Chapter V

Genomic Instability in Breast Cancer

Sigurdur Ingvarsson*
Institute for Experimental Pathology
University of Iceland at Keldur

Abstract

The presence of numerical and structural chromosome aberrations is a common characteristic of tumor cells. Accumulation of these aberrations leads to dramatic changes within the genome. These changes have tumor type and stage specific pattern of segmental losses and gains, resulting in gene losses and gene copy number imbalances. Tumor development and progression is driven by sequential acquisition of specific gene alterations, and chromosome aberrations contribute to these processes. Genomic instability in tumors can be classified as chromosome instability (CIN) and microsatellite instability (MIN). MIN is in general less frequent in tumors than CIN, but is rather frequent in some tumors of the digestive tract. This is mainly due to germline mutations in mismatch repair genes associated with the HNPCC cancer syndrome, but can also be due to somatic mutations and epigenetic mechanism. While MIN is rare in breast tumors, CIN is demonstrated in the majority of breast tumors (about 70%) by aneuploidy, deletions, amplifications and rearrangements. Although the reasons for CIN in breast tumors are not well understood, explanations can partly be obtained from deficient control of genes controlling cell proliferation, apoptosis, DNA repair or chromosome segregation. Among these genes are TP53, MYC, AURKA, BRCA1 and BRCA2. TP53 is relatively well studied and is believed to be a guardian of genome integrity. Myc seems to affect tumor pathogenesis in several ways, including increased proliferation and immortalization of the cancer cells and induction of CIN. The STK15 gene encodes the aurora A kinase, which has been shown to bind to centrosomes and is important in controlling their number and the segregation of correct chromosomes to the daughter cells during mitosis. Genomic instability is high in some hereditary breast cancer, particularly in tumors of BRCA1 and BRCA2 mutation carriers, a finding which is in line with the role of the gene products in DNA repair. Some recent developments in drug

*Tel. 354-5855100; Fax: 354-5673979; Email: siguring@hi.is
therapy are based on molecular and genomic findings about breast cancer pathogenesis. Defects in checkpoint control generate CIN and are believed to facilitate tumorigenesis, but additional disabling of checkpoint signaling is a possible anticancer strategy.

Introduction

Breast cancer has all the hallmarks of a multigenic disease. Although germline mutations in several genes are well known to be involved in breast tumor progression, this is largely a consequence of somatic evolution. Tumors in the breast have, like most other cancer types, several characteristics of abnormal genome. The number of chromosomes is frequently abnormal, as is the amount of DNA.

Genetic instability is typically seen in cancerous cells, as predicted by Boveri, who theorized that tumors may become malignant as the result of abnormal chromosome numbers (Boveri 1914). Later it was postulated that tumor progression is facilitated by genomic instability, with 5-10 essential mutations to establish the malignant phenotype in most solid tumors (Hananah and Weinberg 2000). It has been demonstrated that normal mutation rate is unable to allow for the accumulation of mutations essential for cancer progression, suggesting a need for genomic destabilization (Loeb et al. 2001). Therefore genomic instability can be considered to be the underlying mechanism of tumor evolution. It also provides a means to generate the heterogeneous subpopulation of cells that typifies many solid tumors. Such heterogeneous tumors can be subpopulations of biologically aggressive metastatic and therapy-resistant cancer cells, whose outgrowth can lead to poor clinical outcome. It has been pointed out that, for tumor progression, sufficient genomic instability is required, but it has to be within certain limits, since circumstances causing overwhelming genomic instability probably result in nonviable cells (Cahill et al. 1999). Hence “moderate” genomic instability facilitates tumor development.

The molecular basis of genomic instability in breast tumors is mostly unknown, but some recent developments cast light on some of the pathways and networks involved. The proteins involved participate in cell growth control, cell-cycle checkpoints and genome integrity. Various mechanisms have been described which lead to their deregulation or dysfunction. Defective DNA repair, that is unable to cope with normally occurring damage, provides an indirect means of generating genomic instability. This can arise from deficiencies in repair enzymes or from checkpoint defects, which fail to halt the cell cycle if DNA is damaged. Segregation of chromosomes to daughter cells provides a further opportunity for large losses and perturbations of genetic information. The spectrum of genetic alterations that occur in genetically unstable cells varies considerably. In this chapter on breast cancer progression and genomic instability, the focus will be on proteins involved in DNA repair, centrosome control and telomere maintenance (Table I).
<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein function</th>
<th>Cellular function</th>
<th>Function in tumor progression</th>
<th>Abnormalities in breast cancer</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>Phosphatidylinositol kinase</td>
<td>Response to DNA damage</td>
<td>Signaling to tumor suppressors</td>
<td>Germline variants</td>
<td>10</td>
</tr>
<tr>
<td>AURKA</td>
<td>Protein kinase</td>
<td>Chromosome separation in mitosis</td>
<td>Oncoprotein</td>
<td>Gene amplification</td>
<td>15</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Multifunctional, including DNA</td>
<td>Genome integrity</td>
<td>Tumor suppressor</td>
<td>Mutation*, methylation</td>
<td>5, 15</td>
</tr>
<tr>
<td></td>
<td>repair</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA2</td>
<td>Multifunctional, including DNA</td>
<td>Genome integrity</td>
<td>Tumor suppressor</td>
<td>Mutation*</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>repair</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MYC</td>
<td>Transcription factor</td>
<td>Cell turnover</td>
<td>Oncoprotein</td>
<td>Gene amplification</td>
<td>20</td>
</tr>
<tr>
<td>TERT</td>
<td>Telomerase reverse transcriptase</td>
<td>Telomere maintenance</td>
<td>Cell immortalization</td>
<td>Overexpression</td>
<td>70</td>
</tr>
<tr>
<td>TP53</td>
<td>Transcription factor</td>
<td>Genome integrity, cell turnover</td>
<td>Tumor suppressor</td>
<td>Mutation and other</td>
<td>30</td>
</tr>
</tbody>
</table>

*Germline mutations – somatic mutations are rare. See further details and references in the main text.
Chromosomal Instability

Genomic instability in tumors can be classified as chromosomal instability (CIN) or microsatellite instability (MIN). MIN is in general less frequent in tumors that CIN, but is rather frequent in some tumors of the digestive tract. A possible explanation for this is the special turnover of the cells in the digestive tract and also exposure to the “environment”, i.e. food and food digestion. MIN is mainly due to germline mutations in mismatch repair genes associated with the HNPCC syndrome, but can also be due to somatic mutations and epigenetic mechanism. Frequent mutations are seen within short repetitive sequences in tumors with MIN, which can be within genes that may have a role in tumor progression (Johannsdottir et al. 2000). CIN phenotype is characterized by increased propensity for numerical chromosome aberrations.

CIN is demonstrated in the majority of breast tumors (70%) by aneuploidy, deletions, amplifications and rearrangements, while MIN is rare (Huiping et al. 1999a).

There is a difference in genomic alterations in the two histological types of breast cancer, ductal and lobular. In general the genome is more stable in lobular than ductal breast cancer, with the exception of loss at chromosome arm 16q, which is detected with higher loss in lobular breast cancer (Huiping et al. 1999b). This may be relevant with respect to the rather frequent mutation rate of E-cadherin in lobular breast cancer, not seen in ductal breast cancer.

Alterations at certain chromosome regions are frequently detected in breast tumors with an unstable genome, while alterations at other chromosomes seem to be unrelated to genomic instability. Frequent losses at certain chromosome regions not associated with genomic instability presumably involve a tumor suppressor gene, a loss which gives growth advantage to the cell, resulting in clonal expansion. When losses at certain chromosome regions are associated with genomic instability, a tumor suppressor gene involvement is not as suggestive. Loss at these chromosome regions could reflect the unstable nature of the breast cancer genome, and therefore these losses could serve as markers for genomic instability. But this does not exclude the possibility that the growth advantage of the breast tumor cells, due to tumor suppressor gene elision, may be of importance for the clonal selection of these chromosomal losses. We have shown that chromosome arms 3p, 6q and 8p are the regions most frequently altered in breast tumors with an unstable genome (Ingvarsson 2004). In contrast, loss at chromosome 16q is not associated with an unstable genome, even though this is the chromosome region most frequently altered in sporadic breast cancer (Skirmisdottir et al. 1995). Chromosome 16q loss also differs from chromosomal losses associated with genomic instability with regard to clinicopathological factors, i.e. while chromosome 3p, 6q and 8p losses are associated with aneuploidy, high S-phase and reduced patient survival, chromosome 16 loss does not show this association, and is in contrast associated with low S-phase and elevated patient survival (Bragadottir et al. 1995; Eiriksdottir et al. 1995; Hansen et al. 1998; Sigbjornsottir et al. 2000; Skirmisdottir et al. 1995). No definitive answer has yet been found to the question of whether losses of 3p, 6q, 8q and some other chromosomes are only markers for genomic instability, or whether true tumor suppressor genes of importance for malignant progression of breast cancer are located there.

The 3p region is not only frequently altered in breast cancer, but is also among the most frequently lost regions in many types of cancer (Ingvarsson 2005). However, it has been a
difficult region in which to find a definite tumor suppressor gene, and it has been hypothesized that combined functional loss of several tumor suppressor genes located at 3p contributes to tumor pathogenesis (Huebner 2001). The FHIT gene is located at the most common fragile site in the human genome at 3p14.2, FRA3B, and is frequently altered in breast cancer, particularly if it is of hereditary origin, where DNA repair genes are mutated (Ingvarsson et al. 1999a, 2001). This could merely reflect the unstable nature of the fragile site in the breast tumor cell, but it is also possible that FHIT plays a tumor suppressor role. Specific Fhit pathways have not been identified, but a recent study suggests a role in homologous recombination repair (Hu et al. 2005). The question may be asked, whether the fragile sites in the genome are more sensitive to alterations in a background of germline mutations where DNA repair is dysfunctional. This could be part of the story, but not the only explanation. When comparing losses from chromosomes that carry the most common fragile sites in the genome, FRA3B, FRA16D and FRA6E, only chromosomes 3p and 6q show elevated loss in hereditary tumors associated with DNA repair dysfunction, compared to sporadic breast tumors, but not chromosome 16q (Ingvarsson 2004). Also, there is higher loss at chromosome 8p in hereditary tumors with mutated repair genes, as against sporadic tumors, even though this chromosome region does not contain a defined fragile site (Sigbjörnsdóttir et al. 2000).

**Centrosome Amplification**

A defect in centrosome maturation has been described in several cancer types, including breast cancer (Pihan et al. 2003). Centrosome defects are believed to affect normal segregation of chromosomes and produce aneuploid cells. One of the contributing factors for CIN in breast cancer cells is the presence of more than two centrosomes during mitosis, usually called centrosome amplification. Breast cancer cells frequently display an excess number of centrosomes (D'Assoro et al. 2002, Lingle et al. 2002). Precisely two centrosomes are required for accurate chromosome segregation into daughter cells. Cells are equipped with a mechanism controlling the duplication of centrosomes and DNA in every single cell cycle, and these two events are coordinated, probably to ensure that these two cellular components duplicate only once. If proliferating cells fail to coordinate centrosome duplication with DNA replication, abnormal segregation of chromosomes is provoked. The coupling of the initiation of DNA and centrosome duplication is at least in part achieved by specific activation of cyclin-dependent kinases and their corresponding regulation partner, cyclins (Koff et al. 1992). It has been shown that centrosome amplification is an early event in the development of breast cancer, and amplification of centrosome size and number correlates with CIN (Lingle et al. 2002).

Centrosome amplification is seen in a subset of breast cancer cells harboring mutations in tumor suppressor proteins such as p53, Brca1 and Brca2 or overexpression of oncoproteins such as the aurora kinase A. Among the several centrosome-associated kinases and target substrates implicated in the regulation of centrosome duplication cycle that may become altered during the development of centrosome amplification in breast cancer, the best-documented evidence is for the role of aurora kinase A.
Additional proteins involved in mitotic checkpoints and possibly centrosome amplification are MAD1 and MAD2, both found to be mutated in breast cancer (Percy et al. 2000, Tsukasaki et al. 2001). Also, a recent study describes the prolyl isomerase Pin1, which is overexpressed in breast cancer, as a regulator of centrosome duplication (Suizu et al. 2006).

**The Aurora Kinase A**

The aurora kinase A is overexpressed in a subset of breast cancer (Courjal et al. 1996, Sen et al. 1997). This overexpression can partly be explained by gene amplification, but also by dysregulation at transcriptional or translational level (Bischoff et al. 1998, Jeng et al. 2003).

The aurora kinase A is important for cell-cycle regulation, in particular the passage from G2 to M, and is believed to be involved in a checkpoint network. The aurora kinase A contributes to ensuring that the two daughter cells receive identical copies of the genome in the cell-cycle progression. It is located at centrosomes and microtubules at the spindle poles, and the kinase activity is involved in maturation and separation of centrosomes and assembly and stability of the spindle (Kimura et al. 1997). The deregulation of aurora kinase A leads to defects in centrosome separation and spindle defects (Glover et al. 1996). It has been defined as an oncoprotein, based on an ectopic expression in immortalized fibroblast cell lines resulting in cell transformation (Bischoff et al. 1998, Zhou et al. 1998). Aurora A mutants with defective kinase activity do not induce cell transformation, indicating that the active kinase is oncogenic.

Genetic variants of the aurora kinase A gene have more recently been implicated in cancer risk, including breast cancer (Lo et al. 2005, Cox et al. 2006). One of these genetic variants has been shown to be more effective in transforming cells to a more malignant phenotype (Ewart-Toland et al. 2003). The prevalence of the homozygous state of this allele is higher in Asian populations than Caucasians (Lo et al. 2005). It has been shown that the genetic variants are associated with breast cancer in both populations (Cox et al. 2006).

Several small molecule inhibitors have been designed from the aurora crystal structure (Harrington et al. 2004). Protein kinase inhibitors are among the top new mechanisms for cancer therapy development that are under investigation, with significant progress.

**Cell Cycle Checkpoint Proteins**

One of the most fundamental events in the G1-S cell cycle checkpoint is the stabilization and activation of p53. TP53 is somatically mutated in about 25% of breast tumors, and germline mutations have been described in the Li-Fraumeni Syndrome, where one of the increased susceptibility disease phenotypes is breast cancer (Malkin et al. 1990; Srivastava et al. 1990). P53 is a rather well defined transcription factor, and its role in cell-cycle checkpoint is generally accepted. In the case of DNA aberrations or other defined stress on the cell, the increased amount of p53 due to stabilization of this otherwise unstable protein is
responsible for blocking the cell cycle and inducing apoptosis. Several molecular mechanisms have been described, including the promoter-directed elevated expression of p21, a protein that can block the cyclin-dependent kinase/cyclin function, resulting in halting of the cell cycle at the G1 checkpoint. P53 plays also a central role in the decision of a cell to undergo apoptosis after exposure to diverse stresses, including DNA damage. Interaction of p53 with Brca1 and other proteins important for DNA repair has been reported, but functional evidence for the role of p53 in DNA repair is still limited (Zhang 1998). A feasible model is that p53 is important for blocking the cell from entering the S-phase of the cell cycle upon cell damage, and induces apoptosis if the damage is overwhelming. With failure of p53 normal function due to mutation, there is a risk of accumulation of genomic instability and mutations in additional genes.

The majority of TP53 mutations are missense, in contrast to mutations in several other tumor suppressor genes, where the majority of mutations result in a truncated protein. Some of the TP53 mutations are dominant negative, presumably due to incompetent transcription factor, if one or more mutant copies of the protein are included in the p53 tetrameric form. The germline mutation spectrum is slightly different from the somatic pattern, in line with endogenous mutagenic processes (Olivier 2001). A high frequency of codon 163 mutation of the TP53 is detected in breast tumors, particularly in a BRCA1 mutational background (Crook et al. 1997, 1998; Greenblatt et al. 2001). The mutation spectrum of TP53 in BRCA1 and BRCA2 carriers is different from that of sporadic tumors, which is consistent with a repair function of Brca1 and Brca2 (Greenblatt et al. 2001). The p53 mutants are presumably selected during the malignant progression in the genetic background of BRCA1 and BRCA2 associated tumors.

Somatic and germline mutations in the CHK2 gene have been described in relation to breast cancer, suggesting that loss of Chk2 is functionally equivalent to TP53 mutations, while mutation frequency is lower in CHK2 than in TP53 (Bell et al. 1999; Ingvarsson et al. 2002; Sullivan et al. 2002). Germline mutations of CHK2 have been found in Li-Fraumeni and Li-Fraumeni-like families, and by population screening of breast cancer patients (Bell et al. 1999; Ingvarsson et al. 2002; Sullivan et al. 2002). The germline variants of CHK2 analyzed so far by population screening seem to be low penetrance alleles conferring susceptibility to breast cancer (Ingvarsson et al. 2002; Meijers-Heijboer et al. 2002). Population-based analysis of a mutation that abolishes kinase activity indicated a 5% frequency in individuals with breast cancer, and a twofold and tenfold increased risk of breast cancer in females and males respectively (Meijers-Heijboer et al. 2002). Tumors in BRCA1 carriers have a relatively high frequency of somatic CHK2 mutations, as do tumors in patients with medullary carcinoma (Sullivan et al. 2002). This is of particular interest, since TP53 somatic mutations are also found at a high level in BRCA1 tumors (Crook et al. 1997, 1998; Greenblatt et al. 2001). These findings of somatic mutations in cell-cycle checkpoint genes such as TP53 and CHK2 are in line with the theory that they increase the rate of tumorigenesis in BRCA1 associated tumors.

As p53 is a cell-cycle checkpoint protein in DNA-damaged cells, it is not surprising that cells that lack functional p53 accumulate genomic defects, even though all target genes of this transcription factor and protein networks are not fully characterized.Tp53 mutations
have been implicated as a cause for CIN in breast cancer in some studies, but not all (Sigurdsson et al. 2000, Lingle et al. 2002).

Mouse knockout experiments support the association of dysfunctional p53 and genomic instability (Fukasawa et al. 1996, 1997). Homozygous p53 knockout mouse embryo fibroblasts undergo centrosome amplification (Fukasawa et al. 1996). Further experiments suggested that p53 inactivation induces CIN through centrosome amplification, and a putative mechanism is through loss of transcriptional activation of p21, and subsequent activation of cyclin-dependent kinase 2/cyclin E complexes controlling the centrosome duplication cycle (Mussman et al. 2000). Of interest is the finding that p53 loss induces centrosome amplification and CIN in human cells, in concert with cyclin E overexpression (Kawamura et al. 2004). This may explain some of the discrepancies between different studies, but needs further analysis in breast cancer. Centrosome amplification in p53 negative cells does not necessarily imply a role for p53 in the regulation of centrosome duplication, but instead may reflect the involvement of a p53-dependent checkpoint in the elimination of cells that emerge from aborted divisions. The mechanism for centrosome amplification associated with loss of p53 is poorly understood, but some additional clues come from an experimental system, in which centrosomes undergo multiple rounds of duplication in rodent cells during S-phase (Tarapore et al. 2001). This occurs only if p53 is mutated or lost, and in the presence of wild-type p53 the centrosome re-duplication is blocked. Increased levels of p21 block the initiation of centrosome duplication via inhibition of cyclin-dependent kinase 2/cyclin E. In hydroxyurea-treated cells with mutant p53 there is an abnormal centrosome accumulation, whereas cells with wild-type p53 arrest centrosome duplication under these conditions (Dassoro et al. 2004).

Human and mouse heterozygous ATM mutation carriers are at increased risk for tumors, including breast cancer (Shiloh et al. 2003, Spring et al. 2002). Atm is a key player in the cellular response to DNA damage. It is a phosphatidyl inositol 3-kinase which phosphorylates substrates in response to DNA damage. This includes p53 and results in stabilization and activation of it since it loses the inhibitory interaction of the Mdm2 ubiquitin ligase complex. Brca1 presumably serves as a scaffold for Atm to pass on its phosphorylation to downstream targets required for apoptosis and checkpoint activation. Also, the Chk2 kinase is phosphorylated by Atm.

**DNA Repair, BRCA1 and BRCA2**

BRCA1 is a familial breast and ovarian cancer susceptibility gene (Miki et al. 1994). Brca1 is involved in diverse cellular events and functions, including homologous recombination DNA repair, transcriptional regulation, chromatin remodeling, cell- cycle checkpoint control and ubiquitin ligation (Moyhanan et al. 1999, Welsh et al. 2002, Bochar et al. 2000, Baer et al. 2002). BRCA2 is also a familial breast cancer susceptibility gene that is structurally unrelated to BRCA1, but its protein product plays a partial role in the same pathways (Wooster et al. 1995). The main function of Brca2 is in homologous recombination DNA repair. Both Brca1 and Brca2 bind to Rad51, a protein implicated in recombination and double stranded DNA repair (Ingvarsson 1999). The Brca1 and Brca2 proteins participate in
the BASC (Brca1 associated genome surveillance complex). They are multifunctional proteins involved in complex protein-protein interactions. The factors binding to Brca1 are both specific transcription factors and factors involved in chromatin remodeling. Brca2 is involved in loading of Rad51 to damaged DNA. Mainly active in S and G2 phases of the cell cycle, Brca1 and Brca2 are essential for preserving chromosome structure, suggesting that, in their role as tumor suppressors, they behave as caretakers, suppressing genomic instability. While the role of Brca1 and Brca2 in homologous recombination repair of double-strand DNA breaks is well established, more data are needed to clarify how they act as regulators of cell-cycle events independent of their role in DNA repair.

Even though BRCA1 and BRCA2 are the major genes involved in hereditary breast cancer, they explain only less than 10% of breast cancers. The majority of breast cancers are believed to be sporadic, where somatic mutations have a major role, or are due to the combined effects of low-penetration sequence variants and genetic background. The mechanism of BRCA1 or BRCA2 inactivation in tumors is believed to be a double hit, a germline mutation and a somatic deletion (Smith et al. 1992; Gudmundsson et al. 1995). However, experimental data are lacking to clarify whether losses of the wild-type chromosomes are a prerequisite for non- or abnormal function of the proteins, or whether dominant negative or haplo-insufficient mechanisms can explain the original pathogenesis (Fan et al. 2001). Since germline mutations of BRCA1 and BRCA2 are relatively frequent in relation to familial breast cancer, the rarity of somatic mutations has been regarded as surprising (Khoo et al. 1998; Signori et al. 2001). This situation is different from the TP53 mutation story, where somatic mutations are relatively common and germline mutations are rare. It is not clear whether some prevention of the molecular mechanism leads to somatic mutations of BRCA1 and BRCA2, or whether the mutations do not give growth advantage for the cells. There could be a particular time frame in normal tissue maturation, after which somatic BRCA1 and BRCA2 mutations are not selected during sporadic breast tumor development, although gene silencing mechanisms and large rearrangements and deletions can influence tumor progression. Even though somatic mutations are rare in BRCA1, it is frequently deleted and expression is decreased in breast tumors, although not always by a known mechanism (Thompson et al. 1995; Wilson et al. 1999). Hypermethylations at the promoter region may partly explain the BRCA1 downregulation in sporadic breast tumors (Catteau et al. 1999; Niwa et al. 2000, Rice et al. 1999).

Molecular and pathological data suggest not only a difference between BRCA1 and BRCA2 associated tumors, but also between them and sporadic tumors. BRCA1 and BRCA2 tumors are more aggressive than sporadic tumors, as indicated by S-phase, mitosis, aneuploidy, genomic instability and pathological appearance (Breast cancer linkage consortium 1997). Other characteristics of BRCA1 tumors are low ER content, elevated lymphocyte infiltration and appearance of medullary phenotype (Marcus et al. 1996; Johannsson et al. 1997). The gross genomic instability detected in BRCA1 and BRCA2 tumors fits well with their documented function in DNA repair (Ingvarsson et al. 1998; Tirkkonen et al. 1997). Moreover, the chromosome aberration profiles of BRCA1 and BRCA2 tumors differ from each other and from other breast cancers, suggesting that specific genetic pathways operate in the progression of genomic instability in these inherited tumors (Ingvarsson et al. 1998; Tirkkonen et al. 1997). Functional support for the discrimination
between BRCA1, BRCA2 and sporadic breast tumors is also evident from genome-wide gene expression profiles (Hedenfalk et al. 2002).

It can be hypothesized that in the early stage of BRCA1 and BRCA2 pathogenesis, cells progress through a preliminary crisis phase with massive apoptosis due to accumulation of genetic changes. Further gene alterations, for instance in TP53 or CHK2, rescue the cell from this senescence phase, and progression is towards reduced apoptosis, enhanced cell growth and a fully malignant phenotype (Crook et al. 1997; Greenblatt et al. 2001; Sullivan et al. 2002). Even though TP53 mutations are not as frequent in BRCA2 as in BRCA1 associated tumors, overexpression of p53 is detected, suggesting that in BRCA2 mutation carriers the p53 pathway is deregulated by some other mechanisms in addition to mutation (Crook et al. 1998; Eiriksdottir et al. 1998; Greenblatt et al. 2001; Gretarsdottir et al. 1998). Mouse knockout experiments support the hypothesis of a preliminary crisis phase, and it has been shown that inactivation of p53, or other checkpoint proteins like Bub1 and Mad3L, is of importance in tumor progression in mouse cells lacking Brca (Lee et al. 1999).

In addition to the role of Brca1 and Brca2 in DNA repair, these proteins have multiple functions, one of which seems to be regulation of centrosome number. Brca1 can bind the γ-tubulin which is one of the major centrosomal proteins (Hsu et al. 2001). It has been shown that embryonic fibroblasts derived from mice deficient for a full-length wild-type BRCA1 contain amplified centrosomes (Xu et al. 1999). Similarly, loss of BRCA2 results in centrosome amplification (Tutt et al. 1999). The Brca1 and Brca2 proteins are participants in large protein complexes and, as described earlier, one of the proteins that is considered to be of major importance is Rad51. This refers both to the DNA repair function and to centrosome amplification. The functional inhibition of Rad51 by expression of dominant negative Rad51, or conditional repression of Rad51, results in centrosome amplification (Bertrand et al. 2003, Dodson et al. 2004). This may imply a link between DNA repair and numeral homeostasis of centrosomes. In support of this, centrosome amplification is induced by irradiation, which is otherwise a well-known inducer of DNA breaks (Sato et al. 2000). There seems to be a link between the Brca1 and aurora A kinase, since the former is phosphorylated by the latter, an event that is considered to be important for the regulation of the G2-M transition in the cell cycle (Ouchi et al. 2004). A recent report has suggested that Brca1-dependent ubiquitination activity in concert with Bard1 marks the centrosomes, and inhibits their reduplication (Ko et al. 2006, Starita et al. 2004). Mutations of BARD1 are found at low frequency in breast cancer (Thai et al. 1998, Ghimienti et al. 2002). The role of Bard1 in the Brca1 and Brca2 pathways and genomic stability is further established in knockout mouse experiments (McCarthy et al. 2003).

Fanconi anemia is a rare genetic cancer susceptibility syndrome. In addition to abnormalities in skeleton and skin pigmentation and bone marrow failure, it is characterized by CIN in the form of rearrangements between non-homologous chromosomes, and sensitivity to DNA cross-linking agents (D’Andrea and Grompe 2003). Fanconi anemia patients are also predisposed to developing cancer of several types (Rosenberg et al. 2003). Eight Fanconi anemia genes have been cloned and one of them appears to be BRCA2. The situation is different from typical breast cancer predisposition where one allele is mutated in germline, since Fanconi anemia patients express biallelic BRCA2 mutations (Howlett et al. 2002).
MYC and Telomerase

Another pathway of CIN induction in breast tumors involves telomere maintenance and release of cell senescence, presumably due to elevated expression of the enzyme telomerase. Telomere shortening is a natural consequence of somatic cell proliferation. Loss of telomeres leads to apoptosis unless an event is activating telomerase, leading to maintenance of telomeres and a situation where cells accumulating genomic changes become more viable. Telomerase maintains cell viability and maintenance of telomeres through the addition of repeats to chromosome ends. The reactivation of telomerase through the upregulation of TERT, the telomerase protein subunit, is an important step during cancer development, yet TERT protein function and regulation remain incompletely understood. Telomerase is of importance for cell immortalization and it can establish genomic instability that favors growth advantage of tumor cells. Telomerase overexpression is detected in the majority of breast cancers (Artandi et al. 2003). In mouse models where telomerase is lacking in a p53 heterozygous background, end-joining and unequal translocations between chromosomes are frequent, but these are not detected in mice with long telomeres (Artandi et al. 2000). Analysis of the genome in mouse tumors lacking telomerase shows that uneven translocations lead to a high number of aberrations on chromosome regions, which are not seen in tumors with normal telomeres (O’Hagen et al. 2002).

The well defined oncprotein Myc has elevated expression in a subset of breast cancer cells. It is a transcription factor involved in cell growth and cell turnover control. Myc seems to affect tumor pathogenesis in several ways, including increased proliferation and immortalization of the cancer cells and induction of CIN. Myc overexpression in breast cancer can partially be explained by gene amplification. Myc amplifications are detected in early stage breast cancer, especially in younger patients, but also occur later in tumor development and appear to be associated with disease progression (Aulmann et al. 2006). The effects of Myc on increased CIN in breast cancer have been documented (Rummikainen et al. 2001). Myc can bind to the promoter of the TERT gene and activate transcriptional expression, resulting in elevated telomerase (Wu et al. 1999). It is also of interest that the aurora kinase A upregulates Myc and then indirectly telomerase expression (Yang et al. 2004). This provides further support for the postulated cross-regulation between different pathways leading to genomic instability.

Epigenetics

An epigenetic mechanism may also be of importance for the development of genomic instability in breast cancer progression. It is generally accepted that global DNA hypomethylation leads to genomic instability and increased tumor progression, but there has been little study of the role of epigenetics in genomic instability of breast tumor cells (Feinberg 2006). Evidence for the involvement of hypomethylation in genomic instability comes from an animal model, as global reduction in methylation was shown to increase chromosomal instability in DNA methyltransferase knockout mice (Chen et al. 1998). Also, chromosomal instability events correlate with the extent of global hypomethylation and
expression of MBD2/demethylase in breast cancer cell lines (Vilain et al. 1999). Is it possible to restore genomic stability by affecting global DNA methylation? Further studies will doubtless elucidate the role of global DNA methylation in genomic instability of breast cancer progression, and possibly give some clues on therapeutic intervention.

**Therapeutic Intervention**

Genomic instability is believed to play a role in the mechanism of action of chemotherapy and possibly radiotherapy. In a background of genomic instability, there is an increased rate of acquiring mutations which facilitate tumor progression, but can also accelerate pathways induced by cancer drugs, leading to reduced viability of the cancer cell. The majority of chemotherapeutic agents interfere with DNA metabolism and are mutagens. In a genetically unstable cancer cell, a further increase in mutagenesis due to chemotherapy could lead to accumulation of mutation and extinction of the cell. An obvious side effect of chemotherapy and of radiotherapy is induction of secondary malignancies, and the state of the genome probably affects this progression. In individuals with breast cancer due to predisposition genes such as ATM, BRCA1, BRCA2 and TP53, it is debatable whether radiation should be used, but this will hopefully be clarified through additional follow-up studies. Ionising radiation has a central role, not only in the treatment of breast cancer, but also in diagnosis and surveillance. Valid questions may be asked, about whether defects in the genes that cause inherited predisposition to breast cancer may also affect radiosensitivity, entailing a predisposition to altered radiation-induced effects in tumor tissue, healthy tissue, or both.

Recent developments in understanding of molecular networks involved in genomic instability are already providing some clues in therapy strategies. Can the function of a tumor suppressor protein be restored, as has been suggested for p53, by blocking degradation pathways or using small molecules that reactivate the protein (Issaeva et al. 2003)? To restore Brca1 or Brca2 activity seems more complicated; it may be more relevant to induce further genomic damage in order to make cells less viable, as has been suggested using PARP inhibitors (Farmer et al. 2005). Emerging data on the role of aurora kinases in genomic instability and breast cancer pathogenesis are opportune, and could give some clues on novel kinase inhibitor treatment.

Instability of the genome may also play a role in resistance to cancer therapy. In a background of genomic instability, there might not only be accelerated rate of mutations that are required for tumor progression but it could also in parallel generate an even larger number of mutations that would render cells resistant to chemotherapeutic agents. Within a tumor there may be multiple cells that contain mutations in genes or regulatory sequences that render those cells resistant to specific chemotherapies. In the presence of chemotherapy, the mutant cells would have a growth advantage. Accordingly, treatment with chemotherapy selects resistant mutants, and genomic instability exacerbates this problem.
Conclusion

There is strong evidence that genomic instability has a role in breast cancer pathogenesis, as well as a possible role in sensitivity and resistance to therapy. CIN is frequent in breast tumor cells, and some chromosome regions are more unstable than others, particularly in hereditary breast cancer. Why would a cell with elevated CIN be viable? This must be due to selective pressure of genes involved in cell turnover such as cell proliferation, cell-cycle checkpoints, apoptosis etc. Indeed some experimental data support this hypothesis for breast cancer: both information from human tumors and from mouse models, such as knockout mice. An example of such an explanation derives from the work on Brca1 and Brca2. As genomic instability can result in an increased number of gene abnormalities by growth selection, some of these gene abnormalities can induce development of further genomic instability. Perhaps an autonomous loop of events is induced in breast cancer, as well as in some other cancer types, where gene abnormalities induce CIN, and the induced instability gives rise to further gene abnormalities. There must be some equilibrium in the amount and speed of such events to favor appropriate growth selection for the cancer cell; too much instability could result in poor cell survival. Clearer understanding is being achieved on the networks involved in genomic instability in breast tumors, but molecular pathways need to be studied further. Presumably there is some sort of synergism between the progression of genomic instability and tumor suppressor dysfunction/oncoprotein activation, to facilitate breast tumor development. Genomic instability is probably one of the most common causes of tumor cell evasion of therapy. Hence a complete understanding of the biological basis of genomic instability is essential for effective diagnostic and prognostic evaluation and therapeutic intervention in breast cancer.

References


Bertrand P, Lambert S, Joubert C, Lopez BS. (2003) Overexpression of mammalian Rad51 does not stimulate tumorigenesis while a dominant-negative Rad51 affects centrosome


Ko MJ, Murata K, Hwang DS, Parvin JD. (2006) Inhibition of BRCA1 in breast cell lines causes the centrosome duplication cycle to be disconnected from the cell cycle. *Oncogene* 25, 298-303.


