“Hide-then-Hit” to explain the importance of genotypic polymorphism of DNA repair genes in determining susceptibility to cancer.

Pei-Ei Wu[1] and Chen-Yang Shen[1,2]*

[1]Institute of Biomedical Sciences, Academia Sinica, Taipei, 11529, Taiwan

[2]Graduate Institute of Environmental Science, China Medical University, Taichong, 40402, Taiwan

*Correspondence to:

Chen-Yang Shen (Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; e-mail: bmcys@ibms.sinica.edu.tw; Phone:+886-2-27899036; Fax:+886-2-27823047)

Abstract.

Interindividual variations in DNA repair capacity/efficiency linked to the presence of polymorphisms in DNA repair-related genes have been suggested to account for different risk of developing cancers. In this review article, on the basis of breast cancer formation as a model, we propose a “hide-then-hit” hypothesis indicating the importance of escaping checkpoint surveillance for sub-optimal DNA repair variants to cause cancer. Therefore, only cells with subtle defects in repair capacity arising from low-penetrance variants of DNA repair genes would have the opportunity to grow and accumulate the genetic changes needed for cancer formation, without triggering cell-cycle checkpoint surveillance. Furthermore, distinct from high-penetrance alleles, these polymorphic alleles of DNA repair genes would *predispose* carriers to a higher risk of developing cancer but would not necessarily *cause* cancer. To examine this, we simultaneously genotyped multiple SNPs of cell-cycle checkpoint genes and the DNA repair genes. Support for the hypothesis came from observations that breast cancer risk associated with variant genotypes of DNA repair genes became more significant in the subgroups of women with specific genotypic statuses of checkpoint genes. This “hide-then-hit” hypothesis is certainly needed to be confirmed by biological evidence in which a cause-effect relationship has to be established. However, based on this, possible gene-gene interaction is considered play an important role in modifying the cancer risk associated with genotypic polymorphism of DNA repair gene in different study populations.

Introduction.

Cancer results from a series of genetic alterations leading to progressive disorder of the normal mechanisms controlling growth, differentiation, cell death, or genomic instability. The response of the cell to genetic injury and its ability to maintain genomic stability by means of a variety of DNA repair mechanisms are therefore essential in preventing tumor initiation and progression. Familial cancer syndromes, including xeroderma pigmentosum and hereditary nonpolyposis colorectal cancer, which are, respectively, causally linked to defective nucleotide excision repair and mismatch repair (Sherr, 2004; Vogelstein and Kinzler, 2002), emphasize the importance of DNA repair mechanisms during tumorigenesis. Given this, it is reasonable to speculate that interindividual variations in DNA repair capacity/efficiency linked to the presence of polymorphisms in DNA repair-related genes might account for the different risk of developing cancers. To examine this hypothesis, polymorphisms of several DNA repair-related genes have been found to be associated with the risk of developing different tumor types (e.g. Michiels et al., 2009; Vodicka et al., 2007; Hu et al., 2005; Hosgood et al., 2008), although results are not always consistent. This may be due to the possibility that, distinct from high-penetrance alleles, these polymorphic alleles of DNA repair genes would *predispose* carriers to a higher risk of developing cancer but would not necessarily *cause* cancer. Therefore, possible gene-gene interaction and gene-risk factor interaction may play an important role in modifying the cancer risk associated with genotypic polymorphism of DNA repair gene in different study populations.

"Hide-then-Hit" hypothesis explains polymorphism of DNA repair genes in association with cancer risk.

On the basis of breast cancer as a model, we have proposed a model (i.e., the

hide-then-hit hypothesis) (Figure 1) (Fu et al., 2003) to suggest the importance of genetic polymorphism of DNA repair genes during tumorigenesis and to explain how polymorphic DNA repair genes contribute to breast cancer formation. The “hide-then-hit” hypothesis indicates the importance of escaping checkpoint surveillance for sub-optimal DNA repair variants to cause cancer. Therefore, only cells with subtle defects in repair capacity arising from low-penetrance variants of DNA repair genes would have the opportunity to grow and accumulate the genetic changes needed for cancer formation, without triggering cell-cycle checkpoint surveillance. The rationale underlying this hypothesis is derived from the clues provided by family breast cancer syndromes, in which susceptibility genes, including *p53, ATM, BRCA1* and *BRCA2*, are involved within the common functional pathway of double-strand-break (DSB)-related checkpoint/repair. Specifically, both *BRCA1* and *BRCA2* are involved in the homologous recombination (HR) pathway for DSB repair (O'Donovan and Livingston, 2010) supporting the idea that breast cancer pathogenesis is driven by DSB-initiated chromosome instability, and that the mechanisms involved in DSB repair are of particular etiological importance during breast tumorigenesis. Interestingly, several mutations in the genes encoding for other DSB repair pathway, i.e. nonhomologous end-joining (NHEJ), have recently been identified in breast cancer patients (Wang et al., 2008), which suggests a tumorigenic role of NHEJ in breast cancer formation. .

Germ-line mutations in *Ligase IV*, one of the genes involved in NHEJ, have been identified in patients presenting with a novel syndrome, NBS-like syndrome, which resembles A-T and NBS and is characterized by developmental delay and immunodeficiency (O'Driscoll et al., 2001). An important characteristic of both A-T and NBS is the elevated incidence of cancer, and *ATM* (the gene responsible for A-T) and

NBS1 (the gene responsible for NBS) play critical roles in maintaining a normal checkpoint response to DSB (Khanna and Jackson, 2001; Karran, 2000; Falck, et al., 2002) However, *Ligase IV*-mutated patients is only associated with some levels of cancer predisposition as leukemia was found in some NBS-like syndrome patients (O'Driscoll et al., 2001; O'Driscoll et al., 2004). One possible explanation for this may be that, because NHEJ is crucial for cells to maintain genetic stability, any severe defects (null mutants) in NHEJ-related genes, such as those in NBS-like patients, would result in genomic instability and trigger cell death by cell cycle checkpoint surveillance. Thus, for these high-penetrant NHEJ genes, only subtle defects arising from low-penetrance (risk) alleles (*e.g.*, hypomorphic mutant or polymorphic variant) would escape checkpoint surveillance and accumulate the unrepaired DNA damage required for tumor formation (Fu et al., 2003). The tumorigenic contribution of these alleles would become more obvious if individual putative high-risk genotypes of each NHEJ gene act jointly. Furthermore, this joint effect might be modified by specific environmental factors, and we hypothesized that estrogen exposure might be one such factor because estrogen is suggested to cause DSBs (Cheng et al., 2005), triggering breast tumorigenesis.

We have conducted a genotype-based case-control study (Fu et al., 2003) to examine these hypotheses. Because single nucleotide polymorphisms (SNPs) are the most subtle genetic variation in the genome, we have genotyped SNPs in five major NHEJ genes (*Ku70, Ku80, DNA-PKcs, Ligase IV,* and *XRCC4*). Support for these hypotheses came from the observations that (*a*) SNPs in *Ku70* and *XRCC4* were associated with breast cancer risk; (*b*) a trend toward increased risk of developing breast cancer was found in women harboring a greater number of putative high-risk genotypes of NHEJ genes; (*c*) this association between risk and the number of putative high-risk genotypes was stronger

and more significant in women thought to be more susceptible to estrogen, *i.e.*, those with no history of full-term pregnancy; and (*d*) the protective effect conferred by a history of full-term pregnancy was only significant in women with a lower number of putative high-risk genotypes of NHEJ genes (Fu et al., 2003). This study thus provides new insights to suggest the role of the NHEJ pathway in breast cancer development and supports the possibility that breast cancer is initiated by estrogen exposure, which causes DSBs. More importantly, This observation may also help explain the issue of tissue specificity and why the DSB repair mechanisms are of particular importance in the development of breast cancer, as the risk factors of increased estrogen exposure or increased susceptibility to estrogen exposure presumably reflect the extent of DSB formation or the degree of susceptibility to DSB formation. Consequently, breast cells that have lost DSB-related checkpoint/repair due to the harboring of at-risk genotypes have a growth advantage over DSB-checkpoint/repair-proficient cells and are selected for by the micro-environment imposed by estrogen-related risk factors, resulting in an increased risk of developing breast cancer (Fu et al., 2003). Interestingly, our inference about the interaction between DSB repair genes and estrogen exposure is supported by genetic evidence that reproductive history might influence cancer risk in women with *BRCA1* or *BRCA2* mutation (Narod, 2002).

Similarly, based on a well-known mechanism that the proteins encoded by *Mre11*, *Rad50*, and *Nbs1* form a MRN complex participating in DSB checkpoint, the findings of a SNP-based case-control study (Hsu et al., 2007) supports the role of the MRN pathway in breast cancer development, further strengthening the suggestion that mechanisms regulating DSB repair may play a mutator role driving breast cancer pathogenesis via a “hide-then-hit” model.

DSB repair deficient mice exhibit a relatively long latency or even absence (*Ligase IV* and *XRCC4* knockout mice) of tumorigenesis, which can probably be explained by highly efficient apoptosis because inhibition of apoptosis by a *p53* mutation (Meek DW, 2009; Attardi, 2005), in addition to the DSB repair gene mutation, results in rapid tumor development. The importance of these findings is that escaping checkpoint surveillance is a critical element in the pathogenesis of cancer resulting from defective DNA repair mechanisms (Figure 1*A*) and it is probable that only mild phenotypic defects, such as slightly increased genomic instability resulting from suboptimal repair capacity associated with SNPs of repair genes, could meet this "hide-then-hit" requirement (Figure 1*B*).

"Hide-then-Hit" hypothesis suggests distinct forms of cancer caused by high-penetrance mutant allele and by low-pentrance variant allele of the same DNA repair genes.

Our demonstration of breast tumorigenic contribution of low-penetrance alleles of DSB repair genes is consistent with the suggestion that apparently disparate spectrum of malignancies can be differently caused by the mutated form or by hypomorphic/polymorphic variants of the same genes (Foulkes, 2008; Concannon, 2002). During B- and T-cell differentiation, the genes that encode immunoglobulins and T-cell receptors have to be assembled into active genes by V(D)J recombination, which proceeds through a DSB intermediate and requires DSB repair proteins for completion (van Gent et al., 2001). Accordingly, it appears mechanistically reasonable that B-cell or T-cell tumors are the dominant malignant phenotypes observed in DSB-repair gene knockout mice bearing a *p53* mutation (Khanna and Jackson, 2001; van Gent et al., 2001). In contrast, possible genomic defects resulting from low-penetrance DSB repair variants

are expected to be minor and may not be great enough to initiate tumors at primary sites [*i.e.*, tumors of lymphoid origin (Figure 1)]. Consequently, cancers would develop in other tissues but would require a long period of time to accumulate essential genetic defects, and tumorigenesis would be prompted by selective exogenous or endogenous environmental factors (Elledge and Amon, 2002). Increased exposure of breast epithelium to estrogen may be one such factor, allowing breast cells with a suboptimal DSB repair capacity to accumulate sufficient DSBs in cancer-causing genes and consequently to display a growth advantage, leading to tumors.

"Hide-then-Hit" hypothesis supported by epidemiological evidence: Polymorphism of DSB checkpoint genes modifies breast cancer risk associated with polymorphism of DSB repair genes.

If this hypothesis is correct, the status of cell-cycle checkpoint would be critical in determining the fate of genomically unstable cells and the cancer risk conferred by sub-optimal DNA repair capacity. In a case-control study aimed at examining this question, we similarly used breast cancer as a model and simultaneously genotyped multiple SNPs of checkpoint genes (*ATM* and *p53*) and DSB repair genes involved in NHEJ (*Ku70, Ku80, DNA-PKcs, XRCC4,* and *Ligase IV)* or homologous recombination (HR) (*RAD51, BRCA1*, and *BRCA2*) in 469 primary breast cancer patients and 738 healthy controls. The background information and risk factor profiles of study subjects collected has been described previously (Ding et al., 2010; Yu et al., 2009; Ding et al., 2009). Furthermore, the acquisition of functions making it possible to overcome growth arrest or apoptosis and thus counteract cell cycle checkpoint has been suggested as a key step in allowing cells that already have an unstable genome to progress to tumors (Elledge and Amon, 2002). The breast is the target for estrogen, which has a growth-promoting effect on cells (Dickson and Stancel, 2000; Gompel

et al., 2000), and thus estrogen exposure may be one factor leading to the increased survival of breast cells which will ultimately undergo tumorigenesis (Elledge and Amon, 2002). This possibility was addressed by examining whether the breast cancer risk due to high-risk genotypes of DSB repair genes was increased by additional estrogen-related risk factors, particularly in the subgroup of women harboring susceptibility genotypes of checkpoint genes.

Though statistical power might be a concern to draw conclusion, support for the hypothesis came from observations that women harboring a higher number of high-risk genotypes of the NHEJ or HR genes had a significantly higher risk of developing breast cancer, with adjusted odds ratios (aORs) for one additional high-risk genotype of an NHEJ or HR gene, respectively, of 1.17 [95% confidence interval (CI), 1.01-1.34] or 1.16 (95%CI, 1.00-1.35). However, these risks differed among subgroups of women with different genotypic statuses of the checkpoint genes. Within the stratum of low-risk genotypes of both *ATM* and *p53*, there was only a modest, non-significant increase in risk associated with DSB repair genes. In contrast, in women with one or more high-risk genotypes of *ATM* and *p53*, a significant increase in breast cancer risk related to NHEJ or HR genes was consistently seen (Table 1). Furthermore, the association between risk and the interaction of checkpoint and DSB repair genes was stronger and more significant in those women thought to be more susceptible to estrogen, with the most significant contribution of DSB repair genes to breast cancer being seen in women who became pregnant for the first time at a later age (>25 years of age) and who harbored high-risk genotypes of *ATM* and *p53*; in this group, the aOR associated with the harboring of $\geqq$3 high-risk genotypes of NHEJ genes or $\geqq$2 high-risk genotypes of HR genes, was respectively, 2.02 (95%CI, 1.14-3.58) or 2.63 (95%CI, 1.34-5.16). We finally used a more conservative definition of the joint effect to represent

DSBR, only considering the contribution of genotypic polymorphisms of the two genes, *Ku70* and *RAD51*, that showed the most significant association with breast cancer risk in each repair pathway in our previous analysis. We found that the results using this conservative definition were totally consistent with our "hide-then-hit" hypothesis. Harboring a higher number of putative high-risk genotype of *Ku70* or *RAD51* was significantly associated with an increased breast cancer risk (Figure 2A), averagely one additional high-risk genotype being associated with a 1.37-fold increase in risk (95%CI, 1.09-1.69) (Figure 2B). However, this risk differed among subgroups of women with different genotypic statues of the checkpoint genes, a significant increase in breast cancer risk related to DSB repair genes being consistently seen in women with high-risk genotypes of *ATM* and *p53* or a higher number of high-risk genotypes of these two checkpoint genes (Figure 2B). Furthermore, the association between risk and the interaction of checkpoint and DSBR genes was stronger and more significant in those women thought to be more susceptible to estrogen, with the most significant contribution of DSBR genes to breast cancer being seen in women who had their first full-term pregnancy (FFTP) at a later age and who harbored high-risk genotypes of *ATM* and *p53* (Figure 2C).

The question of how HR and NHEJ are coordinated to repair DSBs is of particular interest. It has been proposed that the two pathways act in competition with each other by suppressing the expression or function of the proteins of the other pathway (Lau et al., 2004; Allen et al., 2002). However, the possibility that the two pathways act in concert to repair DSBs cannot be totally excluded. In animal model studies, mice with defects in both HR and NHEJ display synthetic phenotypes of viability and tumorigenic potential and a synergistic effect of these genes on genomic stability (Couedel et al., 2004), suggesting that these two repair pathways cooperate in DSB repair. In our epidemiological observation, the women

harboring a higher number of high-risk genotype of DSBR genes displayed a higher risk, but this clue is unable to resolve these inconsistencies.

This promoting effect of reproductive risk factors, such as FFTP, can be explained by a dual role of estrogen. As an initiator, prolonged estrogen exposure, resulting in increased DNA strand-break formation, would provide a growth advantage for breast cells with sub-optimal DSB checkpoint/repair function, while, as a promoter, estrogen would promote the survival of breast cells that have escaped checkpoint surveillance to ultimately undergo tumorigenesis. Based on a comprehensive examination of the relationships between checkpoint genes and repair genes and between checkpoint/repair genes and reproductive risk factor, this study provides epidemiological evidence to support the “hide-then-hit” hypothesis. This hypothesis, mainly based on breast cancer as a model, is certainly needed to be confirmed by biological evidence in which a cause-effect relationship has to be established.

Inactivation of checkpoint genes is required to provide a growth advantage for tumor formation.

Given that most DSB repair gene knockout mice, which themselves do not show tumorigenesis, display rapid tumor development when a defective *p53* gene is introduced to inhibit growth arrest or apoptosis (van Gent et al., 2001; Attardi, 2005), an emerging theme is that mutations in *p53* or other DNA damage-sensing/signaling components (e.g. *ATM*) are required to provide a growth advantage to cells harboring mutations in DSB repair genes, allowing cell transformation or tumor development. Consistent with this idea, *p53* is more frequently altered in breast cancers derived from *BRCA1/2* germ-line mutation carriers than in non-*BRCA1/2*-mutated breast cancers (Greenblatt et al., 2001). Our results, showing that the breast cancer risk conferred by DSB repair genes in either the NHEJ or HR pathway is

modified by the genotypic status of *ATM* and *p53*, further expand the concept of the requirement for the checkpoint gene-DSB repair gene interaction in breast cancer development in carriers harboring low-penetrance variants of DSB checkpoint/repair genes, and suggest that this interaction is of significant tumorigenic importance.

The "hide-then-hit" hypothesis suggests the importance of joint effect of low-penetrance alleles in determining cancer risk.

The demonstration of interactions between genotypic polymorphisms of cell-cycle checkpoint genes and DSB repair genes and between genotypic polymorphisms of checkpoint/DSBR genes and reproductive risk factors in the present study, together with our suggestion of the mechanisms by which low-penetrance variants of NHEJ and MRN genes have a tumorigenic effect, has yielded critical insights into how the inactivation of checkpoint/DSB repair genes can lead to the development of sporadic breast cancer. The identification of the *BRCA1* and *BRCA2* genes, which are linked to inherited breast cancer syndromes, prompted a search for somatic mutation of these genes in sporadic breast cancer, since, according to the "two-hit" model proposed by Knudson, tumor suppressor genes often play a role in both the "hereditary" form and the much more common "non-hereditary" form of the same tumor type (Knudson, 1971). However, results showing that somatic mutation of DSB repair genes is rare (Rahman and Stratton, 1998; Elledge and Amon, 2002) raised doubts about their contribution to the development of sporadic cancer. In contrast to the "gatekeeper" type of tumor suppressor gene which directly regulates the growth of tumors by inhibiting growth and promoting death, mutations in these DSB repair genes (considered as the "caretaker" type of tumor suppressor gene) do not cause tumors directly, but only lead to genetic instability, resulting in increased mutation of all genes, and it is only when additional mutations occur in gatekeeper genes that tumors begin to form (Kinzler and Vogelstein,

1997). It has therefore been suggested that sporadic cancers are unlikely to be caused by a defective caretaker pathway, because this indirect mechanism would seem to be highly inefficient, since it requires the accumulation of mutations first in caretaker genes, then in gatekeeper genes (Kinzler and Vogelstein, 1997). This hypothesis explains the absence of somatic mutation in DSB repair genes in sporadic breast cancer and suggests that these genes do not play a major role in the formation of these cancers. However, the observation that DSB formation increases significantly as breast tumors progress to poorer grades or later stages (Shen et al., 2000) prompted us to hypothesize that the genes involved in DSB repair might contribute to breast tumorigenesis by different mechanisms. Instead of genetic mutation, we have focused on subtle changes, i.e. SNPs, and have demonstrated a distinct mechanism by which DSB repair genes contribute to breast tumorigenesis via a cooperative effect of low-penetrance variants of individual genes. Interestingly, the mechanism by which low-penetrance variants manifest their tumorigenic effect is different from that used by high-penetrance mutations of the same genes (Balmain et al., 2003). Concurrent mutations seldom occur at genes participating in the same mechanistic pathway (e.g., genetic defects of *Cyclin D1*, *Rb*, and *p16* frequently occur singly in tumors, but rarely in combination) (Otterson et al., 1994; Shapiro et al., 1995); this can be explained by the functional interdependence of these proteins, which means that a mutation in a single gene is sufficient to inactivate the whole pathway. However, in the findings above, we showed that, in the case of low-penetrance variants, a joint effect of individual genes was important, and that an increase in the number of high-risk genotypes of the DSB repair genes led to an increased risk of developing cancer. Interestingly, the joint effect contributed by individual genes can also explain the incidence of hereditary cancer, as Pharoah *et. al.* (Pharoah et al., 2002) have recently developed a model showing that familial breast cancers that cannot been explained by

mutation in *BRCA1* or *BRCA2* are caused by the joint effect of a large number of codominant alleles of cancer-associated genes, each of which is associated with a small increase in risk.

Consideration of future epidemiological study to examine DNA repair genes in cancer.

Recent success of genome-wide association studies has significantly promoted our interest in using association study approach and the information of genotypic polymorphism to identify genetic loci that determine susceptibility to common human diseases, including cancer. However, genome-wide association studies have so far focused on single-locus analyses, and the importance of genetic interactions for common disease susceptibility is still unexplored on a genome-wide scale. To our knowledge there are no confirmed interactions between common variants for susceptibility to a common disease. Similarly, the contribution of gene-risk factor interaction has remained underappreciated in current genome-wide association study. Thus, the discrepancies regarding the degree and nature of cancer risk related to various genetic polymorphisms among current genome-wide association studies are not surprising. On the basis of the concept we present in this review, cancer risk associated with genotypic polymorphism of DNA repair genes is highly dependent on polymorphisms of the genes participating in the same functional pathways and the genes involved in DNA damage responses. Furthermore, endogenous/exogenous exposures would strongly affect cancer risk associated with DNA repair genes. Therefore, a full understanding of the etiologic role of DNA repair in tumorigenesis will require studies that evaluate both the genes participating in the same DNA repair pathway and the extent to which risk factors modify the associations of the genes with cancer risk.

References.

Allen, C., Kurimasa, A., Brenneman, M.A., Chen, D.J., Nickoloff, J.A. DNA-dependent protein kinase suppresses double-strand break-induced and spontaneous homologous recombination. (2002) Proc. Natl. Acad. Sci. USA *99*,3758-3763.

Attardi, L.D. (2005). The role of p53-mediated apoptosis as a crucial anti-tumor response to genomic instability: lessons from mouse models. Mutation Res, Fundamental Mol Mechanism Mutagenesis *569*, 145-157.

Balmain, A., Gray, J., and Ponder, B. (2003). The genetics and genomics of cancer. Nat. Genet. *33*, 238-244.

Cheng, T.C., Chen, S.T., Yu, J.C., Fu, Y.P., Huang, C.S., Cheng, C.W., Wu, P.E., and Shen, C.Y. (2005). Breast cancer risk associated with genotype polymorphism of the catechol estrogen-metabolizing genes: A multigenic study on cancer susceptibility. Int. J. Cancer *113*, 345-353.

Concannon, P. (2002). ATM heterozygosity and cancer risk. Nat. Genet. *32*, 89-90.

Couedel, C., Mills, K.D., Barchi, M., Shen, L., Olshen, A., Johnson, R.D., Nussenzweig, A., Essers, J., Kanaar, R., Li, G.C., Alt, F.W., abd Jasin, M. (2004) Collaboration of homologous recombination and nonhomologous end-joining factors for the survival and integrity of mice and cells. Genes Dev. *18*,1293-1304.

Dickson, R.B., and Stancel, G.M. (2000). Estrogen receptor-mediated processes in normal and cancer cells. J. Natl. Cancer Inst. Monogr. *27*, 135-145.

Ding, S.L., Yu, J.C., Chen, S.T., Hsu, G.C., Kuo, S.J., Lin, Y.H., Wu, P.E., and Shen, C.Y. (2009) Genetic variants of BLM interact with RAD51 to increase breast cancer susceptibility. Carcinogenesis *30*,43-49.

Ding, S.L., Yu, J.C., Chen, S.T., Hsu, G.C., Hsu, H.M., Ho, J.Y., Lin, Y.H., Chang, C.C., Fann, C.S., Cheng, C.W., Wu, P.E., and Shen, C.Y. (2010) Diverse associations between ESR1 polymorphism and breast cancer development and progression. Clin. Cancer Res. *16*,3473-3484.

Elledge, S.J., and Amon, A. (2002). The BRCA1 suppressor hypothesis: an explanation for the tissue-specific tumor development in BRCA1 patients. Cancer Cell *1*, 129-132.

Falck, J., Petrini, J.H., Williams, B.R., Lukas, J., and Bartek, J. (2002). The DNA damage-dependent intra-S phase checkpoint is regulated by parallel pathways. Nat. Genet.

30, 290-294.

Foulkes, W.D. (2008). Inherited susceptibility to common cancers. N Engl J Med *359*, 2143-53.

Fu, Y.P., Yu, J.C., Cheng, T.C., Lou, M.A., Hsu, G.C., Wu, C.Y., Chen, S.T., Wu, H.S., Wu, P.E., and Shen, C.Y. (2003). Breast cancer risk associated with genotypic polymorphism of the non-homologous end-joining genes: a multigenic study on cancer susceptibility. Cancer Res. *63*, 2440-2446.

Gompel, A., Somai, S., Chaouat, M., Kazem, A., Kloosterboer, H.J., Beusman, I., Forgez, P., Mimoun, M., and Rostene, W. (2000). Hormonal regulation of apoptosis in breast cells and tissues. Steroids *65*, 593-598.

Greenblatt, M.S., Chappuis, P.O., Bond, J.P., Hamel, N., and Foulkes, W.D**.** (2001). TP53 mutations in breast cancer associated with BRCA1 or BRCA2 germ-line mutations: distinctive spectrum and structural distribution. Cancer Res. *61*, 4092-4097.

Hosgood, H.D. 3rd., Menashe, I., Shen, M., Yeager, M., Yuenger, J., Rajaraman, P., He, X., Chatterjee, N., Caporaso, NE., Zhu, Y., Chanock, S.J., Zheng, T., Lan, Q. (2008). Pathway-based evaluation of 380 candidate genes and lung cancer susceptibility suggests the importance of the cell cycle pathway. Carcinogenesis *29*, 1938-1943.

Hsu, H.M., Wang, H.C., Chen, S.T., Hsu, G.C., Shen, C.Y., and Yu, J.C. (2007). Breast cancer risk is associated with the genes encoding the DNA double-strand-break repair Mre11/Rad50/Nbs1 complex. Cancer Epid. Biomarkers Prev. *16*, 2024-2032.

Hu, Z., Ma, H., Chen, F., Wei, Q., and Shen, H. (2005). XRCC1 polymorphisms and cancer risk: a meta-analysis of 38 case-control studies. Cancer Epid. Biomarkers Prev. *14*, 1810-1818.

Karran, P. (2000). DNA double strand break repair in mammalian cells. Curr. Opin. Genet. Dev. *10*, 144-150.

Khanna, K.K., and Jackson, S.P. (2001). DNA double-strand breaks: signaling, repair and the cancer connection. Nature Genet. *27*, 247-254.

Kinzler, K.W., and Vogelstein, B. (1997). Cancer-susceptibility genes. Gatekeepers and caretakers. Nature *386*, 761-763.

Knudson, A.G. Jr. (1971). Mutation and cancer: statistical study of retinoblastoma. Proc. Natl. Acad. Sci. USA *68*, 820-823.

Lau, A., Kanaar, R., Jackson, S.P., O'Connor, M.J. (2004) Suppression of retroviral infection by the RAD52 DNA repair protein. EMBO J. *23*,3421-3429.

Meek, D.W. (2009). Tumour suppression by p53: a role for the DNA damage response? Nat. Rev. Cancer *9*, 714-723.

Michiels, S., Laplanche, A., Boulet, T., Dessen, P., Guillonneau, B., Méjean, A., Desgrandchamps, F., Lathrop, M., Sarasin, A., and Benhamou, S. (2009). Genetic polymorphisms in 85 DNA repair genes and bladder cancer risk. Carcinogenesis *30*, 763-768.

Narod, S.A. (2002). Modifiers of risk of hereditary breast and ovarian cancer. Nat. Rev. Cancer *2,* 113-123.

O'Donovan, P.J., and Livingston, D.M. (2010). BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair. Carcinogenesis *31*, 961-967.

O'Driscoll, M., Cerosaletti, K.M., Girard, P.M., Dai, Y., Stumm, M., Kysela, B., Hirsch, B., Gennery, A., Palmer, S.E., Seidel, J., Gatti, R.A., Varon, R., Oettinger, M.A., Neitzel, H., Jeggo, P.A., and Concannon, P. (2001). DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. Mol. Cell *8*, 1175-1185.

O'Driscoll, M., Gennery, A.R., Seidel, J., Concannon, P., Jeggo, P.A. (2004). An overview of three new disorders associated with genetic instability: LIG4 syndrome, RS-SCID and ATR-Seckel syndrome. DNA Repair 3,1227-1235.

Otterson, G..A., Kratzke, R.A., Coxon, A., Whan Kim, Y., and Kaye, F. (1994). Absence of $p16^{INK4}$ protein is restricted to the subset of lung cancer lines that retains wild type Rb. Oncogene, *9*, 3375-3378.

Pharoah, P.D., Antoniou, A., Bobrow, M., Zimmern, R.L., Easton, D.F., and Ponder, B.A. (2002). Polygenic susceptibility to breast cancer and implications for prevention. Nat. Genet. *3*, 33-36.

Rahman, N., and Stratton, M.R. (1998). The genetics of breast cancer susceptibility. Annu. Rev. Genet. *32*, 95-121.

Shapiro, G.I., Edwards, C.D., Kobzik, L., Goldeski, J., Richards, W., Sugarbaker, D.J., and Rollins, B.J. (1995). Reciprocal Rb inactivation and $p16^{INK4}$ expression in primary lung cancers and cell lines. Cancer Res. *55*, 505-509.

Shen, C.Y., Yu, J.C., Lo, Y.L., Kuo, C.H., Yue, C.T., Jou, Y.S., Huang, C.S., Lung, J.C., and Wu, C.W. Genome-wide search for loss of heterozygosity using laser capture microdissected tissue of breast carcinoma- an implication for mutator phenotype and breast cancer pathogenesis. Cancer Res. *60*, 3884-3892.

Sherr, C.J. (2004). Principles of tumor suppression. Cell *116*, 235-246.

van Gent, D.C., Hoeijmakers, J.H.J., and Kanaar, R. (2001). Chromosomal stability and the DNA double-stranded break connection. Nat. Rev. Genet. *2*, 196-206.

Vogelstein, B., and Kinzler, K.W. (2002). The genetic basis of human cancer. New York: McGraw-Hill.

Vodicka, P,, Stetina, R., Polakova, V., Tulupova, E., Naccarati, A., Vodickova, L., Kumar, R., Hanova, M., Pardini, B., Slyskova, J., Musak, L., De Palma, G., Soucek, P., and Hemminki, K. (2007). Association of DNA repair polymorphisms with DNA repair functional outcomes in healthy human subjects. Carcinogenesis *28*, 657-664.

Wang, X., Szabo, C., Qian, C., Amadio, P.G., Thibodeau, S.N., Cerhan, J.R., Petersen, G.M., Liu, W., and Couch, F.J. (2008) Mutational analysis of thirty-two double-strand DNA break repair genes in breast and pancreatic cancers. Cancer Res. *68*,971-975.

Yu, J.C., Ding, S.L., Chang, C.H., Kuo, S.H., Chen, S.T., Hsu, G.C., Hsu, H.M., Hou, M.F., Jung, L.Y., Cheng, C.W., Wu, P.E., and Shen, C.Y. (2009) Genetic susceptibility to the development and progression of breast cancer associated with polymorphism of cell cycle and ubiquitin ligase genes. Carcinogenesis *30*,1562-1570.

Table 1. Breast cancer risk associated with one additional high-risk genotype of the DNA double-strand-break repair (DSBR) genes in women stratified by different combinations of genotypic status of the cell-cycle checkpoint genes.

Genotype of checkpoint gene		aOR(95%CI) associated with one additional high-risk genotype of the DSBR genes[a]					
ATM	*p53*	NHEJ pathway[b]			HR pathway[c]		
Low-risk	*Pro/Pro, Pro/Arg*	1.07(0.85-1.35)	1.07(0.85-1.35)	1.07(0.85-1.35)	1.06(0.82-1.37)	1.06(0.82-1.37)	1.06(0.82-1.37)
Low-risk	*Arg/Arg*	1.08(0.83-1.41)	1.12(0.91-1.38)		1.06(0.81-1.39)	1.15(0.93-1.42)	
High-risk	*Pro/Pro, Pro/Arg*	1.13(0.80-1.59)		1.22(1.02-1.46)	1.31(0.92-1.86)		1.24(1.03-1.50)
High-risk	*Arg/Arg*	1.56(1.08-2.25)	1.56(1.08-2.25)		1.59(1.02-2.47)	1.59(1.02-2.47)	

[a]The adjusted odds ratio (aOR) for breast cancer development associated with one additional putative high-risk genotype of the DNA DSBR genes was estimated in a multivariate logistic regression model containing age, a family history of breast cancer, a history of full-term pregnancy, body mass index, and the number of putative high-risk genotypes of DSBR genes

[b]Estimated using the genotypic status of the five non-homologous end-joining (NHEJ) genes, *Ku70, Ku80, DNA-PKcs, XRCC4,* and *Ligase IV.*

[c]Estimated using the genotypic status of the three homologous recombination (HR) genes, *RAD51, BRCA1* and *BRCA2*.

Figure 1.The “hide-then-hit” hypothesis of tumorigenic effect contributed by non-homologous end-joining (NHEJ) genes (Fu et al., 2003). ***A***. Since DNA double-strand breaks (DSBs) repair pathways are crucial for cells to maintain genetic stability, any severe defects in these genes would result in high levels of genomic instability and subsequently lead to cell death triggered by cell cycle checkpoint (e.g. *p53*) surveillance. Only in the case of checkpoint inactivation, tumors of lymphoid origin have the chance to develop as these cells depend on proper DSB repair to differentiate and, thus, are particularly susceptible to impaired DSB repair. ***B***. For these high-penetrant DSB repair genes, only mild defects (low levels of DSBs) resulting from sub-optimal repair capacity associated with low-penetrance (risk) alleles would escape checkpoint surveillance and accumulate the unrepaired DNA damage (genetic mutation) required for tumor formation. Tumorigenic effect contributed by “low-penetrance” genes (alleles) would become stronger in the case of a modification, and the modifying factors are probably both genetic (e.g. multiple polymorphic variants of genes of the same repair pathway) and environmental (e.g. exposure to DSB-causing agents, such as estrogen, or cell outgrowth in breast triggered by estrogen).

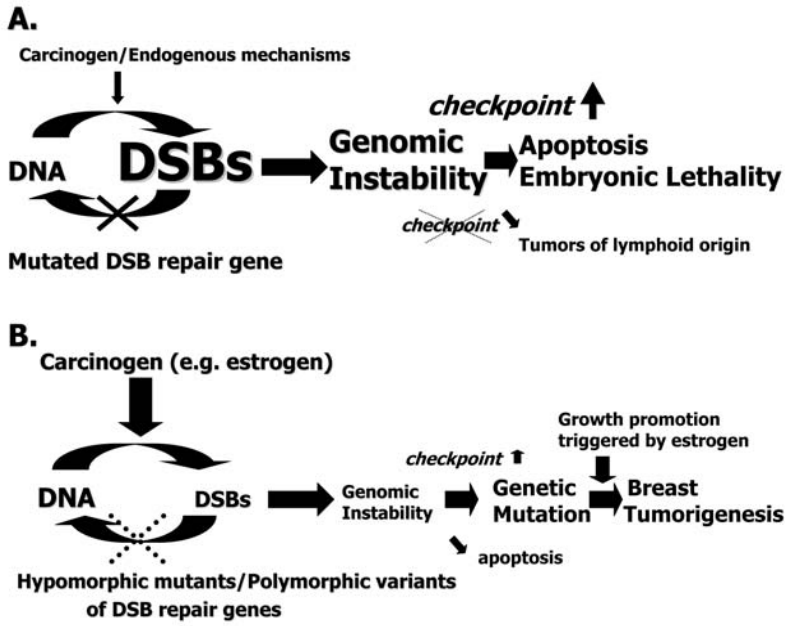

Figure 2. Adjusted odds ratios (●) and 95% of confidence limits (CIs)(*bars*) for breast cancer associated with (A) the number of putative high-risk genotypes of DNA double-strand break repair (DSBR) genes (*Ku70* and *RAD51*); (B) having one additional high-risk genotype of DSBR genes in total women or in total women stratified by genotypic statuses or the number of high-risk genotypes of cell cycle checkpoint genes (*ATM* and *p53*); (C) the combination of the reproductive risk factors [age at first full-term pregnancy (FFTP)] and the number of putative high-risk genotypes of DSBR genes and cell-cycle checkpoint genes.