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REVIEW

Lobular Breast Cancer: Pathology, Biology, and Options for Clinical Intervention

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Abstract Lobular carcinoma is a breast cancer subtype comprising approximately 15 % of all breast cancer cases. Clinical diagnosis of this subtype is difficult due to a characteristic growth pattern that inhibits detection using palpation or standard X-ray mammography. While clinical intervention based on hormone antagonists has proven an effective strategy, hormone receptor negative or nonresponsive disease cannot be treated successfully, indicating the need for alternative curative approaches. In contrast to its well-defined histopathological characteristics that were first recognized a century ago, the surface of the underlying biology has only recently been scratched. Progress was made in understanding the biology of the disease, which will hopefully have its impact on future treatment modalities and initiate development of novel intervention strategies. Here, we review the pathological and molecular features of lobular breast cancer and report on the currently known mechanisms that control disease development and progression. Finally we will reflect on past, present, and future treatment options.

Keywords Lobular breast cancer · Pathological features · Clinical features · Molecular features

Abbreviations

AJ	Adherence junction
ALH	Atypical lobular hyperplasia
DCIS	Ductal carcinoma in situ
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
IDC	Invasive ductal carcinoma
LCIS	Lobular carcinoma in situ
LN	Lobular neoplasia
ILC	Invasive lobular carcinoma
p120	p120-catenin

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Pathology and Molecular Features of Lobular Breast Cancer

Terminology and History

World-wide, breast cancer causes approximately half a million deaths annually (WHO 2010). Lobular carcinoma accounts for approximately 10–15 % of all breast cancer cases, thereby representing the second most common type of breast cancer (Arpino et al. 2004; Li and Daling 2007). At the beginning of the nineteenth century, the general opinion was that all breast tumors were ductal carcinomas (Hajdu and Tang 2009). Broders (1932) was the first to define a non-invasive variant termed lobular "carcinoma in situ" (LCIS). The term "lobular neoplasia" (LN) was subsequently introduced by Haagensen et al. (1978), who used this terminology to cover the whole non-invasive spectrum of the disease: from atypical lobular hyperplasia (ALH) to LCIS. LN was described not only to be associated with invasive lobular breast cancer, but also as a risk factor for subsequent development of invasive cancer (Aulmann et al. 2008; Hwang et al. 2004; Wheeler et al. 1974). This hypothesis was subsequently supported by molecular evidence indicating that LCIS is a precursor of

invasive lobular carcinoma (ILC), which will be discussed later in this review. Also columnar cell lesions can be precursor lesions of ILC (Verschuur-Maes et al. 2012), but they fall beyond the scope of the current review (for review see Abdel-Fatah et al. 2007).

Lobular Breast Cancer Subtypes

Morphological Subtypes

Lobular breast cancer is characterized by a noncohesive growth pattern and can be grouped in non-invasive and invasive subtypes (Fig. 1a, b, respectively). Foote and Stewart (1941) defined LCIS as a population of small monomorphic cells filling and distending from the terminal ducto-lobular unit spreading in a Pagetoid manner through the ductal system. ALH resembles LCIS, but ALH lesions do not completely solidify the acini within the lobular structures of the mammary gland (Harvey and Fechner 1978). In view of the problems when differentiating between ALH and LCIS and the wide overlap in molecular features, the noninvasive forms of lobular cancer are currently grouped as LN. Although usually located in the lobule, cells may spread to the ductal system, denoted "Pagetoid spread" (Liberman 2000; Shin and Rosen 2002; Wheeler et al. 1974). Very often, LN is diffusely dispersed in the affected breast. High-grade variants are pleomorphic LCIS defined by atypical nuclei and occasional apocrine differentiation, and the macroacinar (sometimes denoted "florid") type with hugely distended acini that tend to become necrotic in the center because of severe hypoxia, and may contain micro calcifications (Fig. 1a) (Reis-Filho et al. 2005). These high-grade forms of LCIS are more difficult to discern from ductal carcinoma in situ (DCIS) and tend to behave more as DCIS in the sense that they usually form localized disease, which can be treated surgically. Alternatively, LN has been divided into three progressive groups based on grading: LIN1, LIN2, and LIN3 (Bratthauer and Tavassoli 2002). LIN1 is comparable to ALH, LIN2 to classic (low grade) LCIS, and LIN3 to

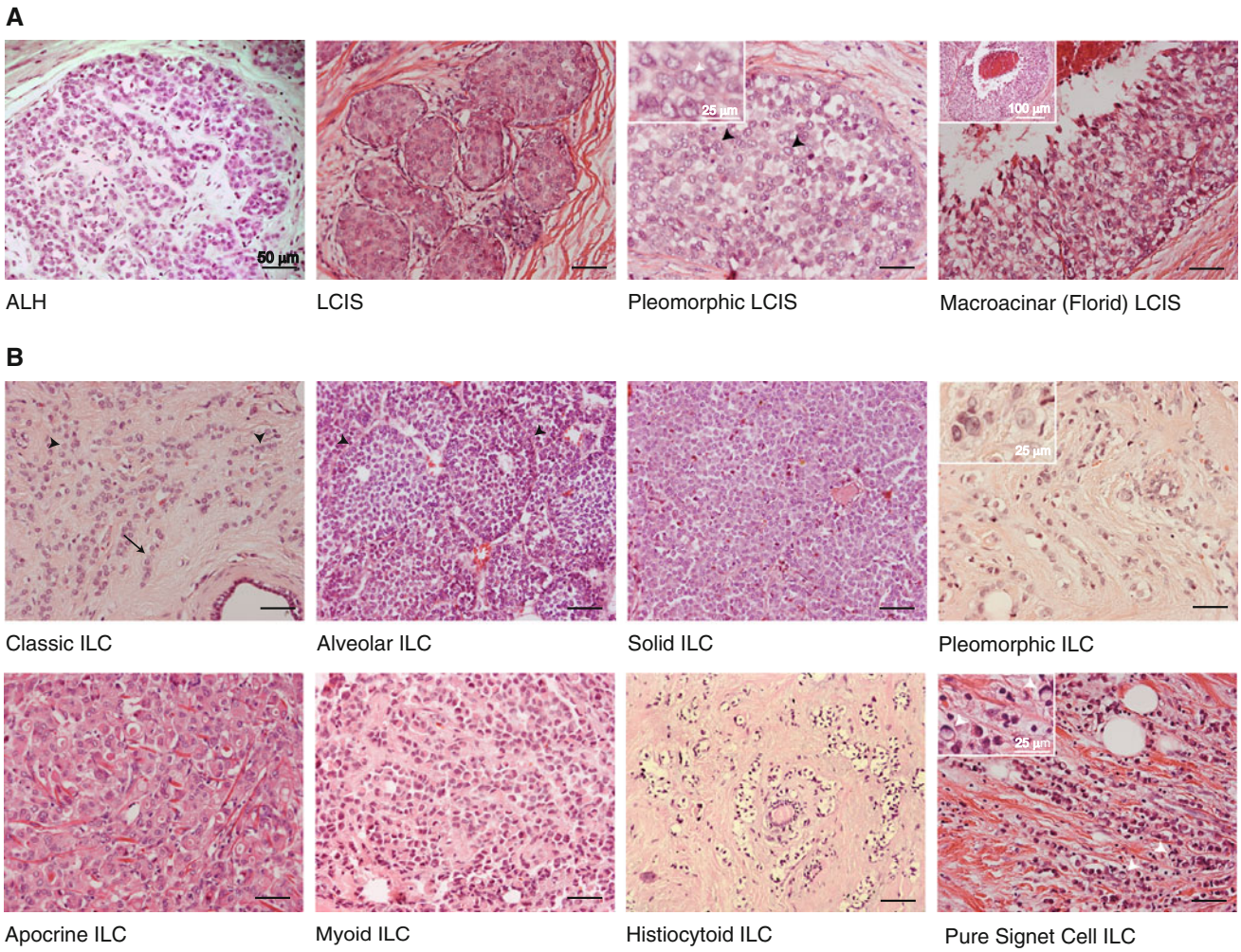

Fig. 1 Labular breast cancer subtypes based on histology

pleomorphic, macroacinar or pure signet ring cell LCIS. While LN lesions are always surrounded by a myoepithelial cell layer and an intact basement membrane, invasive tumors penetrate the basement membrane and invade into the surrounding stroma. Moreover, a rare type called microinvasive lobular carcinoma has been reported. In these tumors LN lesions are present with an intact basement membrane combined with microinvasive foci <1 mm that have penetrated the basement membrane (Nemoto et al. 1998; Ross and Hoda 2011). Classic ILC is characterized by small regular cells, frequently containing cytoplasmic vacuoles (which may induce "signet cell" formation), with small rounded nuclei with no or small nucleoli. The tumor cells grow dyscohesively and infiltrate as single cells or as one-layered strands of cells (so called "trabeculae" or "Indian files"), often in a targetoid pattern around uninvolved ducts (Fig. 1b). The mitotic rate is usually low and there are few apoptotic cells.

Different ILC variants have been described. First, the alveolar and solid subtypes, which show a different architecture, but both harbor a classic lobular cytonuclear appearance. The alveolar variant has an architectural pattern of round, balloon-like aggregates of 20 cells or more, surrounded by thin sheets of intervening stroma (Iorfida et al. 2012). In contrast, solid ILC is characterized by large solid masses of lobular carcinoma cells with little intervening stroma (Fig. 1b, top row) (Fechner 1975). Second, the pleomorphic (Fig. 1b, top row). The pleomorphic variant represents approximately 10 % of the lobular lesions and displays polygonal cells with highly atypical nuclei and harbors more frequent mitoses (Reis-Filho et al. 2005). Some ILC variants show histiocytic, myoid or apocrine differentiation, the latter marked by eosinophilic cytoplasm (Fig. 1b, bottom row).

Immunophenotype

Historically, microscopical examination of fixed and embedded tissue was one of the first tools of classifying breast tumors. The main purpose of classifying breast tumors was to be able to predict patient prognosis. Today markers like hormone receptors and Her2/Neu (ERBB2) are used to predict patient outcome and treatment response. The vast majority of ILC shows estrogen and/or progesterone receptor expression, while lacking expression of ERBB2 (Arpino et al. 2004; Halsted 1898). In contrast, 35–81 % of pleomorphic ILC expresses ERBB2 (Middleton et al. 2000; Monhollen et al. 2012). Compared with classical ILC, pleomorphic ILC is significantly more often p53-positive (Frolik et al. 2001; Middleton et al. 2000; Monhollen et al. 2012), and 46–100 % shows apocrine differentiation based on expression of the apocrine differentiation marker gross cystic disease fluid protein 15 (Eusebi et al. 1992; Middleton et al. 2000; Monhollen et al. 2012). There is very frequent loss of E-cadherin expression and abnormal subcellular localization of p120-catenin (p120) in all ILC subtypes (De Leeuw et al. 1997; Sarrio et al. 2004). (Expression and loss of function of the cadherin-catenin complex will be discussed in part two of this review.) ILC lacks expression of basal markers like cytokeratin (CK)5 and CK14, but expresses the luminal epithelial markers CK8 and CK18 (Fadare et al. 2008; Shao et al. 2012). ILC in general does not show overexpression of the epidermal growth factor receptor (EGFR) (Arpino et al. 2004; Reis-Filho et al. 2005).

Molecular Subtypes

More than a century later, clinicians are still trying to improve patient outcome and treatment response predictions by subgrouping the breast cancer patients. Approximately 10 years ago, Perou et al. (2000) proposed a classification system based on RNA expression profiling. Unsupervised cluster analysis pointed to five novel subtypes of breast cancer: Luminal A, Luminal B, Basal-like, Her2/Neu, and Normal (Sorlie et al. 2001). In this context, the majority of ILC tumors were considered Luminal A-type lesions, based on the absence of basal expression RNA markers (e.g. ERBB2) and hormone receptor presence (Weigelt et al. 2010). Because pleomorphic and apocrine lobular carcinomas tend to be ERBB2-positive, they were grouped in the "HER2" or "Luminal B" subgroups, depending on their hormone receptor status (Iorfida et al. 2012; Weigelt et al. 2010). Pleomorphic tumors with a basal classification are rare, but have also been reported (Monhollen et al. 2012). Recently, the RNA expression profiling was combined with copy number aberration analysis to define DNA-RNA paired profiles, which revealed ten new breast cancer subgroups (Curtis et al. 2012). The subgroups were correlated with differences in survival, but it is still unclear whether these new subgroups will enable better prognosis or treatment strategies. In order to improve treatment efficiency, we will have to extend our knowledge beyond the past profiling studies and reach through to the underlying biology.

Clinical Aspects

Diagnosis

Lobular neoplasia is usually too small and scattered to be detected as a palpable lesion, and presents a challenge for detection by radiologists. Detection limitations and symptomless disease progression render LN difficult to diagnose and estimate disease incidence. Only occasionally, LCIS is accompanied by micro calcifications (Beute et al. 1991;

Hutter et al. 1969; Pope et al. 1988; Sonnenfeld et al. 1991), especially the macroacinar type. Micro calcifications are mostly observed around areas of sclerosing adenosis and apocrine metaplasia juxtaposed to tumor areas and they facilitate diagnosis of LN by radiology. Research indicated that core-needle biopsies can underestimate breast cancer risk, as surgical excision biopsies from women diagnosed with LN by core-needle biopsies, showed invasive disease in 14 % of the cases (Arpino et al. 2004). As a consequence it was concluded that surgical biopsy should be considered to determine the presence of ILC. As for LN, the diagnosis of ILC using physical examination, mammography, sonography, MRI and PET scanning is also often difficult due to the fact that also ILC often does not form a palpable lump (Boetes et al. 2004; Georgian-Smith and Lawton 2001; Krecke and Gisvold 1993; Sapino et al. 2000; Selinko et al. 2004). While MRI is regarded as the most sensitive technique in the detection of ILC, a recent study concluded that there was no statistically significant difference between the sensitivity of mammography, sonography, MRI, and breast-specific gamma imaging (Brem et al. 2009).

Incidence

Lobular carcinoma in situ is found in about 5 % of all breast cancer excision specimens, whereas ILC comprises approximately 15 % of all breast cancers (Arpino et al. 2004; Frykberg 1999; Li et al. 2005). The incidence of LCIS and ILC appears to have increased in the past two decades (Li et al. 2002a, 2005), an increase likely due to the improvement of screening methods, together with the increased awareness of the pathologists about this lesion (Fisher et al. 2004; Liberman et al. 1999). Furthermore, it was suggested that the increase in ILC may be linked to usage of post-menopausal hormone replacement therapy (Biglia et al. 2007). Incidence of the ILC subtypes is as follows: 57 % classic, 19 % alveolar, 11 % solid, and 13 % pleomorphic, signet ring cell, histiocytoid, or apocrine ILC tumors (Orvieto et al. 2008).

Prognosis

Lobular neoplasia The relative risk to develop subsequent invasive cancer was originally estimated to be 4–5-fold for ALH and 8–10-fold for LCIS. The relative risks of the ductal variants, ADH and DCIS are in the same order (Page et al. 2000). More than 50 % of women diagnosed with LCIS present multifocal tumor development in the ipsilateral breast. Surprisingly and specific for lobular breast cancer, 30 % of patients with ipsilateral involvement also develop LCIS in the contralateral breast (Arpino et al. 2004; Urban 1967). The latter case is still a point of debate since there are conflicting reports on the risk of contralateral breast cancer associated with LCIS (Chuba et al. 2005; Li et al. 2006). Despite these differences, both studies showed that a large proportion of women diagnosed with LCIS developed ILC later in their lifetime. In view of the above-described multifocality, and high rate of ipsilateral invasive recurrences, and contralateral disease, the proper surgical treatment for LN would be bilateral mastectomy, but this is generally considered over treatment as only few patients develop ILC. Unfortunately, there are relatively few validated prognostic factors for LN. Bodian et al. (1996) stressed the importance of age at diagnosis of LCIS and family history as reliable prognostic factors (Bodian et al. 1996). Both of these factors were previously reported by others as well (Haagensen et al. 1978). Furthermore, the number of lobules with LCIS within a specimen and nuclear size significantly relate with recurrence of the disease (Ottesen et al. 1993).

Invasive lobular cancer Reported comparisons between the prognoses of ILC versus Invasive ductal carcinoma (IDC) are inconsistent; while some studies report that ILC has a better prognosis (Toikkanen et al. 1997), others conclude that there is no difference (Sastre-Garau et al. 1996) or even that ILC has a worse prognosis (Ashikari et al. 1973; Mate et al. 1986). A common drawback of these studies, however, is the fact that small groups of patients were included. A study on a large cohort of breast cancers (4140 ILC cases versus 45169 IDC cases) showed no prognostic difference between these two biologically different subtypes (5 year survival of 86 and 84 %, respectively), while the metastatic spectrum did differ in ILC compared with IDC (Arpino et al. 2004). Interestingly, another study on a large cohort of patients (767 ILC cases versus 8607 IDC cases) showed that up until 6 years of disease free survival the ILC cohort had an advantage over the IDC cohort; however, after 10 years the ILC cohort had a disadvantage (Pestalozzi et al. 2008). This indicates that the long-term prognosis of ILC patients is worse compared with IDC patients. This is illustrated by a case report of an ILC patient diagnosed at the age of 41, who presented with recurrence 40 years later (Ademuyiwa et al. 2012). Pleomorphic ILC has been reported to be significantly larger then classic ILC tumors (Buchanan et al. 2008). Besides the lymph nodes of pleomorphic ILC patients are more often positive and significantly more patients presented with metastatic disease (Buchanan et al. 2008). Moreover, recurrence in pleomorphic ILC patients is significantly worse compared with classical ILC patients (Weidner and Semple 1992). Overall, this indicates that pleomorphic ILC is a more aggressive form compared with classical ILC. Nothing is known about prognosis of the rare type of lobular carcinoma called microinvasive carcinoma, but in general no lymph node metastases are found in these

patients indicating this rare subtype is probably not very aggressive (Nemoto et al. 1998; Ross and Hoda 2011).

Lobular Breast Cancer Biology

Genetic Features

Genome-wide gene expression profiling indicated that ILC forms a distinct subtype based on hierarchical clustering (Weigelt et al. 2010). ILC displayed downregulation of genes involved in actin cytoskeleton remodeling, ubiquitin conjugation, DNA repair, cell adhesion, and transforming growth factor (TGF)-β signaling. Among the up-regulated genes were those implicated in transcription regulation of immediate early genes, lipid/prostaglandin biosynthesis, and cell migration. Furthermore, comparative genomic hybridization (CGH) showed that IDC carries a higher frequency of copy number alterations compared with ILC (Nishizaki et al. 1997; Richard et al. 2000).

Most lobular carcinomas are E-cadherin negative, which is often due to inactivating mutations and subsequent loss of heterozygosity (LOH) of the unaffected CDH1 allele, which is located on 16q22.1 (Berx and Van Roy 2001). As a consequence, expression of E-cadherin can be used to efficiently differentially diagnose ILC versus IDC, since IDC mostly retains expression (Berx et al. 1995). Apart from genetic alterations in CDH1 at chromosome arm 16q, ILC is characterized by a gain at chromosome arm 1q. Unfortunately, it is unknown which genes at 1q are causal to ILC pathology (Orsetti et al. 2006). Another hallmark of ILC is the presence of somatic PIK3CA mutations, which are found in 33–46 % of ILC. In contrast, IDC shows PIK3CA mutations in approximately 22 %. PIK3CA encodes for a catalytic subunit called p110α, which together with the p85 subunit forms a lipid kinase called phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K). PIK3CA mutations are generally positioned in the interface of p110α and p85 (Huang et al. 2008). PI3K converts phosphatidylinositol (4,5)-diphosphate (PIP-2) into phosphatidylinositol (3,4,5)-triphosphate (PIP-3), which is a prominent substrate for the protein kinase B/AKT1 pathway (Huang et al. 2008). The PIK3CA mutation spectrum seems to vary between breast cancer subtypes (Cancer Genome Atlas Network 2012); the majority of mutations in ILC are positioned in exon 9 (helical domain) and exon 20 (the kinase domain) (Buttitta et al. 2006). Besides mutations in PIK3CA, many other genes in the PI3K signaling pathway were found mutated in breast cancer, like phosphatase and tensin homolog tumor suppressor gene (PTEN), nuclear factor (*NF-κB*), v-akt murine thymoma viral oncogene homolog 1 (AKT1), insulin receptor substrates, and PIK3 regulatory subunits (Kalinsky et al. 2011; Wood et al. 2007). PTEN truncating mutations are found in around 2 % of the ILC tumors (Mercapide et al. 2002). Another gene often affected in breast cancer is ERBB2, which encodes for a protein-tyrosine kinase receptor and has high homology with the EGFR. V-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (ERBB2) amplification can be found in 13 % of the lobular and in 46 % of the ductal tumors, and is associated with poor prognosis (Rosenthal et al. 2002). Activating somatic ERBB2 mutations occur more often in ILC compared with IDC (Cancer Genome Atlas Network 2012). Finally, tumor suppressor protein p53 (TP53) is mutated in about 19 % of the ductal breast cancer samples (Olivier et al. 2006; Soussi 2007), while somatic *TP53* mutations are a rare event in classical ILC (approximately 6 %) (Oliveira et al. 2002). However, the incidence in pleomorphic ILC may be as high as 46 % (Boyault et al. 2012; Ercan et al. 2012a).

Reports on hereditary genetic predispositions in lobular carcinoma patients indicated there might be a slightly higher family incidence of lobular cancer (Cannon-Albright et al. 1994; Rosen et al. 1982). As the frequency of somatic E-cadherin mutations in lobular carcinoma is so high, CDH1 would be a plausible candidate for this hereditary risk. Indeed, E-cadherin germline mutations were observed in lobular breast cancer patients, albeit with a low frequency (Guilford et al. 1998; Masciari et al. 2007; Xie et al. 2011). E-cadherin germline mutations were also found in about 30 % of the hereditary diffuse gastric cancers (HDGC) (Gayther et al. 1998; Guilford et al. 1998; Oliveira et al. 2002). Moreover, patients carrying an inactivating E-cadherin germline mutation have a high cumulative risk to develop gastric cancer (67 % for men and 83 % for women) if they would reach an age of 80 years (Pharoah et al. 2001). Compared with the cumulative risk to develop breast cancer this risk is 39 %, which makes it five times more likely for a female mutation carrier to develop gastric cancer than breast cancer (Pharoah et al. 2001). Interestingly, E-cadherin mutation carrier families are often associated with either breast or gastric cancer, and only a small overrepresentation of lobular breast cancer has been found in HDGC families (Jonsson et al. 2002; Kaurah et al. 2007; Masciari et al. 2007). Many different E-cadherin mutations have been described in lobular and gastric cancer (Berx et al. 1998). Interestingly, almost all of these mutations were found in the extracellular part of the E-cadherin protein. Interestingly, most of the mutations found in lobular breast cancer cause premature stop codons resulting in a complete loss of the E-cadherin protein, while diffuse gastric cancer often contain mutations resulting in exon-skipping and in-frame deletions, rendering expression of a mutant E-cadherin protein (Berx et al. 1998). It is currently unknown whether these differences in mutation patterns differentially affect

signaling and how these are connected to tissue-specific cancer types.

Besides mutational inactivation of E-cadherin, expression of E-cadherin can also be lost due to LOH upon promoter methylation or epigenetic silencing. Methylation of the E-cadherin promoter has been found in a substantial percentage of ILC tumors and LN lesions (Droufakou et al. 2001; Sarrio et al. 2003). Although more rare, E-cadherin can also be epigenetically silenced via the TGF-β/SMAD2 pathway (Da Silva et al. 2008; Fackler et al. 2003). While expression of wild-type E-cadherin is retained in approximately 15 % of the ILC cases (Droufakou et al. 2001), functionality of the adhesion complex seems to be impaired. In these cases, β-catenin expression is lost and p120 is translocated to the cytosol (Da Silva et al. 2008; Rakha et al. 2010). Moreover, biallelic inactivating mutations were identified in α-catenin (CTNNA1), another E-cadherin associated molecule (Hollestelle et al. 2010). While the authors of this study did not observe somatic inactivating mutations in CTNNA1 in patient material, immunohistochemistry revealed loss of protein expression, which correlated with the lobular phenotype. These findings indicate that loss of E-cadherin expression or function can be caused by different genetic alterations. Interestingly, somatic mutations in CTNNB1 or CTNND1 have not been described to date, indicating that mutations in β-catenin or p120 are rare.

Recent genomic advances may also aid to delineate tumor etiology and clonal relationships in lobular cancer. It has long been discussed whether LN is a precursor lesion of ILC. In 1974, a patient follow-up study reported that LN only in some cases leads to invasive carcinomas, often of the lobular kind (Wheeler et al. 1974). A few decades later, genomic analyses showed a clonal relationship between LN and ILC, as patient samples containing LN adjacent to ILC showed identical mutations or genomic alteration patterns in both lesions (Aulmann et al. 2008; Chen et al. 2009; Hwang et al. 2004; Mastracci et al. 2006; Morandi et al. 2006; Vos et al. 1997;). Besides loss of chromosome arm 16q, the majority of the lobular carcinomas harbor a gain of 1q based on whole-arm CGH analysis (Chen et al. 2009; Hwang et al. 2004; Tirkkonen et al. 1998). As these genetic alterations can already be found in LN, they are both thought to be early-onset chromosomal abnormalities. In short, genetic analysis by CGH and mutation analyses show that loss of chromosome arm 16q and gain of 1q are early-onset alterations in lobular breast cancer, confirming that LN is indeed an ILC precursor.

Pleomorphic lobular carcinoma is an interesting lobular carcinoma variant from a cancer progression point of view. Phenotypically it has both lobular and ductal features while displaying apocrine differentiation. It has been under debate whether pleomorphic ILC is an E-cadherin mutated high-grade ductal carcinoma, or if it represents a lobular carcinoma with some ductal features. Genetic studies have indicated that both the in situ and the invasive component can be best classified as a lobular carcinoma, as they also harbor 16q losses and 1q gains (Chen et al. 2009; Palacios et al. 2003; Simpson et al. 2008; Weigelt et al. 2010). Our unpublished data (Ercan et al. submitted) indicate that based on multiplex ligation-dependent probe analysis studies on 21 established breast oncogene and tumor suppressor genes, pleomorphic ILC more frequently harbors copy number changes than classic ILC. Cluster analysis demonstrated classic and pleomorphic ILC to be rather separate entities and pleomorphic ILC as an entity molecularly in between classic ILC and infiltrating ductal cancer (Ercan et al. unpublished data).

Next generation DNA sequencing indicated that mutations in PIK3CA and TP53 may reflect driver mutations (Nik-Zainal et al. 2012). Interestingly, a recent study reported that PIK3CA mutations appear to be more common in the primary tumor than in tumor cells at a metastatic site, suggesting that PIK3CA mutations may not be required for metastases formation (Christgen et al. 2013). Also, a breast cancer case study showed that a specific TP53 mutation was only present in the recurrent tumor and the distant metastases, but not in the original primary tumor, indicating that the p53 mutation had evolved as a secondary event (Christgen et al. 2012). Other research showed that breast cancer can become genetically heterogenic by forming different subclones at early stages of tumor development (Nik-Zainal et al. 2012). During ILC development significant tumor evolution may take place; next-generation sequencing on a single lobular carcinoma sample revealed that 5 of the 32 somatic coding mutations in the metastases could be traced back to the primary tumor that arose 9 years earlier (Shah et al. 2009). Surprisingly, next to genetic alterations in the epithelial tumor cells, genetic alterations in the tumor-associated stroma have also been found. PTEN and TP53 mutations were observed in carcinoma cells as well as in tumor-associated stroma. In most cases mutations were found either in the stromal or in the epithelial compartment and only rarely in both compartments (Kurose et al. 2002). From an evolutionary point of view it would be very interesting to know in which cellular compartment somatic mutations arise first. Little is known about genetic alterations within the tumor microenvironment of lobular breast tumors.

Biological Features

Although loss of E-cadherin expression characterizes lobular tumors, until recently surprisingly little was known about the intracellular signaling pathways that

are affected upon E-cadherin loss and how they promote tumor development and progression. Conditional E-cadherin knock-out mouse models were generated that have provided the tools to understand the underlying cellular biochemistry. Using these models it has become evident that aberrant activation of downstream catenins and the relationship with the actin cytoskeleton are crucial in lobular carcinoma biology and the metastatic phenotype.

The Epithelial Adherens Junction and Its Functional Role in Lobular Breast Cancer

E-cadherin is a calcium-dependent transmembrane protein that controls epithelial integrity by homotypic (cis and trans) interactions. Together with the associated catenins it forms the adherence junction (AJ) on the apical side of the basolateral membrane of the cell (Palacios et al. 1995) (Fig. 2a2). E-cadherin regulates multiple epithelial

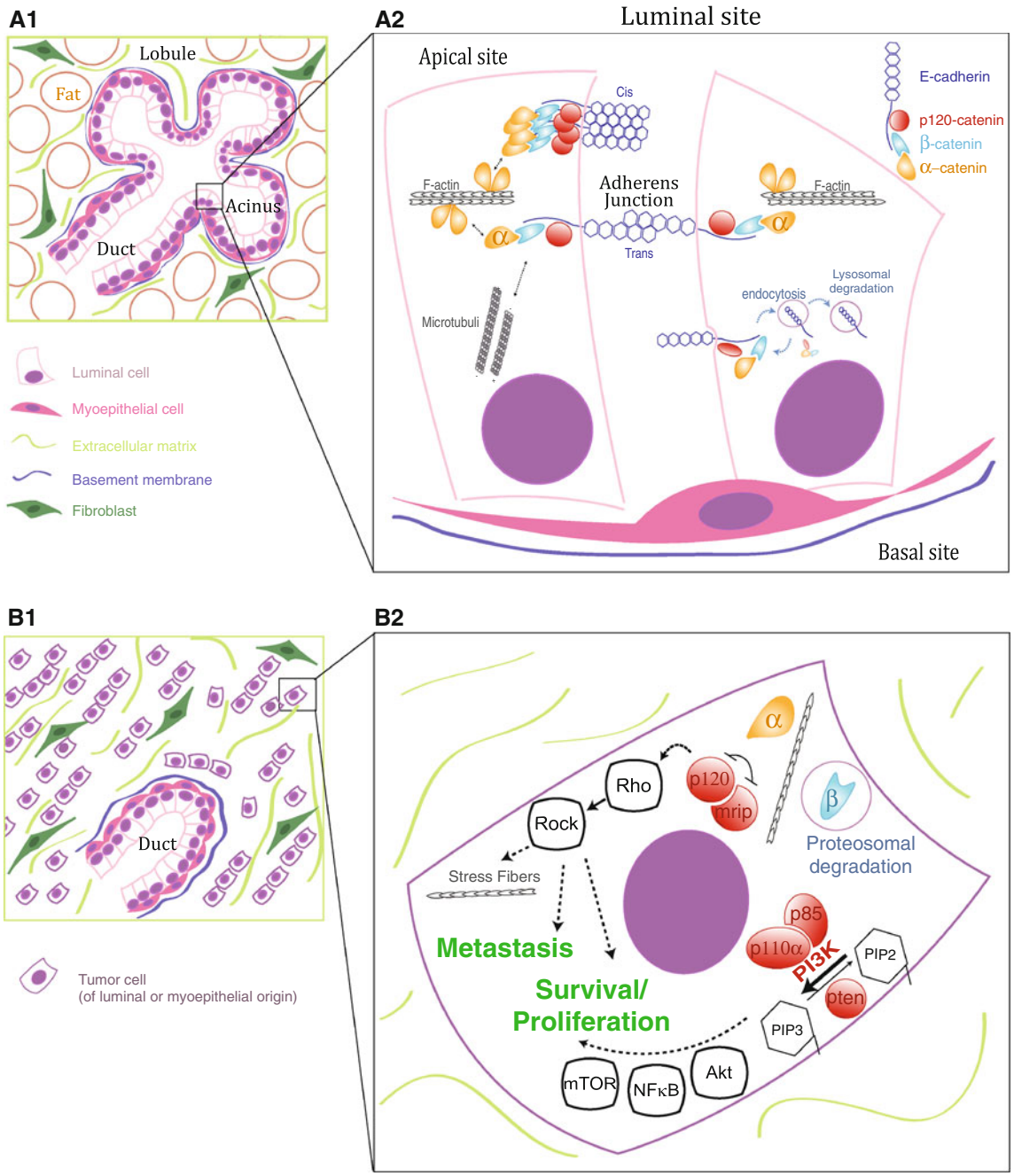

Fig. 2 Schematic representation of breast epithelial and invasive lobular carcinoma cells

processes through linkage to the actin cytoskeleton through β-catenin and α-catenin (Han and Yap 2012). The AJ can also connect to microtubuli via α-catenin, β-catenin or p120 (Ligon et al. 2001; Meng et al. 2008; Ratheesh et al. 2012; Yanagisawa et al. 2004), although these ties remain controversial. The AJ is linked to the actin cytoskeleton via α-catenin, but the exact mechanism still remains unclear. It seems that α-catenin cannot bind β-catenin and actin filaments simultaneously due to its transition between "active" and "inactive" conformations (Drees et al. 2005; Yamada et al. 2005), suggesting highly dynamic and mutually exclusive interactions. The AJ is not a rigid structure; even when cells are grown in confluency and cell–cell junctions are stable, a small pool of E-cadherin is recycled (Le et al. 1999) (Fig. 2a2). Stability and recycling of the AJ complex is mainly dependent on p120-regulated endocytosis (Davis et al. 2003). The p120-binding domain of E-cadherin overlaps with the region responsible for endocytosis (Fujita et al. 2002; Hong et al. 2010; Miyashita and Ozawa 2007). Recent studies indicated that HAKAI-mediated ubiquitination of the E-cadherin juxtamembrane domain inhibits p120-binding and targets E-cadherin for degradation (Hartsock and Nelson 2012). The link between p120 and E-cadherin internalization was further illustrated by the observation that loss of p120 leads to E-cadherin, β-catenin, and α-catenin downregulation (Davis et al. 2003). Moreover, recent data indicated that α-catenin is required to strengthen the association between p120 and E-cadherin and as such may influence E-cadherin internalization (Troyanovsky et al. 2011).

Given that E-cadherin is mostly absent in lobular breast cancer as a result of somatic mutations, conditional knockout mice targeting E-cadherin were generated to model lobular breast cancer. Due to the fact that E-cadherin loss in the mammary gland is not tolerated (Boussadia et al. 2002; Derksen et al. 2011), E-cadherin was ablated in the context of p53 loss. Concomitant inactivation of E-cadherin and p53 in the mouse mammary gland resulted in a shift from noninvasive carcinoma to invasive and metastatic tumors resembling human ILC (Derksen et al. 2006, 2011). Mouse ILC (mILC) tumors grew in a noncohesive manner typical for invasive lobular tumors. Furthermore, different lobular subtypes were observed including LN, classical ILC and solid ILC (Fig. 3). mILC tumors were relatively pleomorphic in appearance and diagnosed as high grade. Similar to human ILC, mILC showed trabecular invasive growth (Indian files), targetoid periductal distribution and stellate "scar"-type lesions. Interestingly, next to common metastatic sites such as bone, lungs and lymph nodes, mILC showed dissemination to the peritoneum and the gastro-intestinal tract, which are typical metastatic sites for human ILC as well. Occasionally, signet ring cells (an occasional characteristic trait of human ILC) were detected (Fig. 3b). While human ILC tumors expressed only cytokeratin 8, mILC cells showed a mixed but mutually exclusive expression of epithelial cytokeratins 8 and 14. Some estrogen receptor (ER) expressing cells were detected in low-grade elements of mILC lesions, but most mILCs were ER-negative suggesting that ER expression is inversely correlated with tumor grade in these mouse models.

Using the mILC model as starting point, Schackmann et al. (2011) confirmed that p120 translocated to the cytoplasm upon E-cadherin loss. The authors went on to show that p120 played a key oncogenic role through regulation of anchorage-independent survival. In contrast, β-catenin was subject to proteasomal degradation, thereby silencing canonical Wnt signaling (Schackmann et al. 2011). These findings were supported by observations in human breast cancer samples showing (i) that missense (NH2)-terminal domain β-catenin mutations were exclusively found in metaplastic breast cancer (Geyer et al. 2011; Hayes et al. 2008) and (ii) that β-catenin expression is very low or absent in most ILC cases (Sarrio et al. 2004). In ILC, p120's Rho GDP-dissociation inhibitor functions are inhibited by binding to the Rho antagonist myosin phosphatase Rho interacting protein. The net result of this interaction is activity of the Rho-Rock signaling axis, which positively controls anchorage-independent tumor growth and subsequent metastasis of ILC cells

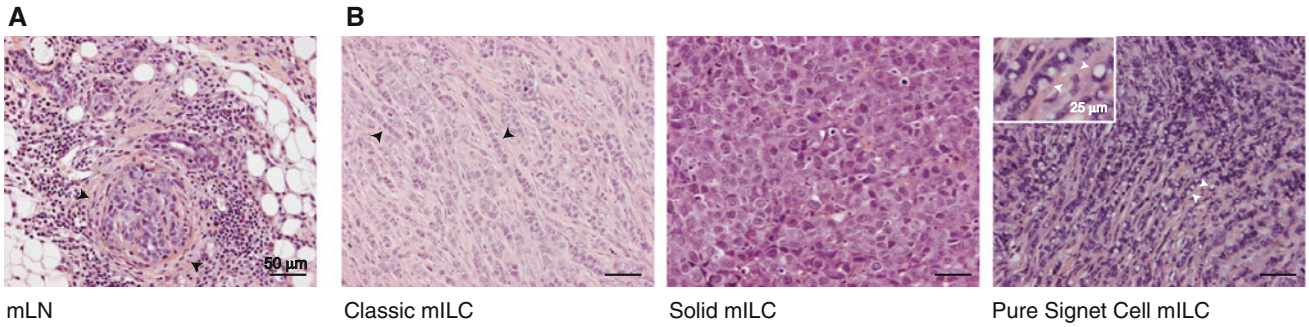

Fig. 3 Subtypes of mouse lobular breast cancer

(Schackmann et al. 2011) (Fig. 2b2). It, however, remains unclear how signals downstream of p120 prevent induction of anoikis. Nonetheless, since Rock is active and susceptible to pharmacological inhibitions, this research opened new avenues for the treatment of metastatic lobular breast cancer. Finally, our unpublished results suggest that conditional homozygous ablation of p120 in the mILC model severely inhibits ILC formation, while tumor incidence, onset, and metastatic potential remained unaffected (Tenhagen, manuscript in preparation).

The contribution of α-catenin to ILC development and progression is less well defined. Mutational analyses in human breast cancer cell lines revealed biallelic inactivating mutations in CTNNA1 resulting in functional inactivation of the protein (Hollestelle et al. 2010). These four mutated cell lines (MDA-MB-468, MDA-MB-330, MDA-MB-157 and HCC1187) retained E-cadherin expression at the membrane and three out four showed a rounded cell morphology suggesting impaired cell–cell adhesion. Interestingly, E-cadherin still seems to form a complex with p120, actin, α-actinin, and high levels of vinculin in MDA-MB-468 (Hazan et al. 1997; Hazan and Norton 1998). Others showed by immunofluorescence that also the tight junctions and desmosomes were affected in this cell line (Troyanovsky et al. 2011). Together, these data suggest that the disturbance of the connection between the AJ and the actin cytoskeleton is crucial for lobular tumor development and that this can be caused by either mutational inactivation of CDH1 or CTNNA1. A lot is still to be learned, as causality between α-catenin loss and lobular development has not been established. Furthermore, it is still unclear how α-catenin mediates the link between the AJ and the actin cytoskeleton. α-catenin contains different domains, each able to bind to different proteins of which many are actin binding proteins themselves (Maiden and Hardin 2011), suggesting the formation of a bigger complex enabling indirect binding of α-catenin to the actin cytoskeleton.

Other Signaling Pathways Involved in ILC

The PI3K/Akt pathway is disturbed in at least one-third of the ILC tumors (Buttitta et al. 2006; Christgen et al. 2013; Mercapide et al. 2002). PIK3CA mutations increase PI3K activity, which stimulates AKT-induced cell survival, cell cycle progression, cell motility, and metabolism (Fig. 2b2). PTEN is a protein/lipid phosphatase that also affects the PI3K/Akt pathway, as it reverses the synthesis of PIP3 (Maehama and Dixon 1998; Sun et al. 1999). Although AKT signaling seems to play a major role in breast cancer, mice carrying activated AKT under the control of the mouse mammary tumor virus promoter crossed with heterozygous p53 mice did not have a different median survival compared with the heterozygous p53 mice (Blanco-Aparicio et al. 2007). This suggested that PI3K and PTEN might promote cancer progression not via the AKT pathway, but via another, like the NF-κB pathway. Supporting this hypothesis is the finding that mutations in NF-κB or in other proteins involved in the NF-κB pathway have been observed in breast cancer (Wood et al. 2007). Mouse models with mammary-specific activation of the PI3K pathway showed excessive ductal branching and tumor development, but give little insight into the role of this pathway in lobular breast cancer development and progression (Li et al. 2002b; Utermark et al. 2012). In conclusion, the high frequency of PIK3CA mutations observed in lobular breast cancer suggests an important role for the PI3K pathway in tumor progression. Although cancer evolution studies advocated PI3K as a key player in tumor initiation (Nik-Zainal et al. 2012), its role in tumor progression remains controversial (Christgen et al. 2013; Dupont Jensen et al. 2011; Kalinsky et al. 2011).

Treatment of Lobular Breast Cancer

Surgical Management

Lobular Neoplasia

In the past, mastectomy was a frequently applied management strategy for the case of LCIS (Ewing 1919; Goldwyn 1977)). Bilateral mastectomy was also suggested as a possible management strategy with the claim that LCIS gives rise to invasive cancer in both breasts with an equal risk (Carter and Smith 1977; Powers et al. 1980). However, LN is usually an incidental finding and recent investigations show only a small percentage of LCIS patients treated with local excision present with recurrence (Fisher et al. 2004). Besides, recurrence developed relatively late as more than 50 % occurred after 15 years or later (Frykberg 1999). These findings led to a more conservative approach (Fisher et al. 2004). An exception to this more conservative attitude is the high-grade forms of LN including pleomorphic LN, which are more often segmental in distribution, have a relatively high local recurrence rate, and should, therefore, be treated with surgery (like DCIS) (Hussain and Cunnick 2011). A strict conservative trend in the management of LN has been favored by some authors to follow the principle of avoiding unnecessary surgery. In this approach patients are only physically examined annually and monitored regularly by mammography (Bauer et al. 2003; Renshaw et al. 2002). Criteria for the choice of surgical excision or another approach are still under discussion and differ between clinical centers. There are certain arguments for choosing surgical excision after diagnosis with LN such as

significant risk of invasive cancer (Cohen 2004; Shin and Rosen 2002), underestimation of the diagnosis due to limitations of core needle biopsies (Elsheikh and Silverman 2005; Londero et al. 2008), presence of suspicious microcalcifications (Berg et al. 2001; Rosenfeld et al. 2001), and specific histopathological features (discussed above). Generally, surgery of the ipsilateral or contralateral breast is not performed after the diagnosis of LN.

Invasive Lobular Cancer

Surgery is usually the first choice of treatment for ILC patients and is performed by a wide local excision for the area of cancer and surrounding healthy tissue. Since the disease may have spread to multiple areas within the breast, a mastectomy can be preferred in selected cases. ILC regularly may metastasize to the axillary lymph nodes and a sentinel node biopsy is, therefore, routinely in place, like for all major epithelial breast cancer subtypes (Borgstein et al. 1998), generally followed by axillary lymph node dissection only in case of macrometastases to the sentinel node. In contrast to IDC, ILC lesions tend to be larger due to their diffuse or multifocal growth and infiltration pattern. As a consequence, breast-conserving surgery and heat ablation are more likely to be irradical in ILC (van Esser et al. 2009). Radiation therapy, chemotherapy, and endocrine therapy are, therefore, also applied individually or as a combination therapy.

Radiotherapy

It has been shown that radiotherapy reduces the risk of local recurrence substantially in LN patients who underwent breast-conserving therapy, but not in patients that underwent mastectomy (Clarke et al. 2005). However, the effect of radiotherapy on long-term survival seems only marginal on patients that underwent breast conserving therapy, while node-positive patients that underwent mastectomy do benefit from radiotherapy. In ILC patients, radiotherapy is generally applied in combination with breast-conserving therapy. However, classic ILC may not be the most sensitive form of breast cancer as it usually shows little proliferation. Pleomorphic ILC, which shows a higher proliferation rate, may, however, be more sensitive (Downs-Kelly et al. 2011; Sneige et al. 2002).

Chemo- and Hormonal Therapy

Systemic treatment (especially with anti-hormonal drugs like tamoxifen) can be used to prevent progression of LN. Unfortunately, we currently have limited evidence showing the efficacy of such adjuvant therapies in the pre-malignant stages of the disease (Cutuli et al. 2005). As most lobular tumors are hormone receptor positive, treatment with tamoxifen or aromatase inhibitors is an option. It has previously been shown that both pre- and postmenopausal women with LCIS benefit from tamoxifen treatment (Fisher et al. 1998; Vogel et al. 2010), although this has been associated with an increased risk of endometrial cancer (Vogel et al. 2006). ILC patient's pathologic response to neoadjuvant therapy tends to be poorer compared with the response of IDC patients, while long-term response to chemotherapy tends to better compared with IDC patients (Cristofanilli et al. 2005; Tubiana-Hulin et al. 2006). The beneficial long-term outcome is most likely due to the often hormone-responsive nature of ILC. Systemic treatment in case of distant metastases is usually based on hormone and HER2 receptor status. Although still controversial, most guidelines (NCCN 2010) indicate that these receptors should preferentially be assessed in biopsies of the metastases (Hoefnagel et al. 2010).

Targeted Intervention

Because little is currently known about the pathways that regulate ILC tumor progression, targeted therapy is still in its infancy. Prominent candidates for clinical intervention are pathways controlling actin remodeling, as ILC cells appear susceptible for Rock inhibition under anchorage-independent conditions (Schackmann et al. 2011; Weigelt et al. 2010). Another important signaling pathway in ILC tumors is the PI3K pathway and the associated AKT, mTOR, and NF-κB pathways, which are currently subject of clinical trials in breast cancer patients (McAuliffe et al. 2010). The majority of lobular breast tumors harbor mutations in multiple pathways (Boyault et al. 2012), which suggests that combined and targeted intervention strategies may be beneficial in these tumors. An example of this is the fact that mutations in proteins involved in the PI3K pathway can affect ER signaling, suggesting that PI3K-targeted therapy may sensitize ILC for hormonal therapy (Hernandez-Aya and Gonzalez-Angulo 2011).

Clinically, the distinction between classic and pleomorphic lobular tumors is important as the latter has a poorer prognosis (Middleton et al. 2000). Especially age and negative hormone receptor status are indicative of worse clinical outcome in pleomorphic ILC patients (Monhollen et al. 2012). What the best treatment options are for pleomorphic lobular tumors and whether they should be treated with trastuzumab, as most of them are ERBB2-positive, are still unknown, but have been suggested by Mahtani and Vogel (2008) as well. Interestingly, enhanced PI3K/AKT signaling in ERBB2-positive tumors correlated with trastuzumab resistance (Nagata et al. 2004), which suggests that ERBB2-positive pleomorphic ILC patients might benefit from combined ERBB2 and

PI3K-targeted therapy. Finally, we recently showed that pleomorphic ILC shows high HIF-1α expression, a feature that is correlated with high mortality (Ercan et al. 2012b). Thus, although drug delivery to hypoxic areas is challenging (Kizaka-Kondoh et al. 2003), pleomorphic ILC may perhaps benefit from treatment with HIF1 inhibitors.

Conclusions and Future Perspectives

Lobular breast carcinoma is a distinct breast cancer subtype based on pathology, genetic aberrations, and cellular biochemistry. Detection and resection of lobular breast cancer is relatively difficult due to its diffuse growth pattern, leaving the pre-existing breast architecture largely intact, and a low tendency to induce a desmoplastic reaction. While ILC tumors harbor a more favorable histological status compared with IDC patients, ILC patients display the same prognosis compared with IDC patients (Arpino et al. 2004). In the past it has been under debate whether LN is an ILC precursor and whether pleomorphic ILC should be considered as a lobular or a ductal tumor. It has now become apparent that based on genetic analysis LN can be classified as a lobular precursor, pleomorphic LN is a pleomorphic ILC precursor, and pleomorphic ILC should be considered as a progressed lobular tumor. Importantly, pleomorphic ILC is characterized by a higher frequency of p53 mutations, hormone receptor negativity, and ERBB2 positivity, suggesting that future treatment of pleomorphic ILC should be designed accordingly. In ILC patients hormone therapy benefits patient survival, but since distant recurrences often occur with long latency (Rakha et al. 2008), additional targeted therapies are needed. Unfortunately, although much progress has been made, little is known about the signaling pathways that drive ILC development and progression. It has become evident that mutations in E-cadherin are causal to ILC development and progression through aberrant activation of Rho and Rock, rendering this pathway eligible for clinical intervention using Rock inhibitors. Moreover, a substantial ILC subgroup harbors mutations affecting the PI3K/AKT pathway. Therapies targeting these pathways represent an attractive option to enhance survival of ILC patients. Finally, advances in sequencing techniques have enabled identification of mutations in systemic tumor cells found in patient plasma samples. This technique might facilitate tumor classification based on genetic aberrations and enable targeted therapy in the future (Leary et al. 2010; McBride et al. 2010).

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