invasive and CIS apocrine cells (results not shown), while another (RH-21990) expressed the enzyme solely in the CIS apocrine cells (Fig. 4F, compare with G) suggesting that in some lesions expression of HMG-CoA reductase may be lost prior to 15-PGDH during cancer progression. Expression of 15-PGDH by five out of the six pure apocrine carcinomas was confirmed by gel-based proteomic analysis of frozen cryostat sections (Fig. 4H). The apocrine tumour that failed to express 15-PGDH (RH-24678), determined both by IHC and 2D PAGE (results not shown) may represent a more advanced apocrine carcinoma.

Given that 15-PGDH is not expressed by any other breast tumour type as judged both by IHC (Fig. 4B and C) and 2D PAGE analysis (results not shown) [20], the results are taken to imply that there is a direct link between benign apocrine metaplasia and apocrine cancer. The results, however, also indicated that most apocrine changes have little intrinsic malignant potential, although some lesions may progress to invasive apocrine cancer. At present we do not know which apocrine lesions will progress to invasive disease, and therefore there is an urgent need to develop novel strategies to reveal and characterize at the molecular level the early spectrum of lesions in the linear histological progression from normal epithelial breast cells to invasive disease. Our initial efforts in this direction are summarized below.

3. Early stages in apocrine cancer progression: a protein expression signature for benign apocrine metaplasia

In order to reveal early precancerous apocrine lesions (atypia and CIS) it is necessary to identify protein markers that are expressed by pure apocrine carcinomas but are absent in benign apocrine lesions. Antibodies against these malignancy specific markers in combination with already available apocrine-specific antibodies are expected to provide powerful tools to identify early apocrine lesions using IHC in combination with immunowalking, i.e staining of consecutive sections with various antibodies [65].

Comparison of the protein profiles of apocrine macrocysts with those of pure invasive apocrine carcinomas have, so far, revealed two proteins expressed by the tumours, albeit at different levels, that are absent in the benign apocrine lesions. These are psoriasin (S100A7) [66] and S100A9 (MRP-14, calgranulin B) [67] (compare Fig. 4I and J), both members of the S100 family of proteins [68,69]. These results have been validated by IHC using specific antibodies (results not shown). Psoriasin has been shown to play a role in the progression of

invasive ductal carcinomas by promoting angiogenesis and enhancing the selection for cells that overcome its anti-invasive function [70]. S100A9 on the other hand is known to be deregulated in several cancers [71–73], but its specific function is at present unknown. Both psoriasin and S100A9 are expressed by other breast tumour types, but the fact that most pure apocrine lesions express 15-PGDH is expected to facilitate the identification of early lesions that are of apocrine origin.

Based on the proteomic and IHC analysis of numerous apocrine cysts, non-malignant breast tissue, and pure apocrine lesions we have defined a protein expression signature that we believe characterises benign apocrine metaplasia. This signature, which consists of 15-PGDH (+), HMG-CoA reductase (+), p53 (−), psoriasin (−), and S100A9 (−) (Fig. 5A–D), may prove valuable for diagnosing benign apocrine metaplasias both in paraffin sections and in fine needle aspiration cytology. Moreover, antibodies against the components of the expression signature offer unique tools to search for precancerous lesions using IHC in combination with immunowalking as described below [65].

3.1. Towards the identification of precancerous apocrine lesions

The assumption that invasive apocrine carcinomas evolve from epithelial cells in TDLU's in a progressive stepwise manner has been a key element in our work, even though we realise that this may be a simplification. An upshot of this assumption is that in the progression from precancerous to invasive lesions, the proteome of cells will inevitably change with expression of

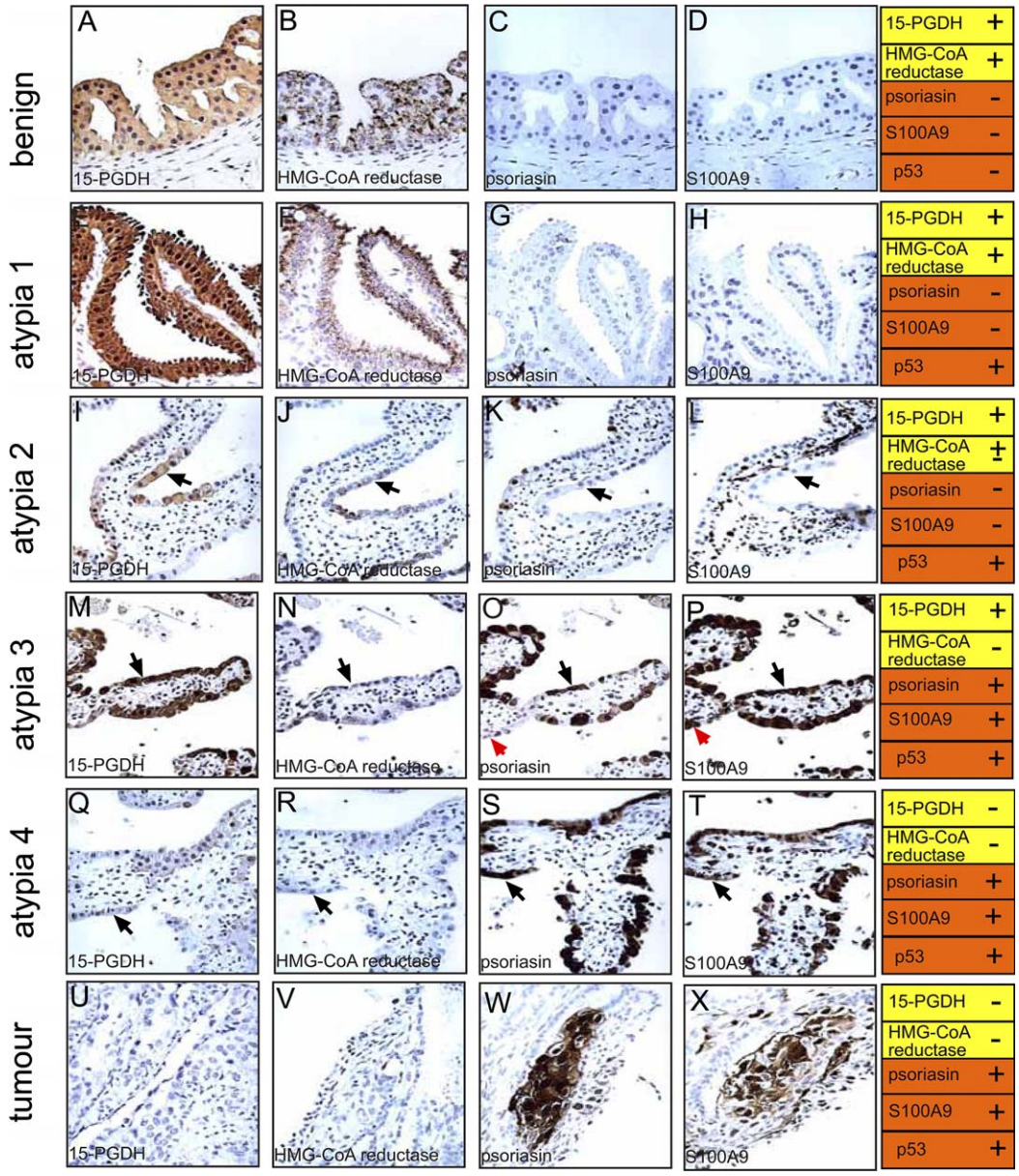

Fig. 5. IHC staining of benign apocrine metaplasia and various apocrine atypias detected in the invasive area of tumour RH-71 using antibodies against components of the expression signature. (A–D) Benign metaplasia. (E–H) Atypia 1. (I–L) Atypia 2. (M–P) Atypia 3. (Q–T) Atypia 4. (U–X) Tumour RH-71. With the exception of U, all others correspond to serial sections. The antibody against S100A9 (EPO 010100) was prepared by Eurogentec. The psoriasin monoclonal antibody has been previously described [85].

some proteins being lost and others gained. Our first indication that levels of apocrine-specific biomarkers change during apocrine cancer progression was provided by the observation that in one pure apocrine tumour examined (RH-21990) the CIS lesions were positive for HMG-CoA reductase (Fig. 4G), while the invasive component was negative (Fig. 4F) [20]. This result encouraged us to carry out a search for early lesions by probing a large number of apocrine lesions with antibodies against the components of the benign apocrine expression signature we had derived, using IHC in combination with immunowalking [65]. Using this approach we expect to be able to characterize the various histological stages involved in apocrine carcinoma progression.

One example of the outcome of this approach and that we believe validates it is shown in Fig. 5E–T which depict atypias present in the invasive area of a breast tumour (RH-71) that was 15- PGDH and HMG-CoA reductase negative, and psoriasin, S100A9 and p53 positive (Fig. 5U–X).

Atypia 1:

Apocrine cells in this atypia stained strongly with the 15-PGDH (Fig. 5E) and HMG-CoA reductase (Fig. 5F) antibodies, while some cells expressed p53 (results not shown). None of the cells however, expressed psoriasin (Fig. 5G) or S100A9 (Fig. 5H).

Atypia 2:

This atypia (arrows in Fig. 5I–L) is very similar to atypia 1, with the exception that cells expressed low levels of HMG-CoA reductase (Fig. 5J) in line with the observation described in Fig. 4F that suggested that the expression of this enzyme may be lost early during apocrine cancer progression in some lesions. Some cells expressed p53 (results not shown), but there was no expression of psoriasin (Fig. 5K) or S100A9 (Fig. 5L).

Atypia 3:

This atypia is like atypia 2, except that the cells express both psoriasin (Fig. 5O) and S100A9 (Fig. 5P). We have also observed cells that express only S100A9 (red arrows in Fig. 5O and P)

Atypia 4:

In this atypia most of the cells were negative for 15-PGDH (arrow in Fig. 5Q) and HMG-CoA reductase (Fig. 5R). Many cells were positive for p53 (results not shown), psoriasin (Fig. 5S), and S100A9 (Fig. 5T).

Given the low number of specimens analyzed so far it is not possible at this stage to draw a reliable conclusion concerning the phenotype continuity of the atypias, or if any of them will progress to invasive disease. To achieve this goal it will be necessary to identify additional biomarkers that are lost or gained during early cancer progression. These studies, which are currently underway, will greatly benefit from the analysis of biopsies obtained from patients with apocrine CIS as these sometime exhibit a whole range of atypias, as well as normal epithelia.

4. Targets for chemoprevention

The identification of differentially expressed proteins that characterize a specific step in the progression from early benign lesions to apocrine cancer has opened a window of opportunity for designing and testing new approaches for pharmacological intervention. Some of the biomarkers we identified as being expressed by apocrine metaplasias such as 15-PGDH, HMGCoA reductase, and COX-2, are well known therapeutic targets [74–78] with pharmacological agents already available (e.g. pravastatin, lovastatin, Ph CL 28A, nafazatrom, celecoxib, and rofecoxib). In at least one case, that of COX-2, one has reason to suspect a causal relationship [79] to the development of fibrocystic changes, making a case for the use of COX-2 inhibitors as chemopreventing agents for breast cyst formation and development. Further studies into the functional role that the various biomarkers identified in this study play in cyst formation are warranted, as they may identify therapeutic targets for fibrocystic changes and premalignant apocrine lesions.

5. Concluding remarks

The studies reviewed here were undertaken in an effort to dissect and gain a better understanding of the steps leading to breast apocrine metaplasia, and ultimately intended to generate specific biomarkers that may enlighten its relationship with cancer phenotype. As a whole, the results indicate that most apocrine changes have little intrinsic malignant potential, although a few lesions may progress to apocrine carcinoma. None of these lesions, however, seems to be a precursor of invasive ductal carcinomas, which accounted for 81% of the tumours analyzed.

All available information indicates that apocrine tumours of the breast comprise a heterogeneous group of lesions that may evolve through diverse molecular pathways. Indeed, studies from Japaze and colleagues [14] indicate that pure apocrine carcinomas correspond to a distinct clinicopathological entity exhibiting a less aggressive behaviour than high grade IDC-NOS, and Farmer and colleagues [13] described a novel group of breast cancer tumours that they designated ‘‘molecular apocrine’’ and that comprised between 8% and 14% of all the tumours analyzed in their microarray study as well as in four published breast cancer datasets [12,80–82]. The latter data implies that apocrine tumours may be more frequent than previously thought from current diagnostic procedures using histopathological criteria alone. Molecular apocrine tumours differ from other tumour types by the expression of several genes, including HMG-CoA reductase in line with our studies. Surprisingly, however, 15-PGDH was not detected in Farmer’s study, a fact that most likely reflects differences between transcriptomics and proteomics approaches [83,84]. Clearly, renewed efforts should be made to identify markers for more advanced apocrine carcinomas as not all of these lesions may be positive for the apocrine specific markers we have identified.

To dissect all of the stages involved in apocrine carcinoma progression., i.e., from benign metaplasia to hyperplasia, atypia, CIS, and invasive cancer, it will be necessary to identify not only those biomarkers that are lost through cancer progression, but also those that appear early in carcinogenesis. This will require the proteomic analysis of many tissue samples in prospective studies, as well as the preparation of specific antibody probes that could be used in immunohistochemical analysis of archival samples. In the long-run the outcome of these studies may provide specific probes that will permit accurate pre-operative diagnosis as well as for complementing the analysis of cells and cyst fluid found by fine needle aspiration cytology of patients attending the mammography clinic.

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