The Distribution of the Earliest Forms of Breast Carcinoma

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6.1 Introduction

The pathological workup of breast specimens has dramatically changed in recent years since the increasing use of large (macro) sections (Foschini et al. 2006, 2007; Tot 2003, 2005, 2007a). Large-format histology sections were applied for the first time to human tissue by Cheatle (1921) and by Ingleby and Holly (1939) to visualize the relationship between neoplastic lesions and the surrounding tissue. Subsequently, the method was improved (Wellings and Jensen 1973; Sarnelli and Squartini 1986; Faverly et al. 1992; Foschini et al. 2002) and studies based on large sections have evidenced important correlations between mammography and pathology, first of all regarding tumor extent (Egan and Mosteller 1977; Faverly et al. 1994; Gallager and Martin 1969).

Large sections are also useful in assessing the status of the excision margins and in facilitating the correct measurement of the size of the tumor (Foschini et al. 2002). Accordingly, the issue was addressed by Jackson et al. (1994) who compared two series of operated breast carcinomas, one studied with conventional histology method and the other with large sections. The size of the lesion could be determined in all cases using large sections, while size could be measured in only 63% of the cases studied with conventional small blocks. Further advantages of using large sections are proper assessment of the extent of the tumors, and assessment of the *unifocality* of in situ and invasive lesions and of multiple (multifocal and multicentric) lesions (Foschini et al. 2006, 2007; Tot 2005).

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6.2 Mural Spread of Neoplastic Cells

The genesis of ductal carcinoma in situ (DCIS) from terminal ductal lobular unit (TDLU) was proposed by Wellings and Jensen (1973) using large sections. This view has been accepted for decades and only recently challenged by Tot (2005).

Since the seminal papers by Going and Moffat (2004), Mai et al. (2000) and Ohtake et al. (1995), it is well established that the breast is constituted of 11–48 lobes which are independent microanatomic structures. Three dimensionally, breast lobes can be depicted as cones with apex directed toward the nipple and their base, which contain most of the lobules, facing the deep fascia. Some lobes (dominant lobes) can be extremely widespread over more than a quadrant and cannot be individually separated from the other lobes because the branches of the ductal system intermingle with those of adjacent lobes. This is well known to radiologists who frequently observe such spread of injected contrast medium into a collecting duct over more than one quadrant. Ohtake et al. (1995) have suggested the existence of branching anastomoses between different lobes, a view that is not confirmed by radiologists who never see retrograde spreading of the contrast medium into branches of different lobes.

Presence of anastomoses would be relevant as it would imply diffusion of neoplastic cells from one lobe to the next without the necessity of invading the stroma. This is pertinent to the knowledge that neoplastic cells from poorly differentiated carcinomas may climb along the duct walls on their way to the epidermis, which is finally cancerized in the form of Paget's cell carcinoma (Marucci et al. 2002). This phenomenon is mostly evident in cells that express HER-2 and show dendritic features (Fig. 6.1), a morphological hallmark of a cell that is capable of
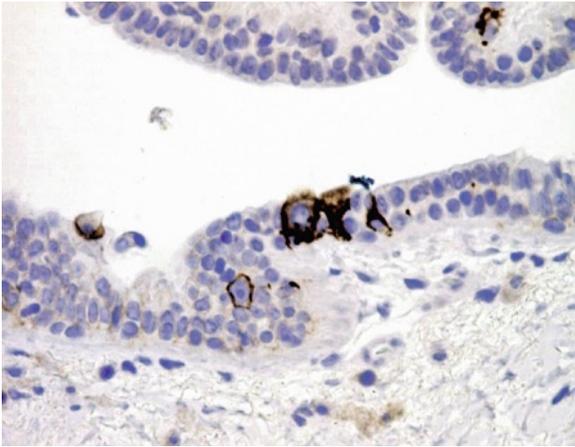
Fig. 6.1 Her-2 positive dendritic cells "climbing" along a galac-tophore duct. These cells were located between a DCIS/DIN3 present deep in the breast and Paget's cell carcinoma in the nipple

movement (Marucci et al. 2002; Tavassoli and Eusebi 2009). An additional feature of intraductal spread is the so-called pagetoid spread, classically observed in lobular carcinomas in situ (LCIS). This form of "mural" ductal spread was described by Fechner (1973), but is not exclusive to lobular carcinomas being present also in duct carcinomas of poorly differ-entiated type (Fig. 6.2a and b) as well as in neuroen-docrine DCIS (Tsang and Chan 1996). The spread of the cells along duct walls is not unanimously accepted (Tot 2005); nevertheless, it would be dif-ficult to justify the presence of individual neoplas-tic cells located in the ducts far away from the main DCIS, a phenomenon that would not been explained even by the field effect theory (Slaughter et al. 1953; Braakhuis et al. 2004).

DCIS have been traditionally classified according to their histological architecture and were named clinging, micropapillary, papillary, cribriform and comedo carci-nomas (Rosen and Oberman 1993). Such subdivision of DCIS, however, was not practically useful as about 50% of the cases were of mixed type (Patchefsky et al. 1989), and in addition, it did not provide any prognostic or pre-dictive information. After the publication of the seminal paper by Holland et al. (1994), the structural criteria to classify DCIS were abandoned and intraductal neo-plasms were mostly classified according to their cyto-architectural differentiation. This led to establishing the category of well-differentiated DCIS when neoplastic nuclei were monotonous and cells were oriented along
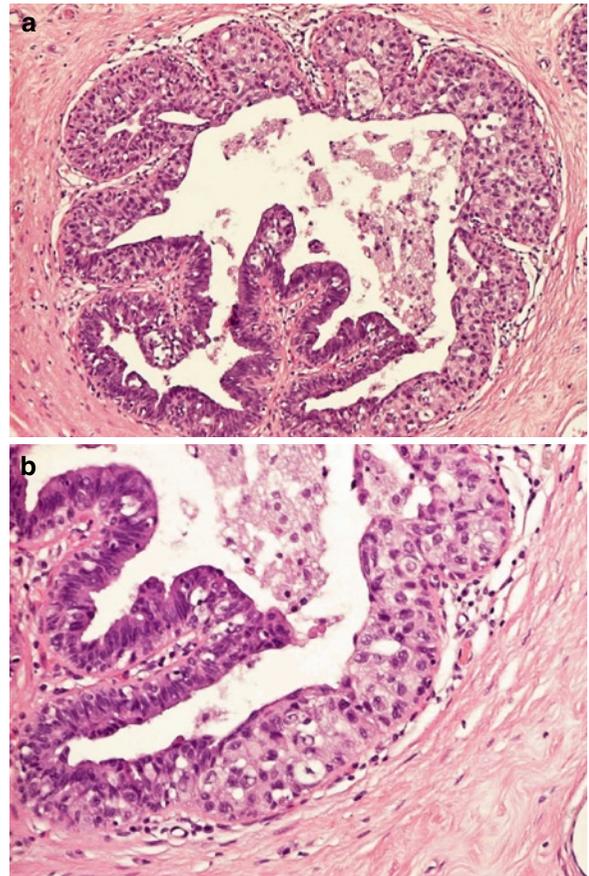

Fig. 6.2 (**a**) Mural spread of neoplastic cells located between the basal lamina and luminal epithelium. (**b**) The neoplastic cells show pleomorphic nuclei (and were Her-2 and e-cadherin posi-tive, which is not illustrated in this figure). A clear-cut DCIS/DIN3 was located nearby

lumina; of poorly differentiated DCIS showing pleo-morphic nuclei and no orientation along lumina, and finally, of intermediately differentiated DCIS with irreg-ular pleomorphic nuclei and cells oriented along lumina. Accordingly, well-differentiated DCIS are estrogen receptor (ER) and progesterone receptor (PR) rich, while poorly differentiated DCIS are ER and PR poor with most of the latter showing HER-2 positivity (Bobrow et al. 1994).

After the paper of Holland et al. (1994), as it fre-quently happens in pathology, classifications of DCIS being mostly variations on the theme of the original proposal flourished, as did the terminological disputes. A classification very similar to the one of Holland et al. (1994) was adopted by WHO (2003) although differ-ent terminologies were used, i.e., DCIS/DIN (ductal

intraepithelial neoplasia) I, II, and III. This classification was also adopted by the AFIP breast tumor fascicle (2009).

6.3 How to Define In Situ Neoplastic Lesion?

Historically, the first definition of in situ carcinoma was provided by Broders (1932) who illustrated a case of what Foote and Stewart (1941) defined later as in situ lobular carcinoma. The first acceptable description of comedocarcinoma was that of Bloodgood (1934). Cheatle (1921) using large sections in 1921 stated for the first time that carcinomas initially existed within ducts. Dawson (1933) also concluded that the majority of cases arise in the terminal, intralobular ducts, a view further expanded by Wellings and Jensen (1973) who stated that intraductal extension of breast carcinoma is a noninvasive continuous proliferation of neoplastic cells originating in ductal or lobular epithelium of TDLUs preserving the basement membrane (Wellings and Jensen 1973). Nevertheless, in the premammographic era, some cases diagnosed as grade 3 DCIS/DIN3 were accompanied by simultaneous presence of lymph node metastases to such an extent that this led to label the primary breast tumors as "infiltrating comedocarcinoma" (Stewart 1950). Stewart himself stated that "comedocarcinoma is invariably infiltrating when its presence is discovered" (1950) and Sirtori and Talamazzi (1967) that in situ carcinomas of the breast hardly exist.

The current classical view is that in situ lesions are invariably surrounded by a continuous layer of myoepithelial cells and basal lamina whereas invasive lesions show discontinuous or fragmented basal lamina (Azzopardi et al. 1979). To this classical view, exceptions probably exist. It has been shown that myoepithelial elements can be absent in normal breast with apocrine changes (Cserni 2008). If a DCIS originates from such structures, it would be devoid of myoepithelial cells. In a case of DCIS of our own in which the in situ nature was undisputable (both structurally and immunohistochemically, i.e., presence of basal lamina), the myoepithelial differentiation of the basal cells could not be proved (Fig. 6.3a–e). Damiani et al. (1999) in an immunohistochemical study designed to assess whether cases in a series of "comedocarcinoma"

were in situ or invasive, employed at the same time three different markers (actin, laminin ,and collagen IV) as only one was not sufficient to establish the correct diagnosis. The presence of one of these, in conjunction with appropriate structural features, was sufficient to regard the given lesion for being in situ. In spite of that, in two cases it was stated that the authors did not reach any conclusion and considered them as indeterminate for invasion. Intracystic papillary carcinomas of large size frequently do not show any myoepithelial layer. These same lesions are equated to DCIS/DIN as practically never generate nodal metastases. Therefore, it seems that the term "in situ" in the breast is a concept of a nonmetastasizing proliferative intraglandular lesion not strictly related to stringent morphologic features. The same applies to invasion. Nerves and vessels including lymphatics, veins, and arteries are occasionally "invaded" by "benign" glandular structures and no harm to the patient ensues (Davies 1973; Eusebi and Azzopardi 1976; Taylor and Norris 1967).

Neoplastic ductoneogenesis is a proliferative not yet morphologically well-defined process. It is characterized by digitiform newly formed tubules filled by neoplastic cells that sprout from ducts in cases of DCIS, most frequently of poorly differentiated type (Tabár et al. 2004). This is probably the neoplastic counterpart of acinar and tubular proliferation seen physiologically in lobules during pregnancy or in benign lesions such as sclerosing adenosis of acinar and periductal types (Tanaka and Oota 1970). Accordingly, newly formed large tubules clump together; they appear distended and filled by neoplastic cells. Most of "neogenetic" tubules show a myoepithelial cell layer and/or a basal lamina. In some of these, the process is defective and consequently myoepithelial elements and/or basal lamina are lacking, which simulates an "invasive comedocarcinoma." This is so true that in a small series of 11 cases of DIN3 with features suggestive of stromal invasion on hematoxylin–eosin (H&E stain), it was found that immunohistochemistry for smooth muscle actin, collagen IV, and laminin assured the correct diagnosis of DIN3 in four cases, of invasive carcinoma in five cases. In two, it was not possible to establish the diagnosis, in spite of immunohistochemistry. This was due to the fact that the "comedo" nests had very heterogeneous staining being variably positive in adjacent clumps for one or another marker while rare
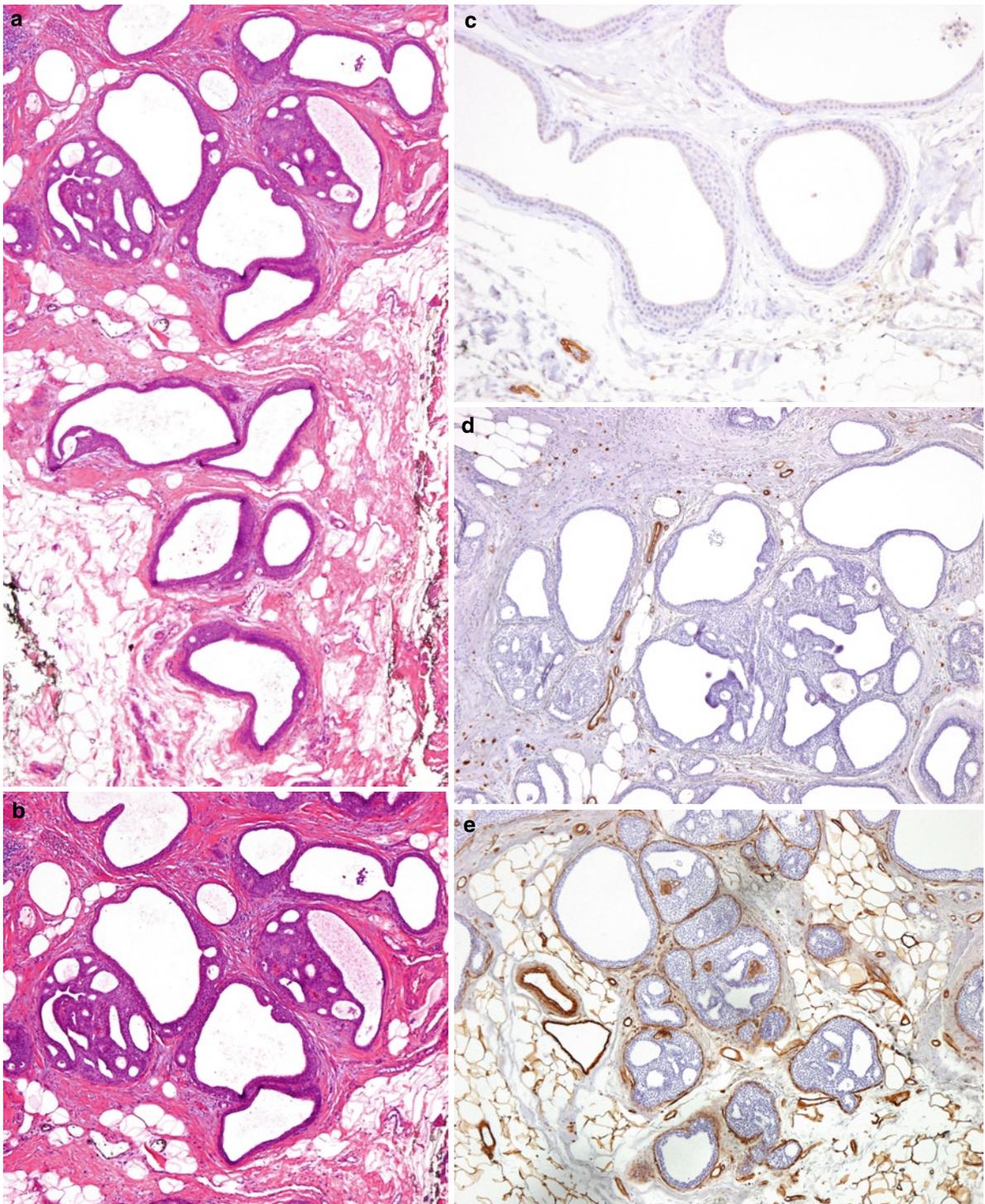
Fig. 6.3 (**a**) Well-differentiated DCIS characterized by flat epithelial atypia and cribriform structures. (**b**) The cells are well oriented; the nuclei are monotonous. A cribriform structure is well evident. (**c**) Keratin 14 immunohistochemistry: The basal cells are present but not expressing keratin 14 (nor p63 or smooth muscle actin, not illustrated in this figure). (**d**) The same case stained on laminin: The glandular neoplastic structures are totally devoid of laminin. (**e**) The same case stained on collagen IV: The neoplastic glandular structures are well outlined by collagen IV

clumps were totally negative for all of them. A final situation is constituted by "blunt invasion" in which carcinoma infiltrates as round or linear nests that simulate ducts distended by carcinoma in situ (Koerner 2009). This type of invasion has probably led Cowen and Bates (1984) to state that the diagnosis of invasion is confounded in some instances as invasive carcinomas can simulate DCIS. Therefore, it is possible to conclude that the diagnosis of in situ lesions, especially in DCIS/DIN3, is occasionally very difficult if not impossible. Immunohistochemistry is often helpful, but the use of large sections is mandatory in order to examine the entire lesion as in these cases the subgross architecture has consistent diagnostic relevance. Ductoneogenesis as described above is a scenario that has not been fully proven; nevertheless, if it is true, it would explain why DCIS forms a lump. One duct only, even if extremely distended by neoplastic cells would hardly be palpable. On the contrary, when several distended neogenetic ducts clump together and are simultaneously distended by neoplastic cells, these would make the lump clinically evident.

6.4 Unifocality, Multifocality, and Multicentricity of DCIS

Faverly et al. (1994) in a seminal paper published in 1994 demonstrated that poorly differentiated DCIS/DIN3 were unifocal proliferations while the opposite was seen in well-differentiated DCIS/DIN1 which were multifocal. Tot (2007a) stratified DCIS in diffuse (24%) (along major ducts), multifocal (40%) (defined as involvement of multiple distant lobules with uninvolved tissue in between), unifocal (32%) (defined as involvement of single or adjacent lobules without uninvolved tissue in between). Tot (2005, 2007b) suggested that the simultaneous and/or asynchronous multiple in situ tumor foci are usually localized in a single lobe of the breast, and he proposed the theory of the sick lobe of one breast stating that the sick lobe itself was genetically malconstructed from birth and that accumulation of genetic changes during the decades following the postnatal period would have led to malignant changes of the epithelial cell in any part of the sick lobe.

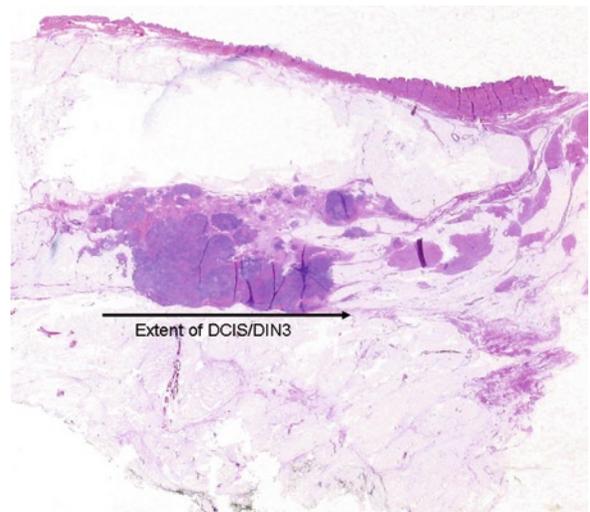Foschini et al. (2006) in a study of 13 cases of lobular intraepithelial neoplasia (LIN) (Tavassoli and

Fig. 6.4 Extent of DCIS/DIN3: This is a nice example of multifocality within the same lobe. Large-format histology slide, H&E stain
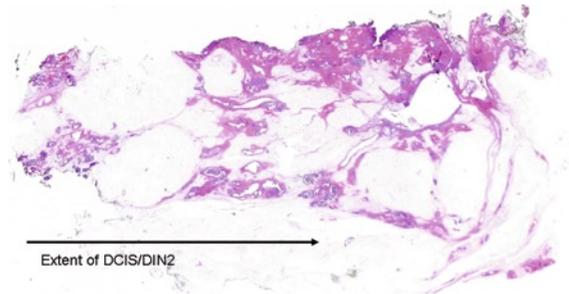

Fig. 6.5 Extent of DCIS/DIN1: The tumor is spread over at least two quadrants. This condition, probably multilobar, might be an example of multicentricity. Large-format histology slide, H&E stain

Eusebi 2009) defined *multifocality* (Fig. 6.4) as multiple foci of LIN present in the same lobe and *multicentricity* (Fig. 6.5) as multiple foci of LIN present in different lobes (Foschini et al. 2006; Tot 2005), a view also shared by Tot (2003). Cases were studied using large sections from mastectomies. The number of neoplastic foci ranged from 2 to 77 (mean 23.92) with 6 cases (46%) showing more than 20 foci. Foschini et al. (2006) also measured the maximum distance among LIN foci which ranged from 5 to 112 mm (mean 35 mm) with 9 cases (69.23%) out of 13 being more than 20 mm. Therefore, it appears that all cases of LIN displayed more than one focus, some foci (30%) clustered within 20 mm, but the majority

were scattered through all breast quadrants. A lobe comprises everything between 2% and 23% of the breast volume (Going and Moffat 2004). Some of the cases studied by Foschini et al. (2006) showed foci distant up to 112 mm. This would make highly unlike the fact that more that 60% of LIN arise within a "dominant" large lobe, while it is more plausible that LIN would arise within different lobes being a multi-focal and/or multicentric disease.

Foschini et al. (2007) also studied large sections from mastectomies of 45 cases of DCIS/DIN. Thirteen cases were DIN1. The number of DIN1 foci ranged from 1 (one case) to over 100 (mean 35.08). The maximum distance among multiple foci ranged from 12 to 55 mm (mean 35.42 mm). In 10 out of 13 cases (76.9%), the maximum distance was superior to 20 mm. Twelve cases were DCIS/DIN3. The number of foci varied from 1 (one case) to over 100 (one case), mean 24. On the all, DIN3 foci were in lower number than DIN1, being in 4 cases out of 12 (33.3%) the number of foci lower than 20. The range of the maximum distance among foci varied from 2 to 51 mm, the mean distance being 22 mm. Five cases only out of 12 (45.4%) displayed a distance superior to 20 mm. The 20 cases of DCIS/DIN2 were similar to those of DIN3. Therefore, it seems that DIN1 is a widespread condition involving more than a quadrant and hence more than one lobe, whereas DIN2 and DIN3 appear to cluster together, probably confined to one lobe. It also appears that DIN1 and LCIS show more similarities than differences than what has been previously recognized.

The fact that LIN and DIN1 are probably multilobar conditions with very distant neoplastic foci appearing almost simultaneously suggests the existence of a
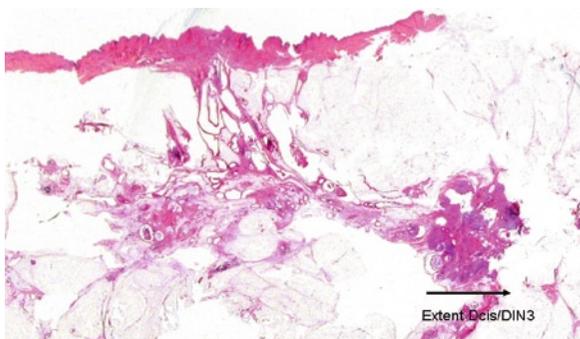

Fig. 6.6 Unilobar, unifocal DCIS/DIN3. Large-format histology slide, H&E stain

genetic "malconstruction" where the oncogenic factors act. DIN3 seem to be more localized, unilobar conditions (Fig. 6.6). These would be more compatible with an acquired neoplastic transformation where "environmental oncogenic factors" would face the tumor.

6.5 Conclusions

Most of the data obtained indicate that DCIS grade 1 and LIN are very often true multicentric (multilobar) diseases, while DCIS grade 2 and 3 are frequently unifocal or at most multifocal (unilobar) diseases. A more widespread use of large sections in routine pathology will give more accurate knowledge on extent and growth patterns of breast in situ neoplasms.

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