Preventing hereditary cancers caused by opportunistic carcinogens

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Running title:

Hereditary cancer prevention

Objectives

Previous studies reported inherited BRCA1/2 deficits can cause cancer by impairing normal protective responses. Opportunistic carcinogens can exploit these deficits by causing chronic inflammation, constant cell death and replacement in a mutagenic environment, DNA crosslinking or double strand breaks. Some of the resulting cancers may be prevented if opportunistic carcinogens are identified.

Methods

The literature was systematically searched for carcinogens capable of exploiting deficits in BRCA1/2 pathways. Search criteria were common exposure, available information, required BRCA1/2 pathway repairs, increased risks for any cancer, and effects on stem cells.

Results

Formaldehyde and acetaldehyde are closely related carcinogens and common pollutants that seem everywhere. Alcohol metabolism also produces acetaldehyde. High levels of either carcinogen overwhelm normal detoxification systems, cause inflammation, inhibit DNA repair and produce DNA cross links as critical carcinogenic lesions. Searching model system studies revealed both carcinogens activate stem cells, BRCA1/2 pathways and connected BRCA1/2 pathways to myeloid leukemia. For example, the BRCA1-BARD1 complex is required for proper nucleophosmin functions. Nucleophosmin prevents a major subset of acute myeloid leukemia (AML). Next, these concepts were independently tested against risks for myeloid leukemia. Epidemiologic results showed that BRCA2 gene defects inherited on both chromosomes increased risks so dramatically that AML occurs in most children. Using data from 14 studies, known/potential heterozygous BRCA1/BRCA2 mutations increased risks for myeloid leukemias by at least 3 fold in 7 studies and by at least 50% in 12.

Acetaldehyde occurs in breast milk. In model studies, excessive acetaldehyde/alcohol exposure affects estrogen metabolism and stimulates alternate alcohol detoxification pathways. These pathways can also cause DNA cross linking by releasing oxygen species and activating procarcinogens. Acetaldehyde in rats' drinking water increased incidence of leukemias, lymphomas, pancreatic tumors and fibroadenomas. Six human epidemiologic studies support an association between alcohol related genotype or alcohol consumption and early onset breast cancers, including those in BRCA1/2 mutation carriers.

Conclusions

Although it is difficult to prove direct causation, BRCA1/2 mutation carriers may reduce cancer risks by avoiding excessive formaldehyde and acetaldehyde. Broader genetic testing and pharmacologic/nutritional detoxification are possible.

Background

Previous explanations for the tissue specificity of hereditary breast cancer. In order to prevent or delay hereditary cancers it is essential to understand why some hereditary cancers seem to target specific organs. BRCA1 and BRCA2 mutations were thought to have an exquisite specificity in causing breast and ovarian cancer. Several explanations for this specific targeting have been published.

One published explanation is because the breast does not have back up systems or redundancy to compensate for the absence of BRCA1/2 functions. Back ups for some BRCA1/2 function are general methods of DNA repair such as non-homologous end joining, or translesion synthesis. When forced into service inappropriately because of abnormal BRCA1/2 pathways, repairs become less accurate and risks for some cancers increase. [e.g. Venkitaraman (2003), Lagerqvist (2008), Friedenson (2007)]. Cancers that depend on gene translocations may occur because double strand breaks are repaired by combining inappropriate pieces of broken chromosomes. One cancer strongly associated with a reciprocal translocation is mantle cell lymphoma. The incidence of mantle cell lymphoma increased 70 fold when the BRCA1/2 pathway molecule ATM was inactivated [Friedenson, 2007]. At least in some cases, losses of whole chromosomes or fragments damage such fundamental and essential systems that alternative mechanisms are unable to compensate without allowing dangerous mutations [Lagerqvist et al. (2008)].

Another proposal is that cancer cells that lose both copies of BRCA1 are only able to survive in breast and ovary but die everywhere else. However there are homozygous defects if not in BRCA1, then in BRCA2, ATM and Fanconi proteins. In these conditions every organ survives.

A different explanation depends on the high proliferation rates in the breast and ovary. Cell proliferation in the absence of BRCA1 or BRCA2 may lead to a higher mutation rate. However breast cancer and leukemias provided foundations for the cancer stem cell hypotheses. This hypothesis states that cancers originate from abnormal stem cells. These retain stem cell ability to self renew and to differentiate asymmetrically. They may be rare and ordinarily do not proliferate.

Another possibility is that tissue specific cofactors, transcription factors, hormones and the context of different tissues determine the tissue specificity of hereditary cancers. However BRCA1/2 mutation carriers have increased relative risks for cancers in a wide variety of tissues and increased risks for cancers in general [Friedenson, (2005)].

Preventing hereditary cancers caused by opportunistic carcinogens. Previous work [Friedenson (2010 a, b, 2011)] suggested that hereditary cancers have defects in processing some carcinogens from the environment because hereditary defects aggravate the effects of some carcinogens. For example there is an increased incidence of cancers related to constant cell death and replacement in a mutagenic environment such as chronic organ specific infections that cause DNA damage requiring BRCA1/2 repairs.

The first defenses against these carcinogen induced cancers are encoded by genes for immune responses, and for processing, detoxifying and metabolizing carcinogens. BRCA1/2 mediated pathways then serve as further defenses to repair certain types of DNA damage

but inherited mutations can cripple these repairs and other BRCA1/2 protective functions. Opportunistic carcinogens that escape detoxification pathways can take advantage of inherited deficiencies in BRCA1/2 pathways. Identifying and minimizing exposure to opportunistic carcinogens is the first step in preventing or delaying some cancers in mutation carriers.

Methods

Identifying opportunistic carcinogens based on model studies. The literature was systematically searched for all available years to identify model studies of common carcinogens that produce DNA cross links or DNA protein cross links. Search criteria were common, unavoidable exposure, available information, required BRCA1/2 pathway repairs, increased risks for any cancer, and effects on stem cells. Formaldehyde and acetaldehyde were identified as two common pollutants and opportunistic carcinogens. Both carcinogens form DNA lesions requiring repairs mediated by BRCA1/2 pathways.

Searches were then conducted for cancers caused by exposure to formaldehyde and acetaldehyde. Cancers caused by both carcinogens were listed.

Cancer risks associated with opportunistic carcinogens tested against cancer risks in carriers of mutations in BRCA1/2 pathways. Risks for cancers associated with exposure to opportunistic carcinogens were then compared to risks associated with mutations in BRCA1/2 pathways. As previously described [Friedenson (2005, 2007, 2010a,b)], the literature was systematically searched for relative risk data on cancers in carriers or likely carriers of mutations in genes encoding pathways that depend on BRCA1/2 genes. Included data was used directly without further statistical calculation or combination. Included data was not corrected for low percentages of mutation carriers in the population, for survival, or for subjects lost to follow-up. Relationships between alcohol consumption and cancer risks can only can be studied in populations with substantial alcohol consumption [Seitz and Becker (2007)]. Studies could not be used if they contained very high percentages of non-drinkers or light drinkers (>=70% to 98%) among potential BRCA1/2 mutation carriers. Substantial differences in numbers of ovariectomies or women on hormone replacement therapy in drinkers vs controls also excluded data.

Studies of second primary cancers after breast cancer provided the most extensive and complete sources of data for BRCA1/2 heteroaygotes. Given the high risk of BRCA1/2 mutation carriers and their involvement with medical oncology personnel, cancer survivors among patients with BRCA1/2 pathway mutations are often monitored for the development of a second primary cancer. This is done more often than observing genetically typed healthy individuals for the development of a first cancer.

Results

Formaldehyde and acetaldehyde were identified as likely opportunistic carcinogens. Exposure is common, virtually unavoidable; exposure increases risks for multiple cancers; much information is available; there are clear connections with BRCA1/2 pathways in model systems, and both carcinogens have effects on stem cells [Zhang et al. (2010), Agency for toxic substances and disease registry (2010), Baan et al. (2009), US Dept Health and Human Services (1999), National Toxicology program (2011)].

Common and widespread exposure. Formaldehyde and acetaldehyde are closely related carcinogens. Widespread human exposures to formaldehyde and acetaldehyde occur during

production and use of many maufactured products. Both carcinogens are also present in tobacco smoke, pollution and foods. Formaldehyde has been illegally used as a food preservative; acetaldehyde forms during alcohol metabolism and high levels pre-exist in some alcoholic beverages and in some foods. Billions of pounds of these carcinogens are produced each year [Agency for toxic substances and disease registry (2010), Baan et al. (2009), Hauptmann et al. (2009), National Toxicology Program (2010)].

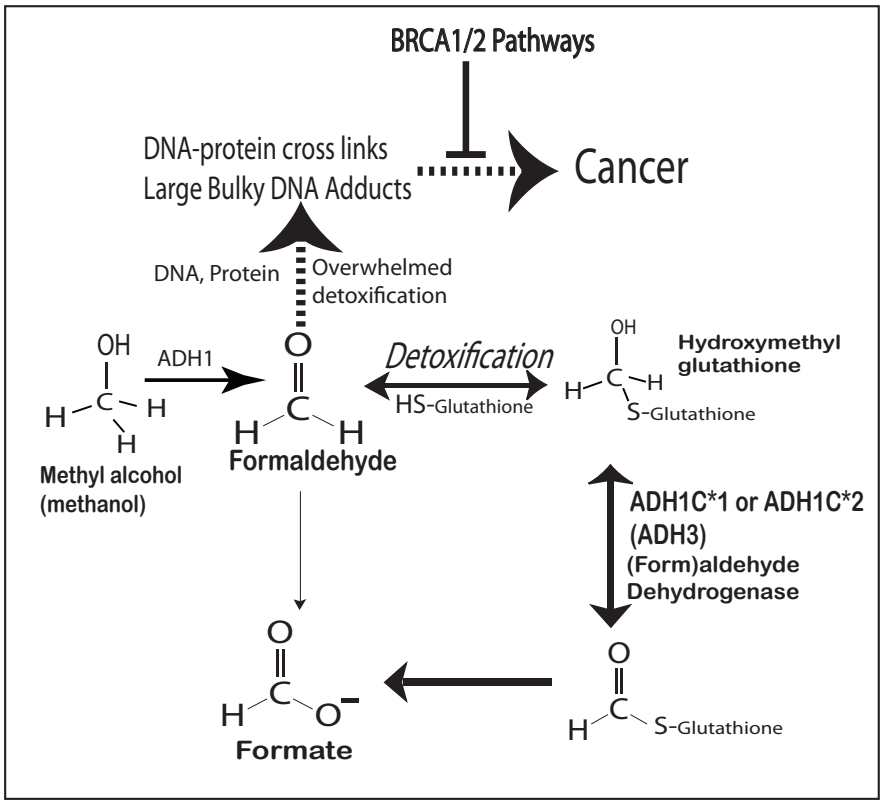


Figure 1 - Carcinogenesis at high levels of formaldehyde competes with formaldehyde detoxification mechanisms. Detoxification of low levels of formaldehyde occurs primarily by a pathway (thicker arrows) involving formaldehyde dehydrogenase (ADH1C*1 or ADH1C*2), an aldehyde dehydrogenase. The pathway converts formaldehyde to formate which is then eliminated in the urine, broken down to CO_2 and water or enters the single carbon pool. Alternate, less used pathways are indicated by thinner arrows. Formate can also generate CO_2^- radicals and can be metabolized to CO_2 via catalase or oxidation of N-formyl tetrahydrofolate. Detoxification of formaldehyde does not involve BRCA1/2 but BRCA1/2 pathways inhibit carcinogenesis.

Model studies: Formaldehyde and acetaldehyde are opportunistic carcinogens causing DNA damage that requires repairs by BRCA1/2 pathways.

Formaldehyde and acetaldehyde chemically cross-link DNA strands and form DNA protein cross links (Fig. 1, Fig. 2). These lesions are probably critical for carcinogenesis [Zhang et al (2010)] and have been demonstrated by a large number of studies in vitro, in exposed animals and in circulating white cells [Zhang et al. (2010), Ishizaka et al (2007), Shaham et al. (1996)]. DNA-protein cross-links exhibit a dose-response relationship to formaldehyde in the respiratory tract of laboratory animals at exposure concentrations relevant to human exposures.

The pathways required to repair DNA damage from formaldehyde and acetaldehye are qualitatively similar to pathways required to repair damage from mitomycin C. Mitomycin C is a known DNA crosslinking agent that has become a functional test for activity of pathways involving Fanconi proteins and including BRCA1/2 proteins [Marietta et al. (2009)].

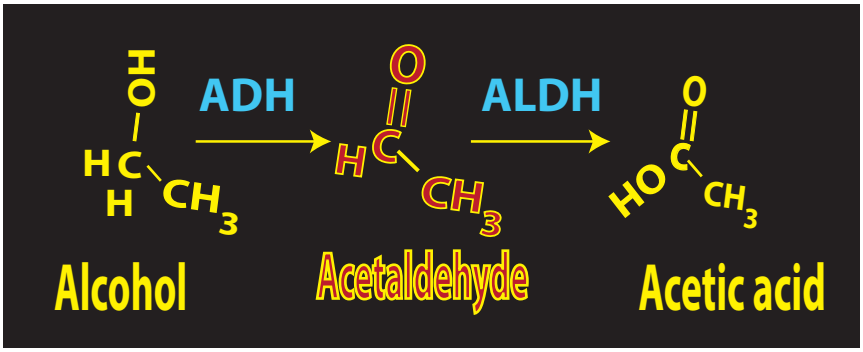
Homologous recombination requiring BRCA1/2 and Fanconi proteins is essential to correct DNA protein cross links [Yamazoe et al.

(2004), Nojima et al. (2005), D'andrea and Grompe (2003),Venkitaraman (2003), Nakano et al (2009)]. Formaldehyde forms protein DNA cross links requiring BRCA1/2 pathway mediated repairs [Nakano et al (2009, 2003)]. In mice exposed to 6 ppm formaldehyde for 1 week, the Brca pathway is among the top 10 most significantly enriched pathways [Andersen et al. (2010)].

BRCA1/2 related pathways also participate in preventing formaldehyde related collateral DNA damage, bone marrow toxicity and immunosuppression (which can reactivate latent viral infections), and inflammation (which can produce cross-links due to oxidative damage). These mutagenic environments increase the probability that cancer stem cells will arise.

Formaldehyde can exist as polymers which may form cross linkers with varying reach. Chemically irreversible and stable cross-linked products form between DNA bases and the amino acids cysteine, histidine and tryptophan. Lysine cross-linked products are labile in solution, supporting widely reported reversibility [Lu et al. (2010)].

Acetaldehyde exposure places demands on BRCA1/2 mediated pathways. Search results found several lines of evidence implicating acetaldehyde as the first product of alcohol metabolism, in alcohol-related carcinogenesis (Fig 2) [Ishizaka et al (2007), Lachenmeier et al (2011)]. Model system searches retrieved several reports that acetaldehyde causes complex DNA adducts and damage



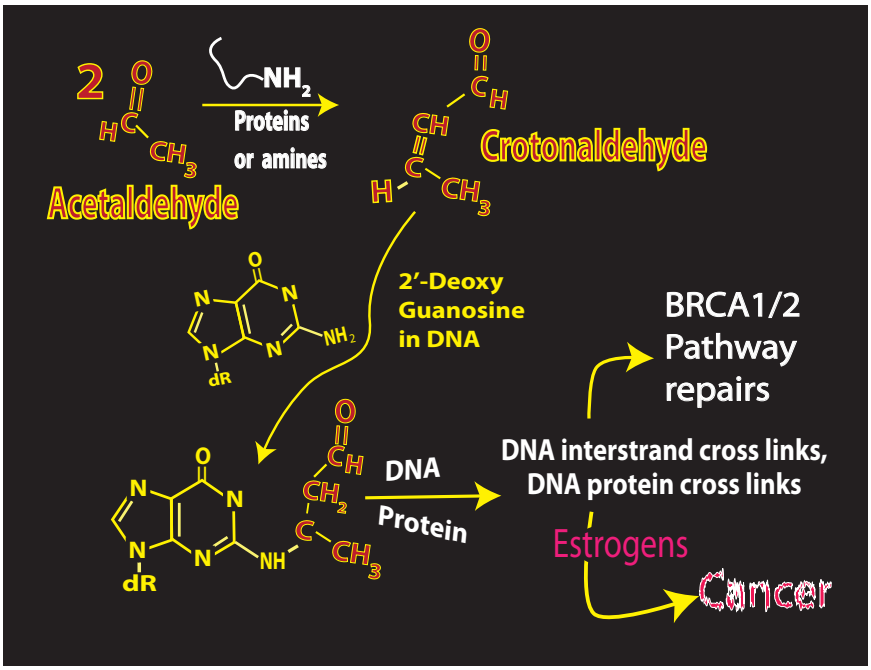


Figure 2a. Normal detoxification of alcohol requires two enzymes, alcohol dehydrogenase (ADH) and then aldehyde dehydrogenase (ALDH). Alcohol dehydrogease produces acetaldehyde, a mutagen and carcinogen. Variations in genes encoding both enzymes influence the susceptibility to alcohol related carcinogenesis. Fig 2b. One mechanism or carcinogenesis. Acetaldehyde that is ingested or escapes alcohol detoxification pathways can form crotonaldehyde, a reaction catalyzed by amine groups from proteins or naturally occurring amines. Crotonaldehyde is genotoxic, mutagenic and carcinogenic and can be derived from beer, wine and liquor. Reactions with deoxyguanosine in DNA produce DNA interstrand cross links and DNA protein cross links [Theravathu et al (2005)]. Some of these lesions require repairs by BRCA1/2 pathways. Inherited mutations for proteins within BRCA1/2 pathways disable cross link repairs and can lead to cancer in exposed organs. Excessive alcohol/acetaldehyde can also generate reactive oxygen species and affect estrogen, adding to cancer risks.

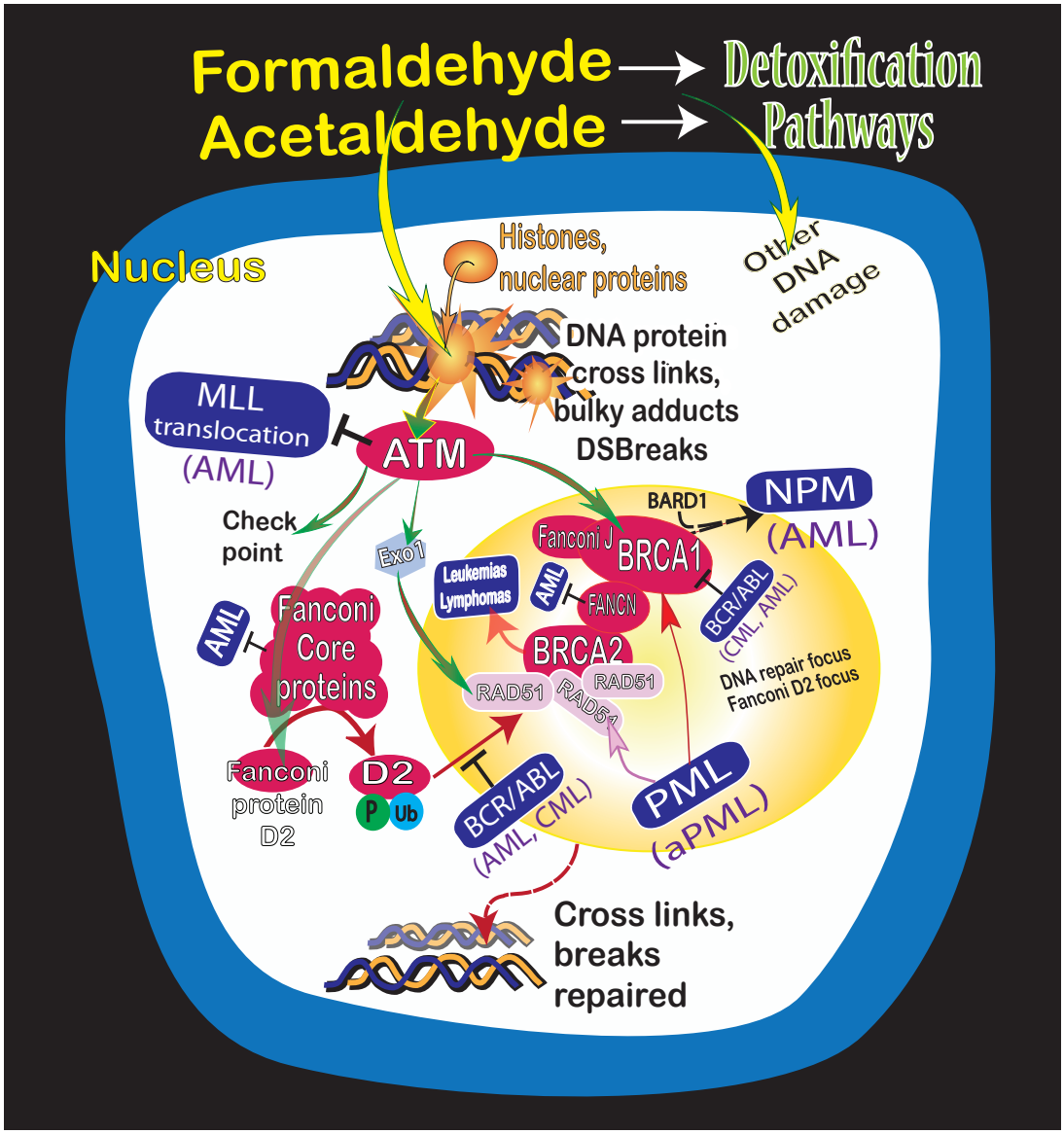


Figure 3 - BRCA1 and BRCA2 in DNA damage repair pathways showing probable links to myeloid leukemia, other leukemias and lymphomas. BRCA1 and BRCA2 are shown in pathways to correct DNA cross links and double strand breaks caused by formaldehyde, acetaldehyde and other agents that cause bulky addition products or DNA cross-links. DNA damage is more likely if the carcinogen escapes metabolic detoxification pathways (Figs. 1, 2a). Hereditary inactivation of a Fanconi gene causes Fanconi anemia, inactivation of the ATM gene causes ataxia-telangiectasia (A-T), and inactivation of BRCA1 or BRCA2 associates with hereditary breast/ovarian cancers. Proteins encoded by genes related to these well known hereditary cancer conditions are colored red. The dark boxes in the figure indicate proven links to myeloid leukemia. The BRCA1-BARD1 complex is required to activate the NPM gene which is lost in a major subset of AML. In AML and CML, the BCR/ABL protein interferes with the formation of nuclear Fanconi protein D2 repair foci. In CML, BRCA1 becomes almost undetectable. The PML protein is required for Rad51 repair focus formation providing another link to leukemia. Family history of leukemia increases risk for breast cancer. Chromosome 13q (encoding BRCA2) is deleted in a subgroup of leukemias and lymphomas. Multiple other pathways participate in repairing DNA damage and members of the pathway shown have been implicated in coordinating repairs.

requiring BRCA1/2 pathway mediated repairs [Marietta et al 2009; Nakano et al (2003, 2009) Langevin et al (2011)]. Histones and naturally occurring polyamines facilitate the formation of these lesions [Theravathu et al. (2005); Sako et al (2003)]. (Fig. 2b). DNA adducts of acetaldehyde were found in the white cells of alcohol abusers. Cells of the myeloid lineage were especially susceptible [Fang and Vaca (1997)]. Chinese hamster ovary cells show a concentration related increase in DNA-protein cross links [Olin et al (1996)]. DNA from ALDH2 deficient Japanese males has low electrophoretic mobility, consistent with DNA cross link formation [Lu and Morimoto (2009)].

Many studies find that acetaldehyde increases the frequency of sister chromatid exchanges and aberrations in mammalian cells. Fanconi anemia cells are much more susceptible than normal cells [Obe and Anderson (1987)]. Acetaldehyde exposure results in a concentration-dependent increase in Fanconi D2 monoubiquitination, which is dependent upon the proper functioning of BRCA1/2 (Fig. 3). Acetaldehyde also stimulates BRCA1 phosphorylation in a dose-dependent manner. Meanwhile ethanol may suppress BRCA1 levels [Fan et al (2000)].

Detoxification by specialized metabolic pathways. Normal metabolism produces both formaldehyde and acetaldehyde. With some exceptions, most background dietary sources are generally too low to cause permanent harm and are managed by reactions and metabolism within the digestive tract.

Formaldehyde is primarily metabolized by glutathione-dependent formaldehyde dehydrogenase (ADH3, existing in two forms renamed as ADH1C*1 or ADH1C*2) and aldehyde dehydrogenases (ALDHs) enzymes in alternative pathways (Fig. 1). Formaldehyde is converted to formate, which is then eliminated in the urine as a sodium salt, broken down to CO_2 and water, or entered into the single-carbon pool [Teng et al. (2001, Andersen et al, (2010)]. Other alcohol and aldehyde dehydrogenases can also contribute. DNA cross-links, chromosome breaks and chromosome abnormalities are favored if formaldehyde or acetaldehyde exceeds the capacity of detoxification pathways (Figs 1 and 2). For formaldehyde, exogenous addition products form with DNA in a highly nonlinear fashion; a 21.7-fold increase in exposure of rats to labeled formaldehyde caused a 286-fold increase in DNA-formaldehyde addition products [Lu et al. (2011)].

Alcohol/Acetaldehyde detoxification. Alcohol metabolism utilizes ADH enzymes to generate acetaldehyde as a first intermediate and then ALDH enzymes to remove acetaldehyde (Fig. 2). Variant enzymes encoded by ADH and ALDH genes are inherited with different activities and lead to differences in acetaldehyde accumulation and thus differences in cancer risks. There are seven types of ADH (Fig. 2), each encoded by a different gene. Two of the seven ADH genes, (ADH1B and ADH1C), exist as more than one variant (allele). In Caucasians, the ADH1C gene has two known alleles: a highly active ADH1C*1 and less active ADH1C*2. The ADH1C*1 allele encodes an ADH isoenzyme which produces 2.5 times more acetaldehyde than the corresponding allele ADH1C*2. In studies with moderate to high alcohol intake, ADH1C*1 is associated with an increased risk for cancer of the upper aerodigestive tract, the liver, the colon and the female breast [Seitz and Maurer (2007)].

Approximately 40% of Japanese, Koreans or Chinese carry one copy of the acetaldehyde dehydrogenase 2*2 (ALDH2*2) allele. This allele codes for an ALDH2 enzyme with poor activity leading to high acetaldehyde concentrations after consuming even small amounts of alcohol. Chronic drinkers with this allele have significantly increased risks for upper alimentary tract and colorectal cancer. These findings underline the

Table 1 Connections between BRCA1/2 containing pathways and leukemias

Leukemia	Evidence for connections to BRCA1/2 pathway	Reference
AML	Childen born with defects on both chromosomes affecting genes encoding BRCA2 (Fanconi protein D-1) or both chromosomes affecting FANCN (PALB2) genes are especially prone to develop AML.	Alter et al (2007, 2003). Tischkowitz et al (2010)
AML	NPM is a substrate for BRCA1-BARD1. In AML in China, where pollution is high, NPM is frequently lost. The NPM1 locus on chromosome 5 is part of a region that modifies breast cancer risk in BRCA1 related breast cancers.	Sato JBC 2004; Wang et al 2010; Nathanson and Weber 2002
AML, CML	BCR/ABL fusion protein inhibits the formation of nuclear FANCD2 foci	Valeri et al 2010
AML	ATM mutations increase the incidence of translocations at 11q23 involving the multi-lineage leukemia gene (MLL). ATM prevents oncogenic translocations	Bredemeyer et al 2006; Sun et al, 2010
Myeloid leukemias	Frequent loss of chromosome 17 [monosomy 17] containing BRCA1 and p53	Zhu et al 2008
Acute promyelocytic leukemia (APL)	PML fusion protein with retinoic acid receptor causes loss of PML protein function. PML is essential for proper localization of RAD51 in nuclear foci and efficient homology directed repair. PML also participates in localizing BRCA1.	Boichuk 2011
Acute promyelocytic leukemia (APL)	BRCA1 expression is reduced 3 to 14.3 fold in APL	Casorelli et al 2006
Fanconi anemia associated cancers especially AML	The model in Fig 3 shows how Fanconi proteins are required for activities of BRCA1 and BRCA2.	

important role of acetaldehyde in ethanol-mediated carcinogenesis [Seitz and Maurer (2007)].

Acetaldehyde produced after heavy drinking (Fig. 2a) can overwhelm natural metabolic systems. Acetaldehyde is a very good substrate for other enzymes present in breast cells: xanthine oxidoreductase (XOR) and aldehyde oxidase (AOX) [Shaw et al (1989), Dumitrescu and Shields (2005)]. Methanol and formaldehyde are inhibitors of XOR. Both enzymes are present and regulated in breast tissues. Breast cancers that lack cytoplasmic XOR have about a 2.5 greater risk of metastases than those with strong XOR expression [Linder et al. (2005)] showing the

importance of this enzyme. Moreover, decreased XOR is associated with worse prognosis in ovarian carcinoma.

As they detoxify acetaldehyde, both XOR and AOX can generate superoxide, hydroxy radical and hydrogen peroxide. These reactive oxygen species can cause complex DNA damage and cross links. The generation of free radicals is also associated with the reaction catalyzed by cytochrome P-4502E1 [Jelski et al (2006)]. This enzyme can also convert procarcinogens to carcinogens.

A model for pathways containing BRCA1/2 has several strong links to leukemias - cancers that can be induced by formaldehyde or acetaldehyde.

Fig. 3 is a model for BRCA1/2 function within a pathway for crosslink repair based on models of D'Andrea and Grompe (2003) and Venkitaraman (2003). In Figure 3, formaldehyde and acetaldehyde are inserted as sources of DNA damage and BRCA1/2 pathway links to myeloid leukemias have been added. Table 1 summarizes the search results in model systems supporting these links. Children born with defects on both chromosomes that affect the BRCA / Fanconi pathway are at high risk for AML. These defects include those in BRCA2 (Fanconi protein D1 [Howlett et al. (2002)] and in PALB2 (Fanconi protein N). Biallelic Fanconi N (PALB2) mutations are associated with AML and other pediatric malignancies [Tischkowitz and Xia (2010)].

Mutations in nucleolar phosphoprotein nucleophosmin/B23 (NPM) are associated with a major subset of AML. The BRCA1-BARD1 heterodimer catalyzes the ubiquitylation of NPM in vitro and in vivo, and BRCA1-BARD1 co-expression in cells stabilizes NPM against degradation [Sato et al (2004)]. Familial AML is linked to the loss of the long arm of chromosome 5 which includes the NPM gene on chromosome 5q33–34. 5q33-34 contains one or more genes that modify breast cancer risk in BRCA1 mutation carriers (Table 1) [Nathanson et al. (2002)]. Loss of genes on chromosome 4 reported byTirkkonen et al. (1997)] also implicates formaldehyde and alcohol metabolism because chromosome 4q encodes all the ADH genes.

A characteristic fusion protein (BCR/ABL) is found in AML (and in CML). BCR/ABL interferes with the formation of nuclear FANCD2 foci (Fig. 3 and Table 1), but this interference can be reversed by the ectopic expression of BRCA1. In CML, BRCA1 becomes virtually undetectable [Valeri et al (2010)].

Fanconi protein A is lost in a subset of sporadic AML [Tischkowitz et al. (2004)] Casorelli et al (2006) found that impaired recombination repair stimulated APL. APL samples all showed major reductions in BRCA1 and RAD51 (Fig 3) expression. The promyelocytic leukemia protein (PML in Fig 3 and Table 1) is critical for forming nuclear bodies with important functions in transcription, apoptosis, DNA repair and antiviral responses. BRCA1 colocalizes with these nuclear bodies [Luciani et al (2006)]. Loss of PML function or expression is associated with acute promyelocytic leukemia (APL) and progression of some solid tumors. PML helps regulate BRCA1 [Boichuk et al, (2011)] and PML is critical for proper localization of the essential repair protein Rad51 (Fig 3 and Table 1) in nuclear foci, and for efficient homology-directed repair. In cells expressing SV40 large T antigen, Rad51 foci depend on PML.

In myeloid leukemias, chromosome 17 (containing BRCA1) is frequently lost or involved in complex chromosomal abnormalities including balanced translocations involving the BRCA1 locus [Zhu et al (2008)] (Table1 and Fig. 3). Some pathogenic mechanisms in leukemias may be related to those in hereditary breast cancers.

Table 2. Risks of leukemia and oral cavity cancers as cancers following breast, ovarian or fallopian tube cancer in proven or potential BRCA1/2 mutation carriers

Row number, Study population and reference	Mutation test status	Risk measurement for leukemias [Confidence interval]
1. 6 children with biallelic BRCA2 mutations [Wagner (2004)].	Biallelic BRCA2 mutations (compound heterozygotes)	All developed leukemia at median age 2.2 years. 4 of 6 AMLs
2. Review of 27 biallelic BRCA2 mutation patients [Alter (2003)].	Biallelic BRCA2 mutations (compound heterozygotes)	79% cumulative probability of leukemia (primarily AML) by age 10 years
3. First breast cancer age <45 in 6958 Connecticut women. [Harvey & Brinton, 1985]	Potential mutation carriers eligible for mutation testing	Acute non-lymphocytic leukemia as 2nd cancer O/E=2.9 at 1-4 years and 6.4 at 5-9 years
4. Breast cancer patients, age 35-49 from 26,617 primary female breast cancers [Teppo et al, 1985]	Potential mutation carriers eligible for mutation testing	Subsequent leukemia (excluding CLL) RR= 3.21 p<.01
5. Female breast cancer surviving >= 10 years (selects 11,273 younger patients) [Ewertz & Mouridsen, 1985]	Potential mutation carriers eligible for mutation testing	Acute non-lymphocytic leukemia as a 2nd cancer RR=2.3
6. 2813 women with 2 breast or ovarian cancers in Thames Cancer Registry. [Evans et al, 2001b]	Potential BRCA1/2 mutation carriers eligible for mutation testing	Myeloid leukemia RR=5.04 [1.85-11.0]
7. 82,520 Women with breast cancer age <=45 in 13 cancer registries. [Mellemkjaer et al, 2006]	Potential mutation carriers eligible for mutation testing	Myeloid Leukemia SIR = 3.02 (2.32–3.85) Leukemia SIR = 2.16 (1.78–2.59).
8. 2,084 Women with primary fallopian tube cancer from 13 cancer registries [Riska et al, 2007]	Probable BRCA1/2 mutation carriers.	Non-lymphoid leukemia RR=3.7 (1.0-9.4)
9. 2nd cancer after Breast Cancer<50. from 32, 799 patients in Thames Cancer Registry. [Evans et al, 2001a]	Potential BRCA1/2 mutation carriers eligible for mutation testing	Myeloid leukemias RR=2.31 [1.52-3.51]
10. 2nd cancer after male breast cancer [Hemminki et al, 2005]	Men at high risk for being (BRCA2) mutation carriers eligible for mutation testing	Myeloid leukemia RR=3.98 [1.46 – 8.67] 1-9 yrs of follow up. RR=3.42 [1.47-6.73] for all periods
11. *534* First degree relatives of BRCA1 probands with ovarian cancer [Risch et al, 2006]	Tested BRCA1 heterozygotes or potential carriers eligible for testing	Leukemias, lymphomas, etc RR=3.7 (1.5 to 9.5)
12. 7/98 multiple primary cancer fami lies with a BRCA1 mutation and 8/98 multiple primary cancer fami lies with a BRCA2 mutation [Shih et al, 2000]	Known BRCA1 and BRCA2 mutation carriers	20% of leukemias in the families occurred in BRCA1 and BRCA2 mutation carriers.
13. 3678 women, 50 men. 1st degree relatives of BRCA2 mutation carriers or of breast or ovarian cancer patients [Breast cancer linkage consortium, 1999]	471 BRCA2 carriers, 390 noncarriers, and 2186 unknown BRCA2 carrier status	Leukemia RR= 1.12 [0.30–4.25]
14. 11847 individuals from 699 families segregating a BRCA1 mutation [Thompson et al, 2002]	18.9% (2245) tested BRCA1 carriers, 9.3% (1106) tested negative, 71.7% (8496) untested.	Leukemia RR = 0.88 [0.37 to 2.14] p=.83
15. 1811 male and female family mem bers [Van Asperen et al, 2005]	50% probability BRCA2 mutation from 139 BRCA2 families.	Leukemia RR= 1.5 [0.5 to 3.5]
16. 728 males & females (BRCA2). 1145 males & females (BRCA1) [Johannson et al, 1999]	From families with an identified BRCA2 or BRCA1 mutation	Acute leukemia SMR=1.54 [0.04±8.59] (BRCA2). 1.01 [0.03±5.62] (BRCA1)

Studies of heterozygotes or potential heterozygotes listed in Table 2

The Breast Cancer Linkage Consortium (1999). Cancer risks in BRCA2 mutation carriers. J. Natl Cancer Inst. 91: 1310-1316.

Evans H, Lewis C, Robinson D, Bell C, Moller H, Hodgson S. (2001a). Incidence of multiple primary cancers in a cohort of women diagnosed with breast cancer in southeast England. Brit J Cancer 84: 435-440

Evans H., Lewis, C., Robinson, D. et al. (2001b) Cancer risks in women with 2 breast or ovarian cancers: clues to genetic cancer susceptibility. Int J. Cancer 94: 758-9

Ewertz, M. & Mouridsen, H. Second cancer following cancer of the female breast in Denmark, 1943-80. Natl Cancer Inst Monogr. 1985; 88:325-9

Harvey EB and Brinton LA. Second cancer following cancer of the breast in Connecticut, 1935–1982. Natl Cancer Inst Monogr 1985; 68, 99–109

Hemminki K, Scélo G, Boffetta P, Mellemkjaer L, Tracey E, Andersen A, Brewster DH, Pukkala E, McBride M, Kliewer EV, Chia KS, Pompe-Kirn V, Martos C, Jonasson JG, Li X, Brennan P. (2005) Second primary malignancies in patients with male breast cancer. Brit. J. Cancer 92 : 1288-92.

Johannsson O, Loman N, Moller T, Kristoffersson U, Borg A, Olsson H. Incidence of malignant tumors in relatives of BRCA1 and BRCA2 germline mutation carriers (1999). Eur J Cancer 35:1248–57

Mellemkjaer L, Friis S, Olsen JH, Scélo G, Hemminki K, Tracey E, Andersen A, Brewster DH, Pukkala E, McBride ML, Kliewer EV, Tonita JM, Kee-Seng C, Pompe-Kirn V, Martos C, Jonasson JG, Boffetta P, Brennan P. (2006). Risk of second cancer among women with breast cancer. Int J Cancer 118(9):2285-92.

Risch HA, McLaughlin JR, Cole DE, Rosen B, Bradley L, Fan I, Tang J, Li S, Zhang S, Shaw PA, Narod SA. (2006) Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. J Natl Cancer Inst. 98(23):1694-706.

Riska A, Pukkala E, Scélo G, Mellemkjaer L, Hemminki K, Weiderpass E, McBride ML, Pompe-Kirn V, Tracey E, Brewster DH, Kliewer EV, Tonita JM, Kee-Seng C, Jonasson JG, Martos C, Boffetta P, Brennan P. (2007) Second primary malignancies in females with primary fallopian tube cancer. Int J Cancer 120(9):2047-51.

Shih H, Nathanson K, Seal S, Collins N, Stratton M, Rebbeck T and Weber B. (2000). BRCA1 and BRCA2 mutations in breast cancer families with multiple primary cancers. Clin Cancer Res 6, 4259-64.

Teppo L, Pukkala E and Saxen E. (1985) Multiple cancer – an epidemiological exercise in Finland. J Natl Cancer Inst 75: 207–217.

Thompson D, Easton DF; Breast Cancer Linkage Consortium (2002). Cancer Incidence in BRCA1 mutation carriers. J Natl Cancer Inst. 94(18):1358-65

van Asperen CJ, Brohet RM, Meijers-Heijboer EJ, Hoogerbrugge N, Verhoef S, Vasen HF, Ausems MG, Menko FH, Gomez Garcia EB, Klijn JG, Hogervorst FB, van Houwelingen JC, van't Veer LJ, Rookus MA, van Leeuwen FE; Netherlands Collaborative Group on Hereditary Breast Cancer (HEBON). (2005) Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. J Med Genet. 42(9):711-9.

Footnotes: *NR= Not reported. Confidence intervals are given in brackets. #RR=1.1 for mouth cancer. * Head/neck and vocal cord cancer reported as "other primary tumors." ** 2 nose cancers reported in addition. *** Nasal sinus cancer reported but RR=0.15 for buccal cavity and pharynx cancer.*

Formaldehyde and acetaldehyde increase risks for leukemias in model studies. Formaldehyde is now a proven cause of human myeloid leukemia which develops at 2 – 5.9 ppm formaldehyde in air. Myeloid leukemia was a prototype in formulating the cancer stem cell theory. Search results in model systems show a connection between formaldehyde and stem cell damage. As measured by increases in characteristic stem cell pathways, formaldehyde activates and damages stem cells during carcinogenesis. [Andersen et al. (2010)].

Rats fed either acetaldehyde or formaldehyde in their drinking water have very high incidence of leukemias and lymphomas [Soffritti et al, (2002)]. BRCA / Fanconi anemia pathways are required to counteract teratogenic and carcinogenic effects of aldehydes in mice [Langevin et al (2011)] Model studies involving acetaldehyde show oxidative DNA damage via the metabolism of ethanol by the ADH1B/ALDH2 pathway [Yan et al. (2011)].

Relative risk data shows human mutation carriers have increased risks for leukemias

Associations between BRCA1/2 mediated pathways and myeloid leukemia predicted from model studies were independently tested against mutation related risks for myeloid leukemia from epidemiologic studies. Table 2 shows this association is especially strong for homozygous or biallelic mutations in BRCA2 (Fanconi protein D1). Six children with biallelic BRCA2 mutations all developed leukemia at median age 2.2 years, with 4 of 6 developing acute myeloid leukemia (AML) [Wagner et al. (2004)]. Biallelic BRCA2 mutation patients have a 79% cumulative probability of leukemia (primarily AML) by age 10 years [Alter (2003)] (Table 2).

Homozygous or biallelic mutations in BRCA1 are incompatible with human life but heterozygous BRCA1 mutations are well known. Carriers of heterozygous mutations in either BRCA1 or BRCA2 have increased risks for myeloid leukemia. Rows 3 through 16 of Table 2 summarize risks for myeloid leukemia from 14 studies of known/potential heterozygous BRCA1/2 mutation carriers or individuals eligible for mutation testing. Most studies reported elevated risks for leukemia or other hematopoietic cancers. Risks may be even greater because none of the heterozygote studies included populations that had all tested positive for mutation and very ill cancer patients may have been lost to follow up.

Mutations in other pathway genes increase risks for leukemias: Fanconi anemia genes. Fanconi anemia is an inherited cancer condition caused by inactivation of one of 13 Fanconi anemia complementation groups. In Fanconi anemia patients, summary relative risks for AML were 703.3 [363.7–1354.5] [Friedenson (2007)]. This suggests that functional Fanconi proteins are essential to prevent generation of cancer stem cells and AML [Tischkowitz et al. (2004)]. Few stringent genotype–phenotype connections have emerged for Fanconi anemia. Other genes and environmental factors may modify the phenotype [Neveling et al. (2009)].

Mutations in Fanconi genes contribute to a subset of sporadic AML [Lensch et al. (2003), Condie et al. (2002), Xie et al. (2000)]. In heterozygous carriers, lymphocytes show increased sensitivity to mutagens, but are not blocked in G2 phase as in full Fanconi Anemia [Rischewski et al (200), Mohseni et al. (2009]. Heterozygous mutation in the Fanconi J gene prevents the encoded helicase from unwinding DNA [Wu et al. (2010]], suggesting one mechanism.

ATM mutations. ATM may prevent oncogenic translocations by preventing excessive loading of recombinational repair proteins onto translocation breakpoint cluster regions ("hotspots") [Sun et al. (2010), Bredemeyer et al. (2006)]. Chromosomal translocations involving the MLL (Multi Lineage

Leukemia) gene on chromosome 11q23 are very common in infantile and secondary leukemias. The ATM gene is near MLL and ATM deficiencies increase the frequency of 11q23 translocations. MLL rearrangements at the 11q23 breakpoint cluster region occur in approximately 15% of patients with AML and ALL and represent a WHO subtype [Zhang, Rowley, (2006)].

More than 70 MLL translocations have been reported in de novo and therapy related AML or ALL. In contrast to most chromosome translocations in leukemia, a strong non-homologous end joining repair signature exists at all of these chromosome translocation breakpoint junctions [Zhang, Rowley, (2006)]. This shows repairs were not BRCA1/2 pathway mediated homologous recombination.

ATM is an important sensor of reactive oxygen species. Activated ATM fails to stimulate checkpoint kinases (Fig 3) in AML [Boehrer et al. (2009)]. Searches of epidemiologic studies found isolated reports of AML associated with ATM mutation, but malignancies in the lymphoid branches of hematopoetic cell development are more common.

Relative risk data linking acetaldehyde/alcohol and leukemias.

Searches of human epidemiologic studies found associations between alcohol and myeloid leukemias. Alcohol consumption during pregnancy caused a 56% increased risk for childhood AML (Latino-Martel et al (2010). In children born to women who drank during the second or third trimester of pregnancy, odds ratios for leukemia were 10.48 [2.8 - 39.1] [Shu et al. (1996)].

Breast cancer risks due to acetaldehyde in model studies.

Acetaldehyde causes mammary cancers in rodents. In Sprague-Dawley rats, the number of malignant mammary tumors increased in all females who consumed acetaldehyde in their drinking water. Some malignant mammary tumors also occurred in treated males [Soffritti et al (2002)]. An exaggerated blood acetaldehyde response that has been reported after giving ethanol to pregnant rats begins a much larger alteration during lactation. At the end of pregnancy, there is a 4-fold increase in acetaldehyde above nonpregnant values after an intragastric dose of 3 g/kg ethanol. During gestational days 1 to 17, the levels did not differ. After delivery, the exaggerated acetaldehyde response to ethanol increased, producing acetaldehyde concentrations 15-fold greater than in nonlactating controls [Gordon et al (1985)].

Whether acetaldehyde exists in human breast milk has been controversial. However Wako (1953) identified acetaldehyde in human milk based on paper chromatography. Adachi et al (1991) identified an adduct of acetaldehyde and trypophan in human milk and in breast-fed infant urine. Breast tissue contains alcohol and aldehyde dehydrogenases and ADH is highly expressed in normal human mammary epithelium. Breast tissue can convert ethanol to acetaldehyde and free radicals [Triano et al. (2003), Maciel et al. (2004)]. This evidence supports the idea that acetaldehyde occurs in breast milk and breast feeding mothers transfer it to infants.

Acetaldehyde/alcohol and breast cancer risks in potential mutation carriers. In the general population >100 published studies find that drinking alcohol increases breast cancer risk. A meta-analysis of data from 322, 647 women including 4335 cases of breast cancer found a 50% higher risk associated with alcohol for women with a history of maternal breast cancer. More specifically, six studies listed in Table 3 support an association between alcohol consumption and early onset breast cancers, including those in BRCA1/2 mutation carriers.

Three studies in Table 3 concluded that the ADH genotype plays a role in early onset breast

Table 3 Epidemiologic studies of acetaldehyde/alcohol exposure in early onset breast cancers

Reference	Patients	Results
Terry et al (2006)	>1000 breast cancer patients and fast alcohol metabolizers based on ADH genotype	Premenopausal breast cancer risk OR=2.9[1.2-7.1] for 1-2 drinks per day
Coutelle et al (2004)	ADH1C genotype in 117 moderate alcohol consumers with breast cancer. The ADH1C*1 allele was significantly more frequent in moderate alcohol consumers with breast cancer vs. age-matched alcoholic controls without cancer (62% vs. 41.9%)	Women with the ADH1C*1,1 genotype had 1.8 times more risk for breast cancer than those with another genotype
Vachon et al (2001)	Daily drinkers who were first degree relatives of breast cancer probands	RR=2.45 [1.2-5.02]
Freudenheim et al (1999)	134 premenopausal, 181 postmenopausal breast cancer patients with an efficient form of ADH vs controls with similar alcohol intakes	Premenopausal breast cancer risk OR=3.6[1.5-8.8]
Moorman et al. (2011)	Females age >=20 diagnosed with breast cancer and tested for BRCA1/2 mutations. 283 BRCA1 and 204 BRCA2 positive cases	BRCA1/2 mutation carriers who consumed alcohol got breast cancer 1.7-2.9 years younger than those who did not drink
Berkey et al. (2011)	Females age 9-15 in 1996. In 2005-2007 surveys, 67 of 6888 women age 18-27 reported benign breast disease and 6741 reported no benign breast disease	Young women whose mothers or aunts had breast cancer were more likely to have benign breast disease (OR=2.34), as were those with maternal benign breast disease (OR=1.59). Adolescents with a family history of breast cancer who consumed 7 alcohol drinks/wk doubled their benign breast disease risk (OR=2.28)

cancer, presumably by increasing acetaldehyde accumulation. This supports acetaldehyde increasing breast cancer risk in BRCA1/2 mutation carriers. Two additional results in Table 3 [Vachon et al (2001), Moorman et al (2011)] show that women who are potential or known mutation carriers have higher risks for breast cancer or get breast cancer years sooner if they consume alcohol. Table 3 also cites a study of young women with benign breast disease, a risk factor for breast cancer. Young women with a family history of breast cancer or benign breast disease who consumed alcohol doubled their risk of benign breast disease [Berkley et al (2011)]. Evidence from

breast cancer clusters on Long Island, New York also suggests that alcohol may increase risk in mutation carriers. Women who lived in this area and developed breast cancer were more likely to use alcohol and to have risk factors associated with BRCA mutations. BRCA mutation related risk factors included Ashkenazi Jewish heritage and a family history of breast cancer.

Adding risks from BRCA1/2 pathway mutations to those associated with variations in alcohol metabolism and estrogen elevation may make some women at especially high cancer risks from alcohol use. Acetaldehyde elevation in drinkers associates with high estrogen menstrual phases and with oral contraceptive use [Eriksson et al (1996), Cannizzaro et al (2010)]. The enzyme encoded by ADH1C may participate in estrogen metabolism. In premenopausal women alcohol associates with higher blood estrogen levels although some studies found this only occurred in women using oral contraceptives. Even small amounts of alcohol given to healthy women led to an increase in estradiol of 27-38% when alcohol was detectable in the blood [Coutelle et al (2004)].

Formaldehyde and breast cancer risks. In contrast to evidence linking alcohol and acetaldehyde to breast cancer, similar links to formaldehyde were difficult to find. However searches found a toxicologic study in Sprague-Dawley rats treated with formaldehyde or methyl alcohol, a precursor. The number of malignant mammary tumors increased in females treated with 100 mg/L formaldehyde or methyl alcohol alone. Some malignant mammary tumors were also found in treated males [Soffritti et al (2002)]

Formaldehyde in the environment has been positively associated with breast cancer risk [Coyle et al. (2005)]. Greater numbers of DNA-protein cross-links were found in the white cells of breast cancer patients than in matched controls [Wu et al. (2002)]. Higher activity of ALDH1 (cytosolic) aldehyde dehydrogenase in BRCA1 breast stem cells and hematopoietic cancer stem cells suggests less ability to remove aldehydes and greater susceptibility to mutagenic effects.

BRCA1/2 mutation carriers have widely varying cancer risks. There is no single breast or ovarian cancer risk associated with BRCA1/2 carrier status [Begg et al. (2008)]. Defective BRCA genes increase risks for cancers in organs other than breast and ovary but individual risks again differ greatly [Friedenson (2005)]. These results implicate an increased susceptibility in organs exposed to environmental carcinogens and/or deficits in additional genes [Friedenson (2010a)].

Discussion

Treatment related risk factors for myeloid leukemia. Fig. 3 suggests that inherited BRCA1/2 pathway defects predispose to myeloid leukemias from any cause. Adding cancers related to known chronic infectious agents stresses the influence of enviromental carcinogens.

Risks also include chemotherapy and radiation treatment for breast cancers. The lack of data separating risk factors from treatment risks makes it difficult to determine risks associated with treatment. Myeloid leukemias are diagnosed at a median age of 67 so they normally occur after early onset breast cancers. Early onset breast cancer patients treated with certain chemotherapy drugs are more likely to develop AML. Drugs linked with these secondary (treatment related) leukemias include mechlorethamine, procarbazine, chlorambucil, melphalan, etoposide, teniposide and cyclophosphamide. Combining these drugs with radia-

tion therapy further increases the risk. [Casorelli et al (2003)]. Infection prophylaxis with myeloid growth factors during chemotherapy could further increase risks.

Several publications in Table 2 addressed treatment related risks directly or provide some data that does. AML occurs with virtual certainty in children and infants with biallelic mutations in BRCA2. AML also occurs at very young ages in Fanconi anemia patients, before any chemotherapy or radiation has been given. Moreover defects in pathways requiring normal BRCA gene function are associated with other hematopoietic malignancies that are not generally considered to be therapy related [Friedenson, 2007].

The first chemotherapy regimen for breast cancer was not published until 1975 with large trial results appearing in 1976 [DeVita and Chu (2008)]. Three 1985 studies in Table 2 include patients diagnosed with breast cancer before adjuvant chemotherapy became widely used. In comparison to later studies, the three pre-chemotherapy studies in Table 2 do not show substantially less risk for AML than the later studies. In the Harvey and Brinton study, women over 55 had no excess risk for any second cancer while women younger than 55 still had over 3 times the risk for myeloid leukemia. Neither group was likely to receive routine chemotherapy. Teppo et al (Table 2) found no difference vs age in subsequent new primary cancers in 1953-79 in any age group. Observations of approximately the same risk for AML before and after 1975 is not compatible with the fact that chemotherapy was given to a larger proportion of breast cancer patients during later periods [Table 2, Mellemkjaer, et al (2006) Evans et al, (2001a)] Hemminki et al. (Table 2) found no trend for increasing myeloid leukemia risk with follow up time.

A few more recent studies in Table 2 show no excess risk for myeloid leukemia, consistent with environmental or additional genetic variation. Although some platinum chemotherapy did significantly raise risks for myeloid leukemias, these risks are only sightly higher than the other studies [(Table 2) and Riska et al (2006)].

Radiation therapy. Therapeutic doses of targeted field radiation cause very little or no increased risk of myeloid cancers. This is consistent with myeloid stem cells being more radiation resistant than non-stem cells [Diehn et al. (2009)]

Despite younger ages, breast cancer patients with BRCAI/2 mutations present at a similar stage, display a normal acute reaction to radiotherapy and have a similar prognosis compared to sporadic breast cancer patients [Gaffney et al (1998)]. An increased risk of radiation therapy related-AML is largely confined to young women with stage III breast cancer, (spread to lymphoid tissue). These patients are more likely to receive radiation in some combination with cytotoxic chemotherapy. In stage 1 disease, radiation is given alone and there is very little excess risk for AML [Martin et al, 2009]. Harvey and Brinton (1985) found only minor differences in AML risks for women given radiotherapy vs those who were not.

Activities of detoxification pathways may further stratify risks. Genetic polymorphisms that affect activities of detoxification enzymes and behavior are potential lurking variables in assigning cancer risks from formaldehyde and acetaldehyde. Activity of dehydrogenases, oxidoreductases, and cytochrome P450 enzymes should further stratify risks among mutation carriers. Broader genetic testing and pharmacologic/nutritional detoxification are possible.

Most epidemiologic studies consider only one polymorphism but cancer risk is determined by variants of many enyzmes in several pathways.

Moreover the statistical power of these studies is low because some polymorphisms are scarce. Despite these limitations, six studies in Table 3 show that alcohol consumption increases breast cancer risk in potential BRCA1/2 mutation carriers and three of these studies link increased risks to variation in alcohol detoxification genes.

Results here imply that carcinogens and differing routes of exposure are major reasons different cells and stem cells are targeted for cancer. Exposure to mutagens from alcohol metabolism occurs primarily in the digestive tract and liver but acetaldehyde, alcohol and detoxification enzymes also occur in the breast. Inherited differences in the intrinsic ability to metabolize alcohol or to detoxify other carcinogens contributes to determining the organs where cancer occurs.

There are unrelated examples of inherited cancer associated conditions that have increased susceptibility to environmental carcinogens. Common findings are increased sensitivity to cancer associated infections, to radiation, and to chemical carcinogens. This shows that the phenomenon of increased sensitivity to environmental carcinogens formaldehyde and acetaldehyde are probably representative of a broad general phenomenon. Further studies of relationships among genetic deficiencies, cancer stem cells and environmental carcinogens are needed.

References

Adachi J, Mizoi Y, Naito T, Ogawa Y, Uetani Y, Ninomiya I (1991) Identification of tetrahydro-beta-carboline-3-carboxylic acid in foodstuffs, human urine and human milk. J Nutr. 121(5):646-52.

Agency for Toxic Substances and Disease Registry, Division of Toxicology and Environmental Medicine, Atlanta, GA 30333. (2010). Addendum to the Toxicological Profile for Formaldehyde.

Alter B (2003) Cancer in Fanconi anemia, 1927-2001. Cancer 97: 425-440.

Alter B, Rosenberg P. Brody L (2007). Clinical and molecular features associated with biallelic mutations in FANCD1/BRCA2. J Med Genet 44:1–9.

Andersen ME, Clewell HJ 3rd, Bermudez E, Dodd DE, Willson GA, Campbell JL, Thomas RS (2010). Formaldehyde: integrating dosimetry, cytotoxicity, and genomics to understand dose-dependent transitions for an endogenous compound. Source-Toxicol Sci. 118(2):716-31.

Baan R, Grosse Y, Straif K, Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Cogliano V; WHO International Agency for Research on Cancer Monograph Working Group (2009). A review of human carcinogens--Part F: chemical agents and related occupations. Lancet Oncol. 10(12):1143-4.

Begg C, Haile R, Borg A, Malone K, Concannon P, Thomas D, Langholz B, Bernstein L, Olsen J, Lynch C, Anton-Culver H, Capanu M, Liang X, Hummer A, Sima C, Bernstein J (2008). Variation of Breast Cancer Risk Among BRCA1/2 Carriers JAMA 299(2):194-201

Berkey CS, Tamimi RM, Rosner B, Frazier AL, Colditz GA. Young women with family history of breast cancer and their risk factors for benign breast disease. Cancer. 2011 Nov 14. doi: 10.1002/cncr.26519.

Boehrer S, Adès L, Tajeddine N, Hofmann WK, Kriener S, Bug G, Ottmann OG, Ruthardt M, Galluzzi L, Fouassier C, Tailler M, Olaussen KA, Gardin C, Eclache V, de Botton S, Thepot S, Fenaux P, Kroemer G (2009). Suppression of the DNA damage response in acute myeloid leukemia versus myelodysplastic syndrome. Oncogene. 28(22):2205-18.

Boichuk S, Hu L, Makielski K, Pandolfi PP, Gjoerup OV (2011) Functional Connection between Rad51 and PML in Homology-Directed Repair. PLoS ONE 6(10): e25814. doi:10.1371/journal.pone.0025814

Bray F, Haugen M, Moger TA, Tretli S, Aalen OO, Grotmol T (2008). Age-incidence curves of nasopharyngeal carcinoma worldwide: bimodality in low-risk populations and aetiologic implications. Cancer Epidemiol Biomarkers Prev. 17(9):2356-65.

Bredemeyer AL, Sharma GG, Huang CY, Helmink BA, Walker LM, Khor KC, Nuskey B, Sullivan KE, Pandita TK, Bassing CH, Sleckman BP. (2006) ATM stabilizes DNA double-strand-break complexes during V(D)J recombination. Nature. 442(7101):466-70.

Cannizzaro C, La Barbera M, Plescia F, Cacace S, Tringali G. Ethanol modulates corticotropin releasing hormone release from the rat hypothalamus: does acetaldehyde play a role? Alcohol Clin Exp Res. 2010 Apr;34(4):588-93.

Casorelli I, Offman J, Mele L, Pagano L, Sica S, D'Errico M, Giannini G, Leone G, Bignami M, Karran P. (2003) Drug treatment in the development of mismatch repair defective acute leukemia and myelodysplastic syndrome. DNA Repair (Amst). 2(5):547-59.

Casorelli I, Tenedini E, Tagliafico E, Blasi MF, Giuliani A, Crescenzi M, Pelosi E, Testa U, Peschle C, Mele L, Diverio D, Breccia M, Lo-Coco F, Ferrari S, Bignami M. (2006) Identification of a molecular signature for leukemic promyelocytes and their normal counterparts: Focus on DNA repair genes. Leukemia. 20(11):1978-88.

Condie A, Powles RL, Hudson CD, Shepherd V, Bevan S, Yuille MR, Houlston RS (2002). Analysis of the Fanconi anaemia

complementation group A gene in acute myeloid leukaemia. Leuk Lymphoma. 43(9):1849-53.

Coutelle, C, Höhn B, Benesova M, et al. (2004) Risk factors in alcohol-associated breast cancer: Alcohol dehydrogenase polymorphism and estrogens. International Journal of Oncology 25:1127–1132.

Coyle YM, Hynan LS, Euhus DM, Minhajuddin AT (2005). An ecological study of the association of environmental chemicals on breast cancer incidence in Texas. Breast Cancer Res Treat. 92(2):107-14.

D'Andrea AD, Grompe M (2003). The Fanconi anaemia/BRCA pathway. Nat Rev Cancer. 3(1):23-34.

DeVita VT Jr, Chu E. A history of cancer chemotherapy (2008). Cancer Res. 68(21):8643-53

Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, Qian D, Lam JS, Ailles LE, Wong M, Joshua B, Kaplan MJ, Wapnir I, Dirbas FM, Somlo G, Garberoglio C, Paz B, Shen J, Lau SK, Quake SR, Brown JM, Weissman IL, Clarke MF. Association of reactive oxygen species levels and radioresistance in cancer stem cells (2009). Nature. 458(7239):780-3.

Dumitrescu R, Shields P (2005) The etiology of alcohol-induced breast cancer. Alcohol 35: 213-25.

Eriksson C, Fukunaga T, Sarkola T, Lindholm H, Ahola L (1996). Estrogen-related acetaldehyde elevation in women during alcohol intoxication. Alcohol Clin Exp Res. 20:1192-1195.

FANG J, .VACA C. (1997). Detection of DNA adducts of acetaldehyde in peripheral white blood cells of alcohol abusers. Carcinogenesis 18: 627–632

Fan S, Meng Q, Gao B, Grossman J., Yadegari M, Goldberg I, Rosen E (2000). Alcohol stimulates estrogen receptor signaling in human breast cancer cell lines. Cancer Res 60, 5635–5639.

Fan, S., Ma, Y.X., Wang, C., Yuan, R.Q., Meng, Q., Wang, J.A., Erdos, M., Goldberg, I.D., Webb, P., Kushner, P.J., et al. (2001). Role of direct interaction in BRCA1 inhibition of estrogen receptor activity. Oncogene; 20, 77–87.

Friedenson B (2011). A common environmental carcinogen unduly affects carriers of cancer mutations: Carriers of genetic mutations in a specific protective response are more susceptible to an environmental carcinogen. Medical Hypotheses (2011), doi:10.1016/j.mehy.2011.07.039

Friedenson B (2010a). A theory that explains the tissue specificity of BRCA1/2 related and other hereditary cancers. J. Med. Med. Sci. 1(8): 372-384

Friedenson B. (2010b) Inflammatory processes inordinately increase tissue specific cancer risks in carriers of mutations in BRCA1, BRCA2, ATM or Fanconi anemia genes. J. Med. Med. Sci. 1(8): 356-371

Friedenson B (2007). The BRCA1/2 pathway prevents hematologic cancers in addition to breast and ovarian cancers. BMC Cancer 7:152.

Friedenson B. (2005). BRCA1 and BRCA2 pathways and the risk of cancers other than breast or ovarian. Medscape General Medicine MedgenMed June 29, 7(2) 2005 Available at http://www.medscape.com/viewarticle/505347.

Freudenheim JL, Ambrosone CB, Moysich KB, Vena JE, Graham S, Marshall JR, Muti P, Laughlin R, Nemoto T, Harty LC, Crits GA, Chan AW, Shields PG. (1999) Alcohol dehydrogenase 3 genotype modification of the association of alcohol consumption with breast cancer risk. Cancer Causes Control. 10(5):369-77.

Gaffney DK, Brohet RM, Lewis CM, Holden JA, Buys SS, Neuhausen SL, Steele L, Avizonis V, Stewart JR, Cannon-Albright LA. (1998) Response to radiation therapy and prognosis in breast cancer patients with BRCA1 and BRCA2 mutations. Radiother Oncol. 47(2):129-36.

Gordon BH, Baraona E, Miyakawa H, Finkelman F, Lieber CS (1985). Exaggerated acetaldehyde response after ethanol administration during pregnancy and lactation in rats. Alcohol Clin Exp Res. 1985 Jan-Feb;9(1):17-22.

Hauptmann M, Stewart PA, Lubin JH, Beane Freeman LE, Hornung RW, Herrick RF, Hoover RN, Fraumeni JF Jr, Blair A, Hayes RB. (2009). Mortality from lymphohematopoietic malignancies and brain cancer among embalmers exposed to formaldehyde. J Natl Cancer Inst. 101(24):1696-708

Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders C, Persky N, Grompe M, Joenje H, Pals G, Ikeda H, Fox EA, D'Andrea A (2002) Biallelic inactivation of BRCA2 in Fanconi anemia. Science. 297(5581):606-9.

Ishikawa H, Ishikawa T, Yamamoto H, Fukao A, Yokoyama K (2007). Genotoxic effects of alcohol in human peripheral lymphocytes modulated by ADH1B and ALDH2 gene polymorphisms. Mutat Res. 2007 Feb 3;615(1-2):134-42.

Jelski W, Chrostek L, Szmitkowski M, Markiewicz (2006) The Activity of clase I, II, II and IV alcohol dehyrogenase isoenzymes and aldehyde dehydrogenase in breast cancer. Clin Exp Med 6:89-93.

Lachenmeier DW, Monakhova YB (2011) .Short-term salivary acetaldehyde increase due to direct exposure to alcoholic beverages as an additional cancer risk factor beyond ethanol metabolism. J Exp Clin Cancer Res. 30:3.

Lagerqvist A, Håkansson D, Prochazka G, Lundin C, Dreij K, Segerbäck D, Jernström B, Törnqvist M, Seidel A, Erixon K, Jenssen D. (2008) Both replication bypass fidelity and repair efficiency influence the yield of mutations per target dose in intact mammalian cells induced by benzo[a]pyrene-diol-epoxide and dibenzo[a,l]pyrene-diol-epoxide. DNA Repair (Amst). 7(8):1202-12.

Langevin F, Crossan GP, Rosado IV, Arends MJ, Patel K (2011) Fancd2 counteracts the toxic effects of naturally produced aldehydes in mice. Nature. 2011 Jul 6;475(7354):53-8. doi: 10.1038/nature10192.

Latino-Martel P, Chan DS, Druesne-Pecollo N, Barrandon E, Hercberg S, Norat T (2010). Maternal alcohol consumption during pregnancy and risk of childhood leukemia: systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev. 2010 May;19(5):1238-60

Lensch MW, Tischkowitz M, Christianson TA, Reifsteck CA, Speckhart SA, Jakobs PM, O'Dwyer ME, Olson SB, Le Beau MM, Hodgson SV, Mathew CG, Larson RA, Bagby GC Jr. (2003) Acquired FANCA dysfunction and cytogenetic instability in adult acute myelogenous leukemia. Blood102(1):7-16.

Linder N, Lundin J, Isola J, Lundin M, Raivio KO, Joensuu H.

(2005) Down-regulated xanthine oxidoreductase is a feature of aggressive breast cancer. Clin Cancer Res. 11(12):4372-81.

Luciani JJ, Depetris D, Usson Y, Metzler-Guillemain C, Mignon-Ravix C, Mitchell MJ, Megarbane A, Sarda P, Sirma H, Moncla A, Feunteun J, Mattei MG. PML nuclear bodies are highly organised DNA-protein structures with a function in heterochromatin remodelling at the G2 phase. J Cell Sci. 2006 Jun 15;119(Pt 12):2518-31

Lu K, Moeller B, Doyle-Eisele M, McDonald J, Swenberg JA. (2011) Molecular Dosimetry of N(2)-Hydroxymethyl-dG DNA Adducts in Rats Exposed to Formaldehyde. Chem Res Toxicol. 24(2):159-61.

Lu K, Ye W, Zhou L, Collins LB, Chen X, Gold A, Ball LM, Swenberg JA. (2010) Structural characterization of formaldehyde-induced cross-links between amino acids and deoxynucleosides and their oligomers. J Am Chem Soc. 132: 3388–3399

Lu Y, Morimoto K (2009). Is habitual alcohol drinking associated with reduced electrophoretic DNA migration in peripheral blood leukocytes from ALDH2-deficient male Japanese? Mutagenesis. 24(4):303-8.

Maciel ME, Castro GD, Castro JA (2004). Inhibition of the rat breast cytosolic bioactivation of ethanol to acetaldehyde by some plant polyphenols and folic acid. Nutr Cancer 49(1):94-9.

Marietta C, Thompson L, Lamerdin J, Brooks P. (2009) Acetaldehyde stimulates FANCD2 monoubiquitination, H2AX phosphorylation in human cells in Vitro: Implications for alcohol related carcinogenesis. Mutat Res 12: 664: 77-83

Martin MG, Welch JS, Luo J, Ellis MJ, Graubert TA, Walter MJ. (2009) Therapy related acute myeloid leukemia in breast cancer survivors, a population-based study. Breast Cancer Res Treat. 118(3):593-8.

Mohseni Meybodi A, Mozdarani H. (2009) DNA damage in leukocytes from Fanconi anemia (FA) patients and heterozygotes induced by mitomycin C and ionizing radiation as assessed by the comet and comet-FISH assay. Iran Biomed J. 3(1):1-8.

Moorman PG, Iversen ES, Marcom PK, Marks JR, Wang F; Kathleen Cuningham Consortium for Research into Familial Breast Cancer, Lee E, Ursin G, Rebbeck TR, Domchek SM, Arun B, Susswein L, Isaacs C, Garber JE, Visvanathan K, Griffin CA, Sutphen R, Brzosowicz J, Gruber S, Finkelstein DM, Schildkraut JM. Evaluation of established breast cancer risk factors as modifiers of BRCA1 or BRCA2: a multi-center case-only analysis. Breast Cancer Res Treat. 2010 Nov;124(2):441-51.

Nakano T, Katafuchi A, Matsubara M, Terato H, Tsuboi T, Masuda T, Tatsumoto T, Pack SP, Makino K, Croteau DL, Van Houten B, Iijima K, Tauchi H, Ide H. (2009) Homologous recombination but not nucleotide excision repair plays a pivotal role in tolerance of DNA-protein cross-links in mammalian cells. J Biol Chem. 284(40):27065-76.

Nakano T, Terato H, Asagoshi K, Masaoka A, Mukuta M, Ohyama Y, Suzuki T, Makino K, Ide H. (2003) DNA-protein cross-link formation mediated by oxanine. A novel genotoxic mechanism of nitric oxide-induced DNA damage. J Biol Chem. 278(27):25264-72.

Nathanson KL, Shugart YY, Omaruddin R, Szabo C, Goldgar D, Rebbeck TR, Weber BL (2002). CGH-targeted linkage analysis reveals a possible BRCA1 modifier locus on chromosome 5q. Hum Mol Genet. 11(11):1327-32.

National Toxicology Program (2010). Final Report on Carcinogens. Background Document for Formaldehyde. Rep Carcinog Backgr Doc. 10-5981:i-512.

National Toxicology Program (2011) Acetaldehyde. Report on carcinogens, Twelfth edition pp. 21-24

Neveling K, Endt D, Hoehn H, Schindler D. (2009) Genotype-phenotype correlations in Fanconi anemia. Mutat Res. 668(1-2):73-91.

Nojima K, Hochegger H, Saberi A, Fukushima T, Kikuchi K, Yoshimura M, Orelli BJ, Bishop DK, Hirano S, Ohzeki M, Ishiai M, Yamamoto K, Takata M, Arakawa H, Buerstedde JM, Yamazoe M, Kawamoto T, Araki K, Takahashi JA, Hashimoto N, Takeda S, Sonoda E. (2005). Multiple repair pathways mediate tolerance to chemotherapeutic cross-linking agents in vertebrate cells. Cancer Res 65:11704–11711.

Obe, G., & Anderson, D. (1987). International Commission for Protection against Environmental Mutagens and Carcinogens. ICPEMC Working Paper No. 15/1. Genetic effects of ethanol. Mutat Res 186, 177–200.

Olin K, Cherr G, Rifkin E, Keen C (1996). The effects of some redox-active metals and reactive aldehydes on DNA-protein cross-links in vitro. Toxicology. 110(1-3):1-8.

Pierce LJ, Haffty BG (2011). Radiotherapy in the treatment of hereditary breast cancer. Semin Radiat Oncol. 21(1):43-50.

Rischewski JR, Clausen H, Leber V, Niemeyer C, Ritter J, Schindler D, Schneppenheim R. (2000). A heterozygous frameshift mutation in the Fanconi anemia C gene in familial T-ALL and secondary malignancy. Klin Padiatr. 212(4):174-6.

Sako M, Inagaki S, Esaka Y, Deyashiki Y (2003). Histones accelerate the cyclic 1,N2-propanoguanine adduct-formation of DNA by the primary metabolite of alcohol and carcinogenic crotonaldehyde. Bioorg Med Chem Lett 13: 3497–3498.

Sato K, Hayami R, Wu W, Nishikawa T, Nishikawa H, Okuda Y, Ogata H, Fukuda M, Ohta T. (2004) Nucleophosmin/B23 Is a Candidate Substrate for the BRCA1-BARD1 Ubiquitin Ligase J Biol Chem 279: 30919–30922,

Seitz H, Becker P (2007). Alcohol metabolism and cancer risk. Alcohol Research and Health 30: 38-47

Seitz H and Maurer B (2007) The relationship between alcohol metabolism, estrogen levels, and breast cancer risk. Alcohol res Health 30 42-43

Shaw S, Jayatilleke E, Herbert V, Colman N. (1989) Cleavage of folates during ethanol metabolism. Role of acetaldehyde/xanthine oxidase-generated superoxide. Biochem J. 257(1): 277–280.

Shaham J, Bomstein Y, Meltzer A, Kaufman Z, Palma E, Ribak J. (1996) DNA--protein crosslinks, a biomarker of exposure to formaldehyde--in vitro and in vivo studies. Carcinogenesis. 17(1):121-5.

Shu XO, Ross JA, Pendergrass TW, Reaman GH, Lampkin B, Robison LL (1996). Parental alcohol consumption, cigarette smoking, and risk of infant leukemia: a Childrens Cancer Group Study. J Natl Cancer Inst. 88(1):24-31.

Soffritti M, Belpoggi F, Lambertin L, Lauriola M, Padovani M, Maltoni C (2002). Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. Ann N Y Acad Sci. 982:87-105.

Smith-Warner SA, Spiegelman D, Yaun SS, van den Brandt PA, Folsom AR, Goldbohm RA, Graham S, Holmberg L, Howe GR, Marshall JR, Miller AB, Potter JD, Speizer FE, Willett WC, Wolk A, Hunter DJ. (1998) Alcohol and breast cancer in women: a pooled analysis of cohort studies. JAMA. 279(7):535-40.

Sun J, Oma Y, Harata M, Kono K, Shima H, Kinomura A, Ikura T, Suzuki H, Mizutani S, Kanaar R, Tashiro S. (2010) ATM modulates the loading of recombination proteins onto a chromosomal translocation breakpoint hotspot. PLoS One. 27;5(10):e13554

Teng S, Beard K, Pourahmad, J, et al. (2001) The formaldehyde metabolic detoxification enzyme systems and molecular cytotoxic mechanism in isolated rat hepatocytes. Chem Biol Interact 130–132:285–296.

Terry MB, Gammon MD, Zhang FF, Knight JA, Wang Q, Britton JA, Teitelbaum SL, Neugut AI, Santella RM. (2006) ADH3 genotype, alcohol intake and breast cancer risk. Carcinogenesis. 2006 Apr;27(4):840-7.

Theruvathu JA, Jaruga P, Nath RG, Dizdaroglu M, Brooks PJ (2005). Polyamines stimulate the formation of mutagenic 1,N2-propanodeoxyguanosine adducts from acetaldehyde. Nucleic Acids Res. 33(11):3513-20.

Tirkkonen M, Johannsson O, Agnarsson B, Olsson H, et al (1997) Distinct Somatic Genetic Changes Associated with Tumor Progression in Carriers of BRCA1and BRCA2 Germ-line Mutations Cancer Research 57: 1222-1227.

Tischkowitz MD, Morgan NV, Grimwade D, Eddy C, Ball S, Vorechovsky I, Langabeer S, Stöger R, Hodgson SV, Mathew CG. (2004) Deletion and reduced expression of the Fanconi anemia FANCA gene in sporadic acute myeloid leukemia. Leukemia 18(3):420-5.

Tischkowitz M, Xia B (2010). PALB2/FANCN - recombining cancer and Fanconi anemia. Cancer Res 70:7353-59.

Triano EA, Slusher LB, Atkins TA, Beneski JT, Gestl SA, Zolfaghari R, Polavarapu R, Frauenhoffer E, Weisz J. (2003) Class I alcohol dehydrogenase is highly expressed in normal human mammary epithelium but not in invasive breast cancer: implications for breast carcinogenesis. Cancer Res. 63(12):3092-100.

U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. (1999). Toxicological profile for Formaldehyde.

Valeri A, Alonso-Ferrero ME, Río P, Pujol MR, Casado JA, Pérez L, Jacome A, Agirre X, Calasanz MJ, Hanenberg H, Surrallés J, Prosper F, Albella B, Bueren JA. (2010) Bcr/Abl interferes with the Fanconi anemia/BRCA pathway: implications in the chromosomal instability of chronic myeloid leukemia cells. PLoS One. 5(12):e15525.

Venkitaraman A (2003). A growing network of cancer-susceptibility genes. N. Engl. J. Med. 348: 1917-9.

Wagner JE, Tolar J, Levran O, Scholl T, Deffenbaugh A, Satagopan J, Ben-Porat L, Mah K, Batish SD, Kutler DI, MacMillan ML, Hanenberg H, Auerbach AD. (2004). Germline mutations in BRCA2: shared genetic susceptibility to breast cancer, early onset leukemia, and Fanconi anemia. Blood 103(8):3226-9.

Wako H (1953) The Isolation and Identification of Methyl Glyoxal The Tohoku Journal of Experimental Medicine, 57:191-7

Wang L, Xu, W-L, Meng H-T , Qian W-B, Mai W-Y, Tong H-Y, Mao L-P, Tong Y, Qian J-J, Lou Y-J, Chen Z-M, Wang Y-G, Jin J (2010) FLT3 and NPM1 mutations in Chinese patients with acute myeloid leukemia and normal cytogenetics Biomed & Biotechnol 11(10):762-770

Welcsh PL, King MC (2001). BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. Hum Mol Genet. 10(7):705-13

Wu FY, Lee YJ, Chen DR, Kuo HW. Association of DNA-protein crosslinks and breast cancer (2002). Mutat Res. 501(1-2):69-78.

Wu Y, Brosh RM Jr. (2010) Helicase-inactivating mutations as a basis for dominant negative phenotypes. Cell Cycle. 9(20):4080-90.

Xie Y, de Winter JP, Waisfisz Q, Nieuwint AW, Scheper RJ, Arwert F, Hoatlin ME, Ossenkoppele GJ, Schuurhuis GJ, Joenje H. (2000) Aberrant Fanconi anaemia protein profiles in acute myeloid leukaemia cells. Br J Haematol. 111(4):1057-64.

Yamazoe M, Sonoda E, Hochegger H, Takeda S. (2004). Reverse genetic studies of the DNA damage response in the chicken B lymphocyte line DT40. DNA Repair (Amst) 3:1175–1185.

Yan Y, Yang JY, Mou YH, Wang LH, Zhang H, Wu CF (2011). Possible metabolic pathways of ethanol responsible for oxidative DNA damage in human peripheral lymphocytes. Alcohol Clin Exp Res. 35(1):1-9.

Zhang Y, Rowley JD. Chromatin structural elements and chromosomal translocations in leukemia. DNA Repair (Amst). (2006) 5(9-10):1282-97.

Zhang L, Tang X, Rothman N, Vermeulen R, Ji Z, Shen M, Qiu C, Guo W, Liu S, Reiss B, Freeman LB, Ge Y, Hubbard AE, Hua M, Blair A, Galvan N, Ruan X, Alter BP, Xin KX, Li S, Moore LE, Kim S, Xie Y, Hayes RB, Azuma M, Hauptmann M, Xiong J, Stewart P, Li L, Rappaport SM, Huang H, Fraumeni JF Jr, Smith MT, Lan Q (2010) Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. Cancer Epidemiol Biomarkers Prev. 19(1):80-8.

Zhang, L, Beane Freeman L, Nakamura J, Hecht S, Vandenberg J, Smith, M, Sonawane B. Formaldehyde and Leukemia: Epidemiology, potential mechanisms and implications for risk assessment. Environmental and Molecular mutagenesis 2010; 51: 181-191

Zhu Y, Xu W, Liu Q, Pan J, Qiu H, Wang R, Qiao C, Jiang Y, Zhang S, Fan L, Zhang J, Shen Y, Xue Y, Li J. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. (2008) [Abnormalities of chromosome 17 in myeloid malignancies with complex chromosomal abnormalities] 25(5):579-82