Breast Cancer Res Treat (2018) 167:9–29
https://doi.org/10.1007/s10549-017-4488-x

REVIEW

Molecular profile of atypical hyperplasia of the breast

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Received: 20 July 2017 / Accepted: 28 August 2017 / Published online: 14 September 2017

Abstract
Purpose Atypical ductal and atypical lobular hyperplasia (AH) of the breast are important proliferative lesions which are associated with a significantly increased risk for breast cancer. The breast cancer which develops in association with AH may occur synchronously, representing local progression, or metachronously at a later date in either the ipsilateral or contralateral breast. These high-risk characteristics of AH suggest they contain significant genomic changes.
Methods To define the genomic changes in AH, a comprehensive review of the literature was conducted to identify the numerical chromosomal and structural chromosomal changes, DNA methylation, and gene expression abnormalities in atypical ductal and atypical lobular hyperplasia.
Results AHs are characterized by advanced genomic changes including aneuploidy, loss of heterozygosity, gross chromosomal rearrangements such as amplifications and large-scale deletions, DNA methylation of tumor suppressor and other genes, and gene expression differences between AH and surrounding normal breast tissue including significant estrogen receptor expression. Many of these changes are shared by an associated synchronous breast cancer, consistent with an important precursor role for AH. At the same time, many of the genomic changes of AHs are also shared by common sporadic breast cancer, consistent with a high risk for future development of metachronous breast cancer.
Conclusions This molecular profile should help clarify the genomic characteristics and malignant predisposition of AH, and aid in the identification of new targets for the prevention of breast cancer

Keywords Atypical hyperplasia · Atypical ductal · Atypical lobular · Breast high risk · Breast cancer · Breast carcinogenesis · Premalignant breast

Abbreviations

ALH	Atypical lobular hyperplasia
ADH	Atypical ductal hyperplasia
AH	Atypical hyperplasia
AI	Allelic imbalance
CGH	Comparative genomic hybridization
DIALH	Ductal involvement by cells of atypical lobular hyperplasia
DCIS	Ductal carcinoma in situ
ER	Estrogen receptor
FISH	Fluorescent in situ hybridization
IDC	Invasive ductal carcinoma
IHC	Immunohistochemistry
LCIS	Lobular carcinoma in situ
LOH	Loss of heterozygosity
MSI	Microsatellite instability
MSP	Methylation-specific PCR
NABT	Normal adjacent breast tissue
NSABP	National Surgical Adjuvant Breast and Bowel Project
PDWA	Poorly differentiated without atypia
UDH	Usual ductal hyperplasia

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Background

Atypical lobular (ALH) and atypical ductal (ADH) hyperplasia are proliferative lesions of the breast which are associated with a significantly increased risk for breast cancer. The histologic characteristics of these lesions are well defined [1, 2], and ADH and ALH each contain early changes of, respectively, DCIS and LCIS, increasing the likelihood that they will contain at least the early genomic changes of malignancy. Estrogen is a major carcinogen for breast cancer, with the ability to cause single base substitutions, single and double DNA strand breaks, and chromosomal rearrangements [3]. ADH and ALH [collectively atypical hyperplasia (AH)] occur more commonly in women over the age of 55 [4, 5]. This allows for a significant (40 plus year) exposure to estrogens and the potential for widespread genomic damage. Individual reports have described the presence of aneuploidy [6], chromosomal gains and losses [7], DNA methylation [8], and gene expression changes [9] in AH lesions, consistent with this exposure. A comprehensive understanding of these genomic changes is needed to further define the nature and spectrum of these genomic alterations and the relationship of these changes to breast cancer.

Atypical ductal and atypical lobular hyperplasia may be associated with the development of synchronous or metachronous breast cancer. Synchronous breast cancer occurs concomitantly with AH and is considered to represent local progression of AH. The incidence of synchronous carcinoma with AH is, on average, 22.0% (Table 1). For this reason, AH lesions are frequently excised at the time of presentation to include these tumors, which provides an important opportunity to define the genomic characteristics of AH and its associated breast cancer. Interestingly, there appears to be a subset of ALH (lesions which are pure ALH, demonstrating radiographic–pathologic concordance and no associated high-risk lesions found on core needle biopsy) in which the incidence of synchronous carcinoma is low (≤3%; [10, 11]). Metachronous breast cancer develops subsequent to the initial AH, potentially out 25 years or more [5], and may occur in either breast (Table 2). Whereas synchronous breast cancer is more commonly DCIS (Table 1), metachronous breast cancers are more often invasive carcinoma (Table 2), suggesting a range of carcinogenic influences for the genomic changes in AH. Atypical hyperplasias are commonly estrogen receptor positive [12], and treatment with antiestrogens may prevent the development of metachronous breast cancer within the remaining normal breast tissue [13]. This indicates that at least one important molecular characteristic of AH (ER positivity) is an indicator of the responsiveness of the remaining normal tissue to systemic therapy. An understanding of the molecular characteristics of these high-risk lesions may therefore clarify the role of AH in breast carcinogenesis, as well as to promote identification of new targets for the development of drugs for breast cancer prevention.

To further define the genomic changes of AH, a comprehensive review of the literature was conducted to include all references describing numerical chromosomal changes, structural chromosomal changes, epigenetic changes, and changes in gene expression in atypical ductal and atypical lobular hyperplasia of the breast. The relationship of these genomic changes to those of associated synchronous breast cancer, and to the development of metachronous breast cancer, is discussed. A model summarizing the developmental pathways of AH is described.

Table 1 Incidence and characteristics of synchronous carcinoma with atypical hyperplasia

Primary atypical hyperplasia	Number of AH cases excised	Overall carcinoma	Invasive carcinoma	Ductal carcinoma in situ	Risk factors	References
ADH	62 cases	9 cases (14.5%)	2 cases (3.2%)	7 cases (11.3%)	Personal/family history breast cancer	[60]
ALH	73	13 (17.8%)	5 (6.8%)	8 (11.0%)		[61]
ADH	104	22 (21.2%)	3 (2.9%)	19 (18.3%)		[62]
ADH	76	21 (27.6%)	3 (3.9%)	18 (23.7%)		[63]
ADH	61	19 (31.2%)	5 (8.2%)	14 ((23.0%)		[64]
ALH	97	21 (21.6%)	6 (6.2%)	15 (15.5%)		[65]
ADH (9 gauge)	74	16 (21.6%)	2 (2.7%)	14 (18.9%)		[66]
ADH	101	20 (19.8%)	3 (3.0%)	17 (16.8%)	Number of ADH foci	[67]
ADH	65	11 (16.9%)	5 (7.7%)	6 (9.2%)	Increasing age	[68]
ALH	40	11 (27.5%)	4 (10.0%)	7 (17.5%)		[69]
Mean		21.97% ± 1.7	5.47% ± 0.84	16.52% ± 1.55		

Table 2 Risk and incidence of metachronous breast carcinoma with atypical hyperplasia

Type of atypical hyperplasia	Number of cases	Follow-up	Overall carcinoma	Invasive carcinoma	DCIS	Relative risk	References
AH	331	Mean—13.7 years	18.4%	16.0%	2.4%	Overall—3.88 ALH—3.67 ADH—3.83 ALH + ADH—7.10	[4]
AH	668	Median—17 years	21.4%	17.4%	3.0%	All AH—4.34 ALH—4.76 ADH—3.93 ALH + ADH—4.36	[5]
ALH	126	Mean—17.5 years	12.0%	12.0%		ALH—4.2	[1]
ADH	150		12.7%	12.7%		ADH—4.3	
ALH	316	Mean—17 years	ALH—12.8% ALH + DIALH—21.3% ALH/no DIALH-7.7% DIALH—6.1%	ALH—12.8% ALH + DIALH—21.3% ALH/no DIALH-7.7% DIALH—6.1%		ALH—4.3 ALH + DIALH-6.8 ALH/no DIALH-2.7 DIALH—2.1	[14]
ADH	82	Mean—12.4 years	9.8%	9.8%			[70]
ALH	ALH all—252 ALH alone—161 ALH + DIALH—76 ALH + ADH—15		ALH all—20% ALH alone—16% ALH + DIALH—24% ALH + ADH—40%	ALH all—20% ALH alone—16% ALH + DIALH—24% ALH + ADH—40%		Invasive carcinoma—3.1	[2]

Methods

Literature search and criteria for identification of tissue specimens

A literature search was conducted through PubMed and cross references to identify all reports of atypical ductal hyperplasia, atypical lobular hyperplasia, or simply atypical hyperplasia of the breast which described studies of molecular changes in four genomic categories: numerical chromosomal changes, structural chromosomal changes, DNA methylation, or gene expression studies (see respective Tables below for references describing methods of analyses). The criteria for diagnosis of ADH and ALH are as previously described [1, 14, 15]. Proliferative epithelium with atypia, such as that acquired through random periareolar fine needle aspirate, or through breast ductal lavage, was also included. The presence of an in situ or invasive carcinoma associated with the AH lesion (such as that removed with an initial excisional biopsy) is noted, and these are referred to as being "synchronous" to indicate they occur at the same time as the primary lesion. This is in contrast to "metachronous" carcinomas which occurred at a later date and in either the ipsilateral or contralateral breast. Molecular changes in an associated breast carcinoma, either in situ or invasive, are included when available; however molecular changes in associated benign lesions such as ductal hyperplasia without atypia, flat epithelial atypia (FEA), or lobular carcinoma in situ (LCIS) are not described, and lobular neoplasia is included only if specific molecular changes in an associated ALH are described.

Results and discussion

Numerical chromosomal changes in atypical hyperplasia

Studies examining chromosomal content by FISH, DNA content, or nuclear morphometry of ADH and ALH showed gains or losses of whole chromosomes compared to

Table 3 Numerical chromosomal abnormalities in atypical hyperplasia

Chromosome	Alteration	Normal breast	Atypical hyperplasia	DCIS	Invasive carcinoma	References
1	Chromosome copy number—FISH	Normal compliment	ALH—no gain or loss	Gain 100%	Gain 100%	[20]
1	Ploidy, FISH	Normal content	ADH—aneusomy, 100.0%	Gain, 72.7% Loss, 9.1%	Gain, 87.5%	[71]
1	Chromosome copy number, FISH	Signal number—1.14	ADH—increased copy number, signal number 1.50	Increased, copy number, signal number 1.95	Increased copy number, signal number 1.74	[23]
16	Chromosome copy number—FISH	Borderline loss	ALH—gain, 50%	Gain, 100%	Gain, 100%	[20]
17	Chromosome copy number—FISH	Normal compliment	ALH—borderline loss, 50%	True gain, 100%	True gain, 100%	[20]
17	Ploidy, FISH	Normal content	ADH—no loss or gain	Gain, 45.5% Loss, 45.5%	Gain, 50.0%	[71]
18	Chromosome copy number—FISH	Borderline loss	ALH—borderline loss 50%	Gain, 100%	Borderline gain, 100%	[20]
Chromosomes 8,11	FISH		Nipple aspirate fluid mild atypia—20% aneusomy			[72]
Chromosomes 1,8,11 or 17.	FISH	Disomy—100%	Nipple aspirate fluid, marked atypia—100% aneusomy		Nipple aspirate fluid, malignant, 100% aneusomy	[72]
Chromosomes 7-12, 17, 18, X	Chromosomal aberrations, cytologic, FISH	40% of non-proliferative lesions	ADH—100%		100% (includes DCIS)	[16]
Chromosomes 1, 7, 8, 16, 17, X	Chromosome copy number—FISH		ADH—no gains or losses ALH, chromosome 8, 3% triploid	70% aneuploidy	100% aneuploidy	[73]
Nucleus—nuclear morphometry	Area	Non-proliferative 25.5	AH—37.4	47.9	54.9	[74]
	Perimeter	Non-proliferative 20.6	AH—24.3	27.2	29.4	[74]
	Maximum diameter	Non-proliferative 7.3	AH—8.52	9.6	10.3	[74]
	Minimum diameter	Non-proliferative 5.1	AH—6.0	6.9	7.4	[74]
	Large dark areas	Non-proliferative 0.022	AH—0.084	0.103	0.099	[74]
	Large light areas	Non-proliferative 0.009	AH—0.038	0.049	0.075	[74]
	Total stain	Non-proliferative 12.52	AH—12.38	23.73	19.72	[74]
DNA content		Normal diploid	ADH—diploid, 33% Type III histogram, 33.3% Aneuploid, 33.3% ALH—diploid, 100%			[75]
DNA content	Nuclear DNA histogram	Diploid	ADH—Type III/IV histogram, 38.1% ALH—diploid			[76]

Table 3 continued

Chromosome	Alteration	Normal breast	Atypical hyperplasia	DCIS	Invasive carcinoma	References
DNA content		Hyperplasia without atypia-diploid	Aneuploid, 33.3%	Aneuploid, 78.2%		[19]
DNA content	Aneuploidy		AH—aneuploid, 30.8%	Aneuploid, 33.3%	Aneuploid, 88.5%	[77]
DNA ploidy	Cytometric assessment	Intraductal proliferation without atypia—euploid	ADH—43% aneuploidy	71.4%—84.4% aneuploidy	54.1%—95.6% aneuploidy	[78]
DNA aneuploidy	DNA histogram		AH—aneuploidy, 71%	Aneuploidy, 71%		[6]
DNA aneuploidy	DNA Index	Non-proliferative—25% aneuploidy	Hyperplasia with atypia—aneuploidy 32%			[79]
Nuclear morphometry	Nuclear area	13.13	AH—24.25	Cribriform—16.16 Comedo—40.23	41.30	[17]
Nuclear morphometry	Nuclear abnormality	0.644	AH—2.261	Cribriform—0.918 Comedo—2.710	1.265	[17]
Monoclonality	X-chromosome inactivation assay	0% monoclonal	AH—51.3% monoclonal	100% monoclonal		[21]
Nucleolar organizer regions	Ag-NOR—IHC	6.0	ADH—8.8 ALH—8.6	9.0	17.7	[18]
DNA quantitation	Aneuploidy	Euploid (NABT)	AEH—aneuploidy 50%		Aneuploidy 50%	[80]
Mitotic figures	Mitoses/HPF	3.5 (proliferative disease without atypia)	ADH—6.7	DCIS—26.5 DCIS in infiltrating carcinoma—51.3		[81]
Chromosomal abnormalities	Cytogenic, FISH		AEH—diploid with structural aberrations			[82]

NABT normal breast tissue adjacent to cancer, *IHC* immunohistochemistry, *FISH* fluorescent in situ hybridization, *AEH* atypical epithelial hyperplasia

normal breast tissue, indicating aneuploidy is a prominent feature of atypical hyperplasia (Table 3). The chromosomal changes seen in atypical hyperplasias are similar to those present in breast cancer [16–18], and are consistent with the proposal that AHs are preneoplastic lesions and part of a continuum in the steps toward breast cancer [19, 20]. Mariuzzi et al. [17] examined the nuclear chromatin pattern in AH and found drastic changes in karyometric features, with these changes similar to those seen in comedo DCIS. Others have noted a high incidence of monoclonality (51.3%) in ADH [21], and an abnormal DNA content [6], consistent with neoplastic transformation. Eriksson et al. [22] considered the DNA cytometric findings in atypical hyperplasias to strongly indicate that, already at this early stage, complex nuclear alterations have occurred. Further evidence for this relationship is found in the studies of Cummings et al. [23] who examined specimens containing both AH and DCIS and found concordance in chromosome 1 aneuploidy between these lesions. They considered these findings to support the concept that benign proliferative breast disease is a biological precursor of in situ and invasive ductal carcinoma, the early histological changes possibly indicating a field effect with further genetic changes required for the development of a malignant phenotype [23].

Aneuploidy is an important indicator of chromosomal instability [24], resulting in significant deregulation of the transcriptome [25], aneuploidy-induced stresses [26], and contributing to further progression in the carcinogenic pathway. The causes of aneuploidy in AH are not clear; however, alterations in multiple genes known to contribute to aneuploidy have been observed in AH (Tables 4, 5, 6).

Table 4 Structural chromosomal abnormalities in atypical hyperplasia

Chromosome	Gene	Alteration	Normal breast	Atypical hyperplasia	DCIS	Invasive carcinoma	References
1p32	*MYC1*	MSI/LOH		8.3%			[33]
1q		CGH		ADH—gain 11.1%			[35]
1q		Chromosome copy number, CGH		ADH—gain 33.3%	Gain, 60%	Gain, 80%	[83]
1q32-42/D1S549, D1S213		LOH (proportion)	2%	ADH with cancer, 29%	52%	44%	[32]
1q32-42/D1S549		MSI/LOH		AH—25.0%			[33]
1q32-qter		CGH		ADH—high-level amplification			[7]
2p11.2		CGH		ALH—gains, 50%			[84]
2q35/D2S362		LOH		ADH non-cancerous breast—none	Non-comedo—6%		[85]
				ADH cancerous breast—6%	Comedo—9%		
3p	*rhoA, cdc25A*	CGH		ADH—gains			[83]
3p24/D3S1298		MSI/LOH		AH—8.3%			[33]
3p22ter		CGH		ADH gain, 67%		Gain, 60%	[83]
3q11-q21		CGH		ADH loss, 11.1%			[35]
5p		CGH		Gain	Gain	Gain	[7]
5p14		CGH		ADH—high-level amplification			[7]
5q32-33.1	*CSF1R*	CGH		ALH—gain			[84]
6q		Chromosomal imbalance, CGH		ALH—gain, 36%	Gain, 22%	Gain, 2%	[86]
6qter/D6S417		LOH		ADH non-cancerous breast—6%	Non-comedo—17%		[85]
				ADH cancerous breast—9%	Comedo—11%		
6g21ter		CGH		ADH—gain, 33.3%	Gain, 40%	Gain, 60%	[83]
6q27-qter	*SEN6*	FISH, cytogenetic		ADH—large deletion present			[87]
7p11.2-p11.1		CGH		ALH—Loss, 83.3%			[84]
7p12-15	*EGFR*	Microsatellite analysis	30.0%	ADH of cancer subject—80%	100.0%	100.0%	[88]
7p22-qter		CGH		ADH—high-level amplification			[7]
7q35ter		CGH		ADH—gain, 33.3%		Gain, 40%	[83]
8p	*NRG1 (just distal to region)*	CGH		ADH—loss	Loss	Loss	[7]
8p/D8S339		LOH		ADH with cancer—$\geq$ 25%		$\geq$35%	[34]
8p12-pter		CGH		ADH—Loss, 11.1%			[35]
8q		CGH		ADH—high-level amplification			[7]
8q21-qter		CGH		ADH—gain, 11.1%			[35]
8q24	*MYC*			ADH—gain, 66.0%	Gain, 60%	Gain, 80%	[83]
8q24	*MTSS1; MYC*	AI	0.0%	ADH—35%	29%	13%—37%	[89]

Table 4 continued

Chromosome	Gene	Alteration	Normal breast	Atypical hyperplasia	DCIS	Invasive carcinoma	References
9p/D9S157		LOH		ADH non-cancerous breast—8%	Non-comedo—10%		[85]
					Comedo—11%		
9p21		AI	5%	ADH—8%	16%	11%–28%	[89]
10q25ter		CGH		ADH—gain, 33.3%	Gain, 40%	Gain, 80%	[83]
10q26		CGH		ADH-high-level amplification			[7]
11p15/D11S988		LOH		ADH non-cancerous breast—15%	Non-comedo—18%		[85]
				ADH cancerous breast—38%	Comedo—19%		
11p15	*THO1*	LOH (proportion)		ADH with cancer—8%	37%	28%	[32]
11q12-13		CGH		ADH—gain, 11.1%			[35]
11q13(PYGM)	*PYGM*	LOH	None	ADH—8.5%	27.6%		[90]
11q13	*INT-2 or PYGM*	LOH		ALH—10.5%		33.3%	[91]
11q13		CGH		ADH—gain, 33.3%	Gain, 40%	Gain, 80%	[83]
11q13.1		AI	0.0%	ADH—8%	9%	21%–30%	[89]
11q13/11q22-23/ D11s1818, D11s1819	*PYGM*	LOH (proportion)		ADH with cancer—11%	35%	57%	[32]
11q23.3		AI	4%	ADH—8%	21%	25%–50%	[89]
11q24ter		CGH		ADH—gain, 33.3%	Gain, 20%	Gain, 40%	[83]
12p13-pter		CGH		ADH—high-level amplification			[7]
12q24		CGH		ADH—gain, 33.3%	Gain, 20%	Gain, 80%	[83]
13q11-22		CGH		ADH—loss, 66%	Loss—100%	Loss—100%	[83]
13q13/D13S137		LOH		ADH non-cancerous breast—13%	Non-comedo—17%		[85]
				ADH cancerous breast—9%	Comedo—13%		
13q32-q34		CGH		ADH—high-level amplification			[7]
14q11.2-q12		CGH		ADH—high-level amplification			[7]
14q24/D14S62		LOH		ADH non-cancerous breast—none	Non-comedo—16%		[85]
				ADH cancerous breast—12%	Comedo—18%		
14q32		CGH		ADH—gain, 33.3%	Gain, 20%	Gain, 40%	[83]
14q32.33	*ATK1*	CGH		ALH- gain			[84]
15q23-25	*c-src -1*	CGH		ADH—gain, 67%			[83]
15q25-qter		CGH		ADH—high-level amplification			[7]
15q26		CGH		ADH—gain, 66.0%	Gain, 40%	Gain, 100%	[83]

Table 4 continued

Chromosome	Gene	Alteration	Normal breast	Atypical hyperplasia	DCIS	Invasive carcinoma	References
16p		Chromosome copy number, CGH		ALH—loss, 57% loss	Loss, 6%	Loss, 1%–13%	[86]
16p		Chromosome copy number/ CGH		ADH—gain, 33.3%	Gain, 80%	Gain, 100%	[83]
16q		LOH		Atypical hyperplasia—50%		71.4%	[92]
16q	*CDH1*	LOH		ALH—7.7%			[93]
		Mutation		ALH—frameshift mutation, 7.7%			
16q	*Matrix metalloproteinase 2, NME3*	CGH		ADH—loss, 33.3%			[83]
16q		CGH		ADH—loss, 55.5%			[35]
16q		CGH		ALH—loss, 36%	Loss, 17%	Loss, 25%–67%	[86]
16q	*CDH1, E2F4, WWOX*	CGH		ADH—loss	Loss	Loss	[7]
16q/D16S413		LOH		Atypical ductal hyperplasia—55.6%			[94]
16q/D16S422	*CDH13*	LOH		ADH with cancer—≥25%		≥35%	[34]
16q21-q23.1	*CDH1*	CGH		ALH—loss, 25%			[84]
16q21-24/ D16s265, D16s402, D16s413, D16s512		LOH (proportion)	2%	ADH—52%	63%	78%	[32]
17p		CGH		ADH—loss 22.2%			[35]
17p	*P53*	CGH		ADH—loss	Loss	Loss	[7]
17p13/D17S796	Close to P53	LOH		ADH in normal adjacent to cancer—33.3%	100%	100%	[95]
17p13/D17S960	P53 candidate gene	LOH		ADH non-cancerous breast—11%	Non-comedo—31%		[85]
				ADH cancerous breast—8%	Comedo—37%		
17p13.1	*TP53*	Microsatellite alterations		AH—16.6%			[33]
17p13.1/D17s796, D17s525	*TP53*	LOH (proportion)		ADH with cancer—6%	42%	59%	[32]
P53	*TP53*	Mutations		ADH—mutations in 50%			[46]
17p13.2/D17S796	Within 2 cM of p53	LOH		ADH—25.0%			[94]
17q	*HER-2/neu, GRB7, TBX2, STARD3, MLN64/*	CGH		ADH—gain			[7]
	CAB1, and ESTIMAGE68400						

Table 4 continued

Chromosome	Gene	Alteration	Normal breast	Atypical hyperplasia	DCIS	Invasive carcinoma	References
17q11/NF1		LOH		ADH non-cancerous breast—14%	Non-comedo—27%		[85]
				ADH cancerous breast—10%	Comedo—15%		
17q21		AI	0.0%	ADH—7%	30%	19%–27%	[89]
17q21/D17S579	Region of *BRCA1*	MSI/LOH		AH—8.3%			[33]
D17S8000	*BRCA1*	LOH		ADH ≥25%			[34]
20p11.2-p13		CGH		ADH—high-level amplification			[7]
20q	*AIB1, TFAP2C, STK15*	CGH		ADH—gain	Gain	Gain	[7]
20q		Chromosomal copy number alterations/ CGH	None	ADH –amplified 100%	100% show amplification	100% show amplification	[96]
20q13		CGH		ADH—gain, 100%	Gain-100%	Gain, 100%	[83]
20q13 region		Chromosomal copy number	None	ADH—amplified 100%	Amplified-100%	Amplified, 100%	[96]
22q		CGH		ADH—gain 67%	Gain- 60%	Gain, 80%	[83]
22q		Chromosomal imbalance/ CGH		ALH—43%	Loss, 28%	13%–20%	[86]
22q11.1		CGH		ALH—loss, 50%			[84]
Xp		CGH		ADH—loss	Loss	Loss	[7]
Xq		CGH		ADH—gain	Gain	Gain	[7]

AI allelic imbalance, *LOH* loss of heterozygosity, *CGH* comparative genomic hybridization

For example, mutations in the tumor suppressor gene APC lead to chromosome mis-segregation as a result of kinetochore–microtubule disconnection during anaphase [27]; p53 mutations result in centrosome hyperamplification leading to multiple spindle poles and mis-segregation of chromosomes [28]; loss of p16 generates supernumerary centrosomes through centriole pair splitting, resulting in aneuploidy [29]; and BRCA1 inactivation leads to microtubule instability or centrosome amplification [30]. While speculative, one might suggest these findings indicate multiple potential pathways to the aneuploidy which is an important advanced genomic change for these lesions. Aneuploidy is also a prominent feature of high-risk normal breast tissue [31], indicating that these chromosomal changes may be a marker in general for high risk for breast cancer.

Structural chromosomal changes in atypical hyperplasia

The genomic progression from normal breast tissue to atypical hyperplasia is accompanied by the development of multiple structural chromosomal abnormalities in the form of loss of heterozygosity [(LOH)/allelic imbalance (AI)] identified by microsatellite markers, and the development of large-scale chromosomal gains and losses identified by comparative genomic hybridization (CGH). These are summarized in Table 4, and it can be seen that these alterations involve multiple chromosomes and a wide range of chromosomal loci. The LOH/AI changes in AH have been shown to involve all informative markers on a chromosome arm, consistent with the pattern found in breast cancer, and in contrast to normal breast tissue and ductal hyperplasia where AI involved only single markers [32]. Atypical hyperplasia lesions are considered to be monoclonal microsatellite alterations involving both length

Table 5 DNA methylation of genes in atypical hyperplasia

Gene	Analysis	Gene function	Novel capability towards carcinogenesis [40, 41]	Normal breast	Atypical hyperplasia	DCIS	Invasive carcinoma	References
APC	MSP	Regulates *myc* and *cyclin D1* expression, cell cycle entry and progression	Tissue invasion and metastases	15%	65%	72%	84%	[8]
APC	MSP		Tissue invasion and metastases	7%	ADH—27%	34%	38%	[43]
β-catenin (CTNNB1)	MSP	Regulates coordination of cell–cell adhesion and gene transcription		10%	19%	34%	44%	[8]
BRCA1	Promoter methylation	Tumor suppressor gene, DNA repair, transcriptional regulation	Limitless replicative potential	18%	22%		29%	[97]
CDH1	Methylation	Epithelial cell–cell adhesion, suppresses invasion and metastasis	Tissue invasion and metastases		ALH—100.0%		92.9%	[42]
CDH1	MSP		Tissue invasion and metastases	15%	ADH—35%	50%	65%	[8]
Cyclin D2	Methylation	Cell cycle regulation	Limitless replicative potential	Unmethylated	ADH—endoscopy, 16.7% ADH—ductal lavage, 30.0%	28.6%	50.0%	[98]
Cyclin D2	Methylation			Unmethylated normal breast	ADH—endoscopy, 16.7%	33.3%	42.1%	[98]
Cyclin D2	Methylation			Benign ductal lavage—6.7%	Atypia—ductal lavage, 30.0%	100%		[98]
DLEC1	Methylation			7%	ADH—33%	37%	40%	[43]
ESR1	MSP	Regulation of cell proliferation	Self-sufficiency in growth signals	40%	ADH—48%	34%	51%	[8]
14-3-3σ	MSP analysis	Cell cycle regulation	Limitless replicative potential	Unmethylated	38%	83%	96%	[99]
GRIN2B	Methylation			0%	3%	23%	32%	[43]
GSTPI	MSP	Drug metabolism		5%	ADH—10%	16%	19%	[8]
HIN-1	Methylation	Tumor suppressor function, regulates cell cycle progression and apoptosis	Insensitivity to antigrowth signals	3%	ADH—23%	34%	36%	[43]
HOXA1	Methylation	Regulates gene expression, morphogenesis, differentiation		13%	ADH—43%	80%	76%	[43]
MINT17	Methylation Index			16%	24%	26%	30%	[100]
MINT31	Methylation Index			5%	10%	21%	13%	[100]

Table 5 continued

Gene	Analysis	Gene function	Novel capability towards carcinogenesis [40, 41]	Normal breast	Atypical hyperplasia	DCIS	Invasive carcinoma	References
MTIG	MSP			0%	ADH—7%	14%	32%	[43]
$p16^{INK4A}$	Methylation	Cell cycle regulation	Limitless replicative potential	7.5% (UDH)	35.0%	Low grade, 50% High grade, 66%	Low grade, 71.4% High grade, 75.0%	[101]
$p16^{INK4A}$ (TSG)	Methylation (MethyLight)			Usual ductal hyperplasia, 7.5%	35.0%	Low grade, 50.0% High grade, 68.2%		[102]
$p16^{INK4A}$ (TSG)	Percentage methylated (MethyLight)	Tumor suppressor gene		Normal breast/ UDH, 7.5%	35.0% %	Low grade, 50.0% High grad, 66.7% %	75.0%	[101]
$p16^{INK4A}$ (TSG)	IHC protein expression			Normal breast/ UDH, 80.0%	47.5%	Low grade, 33.3% High grade, 30.6%	18.8%	[101]
RASSF1A	Methylation	Regulates cell cycle, apoptosis	Self-sufficiency in growth signals	40%	77%	89%	76%	[43]
RASSF1A	Methylation Index			39%	67%	79%	76%	[100]
RARβ	MSP	Cell cycle arrest, growth inhibition, apoptosis	Limitless replicative potential	7%	ADH—7%	31%	26%	[43]
RARβ	Methylation Index		Limitless replicative potential	16%	29%	28%	22%	[100]
RARβ	Methylation		Limitless replicative potential	Unmethylated	ADH—endoscopy, 33.3% ADH—ductal lavage, 30.0%	50%	34%	[98]
RARβ	Methylated	Tumor suppressor gene		Normal breast tissue, unmethylated	ADH—endoscopy, 33.3%	14.3%	60.0%	[98]
RARβ	Methylation			Benign breast—4.4%	Atypia—ductal lavage, 30.0%	100.0%		[98]
SFRP1	Methylation			13%	3%	26%	46%	[43]
SFRP4	Methylation			0%	ADH—7%	6%	18%	[43]
TIMP3	MSP	Inhibit metalloproteinases, tissue remodeling and tumor cell progression	Tissue invasion and metastases	45%	ADH—62%	44%	56%	[8]
TMEFF2	Methylation			40%	ADH—47%	86%	76%	[43]

Table 5 continued

Gene	Analysis	Gene function	Novel capability towards carcinogenesis [40, 41]	Normal breast	Atypical hyperplasia	DCIS	Invasive carcinoma	References
Twist	Methylation	Modulates p53	Evading apoptosis		ADH endoscopy—20% methylated ADH—ductal lavage, unmethylated	28.6%	42%	[98]

MSP methylation-specific PCR; values area percent methylated

variation and allele loss [33], and which may involve multiple genes such as *Myc, EGFR, CDH13, BRCA1, p53* (Table 4). Amari et al. [34] studied 23 synchronous lesions of ADH, DCIS, and invasive carcinoma. ADH tumors with LOH were always accompanied by LOH in DCIS and IDC, consistent with ADH having a high risk of developing malignant transformation. LOH/AI is an important mechanism for loss of tumor suppressor and other genes and would contribute to the development and progression of AH.

AH has been studied by comparative genomic hybridization (CGH) to identify larger-scale structural chromosomal abnormalities. The majority of these studies utilized metaphase spreads with a resolution of $\geq$10 Mb. These studies identified multiple deletions and amplifications both within and between chromosomes of AH (Table 4). Chromosomes 1q, 6q, 8q, 11q, 14q were among the most frequently involved and often exhibited chromosomal gains. Gao et al. [7] reported high-level amplifications at multiple chromosomal sites, and many of these were gains, including those at 1q, 5p, 8q, 12q, 20q, and Xq, which were shared by ADH, DCIS, and invasive carcinoma. Gong et al. [35] on the other hand found five of the nine ADH lesions showing chromosome copy number alterations, with 16q loss and 17p loss being the most frequent changes. Candidate genes that might be associated with some of these losses included *E-cadherin* on 16q and *p53* on 17p. The loss of material from chromosomes 16 and 17 was also consistent with the LOH analyses of AH (Table 4). Together, the presence of large-scale amplifications and deletions is consistent with gross chromosomal rearrangements in atypical hyperplasias. These genomically advanced changes also occur with the transition of normal adjacent breast tissue (a high-risk tissue) to breast cancer, and with the progression of HMEC's to telomere-based crises, and are considered to be the types of chromosomal abnormalities seen in the earliest lesions of breast cancer [31, 36]. It is also noteworthy that DNA double-strand breaks (DSB) are considered to be an important etiologic mechanism in the development of both LOH [37] and GCR [38]. DSBs are an important consequence of estrogen exposure, and the average age of women with AH is over 55 years of age [4, 39], representing forty or more years (from menarche) of estrogen exposure, with the potential to induce these changes.

DNA methylation of genes in atypical hyperplasia

DNA methylation of CpG islands in the promoter region of a gene is an important mechanism for the silencing of tumor suppressor and other genes. Studies of atypical hyperplasia have identified multiple genes which may be methylated in these lesions, including tumor suppressor

Table 6 Gene expression abnormalities in atypical hyperplasia

Gene	Alteration/analysis	Function	Normal breast	Atypical hyperplasia	DCIS	Invasive carcinoma	References
Cox-2	IHC			ALH—61.4% ADH—23.0% ADH + ALH—53.9%			[103]
Cyclin A	ISH	Cell cycle regulation, binds and activates CDK2 and CDK1 kinases, and promotes both cell cycle G1/S and G2/M transitions	Benign breast tissue—35.3%	ADH—62.5%	42.1–46.4%	77.8%	[104]
Cyclin D1	IHC		Normal breast—11.7% PDWA—25.0%	ADH—39.4%	43.6–47.9%	48.3%	[105]
Cyclin D1	Gene amplification		Normal breast—15%	27%	35%	25%	[106]
Cyclin D1	IHC		Normal breast—13%	57%	50%	64%	[106]
Cyclin D2	Methylation	A regulatory subunit of CDK4 or CDK6, whose activity is required for cell cycle G1/S transition; involved in the phosphorylation of tumor suppressor protein Rb	Unmethylated normal breast tissue	ADH—endoscopy, 16.7% methylated	33.3%	42.1%	[98]
Cyclin D2	Methylation		Benign ductal lavage—6.7%	Atypia—ductal lavage, 30.0% methylated	100.0%		[98]
EGFR (HER-1)	IHC	Regulate cell growth, differentiation, and survival	Non-proliferative—21%	Epithelial hyperplasia with atypia—60%			[79]
EZH2	IHC	Transcriptional repression	Normal breast—0.0%	ADH without DCIS—10% ADH with DCIS—40.0%	45%		[52]
FHIT	IHC/H-score	Controls proliferation and apoptosis	Normal adjacent to cancer, strong and uniform, 100%; 2.95/3.0	Loss of FHIT protein vs normal	Marked loss of FHIT protein, 75%	Marked loss of FHIT protein, 54%; 0–1.0/3.0	[107]
FHIT	mRNA		Normal adjacent to ADH, 86%	71%	45%	29%	[108]
FHIT	Western blot		82% Normal adjacent to AH	57%	45%	27%	[108]
HER-2/neu	Amplification FISH		Normal adjacent to ADH, no amplification	ADH—53.8% amplified	95.5% amplified	100% amplified	[109]
HER-2/neu (C-erbB-2)	IHC		Non-proliferative—15%	Epithelial hyperplasia with atypia—40%			[79]

Table 6 continued

Gene	Alteration/analysis	Function	Normal breast	Atypical hyperplasia	DCIS	Invasive carcinoma	References
HER-2/neu (C-erbB-2)	IHC		Ductal hyperplasia without atypia—13.3%	Atypia (mild—severe), 30–56.6%	31.9%	17.8%	[110]
Myc	IHC		Benign lesions, 31%	66.7%	45%	66.7%	[111]
P53	Sequencing, mutations			ADH—28.6%, insertion, deletion		34.1%	[45]
P53	Mutation-gel elect			ADH—1 case	31.5%	55.7%	[47]
P53	SSCP, sequencing; mutation		Normal adjacent to cancer, no mutations	ADH—50% (not adjacent to cancer)	50%		[46]
RB	IHC		Normal breast, weak staining	ADH, mod/strong—11%	Mod/strong—64%	Mod/strong—47%	[112]
Stat3	IHC	Regulates genes that are involved in cell growth and division, cell movement, and apoptosis	Normal breast, 12.8%	ADH, 30.0% ADH adjacent to breast cancer, 31.15%			[113]
Stat5	IHC		Normal breast, 17.1%	ADH—31.65%			[113]
Telomere, anaphase bridges	FISH			ADH—16.7%	18.2%		[114]
Telomerase activity	TRAP signal		Benign breast disease, 14.0%	Atypical hyperplasia, 100%, focal high expression	92% strong	94% strong	[115]
Telomerase	Human telomerase gene (hTR)		Simple hyperplasia, 16.6%	ADH mild—22% ADH moderate—33.3% ADH severe—60.9%	85.7%	91.7%	[116]
Telomerase	Human reverse transcriptase gene (hTRT)		Simple hyperplasia, 0.0%	ADH mild—11.1% ADH moderate—25.0% ADH severe—52.1%	78.6%	83.3%	[116]
α-tubulin	mRNA expression-percent positive	Structural components of centrosomes	Normal breast, 33.3%	ADH—62.5%	82.5%	77.5%	[49]
α-tubulin	Protein expression		Normal breast, 31.7%	ADH—65%	86.3%	87.5%	[49]

Table 6 continued

Gene	Alteration/analysis	Function	Normal breast	Atypical hyperplasia	DCIS	Invasive carcinoma	References
α-tubulin	DNA copy number		Normal breast, 2.05	ADH—4.31	5.54	5.15	[49]
	Centrosome abnormality-frequency		Normal breast, 0%	ADH—30%	52.5%	70.0%	[49]
γ-tubulin	mRNA expression-percent positive		Normal breast, 30.0%	ADH 57.5%	85.0%	82.5%	[49]
γ-tubulin	Protein expression		Normal breast 35.0%	ADH—58.8%	86.3%	85.0%	[49]
Ki67	IHC		Normal breast, 1.5% PBBD without atypia, 3.5%	PBBD with atypia, 16%	Non-high-grade, 6.1% High grade, 17.3%		[54]
Ki67	IHC		Normal breast, 0.1% Usual hyperplasia, 3.3%	ADH—8.2%	8.7%	21.1%	[117]

PDWA proliferative benign breast disease without atypia, *IHC* immunohistochemistry, *ISH* In situ hybridization, *PBBD* Proliferative benign breast disease

genes known to be important in breast carcinogenesis (Table 5; *APC, BRCA1, CDH1, 14-3-3σ, HIN-1, P16, RASSF1A, RARβ*). A number of cellular processes may be altered by inactivation of these genes, including cell cycle control, DNA repair, cell–cell adhesion, cell proliferation, apoptosis, cellular differentiation, and centrosome and mitotic events. Importantly, methylation in AH involves genes which are also instrumental in five of the six capabilities a cell has to acquire to become malignant (tissue invasion and metastases, limitless replicative potential, self-sufficiency in growth signals, insensitivity to anti-growth signals, and evading apoptosis) [40, 41], supporting an early and important role in breast carcinogenesis. A review of Table 5 indicates there is clearly heterogeneity in the incidence of methylation in these lesions, and in some cases methylation may also be monoallelic (*CDH1*) [42], further contributing to a range of gene inactivation. DNA methylation of tumor suppressor and other genes appears to play an important role in the progression of AH to breast cancer. Park et al. [43] examined methylation patterns in synchronous ADH, DCIS, and invasive ductal carcinoma. They found overall methylation levels and frequencies of *APC, DLEC1, HOXA1,* and *RASSF1A* promoter CpG islands were significantly higher in ADH than in normal breast tissue, while *GRIN2B, GSTP1, HOXA1, RARβ, RUNX3, SFRP1,* and *TMEFF2* showed higher methylation levels and frequencies in DCIS than in ADH. This indicated that promoter methylation changed significantly in pre-invasive lesions and suggested that CpG island methylation of tumor-related genes is an early event in breast cancer progression. Hoque et al. [8] studied synchronous ADH, DCIS, and invasive ductal carcinoma. For the genes *APC*, *CDH1*, and *CTNNB1* they found methylation at two or three gene loci in 25% of ADH, 28% of DCIS, and 37% in IDC. They noted atypical ductal hyperplasia and in situ carcinoma showed similar methylation patterns, suggesting that atypical hyperplasia should be considered as a well-differentiated or simply small in situ carcinoma. DNA methylation may also contribute to chromosomal abnormalities in AH. It was seen above that *APC* and *p16INK4a* regulate centrosome duplication and chromosome segregation, and loss of these genes through DNA methylation would be expected to contribute to chromosomal instability and aneuploidy [27, 29]. Together these findings indicate that DNA methylation is not only involved in the formation of AH and contributes significantly to its genomic instability, but also plays an important role in subsequent progression to malignancy.

Gene expression abnormalities

The development of ADH and progression to DCIS and invasive breast cancer is accompanied by the acquisition of multiple gene expression differences. This is demonstrated both by gene expression profiling studies and by multiple individual gene expression studies. Ma et al. [9] examined

gene expression profiling in specimens containing invasive ductal carcinoma (IDC)/DCIS/ADH/adjacent normal tissue. They found that, as compared with the patient-matched adjacent normal epithelium, significant global alterations in gene expression occurred in ADH, and these alterations were maintained in the later stages of DCIS and IDC. All of the ADH samples demonstrated a grade I gene expression signature and clustered with the low-grade DCIS and IDC samples. These three distinct stages of breast cancer (ADH, DCIS, IDC) were thus highly similar to each other at the level of the transcriptome, supporting the idea that the distinct stages of progression are evolutionary products of the same clonal origin [9].

Studies of individual genes in atypical hyperplasia indicate altered expression in multiple genes effecting a wide range of signaling pathways and cellular functions (Table 6), further supporting the gene expression profiling differences between ADH and normal adjacent tissue described above. These genes include estrogen receptor and estrogen-related genes (*ER, EZH2*), cell cycle genes (*cyclin A1, D1, D2*), loss of tumor suppressor genes (*FHIT, p16, p53, RARβ)* increased mitogenic activity of growth factors and oncogenes (*EGFR, Her-2/neu, myc*), and increased expression of transcription factors (*STAT 3,5*). Together these alterations may contribute to increased estrogen responsiveness, increased cell cycle progression, development of aneuploidy, decreased apoptosis, and loss of cell–cell adhesion. Alterations in many of these genes may also be associated with increased proliferation which is confirmed by increased expression of *Ki67* (Table 6), a measure of cellular proliferation. Interestingly, Ki67 has also been found to be a time-varying biomarker of risk of breast cancer in women with atypical hyperplasia [44]. There is evidence that *p53* is mutated in ADH [45–47]. The presence of a dysfunctional p53 could have widespread effects in these cells including loss of cell cycle arrest and apoptosis, altered DNA repair, and genomic instability [48]. Alterations in α-tubulin and γ-tubulin are also observed in AH [49], further disrupting chromosome segregation and contributing to the aneuploidy which is observed in atypical hyperplasia (see above, numerical chromosomal changes). Importantly, it can be seen in Table 6 that for virtually all of these genes (a) the expression in ADH is altered compared with that of normal and/or non-proliferative breast tissue, and (b) this expression difference is maintained or increased in DCIS and invasive breast cancer. This is further evidence that multiple genes regulating multiple cellular processes contribute to formation of ADH and its genomic instability and are instrumental in the progression of ADH to DCIS and invasive breast cancer.

Estrogen receptor in atypical hyperplasia

The expression of ERα in atypical hyperplasia is high [12, 50], and in some series all of the ADH lesion expressed ER [12, 51], consistent with both a prominent sensitivity to estrogens and a clonally expanded population of cells. Estradiol is an important mitogen, and the increased ER content promotes proliferation and clonal expansion, while at the same time increasing the accumulation of mutational changes. The gene *EZH2* is also increased in AH and DCIS [52], and this gene transactivates genes that are commonly targeted by estrogen and *WNT* signaling pathways and promotes cell cycle progression in breast cancer cells [53]. The functional ER and its role in proliferation also makes it an excellent potential target for antiestrogen prevention therapy. Efficacy of tamoxifen in the prevention of breast cancer in women with AH was demonstrated in the NSABP-P1 trial [13]. Closely related to this point, the finding that antiestrogen therapy reduces breast cancer development in the ipsilateral and contralateral breast indicates that this characteristic of AH (ER positivity) is reflected in the genomic characteristics of the remaining normal breast tissue. If other genomic characteristics of AH are also reflected in these normal tissues, then the molecular profile of AH could be important for assessing future risk and responsiveness of these normal tissues. Lastly, the expression of *ERβ* is decreased in atypical hyperplasia [54]. *ERβ* is considered to play an oncosuppressive role in breast cancer [55]. Low *ERβ2* expression, combined with increased ERα expression could further promote progression along the AH-DCIS/invasive carcinoma pathway.

Metachronous breast cancer associated with atypical hyperplasia

Women with AH are at significant risk for the development of metachronous breast cancer (MBC), and it is noteworthy that the characteristics of this event for AH are very similar to those of women with sporadic breast cancer for the development of contralateral breast cancer (CBC): (a) AH contains multiple advanced genomic changes including aneuploidy, gross chromosomal rearrangements, and DNA methylation of tumor suppressor genes, all of which are common in sporadic breast cancer. (b) Both MBC and CBC are more commonly invasive breast cancer, less commonly DCIS (Table 2) [56]. (c) The cumulative incidence of breast cancer appears to increase linearly over time in both MBC [5] and CBC [56, 57]. (d) The annual incidence of breast cancer development of MBC (estimated to be approx. 0.9%/year from Hartmann [5], Fig. 2) is very similar to 0.6–0.7%/year for CBC [56, 58]. (e) The incidence of both MBC and CBC are reduced by antiestrogens

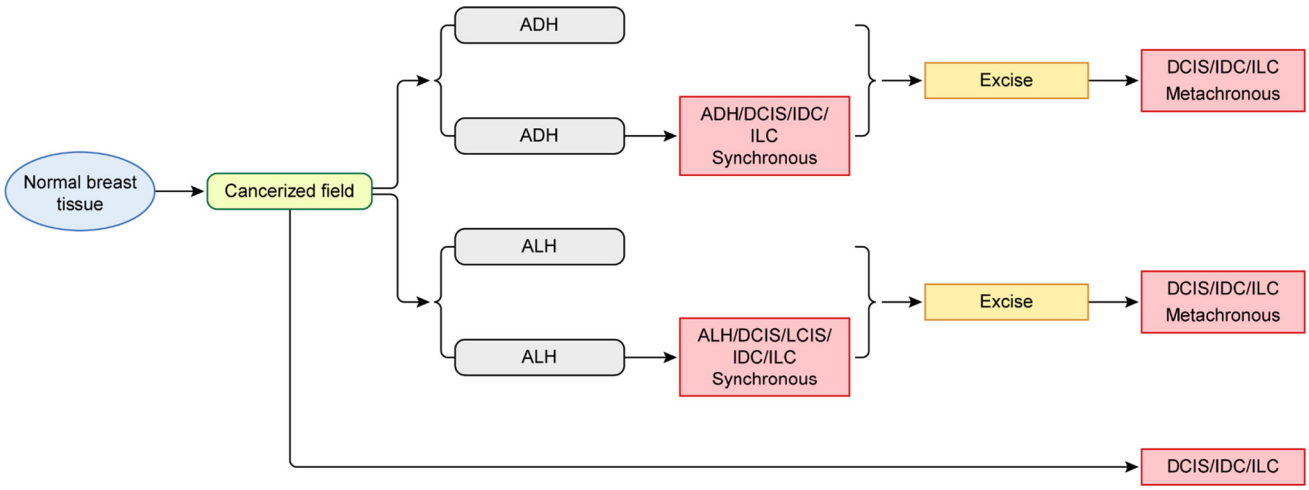

Fig. 1 Development and progression of atypical hyperplasia to breast cancer. Atypical hyperplasia (ALH or ADH) develops within a cancerized field in normal breast tissue (Fig. 1) [31, 118]. AH may present as a solitary lesion (single or multifocal). Local progression of AH results in the development of DCIS/IDC which appear synchronously with the AH. Excision of the primary AH (solitary or with synchronous carcinoma) may then be followed by the development of metachronous breast cancer—DCIS/IDC/ILC. Included in Fig. 1 is a separate pathway in which breast cancer develops without any intervening atypical lesion

[13, 59]. Together, these findings indicate that many of the features of carcinogenesis of AH are shared by sporadic breast cancer, and have strong carcinogenic potential for the future development of metachronous breast cancer.

Summary and conclusions

Atypical ductal and atypical lobular hyperplasia possess a wide range of advanced genomic changes including aneuploidy, loss of heterozygosity, gross chromosomal rearrangement such as amplifications and large-scale deletions, DNA methylation of tumor suppressor and other genes, and gene expression differences which are associated with a significant risk for breast cancer. These genomic changes progress from associated normal breast tissue, indicating an important role in the development of ADH. Many of these genomic charges are also shared by synchronously associated DCIS and invasive carcinoma, suggesting they are an important part of the progression of atypical hyperplasia to breast cancer. At the same time many of these changes, including ER expression, are also shared by standard sporadic breast cancer and thus reflect the propensity of distant normal breast tissue to develop metachronous or contralateral breast cancer. These developmental patterns and relationship of AH to breast cancer are summarized in Fig. 1. Knowledge of a comprehensive profile of the genomic changes of AH should increase our understanding of high-risk lesions of the breast, promote identification of new targets for breast cancer prevention, and clarify progression in the carcinogenic pathway.

Funding This research was supported by the Intramural Research Program, National Cancer Institute, Center for Cancer Research, National Institutes of Health, Bethesda, MD.

Compliance with ethical standards

Conflict of interest The author is employed by the National Institutes of Health which funded this manuscript. The author has no conflicts of interest with the NIH.

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