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Original contribution

Genetic clonal mapping of in situ and invasive ductal carcinoma indicates the field cancerization phenomenon in the breast

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Summary Nearly 80% of well-differentiated in situ duct carcinomas (g1 DCIS) have been shown to be multicentric (multilobar) lesions, while most in situ poorly differentiated duct carcinomas (g3 DCIS) were unifocal (unilobar) lesions. Here we present a clonality study of 15 cases of DCIS, all showing multiple foci. Twelve of these cases were associated with an invasive duct carcinoma. Fifteen cases of female breast cancer patients all showing multiple DCIS foci (5 g1 DCIS, 5 g2 DCIS, 5 g3 DCIS) were randomly selected and histologically studied using large histological sections. Care was taken to laser-microdissect DCIS foci that were most distantly located from one another in the same large section, and pertinent cells were genetically studied. Invasive duct carcinoma and ipsilateral lymph node metastases and/or contralateral lesions, whenever present, were additionally microdissected. DNA of neoplastic cells was purified, and the mtDNA D-loop region was sequenced. Genetic distance of different foci from the same case was visualized by phylogenetic analyses using the neighbor-joining method. Patients ranged in age from 36 to 87 years (mean 65.1). All 9 cases of widely spread DCIS were not clonal. Four of 6 cases that showed multiple adjacent foci were clonally related on mtDNA analysis. In the present series, 11/15 DCIS appeared as multiple synchronous primary breast tumors, genetically not related to one another. The present data enhance the view that breast can also show the field cancerization phenomenon, paralleling what has already been proposed in other organs.

1. Introduction

Large histological sections (LHS) are very useful to study normal and neoplastic breast tissue [1]. LHS allow not only direct visualization of a large part of the breast glandular tree in one plane and enhance not only the correlation between mammography and pathology [2], but also give pertinent reliable information on multifocality, difficult to obtain using conventional paraffin blocks [3,4]. The breast parenchyma is subdivided into lobes, which are individual anatomical structures [5], each formed by a single galactophore duct that branches into segmental, subsegmental and terminal ducts,

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all ending in numerous acini [6]. Terminal ducts together with their relative acini have been named the "terminal duct lobular unit" (TDLU) [7]. Utilizing LHS, it has been shown that in situ and invasive lobular carcinoma is a multifocal [8] neoplastic lesion in over 50% of cases [9]. A study of 45 patients with in situ duct carcinoma (DCIS) using LHS for multifocality [3] showed that grade 1 DCIS was, in nearly 80% of the cases, a widespread multifocal condition involving more than one lobe and/or quadrant, while grades 2 and 3 DCIS were more circumscribed lesions, mostly confined to one lobe. It was then stated that grade 1 DCIS, in terms of multifocality, was more similar to previous observations regarding lobular in situ neoplasia/lobular in situ carcinoma (LIN2/LIN) [3,9].

In a seminal study using LHS on a series of 574 consecutive newly diagnosed breast carcinomas, Tot et al [10] found 75 cases (13%) of pure DCIS, of which 12% were unifocal (involving a single TDLU), 10% multifocal (involving several distant TDLUs with uninvolved breast tissue in between) and 24% diffuse (mainly involving large ducts). In the same study, invasive duct carcinomas (IDC) were unifocal in 62% of cases, multifocal in 24% and widely spread in 5%.

The reported incidence of multifocality (and multicentricity) of DCIS ranges from 0% to 78% according to different authors [11] to the point that multicentricity was denied by Page et al. [12], who stated that it is a misconception mostly related to artifacts. These remarkably different results for assessing the presence of multiple neoplastic foci in the same breast, obtained by different authors, appear to depend on the various methods of study employed, mostly based on traditional multiple-block sampling.

Although multifocality has been interpreted as the simultaneous presence of different neoplastic primaries in different lobes [3], it was felt [9] that some cases escape this rule due to the complexity of the ductal breast tree [5]. In fact, there are lobes that spread over a wide space and can mix with adjacent lobes to such an extent that it is impossible to separate one from the other at the morphological level, even using LHS [5].

The aim of this study was to try to find common or different mitochondrial DNA (mtDNA) mutations among multifocal DCIS that might reflect clonal or nonclonal features. The study was also extended to concurrent invasive carcinomas as well as metastases to axillary lymph nodes. For clonality, point mutations of the hypervariable D-loop region of mtDNA were studied by deep sequencing. Mutations were evidenced with phylogenetic analysis of neighbor-joining (NJ) trees [4,13].

2. Materials and methods

2.1. Patients

Fifteen randomly selected female patients, ranging in age from 36 to 87 years (mean 65.1) were included in the present study. All patients had undergone mastectomies histologically studied with LHS. Criteria for selection of cases were the following: (*a*) multiple TDLUs/ducts involved by DCIS present within the same LHS; (*b*) enough material for microdissection and molecular analysis; (*c*) 5 cases each of g1 DCIS, g2 DCIS and g3 DCIS [14].

DCIS was graded according to current (2012) World Health Organization (WHO) criteria [11]. IDC, when present, was graded according to Elston and Ellis [15]. Nodal metastases, recurrences and contralateral lesions whenever present, were studied for clonal mtDNA analysis.

DCIS, per Tot's criteria [10,16], were considered unifocal when they involved several adjacent TDLUs, multifocal if they involved several distant TDLUs with uninvolved breast tissue in between, possibly containing normal glandular structures, and diffuse when large ducts were involved. IDC was considered unifocal when only one invasive area was observed in the large section, while it was considered multifocal when multiple invasive tumor areas were observed separated by uninvolved nonneoplastic breast tissue [10,16]. No cases of diffuse IDC or invasive lobular carcinoma or LCIS/LIN were present.

2.2. Tissue processing

Parallel large 5 mm thick slices were obtained from each mastectomy specimen, a routine procedure in our laboratory since 1995 [1]. Care was taken to slice each section perpendicularly to the skin, under mammographic guidance. Slices were then fixed in 10% buffered formalin and paraffin embedded as routine. From each large paraffin block, one 8 μm hematoxylin and eosin–stained section was obtained [1].

2.3. Microdissection

Two to 7 foci of DCIS from each case were laser-microdissected for genetic study. Care was taken in capturing foci that were located at the farthest distance from one another in the same LHS.

Pertinent lesions were microdissected using the laser assisted SL µcut Microtest (MMI GmbH distributed by Nikon, Firenze, Italy). Ten-μm-thick sections were obtained, and unstained sections were deparaffinized with Bio-Clear (Bio-optica, Milan, Italy), rinsed in 100% to 80% ethanol and stained with hematoxylin and eosin. Breast glandular or epithelial tissue uninvolved by the neoplastic process and/or lymphocytes from reactive lymph nodes from the same case were also microdissected as control reference DNA. Tissue for microdissection from a pertinent block inclusive of the selected lesion was obtained from LHS. The block was melted and re-embedded to obtain the slide useful for the laser dissecting microscope.

The microdissected cells were placed in SL µcut Transfer Film (Nikon, Firenze, Italy), and the DNA was digested