

Review article

Allelic loss in breast cancer

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Received 9 May 2002; accepted 19 September 2002

Abstract

Breast cancer is the most prevalent cancer type in women and allelic loss constitutes one of the commonest genetic alterations in mammary neoplasias. Frequent detection of Loss of Heterozygosity indicates genes with putative tumour suppressor activity in breast carcinomas. Imbalance between two alleles might also be related with increased expression of an oncogene within a locus. Loci exhibiting frequent allelic loss in breast cancer have been detected, spread throughout the genome, and may contain genes with potential significance in breast carcinogenesis. Loss of Heterozygosity patterns in breast cancer give evidence for multiple clonality of the disease, and that accumulation of such lesions is probably implicated in disease development. Studies on deletions of known breast cancer genes suggest interactions with other common genetic events during disease initiation and progression. Allelic loss has been repeatedly associated with adverse characteristics and poor outcome in breast neoplasms. Detection of allelic loss in the serum of breast cancer patients and in premalignant breast lesions could herald the potential for diagnosis of the disease at an early, and thus curable, stage.

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Keywords: Breast cancer; Loss of heterozygosity; Oncogenes; Tumour suppressor genes

1. Introduction

One of the characteristics of malignant cells is the widespread instability of their genome [1]. Excessive proliferation activity and increased mutation rate both contribute to the genomic dissociation of cancer cells. Allelic imbalance studies constitute one of the main investigative tools for detecting genetic alterations in oncology research. For a given somatic genetic locus, mammalian cells carry two alleles: one of paternal and one of maternal origin, respectively. Detection of dissimilar amounts of DNA between the two alleles in neoplastic cells, compared to normal cells, signifies allelic imbalance for the tumour studied.

Imbalance between alleles can be detected with several methods [2–4]. These may include cytogenetic studies, detection of deletions with PCR and restriction enzyme techniques (RFLP), as well as other methods, such as Southern blotting and comparative genomic hybridization. Proper recognition of allelic alterations requires maximal resolution, together with high specificity to the smallest coding region that a chromosomal aberration may contain.

Allelic imbalance is usually examined at distinct sites throughout the genome. These are repetitive two- or five-nucleotide sequences, called microsatellite DNA [5].

Cellular alleles for a microsatellite locus may differ in the number of repetitions that they contain and, thus, in the size of the microsatellite region. When they differ, the carrier has two alleles of different size and is heterozygous for the given locus. Disproportional intensity between the two alleles may indicate amplification of the allele showing enhanced intensity (i.e. *MYC* amplification at 8q24). More commonly, however, it is due to allelic loss at the site of decreased intensity, at the locus in question. Complete allelic loss at a constitutional heterozygous locus may be covered in part, by the presence of normal cells or by the existence of multiple clones within the same tumour (resulting in decreased intensity of one allele, instead of its disappearance).

Detection of LOH may correspond to the loss of the second allele of a tumour suppressor gene (TSG), according to the Knudson's "two-hit hypothesis" [6]. This second step (leading to loss of the function of the gene, so that the recessive phenotype can be expressed) usually arises from deletions of the region containing the gene, aberrant mitotic recombinations, non-disjunctions and, possibly, other suggested mechanisms [7] (Fig. 1). From this point of view, LOH serves as a molecular tool for identification of sites that could possibly contain tumour suppressor genes. The existence of about 10^5 different microsatellite regions, spread throughout the human genome, offers the possibility to scan for LOH regions, with appropriately selected microsatellite

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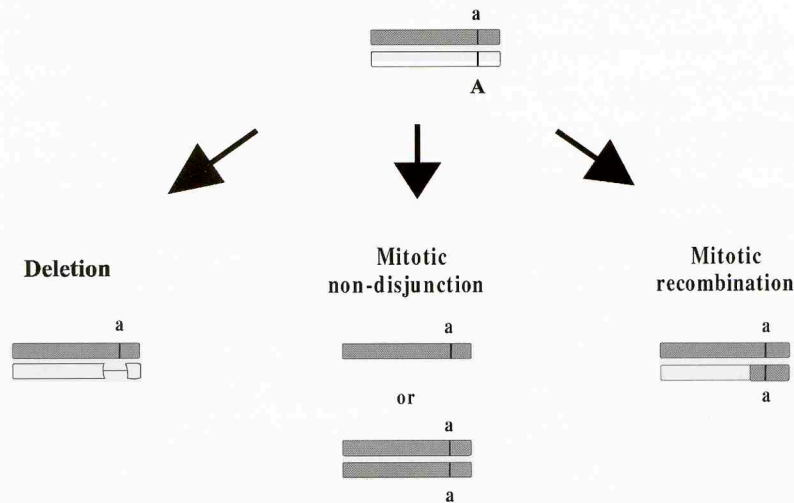


Fig. 1. Possible mechanisms of allelic loss. Loss of Heterozygosity of one (A) of the two autosomal alleles (A and a) usually results from: (1) deletion, either locus restricted or (more commonly) concerning a large segment that contains multiple loci, (2) mitotic non-disjunction, with or without duplication of the remaining chromosome, and (3) mitotic recombination.

markers. This way, new loci containing putative TSG can be identified, or inactivation of an already known gene can be assessed, together with its involvement in the oncogenetic process of a given tumour.

Breast cancer is the most frequent malignancy in women. It is estimated that one in every eight women will develop breast cancer during her lifetime [8]. It is also the second most common cancer-related cause of death among women [9]. High prevalence and mortality of breast carcinoma underscore the necessity for solid clarification of the molecular basis of the disease. LOH has been extensively studied in breast cancer, as it constitutes one of the commonest genetic alterations in this type of cancer [10].

Identification of allelic imbalance at a locus, may signify the existence of a tumour suppressor gene. Recently, the combination of results from different LOH studies in breast cancer led to the recognition of commonly deleted regions for this tumour type [10] (Table 1). Certain TSGs with established involvement in breast carcinogenesis have been identified in some of these regions (i.e. *BRCA1* at 17q21.23, *BRCA2* at 13q12.3, *p53* at 17p13.1, etc.). Most of the loci, however, contain genes that are not yet clearly implicated in the formation and/or progression of breast neoplasias. Furthermore, detection of a region of allelic loss always raises the possibility for the existence of a new gene, with putative tumour suppressor activity, in the locus examined. The existence of another, known gene in the same region does not preclude this option [10].

Several other LOH studies have further elucidated the role of individual TSGs in breast cancer oncogenesis and progression. Noteworthy, low allelic loss rates have been constantly reported at loci that do not contain any putative candidate TSGs [11]. In these cases, detection of LOH probably reflects the extensive (and, non-specific) genomic instability of malignant cells. Table 2 summarises the results

Table 1
Common regions of Loss of Heterozygosity (LOH) in breast cancer

Common regions of LOH	Candidate gene(s)
1p36.1–36.2	<i>p73</i> , <i>MDG1</i> and <i>BRCD2</i>
2q21.3–23.3	<i>FAP</i>
3p14.1	<i>FHIT</i>
5q21.1–21.3	<i>APC</i>
6q15	<i>MYB</i>
6q22.1–23.1	<i>ROSI</i>
6q25.2–27	<i>hZAC</i> and <i>ESR-1</i>
7q31.1–31.31	<i>CAV-1</i>
8q21.3–22	<i>N33</i>
8q24	<i>MYC</i>
9p21	<i>CDKN2A</i> and <i>SDKN2B</i>
10q23.31–23.33	<i>SNCG</i> and <i>PTEN</i>
11p15	<i>ST5</i> , <i>TSG101</i> , <i>HRAS</i> and <i>SRBC</i>
11q13.1	<i>EMS1</i> and <i>INT-2</i>
11q23	<i>ATM</i> and <i>APOC-3</i>
13q12.3	<i>BRCA2</i>
13q14.2–14.3	<i>RB1</i>
14q32.11–31	<i>OGR1</i> and <i>MTA1</i>
16p13	<i>PKD1/TSC2</i>
16q11.2–22.1	<i>CDH1</i>
16q22.3–24.3	<i>CDH13</i>
17p13.1	<i>TP53</i>
17p13.3	<i>BCPR</i>
17q21.23	<i>BRCA1</i>
18p11.3	<i>DAL-1</i>
18q21.1–21.3	<i>DCC</i>
19p13	<i>SAFB/APCL</i>
22q12.3	<i>RRP22</i> and <i>NF2</i>
22q13.1	<i>STI3</i>

Based on [10], with modifications.

of studies performed in our laboratory, concerning allelic loss in breast cancer. Studies of this type add information on the behaviour of genes located at the loci exhibiting allelic loss, during formation, progression and metastasis of breast cancer.

Table 2
Results from studies of allelic loss in human sporadic breast cancer, at various chromosomal loci

Locus	Candidate gene	LOH (%) ^a	Reference ^b
1p32	Not yet identified	11/50 (22)	[56]
1p36–36.2	<i>P73</i>	16/50 (32)	[56]
1q21–23	Not yet identified	17/50 (34)	[56]
1q42–43	Not yet identified	15/50 (30)	[56]
3p25–26	<i>VHL</i>	24/82 (29)	[62]
7p12–q21	Not reported	3/26 (11)	[11]
10q21	Not reported	3/25 (12)	[11]
13q12–13	<i>BRCA2</i>	28/82 (34)	[26]
17p13	<i>TP53</i>	28/82 (34)	[26]
17q11.2–12	Not yet identified	11/37 (30)	[11]
17q21	<i>BRCA1</i>	27/82 (33)	[26]

^a Number of cases exhibiting LOH with at least one marker/number of informative cases studied.

^b Numbers correspond to references within the text. It is of note that low rates of allelic loss were detected at genetic loci (i.e. 7p12, 10q21) where no reports of any putative candidate TSG for breast cancer exist so far in the literature.

2. The *BRCA1* gene

LOH studies performed during the 1980s first raised the possibility for the existence of a gene with important role in breast cancer, at the long arm of chromosome 17 [12]. Allelic loss at the 17q21 locus was linked to families where kindred tended to develop breast cancer at young age. Concurrent association of deletions of the region with ovarian cancer cases implicated this new breast cancer susceptibility gene in the hereditary breast–ovarian syndrome [13]. The gene, called *BRCA1*, was cloned in 1994 [14].

BRCA1 is a large gene. It consists of 22 coding exons, containing 5592 nucleotides, spread over 100 kb of genomic DNA [14]. Evidence from LOH studies suggested that *BRCA1* is a TSG. In genetic linkage studies, loss of the wild-type allele concerned both maternally and paternally derived chromosomes [15]. This finding both excluded the possibility of genomic imprinting and provided further evidence for tumour suppressor identities of *BRCA1*. Retroviral transfer of the wild-type *BRCA1* inhibited growth in vitro of breast and ovarian cancer cell lines [16]. Moreover, experimental inhibition of *BRCA1* expression with antisense oligonucleotides produced accelerated growth of normal and malignant mammary cells [17].

The significance of *BRCA1* alterations in hereditary breast cancer became obvious following a collaborative study, which involved 214 breast cancer families [18]. Cases from 45% of breast cancer families were associated with *BRCA1*. Furthermore, within the subgroup of families with hereditary breast and ovarian cancer, 90% were linked to *BRCA1*. In another study, using LOH and mutation analysis, *BRCA1* alterations could be detected in 63% of familial breast cancer cases [19].

Detection of germline mutations of *BRCA1* as in all putative TSGs enhance its significance as a cancer susceptibility gene, validating also concomitant LOH studies [20].

Such missense mutations of *BRCA1* are relatively prevalent among the general population [21]. Initial studies estimated prevalence of *BRCA1* mutations to be 1/833 [21] or even as high as 1/345 [22]. These estimations, however, were rather overstated, as they were based on families with history of breast and ovarian cancer among pedigrees. A subsequent study, which included women with breast cancer among the general population, concluded that prevalence of *BRCA1* mutations was 0.0011 [23]. In other terms, one in about every 910 females carries a *BRCA1* mutation, which places her at risk for developing breast cancer. This especially concerns women at young age, since mutations were detected in as high as 3.1% of women diagnosed before the age of 36 years [23].

High prevalence of *BRCA1* mutations among the general population implies involvement of the gene in human sporadic breast cancer. Sporadic breast cancer accounts for more than 90% of all cases [24]. In sporadic breast cancer, however, *BRCA1* mutations are absent or extremely rare, and this probably accounts for differences in the biological behaviour between familial and sporadic breast cancer cases [25]. On the contrary, allelic loss at the *BRCA1* locus is frequent, even in sporadic breast cancer. LOH studies, using highly polymorphic markers, revealed that allelic loss at the gene locus occurs in 33% of sporadic breast cancer cases [11,26]. Moreover, allelic imbalance rates as high as 47% have been reported for the 17q21 locus [27]. In another study, LOH at the *BRCA1* locus occurred in 31% of informative cases, significantly correlated with high-grade tumours and negative hormone receptors [28]. Allelic loss of *BRCA1* has been detected with higher frequency in young women (diagnosis before age 36 years), compared to postmenopausal patients [29]. This difference, however, was not statistically significant.

Allelic loss at the *BRCA1* region has been associated with decreased *BRCA1* mRNA expression levels [30]. This association was statistically associated with absence of estrogen receptors in the tumours examined. Moreover, decreased *BRCA1* protein levels have also been reported in sporadic invasive breast carcinomas without loss at the gene locus [28]. Taken together, these results imply for alternative mechanisms of loss of *BRCA1* tumour suppressor activity, apart from loss of gene copies: *BRCA1* expression may be down-regulated, via deletions of regulatory elements in the gene promoter or via interaction with estrogen receptors. Furthermore, sporadic breast cancer cases showing decreased *BRCA1* expression tended also to overexpress *p21*, independently of *mdm2* expression status [30]. These data strongly address the suggestion that inactivation of *BRCA1* may interact with *p21*, for cell cycle arrest at the *p53* checkpoint.

3. Chromosome 13q and the *BRCA2* gene

Allelic loss studies in the mid 1990s revealed that LOH at the 13q12–13 locus was prevalent in breast cancer [31].

Deletions of the locus were linked to familial breast cancer cases without evidence of *BRCA1* involvement, implying the existence of a new breast cancer susceptibility gene [32]. The gene, called *BRCA2*, was cloned 1 year later [33]. Frequent allelic loss at the gene locus, together with detection of homozygous deletions, both suggest that *BRCA2* acts as a TSG [33]. Later LOH studies revealed that *BRCA2* was linked to 32% of hereditary breast cancer cases [19]. Prevalence of *BRCA2* mutations in the general population resembles that of *BRCA1*; 2.4% of young women (under the age of 36 years) suffering from breast cancer and 2.2% of older patients have *BRCA2* mutations [23].

Apart from familial cases, allelic loss of *BRCA2* is also common in sporadic breast cancer. LOH studies estimate that allelic loss at 13q12–13 occurs in more than 30% of cases [26,31], while others report rates as high as 51% [27,34]. Allelic loss at the *BRCA2* locus has been associated with the presence of hormone receptors and with several unfavourable prognostic features [27], as well as with increased risk of recurrence and death [34]. Recent data suggest that, like *BRCA1*, sporadic breast cancer cases in young women tend to present LOH at the *BRCA2* locus more often, compared to cases in older patients [29]. It is not known, however, whether higher allelic loss of these genes is functionally associated with tumours at younger age, or simply reflects widespread genomic distortion, associated with more aggressive behaviour, a common characteristic of breast cancer occurring in young women. The latter assumption arises from the finding that only concomitant losses in both *BRCA1* and *BRCA2* regions were associated with lymph node metastases, higher histologic grade and peritumoral vessel invasion, whereas LOH in either region, with simultaneous heterozygosity in the other, could not be associated with any particular parameter [27]. It seems, therefore, likely that simultaneous allelic loss at *BRCA1* and *BRCA2* loci may define a subset of breast cancer tumours with unfavourable characteristics and aggressive behaviour.

Concomitant allelic loss in *BRCA1* and *BRCA2* loci, however, is not a constant finding. In other relevant LOH studies, no statistically significant correlations between *BRCA1* and *BRCA2* deletions could be identified [26,29], possibly signifying that alterations in each locus are sufficient to give breast cells oncogenic potential. Nevertheless, putative interactions between allelic loss of *BRCA1* and *BRCA2* genes and other genetic events in the formation of human breast cancer have to be determined.

Besides *BRCA2*, two other regions of allelic loss at the long arm of chromosome 13 have been identified in breast cancer [34,35]. Frequent LOH detection at the *RBI* locus (13q14) has been correlated with *RBI* mRNA underexpression, implicating the involvement of the retinoblastoma gene in breast cancer [35]. Furthermore, simultaneous but distinct revealing of LOH patterns for both *BRCA2* and *RBI* indicates that both genes constitute independent targets of allelic loss at 13q in breast cancer [31]. On the other hand, deletion mapping at this chromosome arm has indi-

cated the telomeric 13q31–34 locus as another LOH target [34]. The significance of this finding, however, remains undetermined.

4. Chromosome 17p and the *TP53* gene

Chromosomal locus 17p13 is one of the commonest regions exhibiting allelic loss in breast cancer [10,26]. *TP53* gene, at chromosome 17p13, was the first gene with documented involvement in hereditary breast cancer cases [36]. It exerts cardinal regulatory functions during cell cycle and programmed cell death (apoptosis), so that *TP53* integrity is critical for normal cell growth and division [37]. It is not surprising, therefore, that *TP53* mutations are among the most common genetic alterations in breast cancer, as well as in other human tumours [38]. In sporadic breast cancer, *TP53* mutations have been associated with poor clinical outcome [39,40].

Apart from *TP53* mutations, allelic loss at the gene locus has also been associated with adverse outcome in breast cancer [41,42]. Patients with tumours exhibiting LOH at the *TP53* locus had shorter relapse-free interval and poorer overall survival, and these associations were statistically significant [41]. Since, however, no adjustment for other established prognostic factors was available for those cases, it is not clear whether a causative relationship exists between allelic loss of *TP53* and prognosis. Moreover, in a subset of young breast cancer patients, *TP53* LOH could not be associated with survival, in contrast with specific gene mutations [40]. Taken together, these data emphasize the challenge for future research on the putative prognostic significance of *TP53* LOH in breast cancer.

Allelic loss studies in breast cancer have revealed that other commonly deleted regions exist on the short arm of chromosome 17 [43–45]. Common finding in those studies was the localization of areas of loss, distal to the *TP53* locus. Hence, it has been suggested that a TSG could be the target for allelic loss in that region [45]. Several genes have been identified at 17p, distal to *TP53* [46]. Among them, *HIC-1* seems the most attractive candidate for a TSG in breast cancer [47]. The gene, however, is more commonly inactivated via hypermethylation of a CpG island [48]. Moreover, failure of linkage of breast cancer in several families to LOH at 17p telomeric to *TP53*, argues against causative involvement in disease development [49]. However, this cannot exclude the presence of a breast cancer gene.

Frequent allelic loss at 17p, not related to *TP53*, has been associated with several unfavourable tumour characteristics, such as poor differentiation and high proliferative activity [42]. Furthermore, detection of allelic loss at the short arm of chromosome 17 may have significant prognostic implications [45]. The prognostic power of LOH at 17p13.3-ter for disease-free survival was second only to that of axillary lymph node involvement, being stronger than those of other, well-established, clinical prognostic parameters for breast

cancer [45]. Likewise, allelic loss of the region telomeric to 17p13.3 was a better predictor for overall survival than mutations of the *TP53* gene. These findings demonstrate that LOH studies may serve in the future as prognostic tools in breast cancer treatment decisions.

5. Chromosome 1

Allelic loss has been frequently detected in multiple regions on both the short and long arm of chromosome 1 [10,50]. The region most commonly deleted lies at 1p36, where *p73* TSG, a *p53* homologue, has been identified [51,52]. LOH at the *p73* locus has been prospectively associated with shorter disease-free survival in patients who were lymph node-negative at diagnosis [53]. Moreover, allelic loss of the gene occurs significantly more often in inflammatory breast cancer cases, which is a subset of tumours associated with poor prognosis [54].

On the other hand, in a recent study, allelic loss at the *p73* locus did not correlate with alterations in gene expression, possibly implicating another gene, as the target of allelic imbalance at the locus [55]. Other frequently deleted regions at chromosome 1 have been reported in breast cancer [50,56]. LOH at 1q41–44 is still of unknown significance, together with LOH at 1p32, containing the *RAD54* gene [57]. Allelic loss, however, in region 1q21–23 has been associated with extensive intraductal component and peritumoral angiolymphatic invasion [56]. These are independent markers of local recurrence, while the latter constitutes significant prognostic indicator for distant metastasis. Besides, it seems that a more complex relationship exists between allelic imbalance at 1q21 and breast carcinogenesis; gain in intensity of one allelic band has also been reported, again in correlation with prognostic features for poor survival [58].

6. Chromosome 3p

LOH at chromosome 3p is one of the most common genetic alterations in human neoplasias. Several genes have been identified at this chromosome arm, including *FHIT* at 3p14, and *VHL* at 3p25–26 [59,60]. Allelic loss studies revealed that both these genes are TSGs, and LOH at their loci frequently occurs in sporadic breast cancer [61,62]. The 3p allelic loss has been repeatedly detected in breast carcinoma in situ and even in preneoplastic lesions, such as epithelial hyperplasia [63]. LOH in precursors of breast malignancies usually concerns the same loci on 3p as the cancer cases, implying that allelic imbalance at this chromosome arm is an early event in breast tumorigenesis [63].

Evidence that *FHIT* is the target for LOH at 3p14 was given by coexistence of allelic loss with reduced immunohistochemical expression of the gene product [64]. Allelic loss at the gene locus has been associated with bilateral disease, as well as with *BRCA2* gene defects [65]. The 3p14

LOH correlates also with absence of hormone receptor expression, therefore, it has been postulated that these alterations may play a role in the regulation of differentiation of breast neoplasms [61,66].

Other common regions of 3p allelic loss in breast cancer include 3p12 and 3p24–25 [61,66]. LOH at 3p24–25 might serve as a predictive factor for postoperative survival [66]. There are no reports for any candidate TSG at these loci. On the contrary, allelic loss at the *VHL* gene locus occurs frequently in breast cancer [62,67]. *VHL* is a TSG with involvement in hereditary (*VHL* syndrome), as well as in sporadic cancer forms, mainly of neuroendocrine origin [60]. Allelic loss at the gene locus, at 3p25–26, is frequently detected even in tumour types not involved in the *VHL* disease, such as breast and colon cancer [62,68]. Absence, however, of *VHL* mutations in cases exhibiting LOH at the gene locus strongly implies for alternative methods of gene inactivation, as well as for the existence of a putative, but not identified as yet, TSG at 3p25–26 [62].

7. Chromosome 16 and the *E-cadherin* gene

Chromosome locus 16q21–22.1 contains the *E-cadherin* gene and is one of the most commonly deleted regions in breast cancer [69,70]. Concomitant mutations of the gene with allelic loss at 16q21–22.1 revealed that *E-cadherin* is an important TSG in breast cancer [69]. LOH rates as high as 100% have been reported in the lobular histological type [69], correlating with positive estrogen receptor status [70]. Moreover, possible interactions between allelic loss at other chromosome loci (i.e. 13q) and *E-cadherin* mutations have been hypothesized [69].

Apart from 16q21–22.1, regions also on the short arm of chromosome 16 are often deleted in breast cancer [71]. LOH occurs mainly at 16p13, a region that contains the *PKD1/TSC2* putative TSG. It seems that allelic loss at this locus is restricted to certain histological subtypes of breast cancer, such as apocrine and papillary carcinomas [67,71]. It is of note that these forms of breast malignancies rarely exhibit allelic loss in the most common regions of LOH in breast cancer [71].

8. Other regions of allelic loss in breast cancer

8.1. Chromosome 8

Allelic loss at 8p is frequent in breast cancer, especially in hereditary cases associated with *BRCA2* mutations [72]. It has been associated with LOH at several chromosome loci, as well as with large tumour size [72,73]. Furthermore, strong associations of 8p LOH with other unfavourable tumour characteristics suggest that it may provide an independent prognostic factor in breast cancer [72]. Likewise, allelic loss at the long arm of chromosome 8 has been fre-

quently detected in aggressive forms of breast cancer, such as solid-tubular or scirrhous histological subtypes [74].

8.2. Chromosome 10

LOH at the *PTEN* locus, at 10q23, has been frequently reported in breast cancer, especially in advanced cases, associated with lymph node metastasis and with high histological grade [75,76]. Furthermore, absence of *PTEN* allelic loss in in situ lesions suggests that these alterations constitute late events in breast tumour formation and that are probably related to tumour progression [75].

LOH studies, however, offered also some arguments against the involvement of *PTEN* in breast tumourigenesis [77,78]. Absence of mutations or homozygous deletions in cases exhibiting allelic loss at 10q23 could possibly signify the existence of a new breast cancer TSG at this gene locus [77,78]. Hence, LOH studies are expected to provide further evidence on this issue.

8.3. Chromosome 11

Allelic loss studies have revealed that LOH often occurs in two distinct regions at chromosome 11 in breast cancer [79]. The 11q23 locus contains two candidate TSGs: *ATM* and *APOC-3*. LOH of these genes occur independently, so that they could both be targets of deletions in breast cancer [80]. Allelic loss at 11q23–24 has been correlated with poor survival in different, large studies [81,82]. This correlation does not seem to involve the *APOC-3* gene, weakening thus, its role in breast cancer pathogenesis [83]. On the contrary, *ATM* allelic loss offers a putative predictor of adverse outcome in breast cancer, and this correlation seems to concern young patients also [81,82].

9. Allelic loss in breast cancer prevention, detection and therapy

Allelic loss studies are of particular importance when they are performed on precancerous breast lesions. High rates of LOH at several loci commonly deleted in breast cancer have been detected in apocrine mammary metaplasia and in ductal carcinoma in situ [84,85]. Furthermore, comparison between allelic loss patterns in premalignant situations and in invasive carcinoma may suggest possible causative relationships or clonal divergence during progressive stages of the disease evolution [86,87]. LOH can be detected even in premalignant lesions that are difficult to discriminate from normal epithelium, based on morphological criteria [84]. From this point of view, recognition of allelic loss may assist the classical pathologic examination criteria in early recognition of dangerous lesions.

Allelic loss at specific loci (3p25.1, 8p22, 13q12, 17p13.3 and 22q13) significantly predicts for postoperative recurrence [88]. LOH can be detected in histologically normal

breast tissue at the margins of surgically excised tumours in cases of local recurrence, aiding, thus, in early diagnosis of the complication [89]. Moreover, LOH can be detected in distant metastases, while number of LOH events correlates adversely with survival after metastasis [90]. Therefore, studies on allelic loss can confer valuable information in the process of making appropriate therapeutic decisions in the difficult cases of recurrent or advanced breast cancer.

10. Future perspectives

Studies of allelic loss appear very promising in breast cancer diagnosis and treatment. This results from improvement of techniques used for detection of deletions, as well as from optimization of sample selection [85,91]. Accessibility and feasibility of automated sequencing may probably replace microsatellite marker studies in the future. For the time being, however, progress on mapping of the human genome results in ongoing recognition of novel markers, spanning new sites throughout the genome. Consequently, LOH studies can become more detailed, so that common deletions would be localized in minimal genetic regions. Therefore, new candidate breast cancer TSG may be identified in the near future.

Apart from discriminating normal from premalignant epithelium, refinement of allelic loss studies may contribute to classification of disease subtypes and the assessment of the degree of differentiation, in ambiguous cases [86,92]. Putative recognition of distinct allelic loss patterns in bilateral disease may become an important tool in prevention of such cases, usually resulting from inherited breast cancer predisposition [93]. Finally, detection of LOH in the serum of breast cancer patients in limited disease and in carcinoma in situ may develop into a strong instrument for diagnosis of the disease at an early and, thus, treatable stage [94].

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