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

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Abstract

One way an inherited cancer gene mutation may target specific tissues for cancer is by increasing susceptibility when a tissue is exposed to environmental carcinogens. An example of this may be the increased susceptibility of BRCA1 or BRCA2 mutation carriers to the carcinogen formaldehyde. Formaldehyde is now a proven cause of human myeloid leukemias. Yet millions of tons of formaldehyde are produced every year and it is everywhere. High formaldehyde levels can overwhelm normal enzyme detoxification systems and cause DNA damage. It is known that some types of formaldehyde-associated DNA damage require error-free DNA repairs mediated by pathways containing BRCA1 and BRCA2 proteins. Otherwise some formaldehyde-related DNA damage cannot be properly repaired so mutations may occur. Therefore, carriers of BRCA1 and BRCA2 gene defects should be unduly susceptible to myeloid leukemia. Studies show that inherited biallelic BRCA2 gene defects dramatically increase risks for myeloid leukemia. Heterozygous BRCA1 or BRCA2 mutations also increase risks for myeloid leukemias in most relevant studies. BRCA1/2 mutation carriers may reduce risks for myeloid leukemias by using available precautions to lower their exposure to formaldehyde.

Background

Formaldehyde is a known carcinogen that seems everywhere. Widespread exposures occur during manufacture and use of resins, particle board, plywood, leather goods, paper, pharmaceuticals, cosmetics, baby bath products, nursery furniture, and food. Latex paint, fingernail hardener, and fingernail polish release a large amount of formaldehyde to the air. Tobacco smoking, varnishes, floor finishes, auto exhaust and organic combustion all release formaldehyde. Formaldehyde enters water from industrial wastes and may occur as a by-product of water disinfection. Formaldehyde goods constitute 5% of US gross national product with production of nearly 5 million tons in 2003 [1].

Minimal levels of exposure required to produce cancers and other effects. Exogenous formaldehyde becomes more dangerous at higher levels of exposure. Potentially serious common routes of exposure for exogenous formaldehyde include inhalation, from occupational exposure and environment, dermal, from occupational handling, and intramuscular or subcutaneous from vaccines. Formaldehyde is produced from normal metabolism and occurs naturally in plants, fruits, vegetables, animals and seafood. These sources are generally of insufficient quantity to cause permanent harm and are managed by reactions and metabolism within the digestive tract. Fig. 1 gives minimal levels of inhalation and oral ingestion reported to be associated with cancers and other health effects [2-4]. Human cancers are more likely on exposure to levels that overwhelm normal protective responses or if these responses are lost.

Mechanisms for formaldehyde induced cancers. Formaldehyde is a chemical that can connect strands of DNA to each other and to proteins in the vicinity of DNA. Multiple different amino acids in proteins form covalent cross links with different DNA bases. Some types of formaldehyde cross-links between DNA and protein are irreversible and this damage requires DNA repair pathways. If cells survive but are not able to correctly repair this damage, then the strands of DNA do not reproduce faithfully and the biological instructions on DNA may be misread. Chromosome breaks, gains and losses may result.

Formaldehyde forms a series of polymers of varying length which may form cross linkers with varying reach. To mimic the reactions of formaldehyde with DNA protein mixtures, Lu et al, [5] investigated the formation of formaldehyde-induced cross-links in a model system consisting of deoxynucleosides and amino acids. This provided a rigorous structural identification of formaldehyde induced DNA-protein

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Fig. 1. Health effects of formaldehyde exposure by either inhalation or oral ingestion in animals or humans [2-4] showing that even low levels in normal individuals are associated with several types of cancers. The levels shown indicate the lowest levels at which the indicated health effects have been reported.

cross-links. Cross-linked products that were chemically irreversible, stable and readily isolated were characterized as Cys-CH2-dG, Cys-CH2-dA, and Cys-CH2-dC. The amino acids Histidine and Tryptophan also formed stable cross-links with model deoxynucleosides but in low yield. Low yield products may become significant at higher levels and prolonged exposure. Three lysine cross-linked products were labile in solution, supporting widely reported reversibility of formaldehyde-induced cross-links formed in vivo between lysine rich histones and DNA [5]. Buried within the 3 dimensional structures of protein-DNA adducts, however some lysine cross-links may become more stable.

Formaldehyde is widely used as a biological fixative with the assumptions that most biologically meaningful interactions are preserved by cross-linking and that the fixed structures accurately reflect

Fig. 2. Formaldehyde detoxification occurs primarily by a pathway (thicker arrows) involving formaldehyde dehydrogenase (ADH3), an aldehyde dehydrogenase. The pathway converts formaldehyde to formate which is then eliminated in the urine, broken down to CO₂ and water or enters the single carbon pool. Alternate, less used pathways are indicated by thinner arrows [8]. Initial detoxification does not involve BRCA1/2. In one study, all the genes encoding the ADH enzymes shown were deleted in 81% of BRCA1 associated breast cancers [9] and the region containing ALDH2 was deleted in 40% of BRCA1 associated breast cancers [9].

molecular relationships in the living cell. This assumption becomes invalid when intermolecular contacts are short-lived. In vivo interactions lasting less than five seconds are obvious in live cells but they are invisible in the microscope after formaldehyde fixation [6].

Formaldehyde detoxification by dedicated enzymes. Endogenous formaldehyde is produced by normal metabolism but endogenous formaldehyde is detoxified by enzymes in dedicated pathways. Formaldehyde is primarily metabolized by glutathione-dependent formaldehyde dehydrogenase (ADH3) and aldehyde dehydrogenases (ALDHs) enzymes in these pathways (Fig. 2). The overall pathways also detoxify low levels of ingested formaldehyde. Formaldehyde is converted to formate, which is then

eliminated in the urine as a sodium salt, broken down to CO₂ and water, or incorporated into the single-carbon pool.

The distribution, ontogeny, and regulation of formaldehyde processing enzymes and the genes that encode them have important implications for the clearance of endogenous and low level exogenous formaldehyde [7]. Deficiencies in any of these enzymes in Fig. 2 would increase damage from reactive oxygen species. This is because unmetabolized formaldehyde causes inflammation associated damage. Teng et al. [8] provided evidence that inhibition of the alcohol (ADH) or aldehyde dehydrogenases (ALDH2) in Fig 2 has significant impact on formaldehyde toxicity. The ADH3 pathway in Fig.2 is the major route due to the relative affinities of the enzymes for formaldehyde and the intermediates shown. ADH3 is a member of the alcohol dehydrogenase gene family ubiquitously expressed in organ tissues. There are different frequencies of ADH3 alleles among Chinese, Spanish and Swedish populations [10].

DNA cross links, chromosome breaks and chromosome abnormalities would be favored by saturating formaldehyde metabolizing pathways with environmental formaldehyde. Thus, exogenous addition products form between formaldehyde and DNA in a highly nonlinear fashion; a 21.7-fold increase in exposure caused a 286-fold increase in exogenous adducts [11].

Formaldehyde related cancers. In the general population, the World Health Organization's International Agency for Research on Cancer (IARC) classifies formaldehyde as a direct human mutagen and a known carcinogen [3]. Nasopharyngeal, sinonasal cancer and myeloid leukemia are all linked to formaldehyde and occur beginning at 2 – 5.9 ppm in air (Fig. 1). Myeloid leukemia is a malignancy of the white cells important in the first defenses against disease causing microorganisms. In humans, epidemiological evidence found that embalming was significantly associated with an increased risk for myeloid leukemia. Those who had performed the most embalming and those with the highest estimated formaldehyde exposure had the greatest risk of myeloid leukemia. There were significant trends for cumulative years of embalming (p trend=0.020) and for increasing peak formaldehyde exposure (p trend=0.036) [3]. Fig. 1 indicates myeloid leukemias at the lower range of peak exposures for embalmers (3.7 ppm) although their peak exposure range extended further (to 12.3 ppm)[12].

For years, doubts had been raised about whether formaldehyde caused leukemia. Environmental formaldehyde is a water soluble gas which yields formalin in solution. It was not clear how inhaling

formaldehyde or exposure to formalin could lead to white cell malignancies. The formaldehyde adduct hydroxymethyl deoxyguanine was not detected in the bone marrow by mass spectroscopy. Yet DNA-protein cross links formed by formaldehyde served as a marker for cellular exposure. Then chromosome breaks and leukemia-specific chromosome changes [1, 3] were found in precursors of myeloid white cells. Nasal epithelium in rats contains stem cells capable of forming myeloid white cell producing tissue [13]. This then provided a plausible way carcinogenic damage to nasal stem cells from inhaled formaldehyde could develop into myeloid leukemia. This possibility is further supported by the elevated incidence rate of leukemia and other hematological malignancies in individuals with nasopharyngeal cancer. Identification of genetic susceptibility loci for nasopharyngeal cancer found these loci were also all associated with leukemias. This suggests a partially shared pathogenic mechanism between hematologic malignancy and nasopharyngeal cancer [14].

The Hypothesis vs current thinking

Carcinogens may differentially affect

some members of the population. One way an inherited cancer gene mutation may target specific tissues is by increasing cancer susceptibility when a tissue is exposed to environmental carcinogens [15]. An example of this may be increased susceptibility of BRCA1 or BRCA2 mutation carriers to the carcinogen formaldehyde. BRCA1/2 mutation carriers are unable to correctly repair large DNA protein cross-links caused by formaldehyde [16]. This type of formaldehyde-associated DNA damage requires error-free DNA repairs mediated by pathways containing BRCA1 and BRCA2 proteins (Fig. 3, [16, 17, 15]). Otherwise, mu-

Fig 3. Summary of pathways showing BRCA1, BRCA2 in pathways to correct DNA cross links and double strand breaks caused by formaldehyde that escape metabolic detoxification (as shown in Fig. 2). Fanconi proteins and ATM are also shown within these pathways. Proteins encoded by genes related to well known hereditary cancer conditions are colored black [17, 15].

tations are more likely to occur. Therefore, carriers of BRCA1 and BRCA2 gene defects should be unduly susceptible to myeloid leukemia.

This differs from current thinking which has invoked an exquisite specificity for BRCA1 and BRCA2 mutations in causing breast and ovarian cancer. Ideas for how this occurred were unclear but depended on some intrinsic property of BRCA1 and BRCA2 so that inactivating either gene specifically causes breast and ovarian cancer. Other current ideas assume hormonal or developmental properties of breast and ovaries cause cancers to develop if BRCA1 or BRCA2 genes are inactive.

The hypothesis here grew from an analysis of cancer incidence data showing that cancer targets in BRCA1/2 mutation carriers are in fact not specific for breast and ovary. There were increased risks for a broad range of tissues [18]. In fact people with compromised pathways containing BRCA1/2 and appropriate risk factors are highly prone to develop cancers mediated by some organ specific inflammatory infections. The cancer targets were then determined by organ specific infections and not by the mutation [19].

Evaluation of the Hypothesis.

This prediction can be tested against five different major and separate lines of evidence (Tests 1-5 below).

Test 1. A first test is whether women with defective BRCA1/2 genes show variation in cancer risks sufficient to allow for differences in environmental exposures. However additional genes could account for this variation so this test only provides an essential condition that the hypothesis must meet.

Test 2. The second test is whether hereditary deficits in BRCA1 or in BRCA2 genes increase risks for myeloid leukemias. This may also be true for hereditary deficits in other genes that encode other proteins in pathways containing BRCA1 or BRCA2.

Test 3. The third test is whether excessive formaldehyde exposure causes DNA damage which creates demands on functioning BRCA1/2 pathways for correct repairs.

Test 4. A fourth test is to evaluate measured risks for formaldehyde associated rare cancers of the upper respiratory/ aerodigestive tract in mutation carriers or presumptive mutation carriers.

Test 5. A fifth test is to compare chromosome abnormalities seen in mutation carrier cancers with those caused by formaldehyde and then to chromosome abnormalities in hereditary breast cancers.

Table 1 Risks of leukemia and oral cavity cancers as cancers following breast, ovarian or fallopian tube cancer in proven or potential BRCA1/2 mutation carriers

Study population, <i>reference</i>	Mutation test status	Risk measurement for leukemias [Confidence interval]	Elevated risk for pharynx, sinus or nose cancer
6 children with biallelic BRCA2 mutations <i>Wagner et al, 2004 [21]</i>	Biallelic BRCA2 mutations (compound heterozygotes)	All developed leukemia at median age 2.2 years. 4 of 6 AMLs	
Review of 27 biallelic BRCA2 mutation patients <i>Alter et al, 2007 [22]</i>	Biallelic BRCA2 mutations (compound heterozygotes)	79% cumulative probability of leukemia (primarily AML) by age 10 years	
First breast cancer age <45 in 6958 Connecticut women <i>Harvey & Brinton, 1985 [23]</i>	Potential mutation carriers eligible for mutation testing	Acute non-lymphocytic leukemia as 2 nd cancer O/E=2.9 at 1-4 years and 6.4 at 5-9 years	+
82,520 Women with breast cancer age <=45 in 13 cancer registries. <i>Mellemkjaer et al, 2001, [24]</i>	Potential mutation carriers eligible for mutation testing	Myeloid Leukemia SIR = 3.02 (2.32–3.85) Leukemia SIR = 2.16 (1.78–2.59).	
2,084 Women with primary fallopian tube cancer, <i>Riska et al IntJ Cancer 2007, [25]</i>	Probable BRCA1/2 mutation carriers.	Non-lymphoid leukemia RR=3.7 (1.0-9.4)	+
Women with 2 breast or ovarian cancers in Thames Cancer Registry <i>Evans et al, 2001 [26]</i>	Potential mutation carriers eligible for mutation testing	Myeloid leukemia RR=5.04 [1.85-11.0]	+
2 nd cancer after Breast Cancer<50 <i>Evans et al, 2001 [27]</i>	Potential mutation carriers eligible for mutation testing	Myeloid leukemias RR=2.31 [1.52-3.51]	
2 nd cancer after male breast cancer <i>Hemminki et al, 2005 [28]</i>	Men at high risk for being mutation carriers eligible for mutation testing	Myeloid leukemia RR=3.42 [1.47-6.73] Lymphohematopoietic system RR=1.63 [1.12-2.29]	+
Primary breast cancer patients, age 35-49, Finnish Cancer Registry (1953-1979) <i>Teppo et al, 1985 [29]</i>	Potential mutation carriers eligible for mutation testing	Subsequent leukemia (excluding CLL) RR= 3.21 p<.01	
291 first degree relatives of BRCA1 probands with ovarian cancer <i>Risch et al, 2006; Risch et al, 2001 [30,31]</i>	Tested BRCA1 heterozygotes or potential carriers eligible for testing	Leukemias, lymphomas, etc RR=3.7 (1.5 to 9.5) (2006); RR= 2.6 [1.02-6.6] (2001).	
Female breast cancer in Denmark (1943-80) surviving >= 10 years (selects 11,273 younger surviving patients) <i>Ewertz & Mouridsen, 1985 [32]</i>	Potential mutation carriers eligible for mutation testing	Acute non-lymphocytic leukemia as a 2 nd cancer RR=2.3	+#
BRCA1+BRCA2 families <i>Shi et al, 2000 [33]</i>	Known BRCA1 and BRCA2 mutation carriers	20% of leukemias occurred as 2 nd primary cancers in BRCA1 and BRCA2 carriers	+?
3678 women, 50 men 1st degree relatives of mutation carriers or of breast or ovarian cancer patients <i>BCLC 1999 [34]</i>	471 carriers, 390 noncarriers, and 2186 unknown carrier status	Leukemia RR= 1.12 [0.30–4.25]	+
11 847 individuals from 699 families segregating a BRCA1 mutation <i>Thompson et al, 2002 [35]</i>	18.9% (2245) tested positive, 9.3% (1106) tested negative, 71.7% (8496) untested.	Leukemia RR = 0.88 [0.37 to 2.14] p=.83	+*
1811 male and female family members <i>van Asperen et al, 2005 [36]</i>	50% probability BRCA2 mutation from 139 BRCA2 families. 66 different pathogenic mutations	Leukemia RR= 1.5 [0.5 to 3.5]	+

? Head/neck and vocal cord cancer reported as “other primary tumors”

RR=1.1 for mouth cancer

* Nasal sinus cancer reported. Buccal cavity and pharynx RR=0.15

Test 1. Mutation carriers have widely varying cancer risks. Female mutation carriers have high risks for breast /ovarian cancer as a group but individual risks within this group vary greatly [20]. Results support the conclusion that there is no single risk associated with BRCA1 or BRCA2 carrier status. This is consistent with BRCA1/2 mutations causing an increased sensitivity to environmental carcinogens and/or modification of cancer risks by contributions from additional genes.

Defective BRCA genes also increase risks for cancers in organs other than breast and ovary but individual mutation carriers again differ greatly in where these other cancers occur [18]. These individual differences may also implicate an increased susceptibility in organs exposed to environmental carcinogens and/or additional genes [15].

There are hereditary cancer conditions related to any of multiple genes encoding the pathway in Fig. 3. BRCA1/2 mutations are associated with hereditary breast/ovarian cancer syndrome but other hereditary cancer related conditions are also caused by homozygous or biallelic mutations in genes encoding proteins within the model pathway (Fig.3). These conditions are ataxia-telangiectasia caused by inactivation of the ATM gene and Fanconi anemia caused by inactivation of one of the Fanconi anemia genes. Both conditions are associated with familial cancers and cancer incidence rates are available.

Test 2. BRCA1/2 gene mutations are associated with myeloid leukemia. Inherited mutations in BRCA1/2, Fanconi anemia and ATM genes share the ability to compromise the pathways in Fig. 3 and to hinder complex repairs needed to remove DNA cross links. These mutations are all associated with leukemias, especially myeloid leukemias. This association is especially strong and very clear 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) [21]. A review of all biallelic BRCA2 mutation patients found a 79% cumulative probability of leukemia (primarily AML) by age 10 years [22].

Homozygous or biallelic mutations in BRCA1 are thought to be incompatible with life but heterozygous BRCA1 mutations are well known. Carriers of heterozygous mutations in either BRCA1 or BRCA2 have increased risks for myeloid leukemia. Table I summarizes risks for myeloid leukemia from a total of 16 studies of BRCA1/2 mutation carriers or individuals eligible for mutation testing [21-36]. Fifteen of the

16 studies reported elevated risks for leukemia or myeloid leukemia (Table I). These increased risks were noted despite the fact that individuals with multiple cancers are more likely to die and to be lost to follow-up. Some studies selected for survivors and most excluded individuals with myeloid leukemia if it occurred before breast cancer.

Test 2. Mutations in Fanconi anemia genes are associated with myeloid leukemia.

Complete inactivation of one of 13 Fanconi anemia genes is also well known. In Fanconi anemia patients, summary relative risks for AML were 703.3 [363.7–1354.5] [37]. The high incidence of AML in Fanconi anemia homozygotes agrees with evidence for deficiency of Fanconi proteins and their complexes in sporadic AML. This suggests that functional Fanconi proteins are essential to prevent AML [38]. A model of AML pictures loss of Fanconi protein A (encoded by a major Fanconi anemia gene product) as promoting cytogenetic instability and clonal progression in patients with initiated cells [39]. Mutations in this major Fanconi gene may contribute to the development of the disease in a subset of AML [40].

Xie et al suggested the ‘Fanconi anemia pathway’ helps prevent AML in non-Fanconi individuals [41]. A significant proportion of general AML has a disturbance of the ‘Fanconi pathway’ that may represent an early event in the development of this type of leukemia. Lymphocytes from carriers of a Fanconi anemia C frame shift mutation show greater than normal sensitivity to mutagens, but do not get blocked in G2 phase as in full Fanconi Anemia [42]. Baseline DNA damage in leukocytes of patients and heterozygotes was higher than in controls [43]. Fanconi anemia protein J is a helicase and heterozygous mutation is dominant negative because it prevents the protein from unwinding DNA [44]. Several early epidemiologic studies in probable Fanconi heterozygotes reported increased risks for leukemias and multiple cancers [45].

Test 2. Mutations in the ATM gene (Fig. 3) may increase risks for myeloid leukemias.

In addition to requiring BRCA and Fanconi proteins, complex DNA damage (and replication fork stalling) also activate ATM and other kinases. In 1976 it was widely recognized that ataxia telangiectasia patients (bi-allelic ATM mutation) are very likely to develop leukemias. ATM modulates the loading of recombinational repair proteins onto translocation breakpoint hotspots. This protects against inappropriate recombination and translocations characteristic of myeloid leukemias [45].

Among 56 patients with A-T, Olsen et al observed six cases of cancer (four leukemias and two

non-Hodgkin's lymphomas) compared with 0.16 expected, yielding a standardized incidence ratio of 37 (95% confidence interval = 13 to 80)[46]. Hematologic malignancies that caused premature deaths in blood relatives from 27 families of ataxia-telangiectasia patients included acute myeloid leukemia and monocytic leukemia. Five deaths from leukemias and lymphomas occurred below age 45 in comparison to 1 death expected [47]. There are also isolated reports of AML associated with ATM mutation.

Test 3. Formaldehyde causes some DNA damage that requires the pathway in Fig. 3.

Many studies show that carriers of mutations in BRCA1/2 dependent pathways impair the ability to repair damage, specifically damage from DNA cross-linking agents. Laboratory experiments with cells show that one of these cross linking agents is formaldehyde or formalin in aqueous solution. Repairing complex DNA damage caused by formaldehyde requires repairs mediated by BRCA1, BRCA2 (Fanconi protein D1), and other Fanconi proteins (Fig. 3) [16]. DNA-protein cross-links exhibit a dose-response relationship to formaldehyde exposure in the respiratory tract of laboratory animals and are observed at exposure concentrations relevant to human exposures (0.3 ppm, 0.7 ppm, Fig. 1) Shaham et al. measured the formation of DNA-protein cross-links in peripheral white blood cells of occupationally exposed workers (n=12) and unexposed controls (n=8). Formaldehyde concentrations were 2.8–3.1 ppm during peak work times and an average concentration of 1.46 ppm at times when work was usually completed [Fig. 1]. Exposure to formaldehyde resulted in a significant increase in the incidence of DNA-protein cross-links. A linear relationship between years of exposure and DNA-protein cross links formation was also detected [48].

Other potential mechanisms of carcinogenesis by formaldehyde involve collateral damage. These mechanisms include increased cell proliferation (which increases the probability that damaged cells will multiply and acquire further errors), bone marrow toxicity and immunosuppression (which can reactivate latent viral infections), and inflammation (which can produce cross links due to oxidative damage). BRCA1/2 related pathways participate in the prevention and repair of these kinds of collateral DNA damage as well.

Test 4. Pharynx associated cancers in mutation carriers. The IARC implicated formaldehyde in very rare nasopharyngeal and sinonasal cancers [3,13]. Computational models predict approximately 90% of inhaled formaldehyde is absorbed in the nose at resting inspiration. As the inspiratory rate increases, this percentage drops to about 58% during heavy exercise [50]. Over half the airflow then be-

comes oral so the mouth and pharynx, are also among the first structures exposed.

The annual incidence of nasal tumors in the United States is estimated to be less than 1 in 100,000 people per year with females having half the incidence of males. Rare outcomes are difficult to study due to a lack of statistical power to identify risks. In Table 1, most studies report some increase in risks for cancers within the oral cavity or upper respiratory tract. Cohort studies that included typed mutation carriers are consistent with elevated risks among BRCA1 or BRCA2 heterozygous carriers (Table 1). Exceedingly rare nasal sinus cancer occurred among 11847 members of BRCA1 families [35]. Two “nose cancers” were found among 3728 BRCA2 family members [34]. One of two buccal cavity cancers in 1160 relatives of A-T patients was nasopharyngeal cancer in the 6-year-old brother of an ataxia-telangiectasia patient; this was the only juvenile cancer in a relative [49]. Human papilloma virus infection has also been implicated in oropharyngeal cancer, a site where nasal sinus cancer originates. EBV infection has been linked to nasopharyngeal cancer. In addition to causing cancer itself, formaldehyde might facilitate viral infections because formaldehyde may impair both the epithelial barrier and immunity in the structures that receive the first exposure.

Test 4, Test 1. Different incidences of cancers linked to formaldehyde in different environments. The distinct geographic variation in the global incidence of nasopharyngeal carcinoma reflects a complex etiology of several subtypes involving not only environmental, but also viral (Epstein Barr virus), and genetic components as first or second hits. The high to intermediate rates observed in endemic areas contrast markedly with the uniformly rare rates seen in most of the world [51]. There are wide differences in the amount of formaldehyde in the air of different environments but this cannot now be separated from genetic or infectious influences. Leukemia rates also differ substantially for different racial and ethnic groups that live among different levels of environmental formaldehyde. Leukemia incidence is highest among whites (12.9 per 100,000) and lowest among American Indians/Alaskan natives (6.5 per 100,000), Asian and Pacific Islander populations (7.2 per 100,000). Cancer incidence in mutation carriers with high level formaldehyde exposure should be compared to cancer incidence in mutation carriers with low level formaldehyde exposure.

Test 5. Comparisons of chromosome abnormalities. Certain cytogenetic abnormalities are commonly seen in Fanconi anemia patients with AML and myelodysplastic syndrome, including monosomy 7 and monosomy 5. These clonal abnormalities are more often found in AML that develops from sporadic myelodysplastic syndrome after treatment with chemical alkylating agents. Zhang et al. [1] examined formaldehyde associated loss of chromosome 7 because it is one of the most frequent cytogenetic changes observed in myeloid leukemia. This cytogenetic change is also affected by exposure to the established human leukemogen benzene[1]. Thus, formaldehyde exposure was associated with increased levels of specific chromosome aberrations related to myeloid leukemia in the stem/progenitor cells that may be sites where leukemia originates.

Test 5. Formaldehyde and breast cancer chromosomes. Greater numbers of DNA protein cross-links were found in breast cancer patients than in a matched control group [52] and formaldehyde has been positively associated with breast cancer risk [53]. It is well known that BRCA1/2 related pathways help protect against chromosome losses. 81% of BRCA1 breast tumors lose chromosome 4 [9]. The human alcohol dehydrogenase genes essential for formaldehyde detoxification (Fig. 2) are found in a cluster on chromosome 4, so loss of chromosome 4 might accelerate formation of DNA cross links and mutations in breast tumors. Similarly 40% of BRCA1 associated breast cancers have lost chromosome 12q [9] which contains the ALDH2 gene (Fig. 2). These losses were significantly more common than in control tumors. These losses remove the main protective metabolic pathways (Fig. 2) from latent or occult breast tumors so that formaldehyde then accelerates their growth.

Losses of genetic material from chromosome 5 and chromosome 7 common in myeloid leukemias are also associated with hereditary breast cancers. 86% of BRCA1 associated breast tumors showed loss of chromosome 5q [9] and 27% (4/15) BRCA2 breast cancers had lost a marker on chromosome 7 [54]. These observations suggest there may be some relationship between pathogenic mechanisms in leukemias and hereditary breast cancers.

Implications of the hypothesis. It should be stressed that the above evidence is not incontrovertible proof that formaldehyde exposure contributes to excess cancer risks in mutation carriers. If the hypothesis is true, it means that a change in lifestyle by taking greater care in avoiding specific mutagens can reduce cancer risk in this highly susceptible population. This population otherwise has very limited options.

Recently, the President's Cancer Panel [2008-2009] protested that preventive action is not taken when uncertainty exists about potential harm from a chemical, because the US regulatory approach demands that a hazard be incontrovertibly demonstrated. It is now incontrovertible that formaldehyde increases risks for leukemias. Evidence is also strong that formaldehyde causes some types of DNA damage in humans that are known to require repairs mediated by BRCA1/2 containing pathways which also require, Fanconi and ATM proteins.

Conclusions. Formaldehyde is a pervasive environmental carcinogen that is theoretically more likely to cause malignancy in carriers of mutations that disable protective repair pathways. Because of this potential for harm, it is prudent for those with mutations that affect pathways mediated by BRCA1/2, Fanconi and ATM proteins to immediately avoid high level exposure to formaldehyde. The EPA recommends four basic "Steps to Reduce Exposure" for everyone.

- Use "exterior-grade" pressed wood products (lower-emitting because they contain phenol resins, not urea resins).
- Use air conditioning and dehumidifiers to maintain moderate temperature and reduce humidity levels.
- Increase ventilation, particularly after bringing new sources of formaldehyde into the home.
- Ask about the formaldehyde content of pressed wood products, including building materials, cabinetry, and furniture before you purchase them.

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References

1. 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. Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. *Cancer Epidemiol Biomarkers Prev*. 2010; 19(1):80-8.
2. Agency for Toxic Substances and Disease Registry, Division of Toxicology and Environmental Medicine, Atlanta, GA 30333. 2010. Addendum to the Toxicological Profile for Formaldehyde.
3. Baan R, Grosse Y, Straif K, Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Coglian V; WHO International Agency for Research on Cancer Monograph Working Group. A review of human carcinogens--Part F: chemical agents and related occupations. *Lancet Oncol*. 2009; 10(12):1143-4.
4. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. July 1999. Toxicological profile for Formaldehyde,
5. Lu K, Ye W, Zhou L, Collins LB, Chen X, Gold A, Ball LM, Swenberg JA. Structural characterization of formaldehyde-induced cross-links between amino acids and deoxynucleosides and their oligomers. *J Am Chem Soc*. 2010; 132, 3388–3399
6. Schmiedeberg L, Skene P, Deaton A, Bird A. A Temporal Threshold for Formaldehyde Crosslinking and Fixation. *PLoS ONE* 2009; 4(2): e4636
7. Thompson C, Sonawane B, Grafstrom R. The Ontogeny, Distribution, and Regulation of Alcohol Dehydrogenase 3: Implications for Pulmonary Physiology. *Drug metabolism and disposition*. 2009; 37:1565–1571.
8. Teng S, Beard K, Pourahmad, J, et al. The formaldehyde metabolic detoxification enzyme systems and molecular cytotoxic mechanism in isolated rat hepatocytes. *Chem Biol Interact* 2001; 130–132:285–296.
9. Tirkkonen M, Johannsson O, Agnarsson B, Olsson H, et al. Distinct Somatic Genetic Changes Associated with Tumor Progression in Carriers of BRCA1 and BRCA2 Germ-line Mutations. *Cancer Research* 1997; 57, 1222-1227.
10. Hedberg, JJ; Backlund, M; Stromberg, P; et al. Functional polymorphism in the alcohol dehydrogenase 3 (ADH3) promoter. *Pharmacogenetics* 2001; 11:815–824.
11. Lu K, Moeller B, Doyle-Eisele M, McDonald J, Swenberg JA. Molecular Dosimetry of N(2)-Hydroxymethyl-dG DNA Adducts in Rats Exposed to Formaldehyde. *Chem Res Toxicol*. Feb 2011; 18; 24(2):159-61.
12. Hauptmann M, Stewart PA, Lubin JH, Beane Freeman LE, Hornung RW, Herrick RF, Hoover RN, Fraumeni JF Jr, Blair A, Hayes RB. Mortality from lymphohematopoietic malignancies and brain cancer among

embalmers exposed to formaldehyde. *J Natl Cancer Inst.* 2009; 101(24):1696-708

13. National Toxicology Program. Final Report on Carcinogens. Background Document for Formaldehyde. *Rep Carcinog Backgr Doc.* 2010; (10-5981):i-512.
14. Bei JX, Li Y, Jia WH, Feng BJ, Zhou G, Chen LZ, Feng QS, Low HQ, Zhang H, He F, Tai ES, Kang T, Liu ET, Liu J, Zeng YX. A genome-wide association study of nasopharyngeal carcinoma identifies three new susceptibility loci. *Nat. Genet.* 2010; 42(7):599-603.
15. Friedenson B. A theory that explains the tissue specificity of BRCA1/2 related and other hereditary cancers. *J. Med. Med. Sci.* 2010; 1(8): 372-384
16. 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, Tsuchi H, Ide H. Homologous recombination but not nucleotide excision repair plays a pivotal role in tolerance of DNA-protein cross-links in mammalian cells. *J Biol Chem.* 2009; 284(40):27065-76.
17. D'Andrea AD, Grompe M. The Fanconi anaemia/BRCA pathway. *Nat Rev Cancer.* 2003 Jan; 3(1):23-34.
18. 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>.
19. Friedenson B., Inflammatory processes inordinately increase tissue specific cancer risks in carriers of mutations in BRCA1, BRCA2, ATM or Fanconi anemia genes. *J. Med. Med. Sci.* 2010; 1(8): 356-371
20. 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. Variation of Breast Cancer Risk Among BRCA1/2 Carriers *JAMA.* 2008; 299(2):194-201
21. Wagner JE, Tolar J, Levrant O, Scholl T, Deffenbaugh A, Satagopan J, Ben-Porat L, Mah K, Batish SD, Kutler DI, MacMillan ML, Hanenberg H, Auerbach AD. Germline mutations in BRCA2: shared genetic susceptibility to breast cancer, early onset leukemia, and Fanconi anemia. *Blood* 2004; 103(8):3226-9.
22. Alter B, Rosenberg P, Brody L. Clinical and molecular features associated with biallelic mutations in FANCD1/BRCA2. *J Med Genet* 2007; 44:1-9.
23. Harvey EB and Brinton LA. Second cancer following cancer of the breast in Connecticut, 1935-1982. *Natl Cancer Inst Monogr* 1985; 68, 99-109
24. Møller M, Friis S, Olsen JH, Scélo G, Hemminki K, Tracey E, Andersen A, Brewster DH, Pukkala E, McBride ML, Kliever EV, Tonita JM, Kee-Seng C, Pompe-Kirn V, Martos C, Jonasson JG, Boffetta P, Brennan P. Risk of second cancer among women with breast cancer. *Int J Cancer* 2006; 118(9):2285-92.
25. Riska A, Pukkala E, Scélo G, Møller M, Hemminki K, Weiderpass E, McBride ML, Pompe-Kirn V, Tracey E, Brewster DH, Kliever EV, Tonita JM, Kee-Seng C, Jonasson JG, Martos C, Boffetta P, Bren-

- nan P. Second primary malignancies in females with primary fallopian tube cancer. *Int J Cancer* 2007; 120(9):2047-51.
26. Evans H., Lewis, C., Robinson, D. et al. Cancer risks in women with 2 breast or ovarian cancers: clues to genetic cancer susceptibility. *Int J. Cancer* 2001; 94: 758-9
 27. Evans H, Lewis C, Robinson D, Bell C, Moller H, Hodgson S. Incidence of multiple primary cancers in a cohort of women diagnosed with breast cancer in southeast England. *Brit J Cancer* 2001; 84: 435-440
 28. Hemminki K, Scélo G, Boffetta P, Møller H, Tracey E, Andersen A, Brewster DH, Pukkala E, McBride M, Kliever EV, Chia KS, Pompe-Kirn V, Martos C, Jonasson JG, Li X, Brennan P. Second primary malignancies in patients with male breast cancer. *Brit. J. Cancer* 2005; 92 : 1288-92.
 29. Teppo L, Pukkala E and Saxen E. Multiple cancer – an epidemiological exercise in Finland. *J Natl Cancer Inst* 1985; 75: 207–217.
 30. Risch HA, McLaughlin JR, Cole DE, Rosen B, Bradley L, Fan I, Tang J, Li S, Zhang S, Shaw PA, Narod SA. Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. *J Natl Cancer Inst.* 2006; 98(23):1694-706.
 31. Risch H, McLaughlin J, Cole D, Rosen B et al. Prevalence of Germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. *Am J Hum Genet* 2001; 68, 700-710.
 32. Ewertz, M. & Mouridsen, H. Second cancer following cancer of the female breast in Denmark, 1943-80. *Natl Cancer Inst Monogr.* 1985; 88:325-9
 33. Shih H, Nathanson K, Seal S, Collins N, Stratton M, Rebbeck T and Weber B. BRCA1 and BRCA2 mutations in breast cancer families with multiple primary cancers. *Clin Cancer Res* 2000; 6, 4259-64.
 34. The Breast Cancer Linkage Consortium. Cancer risks in BRCA2 mutation carriers. *J. Natl Cancer Inst.* 1999; 91: 1310-1316.
 35. Thompson D, Easton DF; Breast Cancer Linkage Consortium. Cancer Incidence in BRCA1 mutation carriers. *J Natl Cancer Inst.* 2002; 94(18):1358-65
 36. 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 Houtwelingen JC, van't Veer LJ, Rookus MA, van Leeuwen FE; Netherlands Collaborative Group on Hereditary Breast Cancer (HEBON). Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. *J Med Genet.* 2005; 42(9):711-9.
 37. Friedenson B. The BRCA1/2 pathway prevents hematologic cancers in addition to breast and ovarian cancers. *BMC Cancer* 2007; 7:152.
 38. Tischkowitz MD, Morgan NV, Grimwade D, Eddy C, Ball S, Vorechovsky I, Langabeer S, Stöger R, Hodgson SV, Mathew CG. Deletion and reduced expression of the Fanconi anemia FANCA gene in sporadic acute myeloid leukemia. *Leukemia* 2004; 18(3):420-5.
 39. Lensch MW, Tischkowitz M, Christianson TA, Reifsteck CA, Speckhart SA, Jakobs PM, O'Dwyer ME, Ol-

- son SB, Le Beau MM, Hodgson SV, Mathew CG, Larson RA, Bagby GC Jr. Acquired FANCA dysfunction and cytogenetic instability in adult acute myelogenous leukemia. *Blood* 2003;102(1):7-16.
40. Condie A, Powles RL, Hudson CD, Shepherd V, Bevan S, Yuille MR, Houlston RS. Analysis of the Fanconi anaemia complementation group A gene in acute myeloid leukaemia. *Leuk Lymphoma*. 2002;43(9):1849-53.
 41. Xie Y, de Winter JP, Waisfisz Q, Nieuwint AW, Scheper RJ, Arwert F, Hoatlin ME, Ossenkoppele GJ, Schuurhuis GJ, Joenje H. Aberrant Fanconi anaemia protein profiles in acute myeloid leukaemia cells. *Br J Haematol*. 2000; 111(4):1057-64.
 42. 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*. 2000; 212(4):174-6.
 43. Mohseni Meybodi A, Mozdarani H. 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*. 2009; 3(1):1-8.
 44. Wu Y, Brosh RM Jr. Helicase-inactivating mutations as a basis for dominant negative phenotypes. *Cell Cycle*. 2010;9(20):4080-90.
 45. Sun J, Oma Y, Harata M, Kono K, Shima H, Kinomura A, Ikura T, Suzuki H, Mizutani S, Kanaar R, Tashiro S. ATM modulates the loading of recombination proteins onto a chromosomal translocation breakpoint hotspot. *PLoS One*. 2010 Oct 27;5(10):e13554
 46. Olsen JH, Hahnemann JM, Børresen-Dale AL, Brøndum-Nielsen K, Hammarström L, Kleinerman R, Kääriäinen H, Lönnqvist T, Sankila R, Seersholm N, Tretli S, Yuen J, Boice JD Jr, Tucker M. Cancer in patients with ataxia-telangiectasia and in their relatives in the nordic countries. *J Natl Cancer Inst*. 2001; 93(2):121-7.
 47. Swift M, Sholman L, Perry M, Chase C. Malignant neoplasms in the families of patients with ataxia-telangiectasia. *Cancer Res*. 1976; 36(1):209-15.
 48. Shaham J, Bomstein Y, Meltzer A, Kaufman Z, Palma E, Ribak J. DNA--protein crosslinks, a biomarker of exposure to formaldehyde--in vitro and in vivo studies. *Carcinogenesis*. 1996; 17(1):121-5.
 49. Thompson D, Duedal S, Kirner J, McGuffog L, Last, Reiman J, Byrd P, Taylor M, Easton D. Cancer Risks and Mortality in Heterozygous ATM Mutation Carriers *J Natl Cancer Inst* 2005; 97: 813-822,
 50. Kimbell, JS; Overton, JH; Subramaniam, RP; et al. Dosimetry modeling of inhaled formaldehyde: binning nasal flux predictions for quantitative risk assessment. *Toxicol Sci* 2001; 64:111-121.
 51. Bray F, Haugen M, Moger TA, Tretli S, Aalen OO, Grotmol T. Age-incidence curves of nasopharyngeal carcinoma worldwide: bimodality in low-risk populations and aetiological implications. *Cancer Epidemiol Biomarkers Prev*. 2008;17(9):2356-65.

52. Wu FY, Lee YJ, Chen DR, Kuo HW. Association of DNA-protein crosslinks and breast cancer. *Mutat Res.* 2002; 501(1-2):69-78.
53. Coyle YM, Hynan LS, Euhus DM, Minhajuddin AT. An ecological study of the association of environmental chemicals on breast cancer incidence in Texas. *Breast Cancer Res Treat.* 2005; 92(2):107-14.
54. Eiriksdottir G, Barkardottir RB, Agnarsson BA, Johannesdottir G, Olafsdottir K, Egilsson V, Ingvarsson S. High incidence of loss of heterozygosity at chromosome 17p13 in breast tumours from BRCA2 mutation carriers. *Oncogene.* 1998; 16(1):21-6.