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Stem Cell and Benzene-Induced Malignancy and Hematotoxicity

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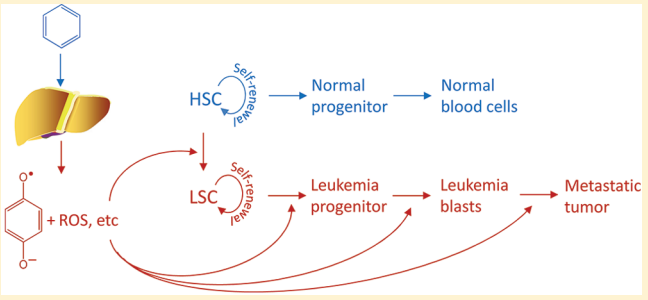
ABSTRACT: The biological effect of benzene on the hematopoietic system has been known for over a century. The rapid advancement in understanding the biology of hematopoietic stem cells (HSCs) and cancer stem cells (CSCs) in recent years has renewed interest in investigating the role of stem cells in benzene-induced malignancy and bone marrow depression. The interplay between benzene and stem cells is complex involving the stem cell, progenitor, and HSC niche compartments of the bone marrow. In this prospect, benzene metabolites formed through metabolism in the liver and bone marrow cause damage in hematopoietic cells via multiple mechanisms that, in addition to traditionally recognized chromosomal aberration and covalent binding, incorporate oxidative stress, alteration of gene expression, apoptosis, error-prone DNA repair, epigenetic regulation, and disruption of tumor surveillance. However, benzene-exposed individuals exhibit variable susceptibility to benzene effect that arises, in part, from genetic variations in benzene metabolism, DNA repair, genomic stability, and immune function. These new studies of benzene leukemogenesis and hematotoxicity are expected to provide insights into how environmental and occupational chemicals affect stem cells to cause cancer and toxicity, which impact the risk assessment, permissible level, and therapy of benzene exposure.



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1. INTRODUCTION

Benzene (C_6H_6), a simple, single-ringed, and volatile aromatic hydrocarbon, is an established human leukemogen and hematotoxicant.[1−3] Benzene is commonly used in industries worldwide as a general purpose solvent or as a starting material for the synthesis of other chemicals, such as plastics and polymers, detergents, pesticides, rubbers, dyes, drugs, and explosives. Benzene occurs naturally in petroleum. In the US, benzene is ranked among the top 20 chemicals for production

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volume.[3] Occupational exposure to benzene takes place in the rubber industry, oil refineries, oil shipping, coke and chemical plants, and shoe making and gasoline-related professions. The general population is exposed to benzene from cigarette smoke, gasoline vapors, automobile exhaust, and benzene-contaminated water and soil. The current occupational exposure standard in the US by OSHA (Occupational Safety and Health Administration) is 1 ppm (8-h of a 40-h week total weight average permissible exposure limit, TWA). However, exposure to potentially high levels of benzene continues to exist in both developing and developed countries.[4] Moreover, the possibility that exposure to benzene at or below 1 ppm may still cause toxicity to the hematopoietic system is an ongoing concern.[5]

Chronic exposure to benzene typically causes bone marrow depression that often initially displays clinically decreased peripheral blood cell counts (anemia, leukopenia, and thrombocytopenia), but may manifest pancytopenia, aplastic anemia, myelodysplasia, or myelogenous leukemia.[6,7] The mechanism by which benzene induces hematopoietic malignancy and toxicity remains elusive. Because benzene specifically suppresses hematopoietic functions and causes leukemia, it has long been suspected to have an effect on hematopoietic stem cells (HSCs). However, solid evidence supporting the notion has been lacking. The rapid development in the fields of HSC and cancer stem cell (CSC, or LSC in the case of leukemia) in recent years has spurred the interest of investigating the role of HSC and LSC in benzene hematotoxicity and carcinogenesis. Indeed, emerging evidence has provided new insights into the interplay between benzene and stem cell in the hematopoietic system that potentially underlies the adverse effects of benzene. In this review, we examine recent progress in the understanding of the mechanism of benzene leukemogenesis and hematotoxicity with a focus on the role of stem cell.

2. STEM CELL, HEMATOPOIESIS, AND LEUKEMIA

2.1. HSC and Bone Marrow Failure. The hematopoietic system is responsible for the development of all blood cells that, in turn, control the constant maintenance and immune protection of every cell type of the body. This relentless work requires that the hematopoietic cells have a great power of self-renewal and differentiation, which are now recognized as the hallmarks of stem cells (Figure 1).

The stem cells that form blood and immune cells are HSCs.[8] HSCs consist of long-term stem cells that are capable of self-renewal for the lifespan of an organism, and short-term stem cells that regenerate different types of blood cells but cannot renew themselves over a long-term. HSCs differentiate into committed multipotent, oligopotent, and unipotent progenitors that have limited capacities to differentiate into various types of blood cells. The "lymphoid" line progenitors form lymphocytes, whereas the "myeloid" lineage gives rise to granulocytes, monocytes, megakaryocytes, and erythrocytes. Among the myeloid cells, granulocytes and monocytes come from a common precursor, the "CFU-GM" (colony forming unit-granulocyte/macrophage progenitor) cell.

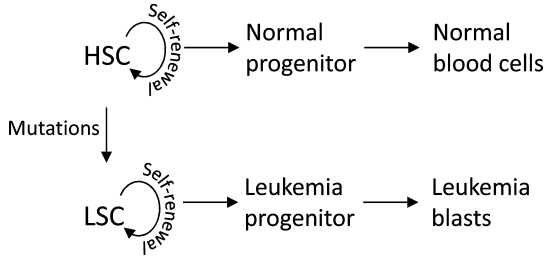

Figure 1. Simplified CSC model for leukemia. A HSC (hematopoietic stem cell) gives rise to normal progenitors and mature blood cells. HSC undergoes oncogenic mutations to form LSC (leukemia stem cell). LSC retains some degree of developmental potential giving rise to leukemia progenitor and blast cells. Both HSC and LSC maintain their ability to undergo self-renewal.

The microenvironment of HSCs also plays an important role in hematopoiesis. The bone marrow is an ordered environment with HSCs being in close proximity to stromal cells that are supportive to HSCs. Mature B and T cells are present and may have significant effects on the stem cell compartment. The differentiating myeloid precursors are rich in myeloperoxidase (MPO) that can promote oxidative stress in the bone marrow wherein a rich supply of blood and oxygen is provided.

Bone marrow failure, such as MDS (myelodysplastic syndromes), is a disorder of HSCs or later stage precursor cells. In MDS, hematopoiesis is disorderly and inefficient resulting in cytopenias in the blood and, ultimately, aplastic anemia. One third of the patients with MDS develop acute myelogenous leukemia (AML) within months to a few years.

2.2. CSC and Leukemia. The cancer stem cell paradigm denotes that a subpopulation of cancer cells have the ability to initiate tumorigenesis by undergoing self-renewal and differentiation, much like normal stem cells, whereas the remaining majority of the cancer cells are "differentiated" and do not have these properties [9] (Figure 1). The CSC concept originated from a study on leukemia, where human acute myeloid leukemia cells were found to be organized as a hierarchy that stems from a primitive hematopoietic cell, that is, LSC.[10] The study used fluorescence-activated cell sorting (FACS) to isolate primary leukemia cells with antibodies directed against defined cell-surface markers; this was followed by limiting dilution transplantation into an orthotopic site in immunocompromised mice to induce tumors of the same type. This approach was soon adopted for solid tumors, wherein a similar model with CSCs at the top of a hierarchical pyramid of tumor cells was unveiled.[11]

One major challenge in the study of CSCs concerns the cell of origin of CSCs. The similar cell surface phenotypes of LSC and HSC supported the notion that primitive cells are the cell of origin for AML; however, this does not necessarily mean that LSCs derive from HSCs that have become cancerous.[9] In the multistep leukemogenesis, the concept of "preleukemic stem cells" (PLSCs) was suggested, in which an initial oncogenic event may occur in a primitive stem cell, but subsequent events take place in the committed progenitor pool giving rise to LSCs. Tumor cells undergo "epithelial-to-mesenchymal transition" (EMT) to disseminate from the primary tumor mass and the reverse process "mesenchymal-to-epithelial transition" (MET) to colonize at remote sites. During EMT and MET, tumor cells acquire a stem-like phenotype upon stimulation with appropriate environmental cues and become "migratory cancer stem cells" (mCSCs).[12] mCSCs may be responsible for tumor invasion, metastasis, and even drug resistance. Last but not least, CSCs isolated from both leukemia and solid tumors can vary widely from tumor to tumor in their relative frequencies and properties. The variation is a function of both tumor type and specific experimental system used, suggesting that both intrinsic factors, such as the oncogenes activated in CSCs, and extrinsic signals, such as the micro-

environmental cues, influence CSCs. Thus, as more tumors are examined, the CSC paradigm has evolved and become more complex.[13]

Studies on AML blasts have provided some insights into the molecular lesions that may underlie the mechanism by which HSCs develop into LSCs. These include balanced translocations, interstitial deletions, mutations, and DNA methylation.[14,15] Some AML are characterized by balanced translocations. In these cases, translocation is necessary for the development of leukemia, and the rate of acquisition is relatively constant in a life span. Some AML are marked by deletions (5q7q-). This type of leukemia requires multiple genetic hits, increases with age, and is often associated with environmental exposures.[16] Balanced translocations lead to deregulation of a number of oncogenes, among which disruption of the CBFβ/AML 1 complex is well characterized and is shown to interfere with differentiation of HSCs; examples include TEL/AML 1 in t(12;21), EVI 1/AML 1 in t(3;21), and AML 1/ETO in t(8;21). The inversion leads to the generation of a CBFβ/MYH 11 gene, a binding partner of AML 1, which acts as a dominant negative inhibitor of transcription. Translocations also alter the chromatin status at the binding site of the complex indicating epigenetic mechanisms in the control of gene expression in AML. Hypermethylation is observed for some genes: up to 80% of AML cases for P15, 5% for MGMT (*O*-6-methylguanine-DNA methyltransferase), and 28% for cadherin.[17] Mutations of C/EBPα are found to be frequent in AML and may affect proliferation and differentiation of AML cells. Another frequent mutation, mutation and internal tandem duplication of the flt-3 tyrosine kinase, is associated with AML progression with poor prognosis.[18]

As discussed below, environmental factors, such as exposure to benzene, radiation, and cancer chemotherapy, can affect all components of the bone marrow, including stem cells (HSCs and CSCs), progenitors, and stromal cells through both genetic and nongenetic means to cause hematopoietic dysplasia and malignancy.

3. BENZENE-INDUCED LEUKEMIA, OTHER MALIGNANCIES, AND HEMATOTOXICITY

3.1. Early Observations. The recognition of the adverse effect of benzene on the hematopoietic system dated back more than a century ago, when Santesson reported that four female workers exposed to benzene solvent in a tire factory suffered from "purpura haemorrhagica" caused by a severe defect in blood clotting.[19] In the US, the hematotoxic effect of benzene in humans was first documented by Selling who described a few cases of chronic benzene poisoning from a can factory where rubber dissolved in benzene was used as a sealing fluid.[20] Benzene toxicity to hematopoietic organs was subsequently demonstrated in experimental animals.[21,22] However, it was through the efforts of Alice Hamilton, a pioneer in occupational medicine, that the medical field was alerted about the health hazard of chronic exposure to benzene in workers.[23−25]

Association of benzene exposure with leukemia was first noted in 1928.[26] Since then, many reports linking benzene with hematopoietic malignancy have been made. The studies by Vigliani et al.[27] and by Aksoy et al.[6,28] in the 1960s and 1970s, which demonstrated multiple cases of leukemia and hematotoxic effects among shoe-making workers in Italy and Turkey, confirmed that benzene is a bone marrow carcinogen that could cause one or more types of leukemia as well cytopenias and aplastic anemia.

3.2. Quantitative Study in Human Populations. Epidemiological studies provided quantitative estimates of leukemia risk from benzene exposure, which greatly facilitated both the etiological understanding of benzene hematopoietic effects and regulation of industrial benzene exposure. The Pliofilm study reported by Infante et al. first demonstrated a statistically significant increase in leukemia incidence among workers in the US rubber industry.[29] The retrospective cohort revealed that workers exposed occupationally to benzene between 1940 and 1949 had a 5-fold increased risk of all leukemias and a 10-fold increase of deaths from myeloid and monocytic leukemias combined in comparison with controls. Subsequent follow-up of the cohort reconfirmed the carcinogenic effects of benzene exposure.[30,31] These studies played a major role in lowering the benzene permissible exposure level from 10 to 1 ppm (TWA) by US OSHA.

More powerful studies involving large numbers of workers exposed to benzene or benzene-containing mixtures in China were conducted by Yin et al. from the Chinese Academy of Preventive Medicine (CAPM)[32,33] in collaboration with a team from the US National Cancer Institute (NCI).[34,35] The NCI-CAPM studies confirmed increased risk of AML as well as other malignant and nonmalignant hematopoietic disorders, that is, non-Hodgkin lymphoma (NHL) and MDS. Moreover, the studies revealed excess risk at relatively low levels of benzene exposure (<10 ppm average and <40 ppm-years cumulative).

A nested case-control study was conducted in Australia in which 79 cases were each age-matched with 5 control subjects, and benzene exposure was estimated using occupational history, local site information, and the Australian petroleum industry-monitoring data.[36−38] The study found increased risks of leukemia associated with cumulative benzene exposure lower than previously reported; for instance, a cumulative exposure of >8 ppm-years increased the risk for ANLL (acute non-lymphocytic leukemia) by 7-fold. The study also indicated that there is no evidence of a threshold of cumulative exposure, below which there is no risk from benzene exposure.

3.3. Other Forms of Malignancy. The above and many other epidemiological studies demonstrated consistently that exposure to benzene causes AML/ANLL and MDS, even at relatively low doses; in many cases, MDS precedes AML. Other forms of malignancy, such as lymphoproliferative neoplasia, were also increasingly associated with chronic exposure to benzene. However, the diversity of this group and the rarity of some forms of the tumors have made it difficult to assess the risk in epidemiological studies.[39]

The study by Glass et al. identified an association of benzene exposure with CLL (chronic lymphocytic leukemia),[36] which is now classified as a form of NHL. The NCI-CAPM study found a 3-fold increase in risk for NHL among the Chinese benzene-exposed workers, with risk rising to 4-fold for workers with 10 or more years of benzene exposure.[40] Meta-analyses of studies on NHL and benzene exposure in industries other than refineries (a refinery was typically associated with benzene exposure in the past) and studies on NHL and refineries, confirmed that both benzene exposure and refinery work were associated with increased risks of NHL.[41] A few studies have linked multiple myeloma with benzene exposure.[31,42−44] Experimental animal studies demonstrated a causal relationship between benzene and lymphomas. In recent years, an

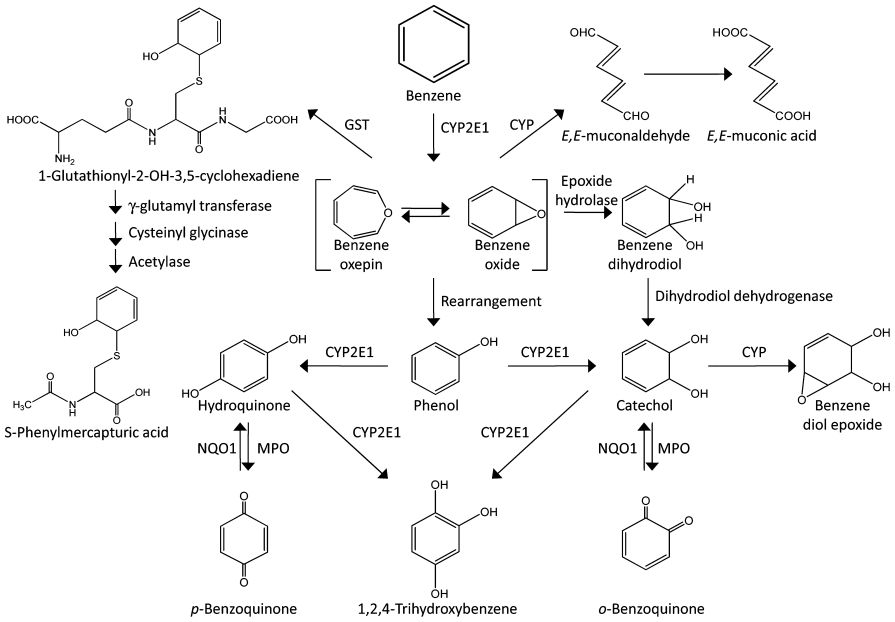

Figure 2. Summary of benzene metabolism. CYP, cytochrome P450; GST, glutathione *S*-transferase; MPO, myeloperoxidase; NQO1, NAD(P)H:quinone oxidoreductase 1.

association of childhood leukemia with air pollution sources, such as gas stations, traffic, and automobile repair garages that emit benzene, was suggested.[45,46] The finding that childhood ALL and AML are probably initiated in utero supports the notion that exposure of the mother and/or father to benzene is also important in the development of childhood AML and ALL from benzene.[47,48] Animal studies revealed that in utero exposure to benzene indeed increased micronuclei frequency and DNA recombination events in fetal and postnatal hematopoietic tissues.[49,50] Because all hematopoietic malignancies arise from damaged stem cells, some researchers believed that all kinds of myeloid and lymphoid malignancies, including their prestages, can be caused by occupational exposure to benzene.[51]

4. STEM CELL AS A CENTRAL TARGET FOR BENZENE HEMATOPOIETIC EFFECT

4.1. Benzene- versus Cancer Therapy-Induced AML and MDS. AML and MDS are closely related diseases of the bone marrow: both arising from $CD34^+$ hematopoietic stem or progenitor cells, both characterized with recurrent chromosomal aberrations, and both occurring de novo or as a result of exposure to benzene and cancer therapeutic drugs. AML associated with exposures is classified as "secondary" AML (i.e., the third group from classification in conformity with World Health Organization recommendation, http://atlasgeneticsoncology.org/Anomalies/ClassifAMLID1238.html) that includes both therapy-related (tAML) and environmental exposure-associated cases; in both scenarios, MDS often precedes AML.[52] tAML has two distinct phenotypes: those following topoisomerase (topo) II inhibitors are characterized with 11q23 chromosomal abnormalities and occur 2−3 years after exposure, whereas those occurring after alkylating agent treatment often have a prior MDS, require longer time to develop (5−8 years after exposure), and have interstitial deletions (5q7q-).[15,53,54] Benzene-induced AML is similar to alkylating agent-related AML in that (a) MDS or other hematotoxicity often precedes AML; (b) longer latency between exposure and leukemia may be necessary (4 months to 10 years); (c) metabolism is required; and (d) chromosomal changes include monosomy of chromosomes 5 and 7 together with interstitial deletions of chromosomes 5 and 7 as well as translocation into 21q22.[16,55,56] These findings strongly support a causative relationship between benzene-induced damage to HSCs (or progenitors) and benzene-induced AML.

4.2. Role of Metabolism. Benzene is an unreactive chemical due to its closed aromatic ring structure. Metabolism is required for benzene to become toxic and carcinogenic to the hematopoietic system.[57,58] The first step in benzene metabolism occurs in the liver, where cytochrome P450 (CYP, P450) catalyzes mono-oxygenation of benzene to benzene oxepin and benzene oxide, which are tautomers and exist in equilibrium (Figure 2). Most benzene oxide rearrange to phenol. CYP2E1 is the major P450 for the oxidation of benzene. These conclusions were supported by several lines of evidence: (a) partial hepatectomy reduces benzene metabolism and toxicity in rats; (b) knockout (KO) of CYP2E1 decreases benzene metabolism and toxicity; and (c) coadministration of toluene or phenol inhibits benzene metabolism competitively.[57]

Phenol can be further metabolized to hydroquinone (HQ), catechol, and 1,2,4-trihydroxybenzene via CYP2E1. Hydroquinone and catechol can be oxidized to *p*- or *o*-benzoquinone (BQ). Catechol can also be oxidized by P450 to benzene diol epoxide. Benzene oxide can be converted to dihydrodiol via microsomal epoxide hydrolase (mEH, EPHX1); it also reacts with glutathione to form 1-glutathionyl-2-OH-3,5-cyclohexadiene that is further metabolized to *S*-phenylmercapturic acid (SPMA) via sequential reactions catalyzed by γ-glutamyl transferase, cysteinyl glycinase, and acetylase. Formation of open ring products, including the reactive *E,E*-muconaldehyde and *E,E*-muconic acid (MA), is believed to occur via benzene oxepin. About one-third of exposed benzene is excreted in the urine as metabolites, the majority of which is phenol (70−85%), followed by HQ, MA, catechol (each 5−10%), and SPMA (<1%). Many of the metabolites in urine are sulfate or glucuronide conjugates.

Benzene oxide, *p*- and *o*-BQs, benzene diol epoxide, and open ring muconaldehyde are electrophiles that can interact with macromolecules and cause damage to cells. Presumably, a mechanism of transportation is necessary for the metabolites formed in the liver to reach the bone marrow and damage the hematopoietic cells. However, MPO is richly expressed in myeloid precursor cells in the bone marrow and oxidizes HQ to BQ (Figure 3).[59] BQ can be reduced to HQ through two one-

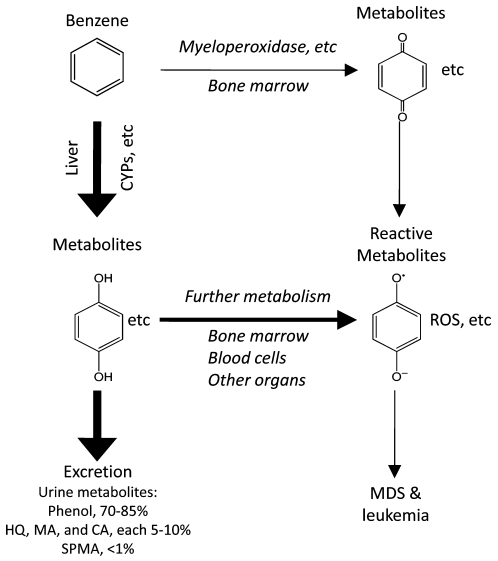

Figure 3. Metabolism of benzene in the liver and bone marrow. The *p*BQ semiquinone radical was used to represent reactive, toxic benzene metabolites. Arrow thickness indicates the relative importance of pathways.

electron reduction steps or one two-electron reduction reaction. A number of reductases, such as cytochrome P450 reductase, catalyze one-electron reduction reactions that involve the formation of an intermediate semiquinone radical; the semiquinone radical is unstable and cycles back to quinone forming a redox cyclic reaction accompanied by the production of a superoxide anion radical. Bone marrow stromal cells express NAD(P)H:quinone oxidoreductase 1 (NQO1) that catalyzes the obligatory two-electron reduction of BQ to HQ bypassing semiquinone radical formation.[60] Knockout of Nqo1 in mice caused myelogenous hyperplasia.[61] An NQO1 polymorphism (C609T) is associated with increased hematological malignancy in humans exposed to benzene.[62] These findings underscore the importance of further metabolism of benzene metabolites and the redox cycling of BQ and HQ in the bone marrow for benzene-induced hematopoietic effects.

4.3. Emerging AhR Connection. The aryl hydrocarbon receptor (AhR) is a xenobiotic-activated receptor and transcription factor of the basic region helix−loop−helix Per-Arnt-Sim-homology (bHLH-PAS) family.[63,64] AhR is known to mediate the toxic and adaptive responses to environmental contaminants including the carcinogenic polycyclic aromatic hydrocarbons (PAHs) and halogenated aromatic hydrocarbons (HAHs). The AhR action was best studied for induction of the CYP1 enzymes (CYP1A1, 1A2, and 1B1).[65] AhR is also activated by endogenous ligands, such as the tryptophan photoproduct 6-formylindolo[3,2-*b*]carbazole (FICZ), to mediate a range of endogenous functions under physiological and disease conditions.[66] AhR is expressed in HSCs, and considerable evidence supports the fact that AhR has an important function in the regulation of HSCs.[67] Treatment of mice with TCDD (2,3,7,8-tetrachlorodibenzo[p]dioxin), a stable high affinity HAH ligand of AhR, affected hematopoiesis, causing decreased thymic seeding, reduced numbers of immature B cells and CFU-pre-B-progenitors in the bone marrow, and decreased reconstitution activity of $Lin^-/Sca\text{-}1^+/c\text{-}kit^+$ (LSK, HSC-enriched) bone marrow cells.[68,69] Knockout of AhR increased proliferation of HSCs, whereas aged AhR KO mice showed characteristics of premature bone marrow senescence and were prone to hematopoietic disease.[67] Inhibition of AhR in cultured stem cells with a synthetic ligand of AhR, StemRegenin 1 (SR-1), promoted the ex vivo expansion of human $CD34^+$ HSCs.[70] In addition, AhR appeared to regulate a number of signaling pathways that control hematopoiesis including HES-1, c-MYC, C/EBP, Pu.1, β-catenin, CXCR4, and STAT5-dependent processes.[71]

A role of AhR in benzene hematotoxicity was suggested when AhR KO mice were found to be resistant to benzene-induced toxicity to hematopoietic cells in mice.[72] Furthermore, mice that had been lethally irradiated but repopulated with bone marrow cells from the AhR KO mice did not show impairment of CFU-GMs in the bone marrow upon treatment with benzene, whereas reconstitution of irradiated mice with wild type marrow cells developed bone marrow depression as expected.[73] AhR may influence the hematopoietic effects of benzene by several means, including induction of drug metabolizing enzymes, such as CYPs, to influence the metabolic activation of benzene, and alteration of HSC proliferation and functional status to increase HSC sensitivity to toxic metabolites of benzene. However, it was shown that benzene metabolites, such as HQ and BQ, did not activate AhR, and the lack of AhR in mouse hepatoma cells did not affect ROS production induced by the exposures.[74] Therefore, at least from a mechanistic viewpoint, it remains a challenge to define the role of AhR in benzene hematotoxicity. Nonetheless, exploring the interplay among AhR, HSCs, and benzene metabolites provides a new opportunity for understanding both AhR biology and benzene effect on HSCs.

4.4. HSC Niche. The HSC compartment is an ordered environment in which HSCs are in close proximity to supportive stromal cells.[75] Adult HSCs are mostly in quiescent G_0/G_1 phase of the cell cycle during steady-state conditions but respond quickly to hematopoietic stress to proliferate and differentiate in order to replenish the hematopoietic system in a highly regulated manner. Lesions to either the HSCs or the bone marrow microenvironment (i.e., the HSC niche) can damage this response axis leading to bone marrow failure or uncontrolled proliferation and clonal expansion of LSCs.

Cumulative evidence showed that benzene metabolites can damage the marrow microenvironment. *E,E*-Muconaldehyde interferes with gap junctions of stromal cells in vitro to affect intercellular communications.[76] HQ inhibits tube formation from human bone marrow endothelial cells, which potentially blocks angiogenesis of the HSC vascular microenvironment.[77] As discussed above, oxidation of HQ to BQ by MPO and redox cycling of BQ occur in bone marrow cells leading to oxidative stress, particularly in stromal cells where multiple cell types exist and express varying levels of NQO1 that protects against oxidative stress from BQ. Individuals lacking functional NQO1 have increased sensitivity to benzene toxicity, which is, in part, attributed to decreased $CD34^+$ cell adhesion.[78] At a molecular

level, HQ modulates the phosphorylation and functions of PU.1, thereby promoting the clonal expansion of $CD34^+$ cells.[79]

4.5. Bone Marrow versus Peripheral Effects of Benzene. Benzene metabolites, such as HQ and BQ, induce apoptosis in blood cells or cell lines in vitro.[80] In the mice that had been lethally irradiated but repopulated with AhR KO bone marrow cells discussed above, benzene induced toxicity in mature white blood cells but not bone marrow cells; in this scenario, benzene metabolites formed in the liver (wild type) may have directly damaged circulating blood cells.[73] However, benzene-induced toxicity to mature white blood cells was not observed in AhR KO mice that had been similarly irradiated but repopulated with wild type bone marrow cells; in this case, the lack of AhR in the liver may be responsible for reduced toxicity in mature blood cells. These results suggest that AhR is required for the toxic effect of benzene to peripheral blood cells, in addition to affecting HSCs and marrow stromal cells. Whether the peripheral toxicity of benzene contributes to cytopenias observed in vivo significantly is questionable because most peripheral blood cells have limited life spans under physiologic conditions. In the case of cancer, benzene and metabolites may affect invasion and metastasis but not the initiation of leukemic blasts outside the bone marrow.

5. MECHANISTIC CONSIDERATIONS OF BENZENE ACTION

Benzene and metabolites cause multiple effects in hematopoietic cells through a number of mechanisms that include traditionally recognized benzene actions, such as chromosomal aberration, covalent binding, and gene mutation, as well as newly identified means, such as alteration of gene expression, oxidative stress, apoptosis, error-prone DNA repair, epigenetic regulation, and immune suppression.

5.1. Chromosomal Aberration and Genomic Instability. As discussed, benzene induced-AML and MDS have multiple chromosomal abnormalities that resemble those of therapy-related AML and MDS. Chronic exposure to benzene is associated with high levels of chromosomal changes including 5q−/−5 or 7q−/−7, +8, and t(8:21) in peripheral blood cells from highly exposed workers.[81−83] These changes were also observed in human cell cultures including $CD34^+$ progenitors exposed to benzene metabolites.[84]

Detection of chromosomal aneuploidy in the peripheral blood lymphocytes of benzene-exposed workers can be done with classical cytogenetic methods but was greatly enhanced by new approaches including the OctoChrome fluorescent in situ hybridization, a novel chromosome-wide aneuploidy study approach, and the micronucleus−centromere assay in conjunction with fluorescent in situ hybridization and chromosome-specific centromeric probes.[85−87] Using these new methods, monosomy of chromosomes 5, 6, 7, and 10 and trisomy of chromosomes 8, 9, 17, 21, and 22 were found in one study, whereas monosomy of 5, 6, 7, 10, 16, and 19, and trisomy of 5, 6, 7, 8, 10, 14, 16, 21, and 22 were detected in another study. Benzene metabolites HQ and 1,2,4-trihydroxybenzene are known inducers of chromosome breaks through inhibition of microtubule assembly leading to the formation of micronuclei.[88] Benzene could cause leukemias with chromosomal translocations and inversions known to be induced by topo II inhibitors, such as t(21q22), t(15;17), and inv(16). Benzene-induced chromosomal translocations and inversions are likely due to the inhibition of topo II through HQ and BQ.[83] Conversion of HQ to BQ by peroxidase increases topo II inhibition, and BQ is more potent than HQ in the inhibition of topo II in vitro.

Genomic instability is a hallmark of cancer and is important for CSCs to acquire sufficient mutations for tumor progression and evolution of tumor phenotypes. Genomic instability broadly includes microsatellite instability (MIN), which is associated with mutator phenotype and involves DNA repair functions, and chromosome instability (CIN), which is identified by gross chromosomal abnormalities. Benzene and metabolites cause chromosomal aberrations (discussed above) and affect DNA repair functions (discussed below), thereby increasing genomic instability that further promotes tumor formation.

5.2. Covalent Binding. Treating mice with C^{14}-labeled benzene led to covalently bound radioactivity in multiple organs including the liver, brain, kidneys, spleen, and lungs, and skeletal muscle, blood, fat, and bone marrow.[89−91] Covalent binding was likely the result of reactive metabolites formed in the liver and transported to the organs via the circulation. As an example, *S*-phenylcysteine was found in circulating albumin and hemoglobin that were formed from reactive metabolites released from the liver into the blood.[92] Benzene oxide and *p*-BQ are thought to be two important benzene metabolites for covalent binding. BQ adducts were found in greater quantity in bone marrow than benzene oxide adducts in rats treated with benzene orally.[93] A study showed that workers exposed to benzene at or less than 31 ppm had 32 pmol of benzene adducts/g globin, whereas those exposed to higher levels had a mean value of 129 pmol/g globin.[94] Incubation of benzene with microsomal proteins or of benzene metabolites with enzymes, such as myeloperoxidase from bone marrow, also leads to adduct formation. Formation of protein adducts causes inhibition of enzymes including microsomal enzymes, mtDNA polymerase, and topo II, and blocks mitosis by way of inhibiting tubulin function.

Binding of benzene metabolites to DNA was demonstrated in vivo in the liver and bone marrow.[95,96] Binding to DNA was readily demonstrated in vitro, and several possible structures of DNA adducts from benzene have been postulated, including N^7-phenylguanine and 3′-OH-1,N^2-benzetheno-2′deoxyguanosine.[97−99] However, in most in vivo studies, binding of benzene metabolites to DNA was at very low levels,[100] suggesting that covalent binding itself does not readily explain benzene hematotoxicity and carcinogenesis and that other mechanisms should be considered.

5.3. Mutation. Benzene and metabolites are weakly or nonmutagenic in most simple gene mutation assays. Nonetheless, several studies showed mutagenic effects of benzene in vivo and in vitro. The glycophorin A (GPA) gene loss mutation assay identifies stem cell or precursor erythroid cell mutations expressed in peripheral erythrocytes. By using the assay, gene-duplicating but not gene-inactivating mutations were found at the GPA locus in humans exposed to high levels of benzene.[101] The spectrum of *p53* mutations induced by benzene was determined by using Functional Analysis of Separated Alleles in Yeast (FASAY) in human cells. A > G and G > A transitions were found to be the most prevalent (23.5% for both), consistent with the notion that A > G transitions of *p53* are fingerprints of AML.[102] Inhalation exposure of mice harboring a bacteriophage lambda *lacI* transgene to high levels of benzene increased the mutation frequency of *lacI* by 1.8-fold in the lungs compared with that of the untreated control.[103]

Table 1. Microarray Analysis of Differential Gene Expression between Benzene Exposed Workers and Controls

study	subjects[a]	exposure ppm	major findings	examples	ref
1	6, 6	≥10	29 genes differentially expressed	CXCL 16, ZNF331, JUN, PF4	104
2	8, 8	≥10	overrepresentation of genes in apoptosis and lipid metabolism by two platforms of microarray	CXCL 16, ZNF331, JUN, PF4	105
3	83, 42	<1, <10, >10	16-gene expression signature associated with all levels of exposure	AML pathway and immune response pathways identified	106
4	7, 7	2.9 −60.3	22 genes up-regulated and 18 down-regulated	CYP4F3A and DNA-PKcs up-regulated	107

[a]Represent benzene exposed workers and matched controls.

5.4. Alteration of Gene Expression. The chronic and progressive nature of benzene hematotoxicity and tumorigenicity suggests genomic reprogramming that would result in aberrant gene expression during the development of myelotoxicity and leukemia by benzene. Nevertheless, recognition of gene regulation as a mechanism of benzene action was made possible only in recently years when several microarray studies showed genome-wide differential expression of genes in the peripheral blood cells from humans exposed to benzene.[80,104−107] It is prudent to point out that altered gene expression in peripheral blood cells does not necessarily reflect changes of gene expression in HSCs, CSCs, and progenitors. However, these studies demonstrated that human exposure to benzene indeed results in altered patterns of gene expression of several pathways important for hematopoietic development, immune response, apoptosis, oncogene signaling, and DNA repair (Table 1). These findings provide measurable molecular targets for analysis of benzene action relevant to human exposure. Identification of benzene-specific biomarkers or finger prints of gene expression in peripheral blood cells would facilitate epidemiological studies of occupational and environmental exposure to benzene. Benzene and metabolites may modulate gene expression via several mechanisms including chromosomal changes, oxidative stress, apoptosis, and epigenetic regulation.

5.5. Oxidative Stress. Several benzene metabolites have high potentials of redox cycling that is generally accompanied by the production of reactive oxygen species (ROS) and depletion of reducing equivalents such as reduced glutathione (GSH) causing oxidative stress in cells.[57] As discussed, HQ is readily oxidized to BQ by MPO in the bone marrow. *p*- and *o*-BQs can undergo sequential one-electron reductions to the semiquinone radical and, finally, HQ. The semiquinone radical is not stable and cycles back to BQ, passing an electron to oxygen to form superoxide anion radical ($O_2^{\bullet-}$) in the bone marrow. $O_2^{\bullet-}$ is dismutated to H_2O_2 by superoxide dismutase and H_2O_2 to $^{\bullet}OH$ via the Fenton reaction in the presence of iron. However, the two-electron reduction of BQ to HQ by NQO1 in the stroma cells bypasses the formation of semiquinone radical and superoxide. The observation that loss of NQO1 increased benzene toxicity confirms the importance of the BQ-HQ redox conversions in benzene toxicity. *p*-BQ can also be oxidized to 2,3-oxide and 1,2,4-trihydroxybenzene, or to glutathionyl 1,2,4-trihydroxybenzene if *p*-BQ 2,3-oxide reacts with GSH. Glutathionyl 1,2,4-trihydroxybenzene is highly capable of autoxidation and redox cycling spontaneously producing superoxide and other radicals, thus playing a significant role in benzene-induced oxidative stress. The open ring metabolite muconaldehyde may promote oxidative stress from its reactivity with cysteine thiols of the redox pools in cells.

Exposure to benzene in humans at several levels including <1 ppm caused dysregulation of the oxidative phosphorylation pathway in peripheral blood cells.[106] Benzene increased oxidative damage as measured by 8-hydroxy-2′-deoxyguanosine (8-OHdG) in mouse bone marrow.[108] Benzene-induced oxidative stress may damage macromolecules via several mechanisms. These include (a) DNA base oxidation in which BQ, HQ, and 1,2,4-trihydroxybenzene induce the formation of 8-OHdG; (b) DNA strand breaks and mutations; (c) induction of homologous recombination; and (d) damage to mitochondria.[108−111]

5.6. Apoptosis. The apoptotic effect of benzene and metabolites is readily measurable in vitro. HQ, BQ, and, to a lesser extent, phenol induced apoptosis in human leukemic cells HL-60 and K562, human $CD34^+$ bone marrow progenitor cells, and rat lymphocytes.[80,112−115] A role of apoptosis in benzene hematotoxicity in vivo was suggested by the observations that (a) overexpression of pro-apoptotic genes and down-regulation of antiapoptotic genes were found in peripheral blood cells of benzene-exposed individuals by microarray analysis;[105−107] and (b) dose-dependent decreases in myeloid progenitor cell colony formation and multilineage involvement suggested that bone marrow toxicity (possibly through apoptosis) is, in part, responsible for the widespread hematotoxicity of individuals exposed to benzene at low levels (<1 ppm).[5,116] The AhR-dependent, peripheral toxic effect of benzene is likely a reflection of the apoptosis of blood cells induced by benzene metabolites.

5.7. Error-Prone DNA Repair. DNA-dependent protein kinase (DNA-PK) is activated by DNA double strand breaks (DSB) to initiate nonhomologous end joining (NHEJ) for DSB repair [117] (Figure 4). Cells defective in DNA-PK regulatory subunits Ku70 and Ku80, or catalytic subunit DNA-PKcs, are highly sensitive to DSB-causing agents, such as ionizing radiation. However, NHEJ is error-prone and increases mutations in chromosomes contributing to genomic instability and cancer. A microarray of gene expression in peripheral mononuclear blood cells of workers diagnosed with chronic benzene poisoning revealed elevated expression of DNA-PKcs compared with matched controls.[107] In vitro studies revealed that benzene metabolites phenol, HQ, and BQ induced DNA-PKcs and the formation of γ-H2AX foci, a marker of DNA DSB, dose-dependently.[107,113,118] Therefore, benzene promotes NHEJ by inducing both DSB and DNA-PKcs, which, in turn, increases genomic instability (Figure 4). Quiescent human HSCs preferentially undergo NHEJ for DNA repair for survival in the presence of DNA damage instead of initiating apoptosis, accounting, in part, for the susceptibility of HSCs to benzene leukemogenesis and bone marrow toxicity.[119]

5.8. Epigenetic Regulation. Epigenetics has been implicated in the regulation of gene expression, proliferation and differentiation of stem cells, and tumorigenesis.[120−122]

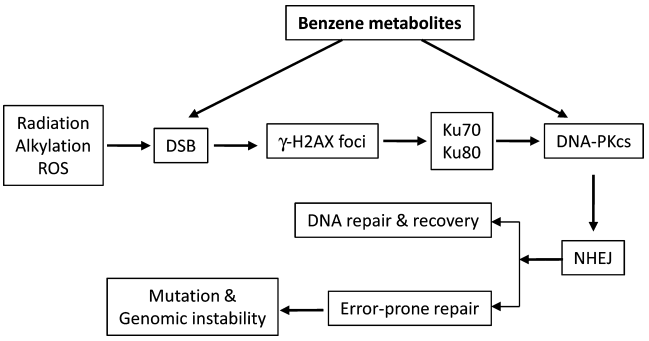

Figure 4. Promotion of error-prone DNA repair by benzene. Benzene metabolites promote nonhomologous end joining (NHEJ) to repair DNA double strand break (DSB) by inducing both DSB and expression of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). The error-prone NHEJ helps cells survive in the presence of DNA damage but promotes mutations and genomic instability for tumorigenic transformation. Formation of γ-H2AX foci and activation of Ku70 and Ku80, the regulatory subunits of DNA-PK, facilitate activation of DNA-PKcs.

Because benzene affects all of these processes, it is believed that epigenetic mechanisms play a role in benzene-induced hematotoxicity and malignancy.[123] Alteration in DNA methylation patterns were observed in subjects exposed to low-doses of benzene, including gas station attendants and traffic police officers.[124] DNA promoter methylation may account for the down-regulation of p15 and p16 gene expression in workers diagnosed with benzene poisoning.[125] Benzene exposure also caused changes in microRNA expression profiles in addition to DNA methylation.[126] In cultured TK6 cells, HQ induced global DNA methylation.[127]

5.9. Immunosuppression. Suppression of immune functions by benzene was noticed in early studies of benzene toxicity, in which exposure to benzene at levels that caused bone marrow depression in humans markedly increased susceptibility to tuberculosis and other infections in experimental animals.[128,129] In addition to causing bone marrow depression and apoptosis of blood cells, benzene alters the expression and secretion of immune and inflammatory cytokines and growth factors, which may contribute to its immuno-suppressive effects at low doses.[100] Microarray analysis of gene expression in the blood of workers exposed to benzene or diagnosed with benzene poisoning revealed apparent effects on immune-related pathways. As an example, cytochrome P450 4F3A (CYP4F3A) encodes the leukotriene B4 (LTB_4) hydroxylase in neutrophils responsible for inactivation of LTB_4, a major chemoattractant for neutrophils (Figure 5). CYP4F3A expression was elevated significantly in workers with benzene poisoning, which potentially reduced neutrophil functions by inactivating LTB_4.[80,112] Exposure to benzene also altered the ratios of immune cell subsets in humans, even at low doses. In addition, benzene and metabolites, such as HQ, *p*BQ, and 1,2,4-trihydroxybenzene, modulate macrophage functions, such as increasing the sensitivity of bone marrow macrophages to inflammatory and NO (nitric oxide) stimulants lipopolysaccharide and interferon γ.[130] In addition to decreasing the defense to microbe infection, inhibition of the immune and inflammatory functions by benzene also damages the surveillance of CSCs and non-CSC tumor cells through immune cells, thereby promoting CSC clonal expansion and metastasis.

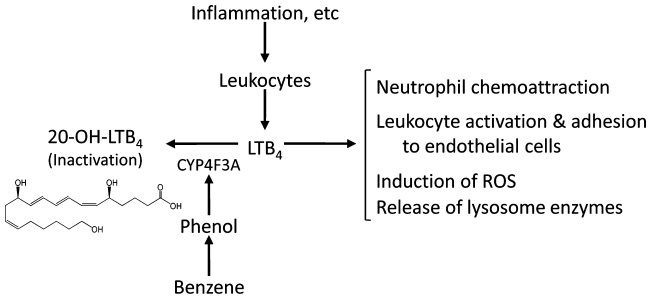

Figure 5. Regulation of LTB_4 metabolism by benzene and metabolites. LTB_4 (leukotriene B_4) is inactivated by CYP4F3A in neutrophils. Benzene and metabolites induce CYP4F3A to inhibit LTB_4 functions.

6. INDIVIDUAL VARIABILITY

The effect of exposure to benzene in humans can vary significantly from individual to individual, ranging from no observable effect or mild and transient reduction of blood cell counts to cytopenia, pancytopenia, MDS, aplastic anemia, and AML.[28] Some variations are exposure-related (dose, frequency, route of exposure, and coexposed chemicals); however, many are due to individual differences in genetics, age, gender, history of smoking and alcoholic use, and physical and dietary habits. Understanding individual variability and identifying individual workers susceptible to benzene toxicity are among the major challenges in occupational safety assessment.

A number of studies showed that genetic factors play an important role in individual variability of benzene toxicity. Drug metabolizing enzymes including CYP2E1, NQO1, myeloperoxidase, mEH, and GSTs determine the formation and elimination of active metabolites of benzene as well as the formation of ROS.[131] Polymorphisms of the enzymes, such as GSTM1 and GSTT1, have been consistently associated with biomarkers of benzene exposure and effect in humans. Polymorphisms of genes important for DNA repair and genomic maintenance, such as BLM, TP53, RAD51, WDR79, and WRN, were significantly associated with benzene-induced decrease in white blood cell counts.[132] Finally, genetic polymorphisms of cytokines and chemokines have also been associated with chronic benzene poisoning.[133,134]

7. "MULTI-HIT" MODEL FOR BENZENE LEUKEMOGENESIS AND HEMATOTOXICITY

From the above discussions, it is reasonable to postulate that benzene and metabolites have multiple effects on multiple cell components of the hematopoietic system to cause malignancy and hematotoxicity. The potential pathways leading to the formation of CSCs and progression of the tumor are depicted as a "multi-hit" model (Figure 6). CSCs may arise via tumorigenic changes initiated in normal, self-renewing HSCs or downstream progenitors by reactive benzene metabolites, resulting in the expansion of the stem cell and progenitor pools and generation of PLSCs. Secondary events may occur in expanded pools of target cells producing CSCs. Reactivation of self-renewal is necessary if the oncogenic events occur in, otherwise short-lived, progenitor cells. Intratumoral genomic instability and acquisition of additional changes by CSCs or by nontumorigenic cells including stroma cells and immune cells promote tumor progression and, eventually, metastasis. Evolution of tumor phenotypes, such as genetic and epigenetic signatures, may occur during these later two stages. If the attack by benzene metabolites results in apoptosis and/or blockade of

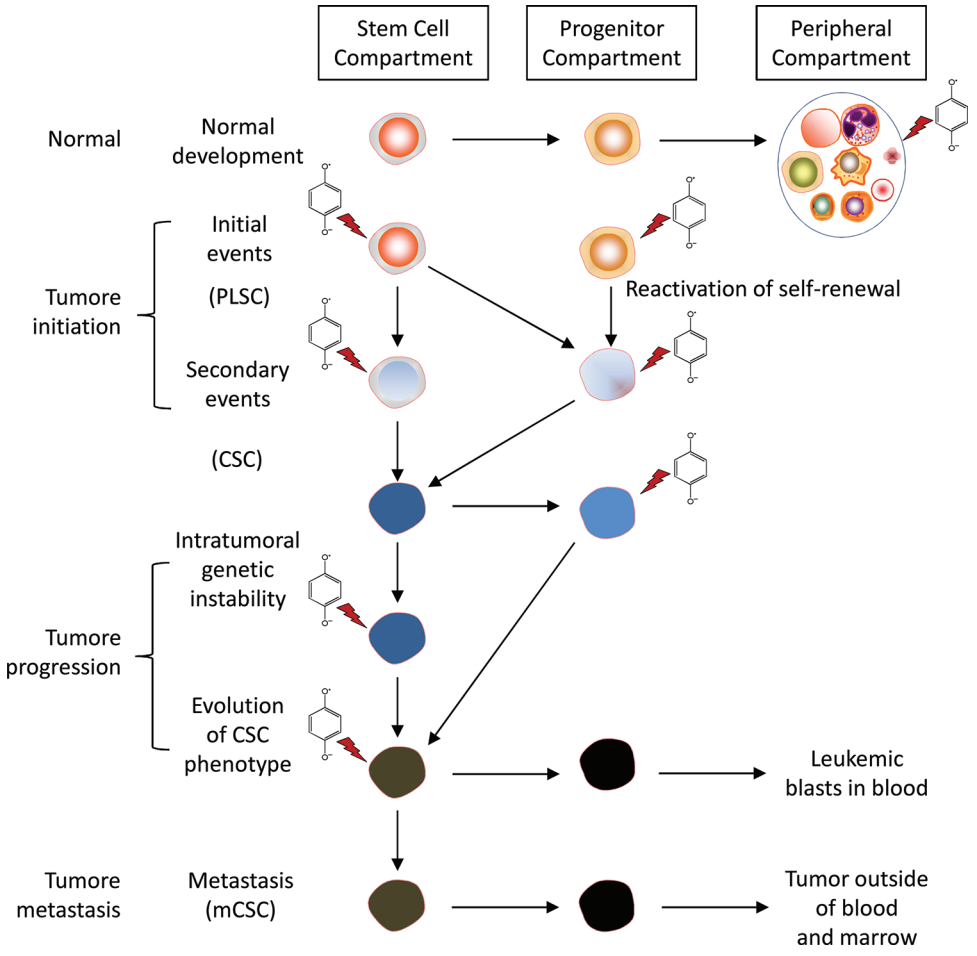

Figure 6. "Multi-hit" model for benzene leukemogenesis and hematotoxicity. PLSC, preleukemic stem cell; CSC, cancer stem cell; mCSC, migratory cancer stem cell. The *p*-BQ semiquinone radical was used to represent reactive and toxic benzene metabolites.

differentiation of HSCs and progenitors, bone marrow depression will take place. Benzene metabolites may also have effects on peripheral blood cells and leukemic blasts. Genetic variations modulate benzene metabolism, DNA repair, genomic stability, and immune surveillance of tumor cells to influence the development of benzene-induced malignancy and toxicity. In light of a considerable overlap of the clinical manifestation and mechanism among "secondary" MDS and AML, this "multi-hit" model of stem cell–benzene metabolite interaction may also underlie the pathogenesis of MDS and AML caused by therapeutic agents and other environmental leukemogens and hematotoxicants.

8. CONCLUSIONS

Benzene toxicity, which mainly involves bone marrow depression and leukemogenesis, is reasonably believed to develop through interactions with hematopoietic stem cells. The emerging understanding of the biology of HSC and CSC in the recent 5 to 10 years is beginning to shed new light into such interactions. Toxic metabolites of benzene appear to target several cell types including HSCs, progenitors, and niche cells through multiple hits leading to the generation of CSCs and the evolution of tumor phenotypes. New study tools and approaches, such as microarray analysis, epigenetics, DNA repair, and toxicogenomics, also helped to uncover new mechanisms of benzene action, along with potential sources of individual variability to benzene toxicity. These findings are likely to significantly impact the understanding of benzene biological effects and the prevention and therapy against cancer and hematotoxicity caused by occupational and environmental exposure to benzene.

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Notes

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ABBREVIATIONS

AhR, aryl hydrocarbon receptor; AML, acute myeloid leukemia; BQ, benzoquinone; CPU-GM, colony forming unit-granulocyte and macrophage progenitor; CSC, cancer stem cell; CYP, cytochrome P450; DSB, double strand break; mEH, micro-

somal epoxide hydrolase or EPHX1; EMT, epithelial-to-mesenchymal transition; GST, glutathione *S*-transferase; HQ, hydroquinone; HSC, hematopoietic stem cell; LSC, leukemia stem cell; LTB_4, leukotriene B_4; MA, *E,E*-muconic acid; mCSC, migratory cancer stem cell; MDS, myelodysplastic syndromes; MET, mesenchymal-to-epithelial transition; MPO, myeloperoxidase; NHEJ, nonhomologous end joining; NHL, non-Hodgkin lymphoma; NQO1, NAD(P)H:quinone oxidoreductase 1; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; PLSC, preleukemic stem cell; ROS, reactive oxygen species; topo, topoisomerase

REFERENCES

(1) NTP (2011) *Report on Carcinogens*, 12th ed., U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, http://Ntp.Niehs.Nih.Gov/Ntp/Roc/Twelfth/Roc12.Pdf (accessed 2012).

(2) IARC (1987) Summaries and Evaluations: Benzene, Supplement 7, pp 120−122, *International Agency for Research on Cancer Publication*, IARC, Lyon, France.

(3) ATSDR (1997) *Toxicological Profile for Benzene*, Public Health Service, Atlanta, GA.

(4) Smith, M. T. (2010) Advances in Understanding Benzene Health Effects and Susceptibility. *Ann. Rev. Public Health 31*, 133−148.

(5) Lan, Q., Zhang, L., Li, G., Vermeulen, R., Weinberg, R. S., Dosemeci, M., Rappaport, S. M., Shen, M., Alter, B. P., Wu, Y., Kopp, W., Waidyanatha, S., Rabkin, C., Guo, W., Chanock, S., Hayes, R. B., Linet, M., Kim, S., Yin, S., Rothman, N., and Smith, M. T. (2004) Hematotoxicity in Workers Exposed to Low Levels of Benzene. *Science 306*, 1774−1776.

(6) Aksoy, M. (1989) Hematotoxicity and Carcinogenicity of Benzene. *Environ. Health Perspect. 82*, 193−197.

(7) Snyder, R., Witz, G., and Goldstein, B. D. (1993) The Toxicology of Benzene. *Environ. Health Perspect. 100*, 293−306.

(8) Guo, Y., Lubbert, M., and Engelhardt, M. (2003) Cd34-Hematopoietic Stem Cells: Current Concepts and Controversies. *Stem Cells 21*, 15−20.

(9) Dick, J. E. (2008) Stem Cell Concepts Renew Cancer Research. *Blood 112*, 4793−4807.

(10) Lapidot, T., Sirard, C., Vormoor, J., Murdoch, B., Hoang, T., Caceres-Cortes, J., Minden, M., Paterson, B., Caligiuri, M. A., and Dick, J. E. (1994) A Cell Initiating Human Acute Myeloid Leukaemia after Transplantation into Scid Mice. *Nature 367*, 645−648.

(11) Visvader, J. E., and Lindeman, G. J. (2008) Cancer Stem Cells in Solid Tumours: Accumulating Evidence and Unresolved Questions. *Nature Rev. Cancer 8*, 755−768.

(12) Brabletz, T., Jung, A., Spaderna, S., Hlubek, F., and Kirchner, T. (2005) Opinion: Migrating Cancer Stem Cells - an Integrated Concept of Malignant Tumour Progression. *Nature Rev. Cancer 5*, 744−749.

(13) Rosen, J. M., and Jordan, C. T. (2009) The Increasing Complexity of the Cancer Stem Cell Paradigm. *Science 324*, 1670−1673.

(14) Haferlach, T., Schnittger, S., Kern, W., Hiddemann, W., and Schoch, C. (2004) Genetic Classification of Acute Myeloid Leukemia (Aml). *Ann. Hematol. 83* (Suppl 1), 97−100.

(15) Mrozek, K., Heerema, N. A., and Bloomfield, C. D. (2004) Cytogenetics in Acute Leukemia. *Blood Rev. 18*, 115−136.

(16) Morgan, G. J., and Alvares, C. L. (2005) Benzene and the Hemopoietic Stem Cell. *Chem.-Biol. Interact. 153−154*, 217−222.

(17) Esteller, M. (2003) Profiling Aberrant DNA Methylation in Hematologic Neoplasms: A View from the Tip of the Iceberg. *Clin. Immunol. 109*, 80−88.

(18) Gilliland, D. G., and Griffin, J. D. (2002) The Roles of Flt3 in Hematopoiesis and Leukemia. *Blood 100*, 1532−1542.

(19) Santesson, C. G. (1897) Uber Chronische Vergiftungen Mit Steinkohlenteerbenzin. Vier Todesfalle. *Arch. Hyg. 31*, 336−376.

(20) Selling, L. (1910) A Preliminary Report on Some Cases of Purpura Haemorrhagica Due to Benzol Poisoning. *Johns Hopkins Hosp. Bull. 21*, 33−37.

(21) Selling, L. (1916) Benzol as a Leucotoxin. Studies on the Degeneration and Regeneration of the Blood and Haematopoietic Organs. *Johns Hopkins Hosp. Rep. 17*, 83−148.

(22) Weiskotten, H. G., C.B.F., G., Boggs, E. O., and Templeton, E. R. (1920) The Action of Benzol. Vi. Benzol Vapor Leucopenia (Rabbit). *J. Med. Res. 41*, 425−438.

(23) Hamilton, A. (1922) The Growing Menace of Benzene (Benzol) Poisoning in American Industry. *J Am Med Assoc 78*, 627−630.

(24) Hamilton, A. (1928) The Lessoning Menace of Benzol Poisoning in American Industry. *J. Ind. Hyg. 10*, 227−233.

(25) Hamilton, A. (1931) General Review: Benzene (Benzol) Poisoning. *Arch. Pathol. 11*, 434−647.

(26) Delore, P., and Borgomano, C. (1928) Leucemie Aigue Au Cours De La Intoxication Benzenique. *J. Med. Lyon 9*, 227−233.

(27) Forni, A. M., Cappellini, A., Pacifico, E., and Vigliani, E. C. (1971) Chromosome Changes and Their Evolution in Subjects with Past Exposure to Benzene. *Arch. Environ. Health 23*, 385−391.

(28) Aksoy, M. (1988) Benzene Hematotoxicity, in *Benzene Carcinogenicity* (Aksoy, M., Ed.), CRC Press, Boca Raton, FL.

(29) Infante, P. F., Rinsky, R. A., Wagoner, J. K., and Young, R. J. (1977) Leukaemia in Benzene Workers. *Lancet 2*, 76−78.

(30) Rinsky, R. A., Hornung, R. W., Silver, S. R., and Tseng, C. Y. (2002) Benzene Exposure and Hematopoietic Mortality: A Long-Term Epidemiologic Risk Assessment. *Am. J. Ind. Med. 42*, 474−480.

(31) Rinsky, R. A., Smith, A. B., Hornung, R., Filloon, T. G., Young, R. J., Okun, A. H., and Landrigan, P. J. (1987) Benzene and Leukemia. An Epidemiologic Risk Assessment. *N. Engl. J. Med. 316*, 1044−1050.

(32) Yin, S. N., Li, Q., Liu, Y., Tian, F., Du, C., and Jin, C. (1987) Occupational Exposure to Benzene in China. *British Journal of Industrial Medicine 44*, 192−195.

(33) Yin, S. N., Li, G. L., Tain, F. D., Fu, Z. I., Jin, C., Chen, Y. J., Luo, S. J., Ye, P. Z., Zhang, J. Z., Wang, G. C., et al. (1987) Leukaemia in Benzene Workers: A Retrospective Cohort Study. *Br. J. Ind. Med. 44*, 124−128.

(34) Hayes, R. B., Yin, S. N., Dosemeci, M., Li, G. L., Wacholder, S., Chow, W. H., Rothman, N., Wang, Y. Z., Dai, T. R., Chao, X. J., Jiang, Z. L., Ye, P. Z., Zhao, H. B., Kou, Q. R., Zhang, W. Y., Meng, J. F., Zho, J. S., Lin, X. F., Ding, C. Y., Li, C. Y., Zhang, Z. N., Li, D. G., Travis, L. B., Blot, W. J., and Linet, M. S. (1996) Mortality among Benzene-Exposed Workers in China. *Environ. Health Perspect. 104* (Suppl 6), 1349−1352.

(35) Yin, S. N., Hayes, R. B., Linet, M. S., Li, G. L., Dosemeci, M., Travis, L. B., Zhang, Z. N., Li, D. G., Chow, W. H., Wacholder, S., and Blot, W. J. (1996) An Expanded Cohort Study of Cancer among Benzene-Exposed Workers in China. Benzene Study Group. *Environ. Health Perspect. 104* (Suppl 6), 1339−1341.

(36) Glass, D. C., Gray, C. N., Jolley, D. J., Gibbons, C., Sim, M. R., Fritschi, L., Adams, G. G., Bisby, J. A., and Manuell, R. (2003) Leukemia Risk Associated with Low-Level Benzene Exposure. *Epidemiology 14*, 569−577.

(37) Glass, D. C., Sim, M. R., Fritschi, L., Gray, C. N., Jolley, D. J., and Gibbons, C. (2004) Leukemia Risk and Relevant Benzene Exposure Period-Re: Follow-up Time on Risk Estimates. *Am. J. Ind. Med. 42*, 481−489, (2004) 222−223 author reply pp 224−225.

(38) Glass, D. C., Gray, C. N., Jolley, D. J., Gibbons, C., and Sim, M. R. (2005) Health Watch Exposure Estimates: Do They Underestimate Benzene Exposure? *Chem.-Biol. Interact. 153−154*, 23−32.

(39) Goldstein, B. D. (2010) Benzene as a Cause of Lymphoproliferative Disorders. *Chem.-Biol. Interact. 184*, 147−150.

(40) Hayes, R. B., Yin, S. N., Dosemeci, M., Li, G. L., Wacholder, S., Travis, L. B., Li, C. Y., Rothman, N., Hoover, R. N., and Linet, M. S. (1997) Benzene and the Dose-Related Incidence of Hematologic Neoplasms in China. Chinese Academy of Preventive Medicine--National Cancer Institute Benzene Study Group. *J. Natl. Cancer Inst. 89*, 1065−1071.

(41) Steinmaus, C., Smith, A. H., Jones, R. M., and Smith, M. T. (2008) Meta-Analysis of Benzene Exposure and Non-Hodgkin Lymphoma: Biases Could Mask an Important Association. *Occup. Environ. Med. 65*, 371−378.
(42) Decoufle, P., Blattner, W. A., and Blair, A. (1983) Mortality among Chemical Workers Exposed to Benzene and Other Agents. *Environ. Res. 30*, 16−25.
(43) Infante, P. F. (2006) Benzene Exposure and Multiple Myeloma: A Detailed Meta-Analysis of Benzene Cohort Studies. *Ann. N.Y. Acad. Sci. 1076*, 90−109.
(44) Goldstein, B. D. (1990) Is Exposure to Benzene a Cause of Human Multiple Myeloma? *Ann. N.Y. Acad. Sci. 609*, 225−230 discussion 230−224.
(45) Brosselin, P., Rudant, J., Orsi, L., Leverger, G., Baruchel, A., Bertrand, Y., Nelken, B., Robert, A., Michel, G., Margueritte, G., Perel, Y., Mechinaud, F., Bordigoni, P., Hemon, D., and Clavel, J. (2009) Acute Childhood Leukaemia and Residence Next to Petrol Stations and Automotive Repair Garages: The Escale Study (Sfce). *Occup. Environ. Med. 66*, 598−606.
(46) Whitworth, K. W., Symanski, E., and Coker, A. L. (2008) Childhood Lymphohematopoietic Cancer Incidence and Hazardous Air Pollutants in Southeast Texas, 1995−2004. *Environ. Health Perspect. 116*, 1576−1580.
(47) Pyatt, D., and Hays, S. (2010) A Review of the Potential Association between Childhood Leukemia and Benzene. *Chem.-Biol. Interact. 184*, 151−164.
(48) Smith, M. T., Zhang, L., McHale, C. M., Skibola, C. F., and Rappaport, S. M. (2011) Benzene, the Exposome and Future Investigations of Leukemia Etiology. *Chem.-Biol. Interact. 192*, 155−159.
(49) Badham, H. J., and Winn, L. M. (2010) In Utero and in Vitro Effects of Benzene and Its Metabolites on Erythroid Differentiation and the Role of Reactive Oxygen Species. *Toxicol. Appl. Pharmacol. 244*, 273−279.
(50) Lau, A., Belanger, C. L., and Winn, L. M. (2009) In Utero and Acute Exposure to Benzene: Investigation of DNA Double-Strand Breaks and DNA Recombination in Mice. *Mutat. Res. 676*, 74−82.
(51) Beelte, S., Haas, R., Germing, U., and Jansing, P.-J. (2009) Paradigm Change in The Assessment of Myeloid and Lympoid Neoplasms Associated with Occupational Benzene Exposure. *Med. Klin. 104*, 197−203.
(52) Pedersen-Bjergaard, J., Andersen, M. K., Andersen, M. T., and Christiansen, D. H. (2008) Genetics of Therapy-Related Myelodysplasia and Acute Myeloid Leukemia. *Leukemia 22*, 240−248.
(53) Pedersen-Bjergaard, J., Christiansen, D. H., Desta, F., and Andersen, M. K. (2006) Alternative Genetic Pathways and Cooperating Genetic Abnormalities in the Pathogenesis of Therapy-Related Myelodysplasia and Acute Myeloid Leukemia. *Leukemia 20*, 1943−1949.
(54) Mrozek, K., and Bloomfield, C. D. (2008) Clinical Significance of the Most Common Chromosome Translocations in Adult Acute Myeloid Leukemia. *J. Natl. Cancer Inst. Monographs*, 52−57.
(55) Irons, R. D., and Stillman, W. S. (1996) The Process of Leukemogenesis. *Environ. Health Perspect. 104* (Suppl 6), 1239−1246.
(56) Zhang, L., Rothman, N., Li, G., Guo, W., Yang, W., Hubbard, A. E., Hayes, R. B., Yin, S., Lu, W., and Smith, M. T. (2007) Aberrations in Chromosomes Associated with Lymphoma and Therapy-Related Leukemia in Benzene-Exposed Workers. *Environ. Mol. Mutagen. 48*, 467−474.
(57) Snyder, R., and Hedli, C. C. (1996) An Overview of Benzene Metabolism. *Environ. Health Perspect. 104* (Suppl 6), 1165−1171.
(58) Ross, D. (2000) The Role of Metabolism and Specific Metabolites in Benzene-Induced Toxicity: Evidence and Issues. *J. Toxicol. Environ. Health, Part A 61*, 357−372.
(59) Subrahmanyam, V. V., Doane-Setzer, P., Steinmetz, K. L., Ross, D., and Smith, M. T. (1990) Phenol-Induced Stimulation of Hydroquinone Bioactivation in Mouse Bone Marrow in Vivo: Possible Implications in Benzene Myelotoxicity. *Toxicology 62*, 107−116.
(60) Iskander, K., and Jaiswal, A. K. (2005) Quinone Oxidoreductases in Protection against Myelogenous Hyperplasia and Benzene Toxicity. *Chem.-Biol. Interact. 153−154*, 147−157.
(61) Long, D. J., II, Gaikwad, A., Multani, A., Pathak, S., Montgomery, C. A., Gonzalez, F. J., and Jaiswal, A. K. (2002) Disruption of the Nad(P)H:Quinone Oxidoreductase 1 (Nqo1) Gene in Mice Causes Myelogenous Hyperplasia. *Cancer Res. 62*, 3030−3036.
(62) Rothman, N., Smith, M. T., Hayes, R. B., Traver, R. D., Hoener, B., Campleman, S., Li, G. L., Dosemeci, M., Linet, M., Zhang, L., Xi, L., Wacholder, S., Lu, W., Meyer, K. B., Titenko-Holland, N., Stewart, J. T., Yin, S., and Ross, D. (1997) Benzene Poisoning, a Risk Factor for Hematological Malignancy, Is Associated with the Nqo1 609c→T Mutation and Rapid Fractional Excretion of Chlorzoxazone. *Cancer Res. 57*, 2839−2842.
(63) Ma, Q. (2012) Overview of Ahr Functional Domains and the Classical Ahr Signaling Pathway: Induction of Drug Metabolizing Enzymes, in *The Ah Receptor in Biology and Toxicology* (Pohjanvirta, R., Ed.) pp 35−45, John Wiley & Sons, Inc., New York.
(64) Ma, Q. (2008) Xenobiotic-Activated Receptors: From Transcription to Drug Metabolism to Disease. *Chem. Res. Toxicol. 21*, 1651−1671.
(65) Ma, Q., and Lu, A. Y. (2007) Cyp1a Induction and Human Risk Assessment: An Evolving Tale of in Vitro and in Vivo Studies. *Drug Metab. Dispos. 35*, 1009−1016.
(66) Ma, Q. (2011) Influence of Light on Aryl Hydrocarbon Receptor Signaling and Consequences in Drug Metabolism, Physiology and Disease. *Expert. Opin. Drug Metab. Toxicol. 7*, 1267−1293.
(67) Gasiewicz, T. A., Singh, K. P., and Casado, F. L. (2010) The Aryl Hydrocarbon Receptor Has an Important Role in the Regulation of Hematopoiesis: Implications for Benzene-Induced Hematopoietic Toxicity. *Chem.-Biol. Interact. 184*, 246−251.
(68) Thurmond, T. S., and Gasiewicz, T. A. (2000) A Single Dose of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin Produces a Time- and Dose-Dependent Alteration in the Murine Bone Marrow B-Lymphocyte Maturation Profile. *Toxicol. Sci. 58*, 88−95.
(69) Murante, F. G., and Gasiewicz, T. A. (2000) Hemopoietic Progenitor Cells Are Sensitive Targets of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin in C57bl/6j Mice. *Toxicol. Sci. 54*, 374−383.
(70) Boitano, A. E., Wang, J., Romeo, R., Bouchez, L. C., Parker, A. E., Sutton, S. E., Walker, J. R., Flaveny, C. A., Perdew, G. H., Denison, M. S., Schultz, P. G., and Cooke, M. P. (2010) Aryl Hydrocarbon Receptor Antagonists Promote the Expansion of Human Hematopoietic Stem Cells. *Science 329*, 1345−1348.
(71) Singh, K. P., Casado, F. L., Opanashuk, L. A., and Gasiewicz, T. A. (2009) The Aryl Hydrocarbon Receptor Has a Normal Function in the Regulation of Hematopoietic and Other Stem/Progenitor Cell Populations. *Biochem. Pharmacol. 77*, 577−587.
(72) Yoon, B. I., Hirabayashi, Y., Kawasaki, Y., Kodama, Y., Kaneko, T., Kanno, J., Kim, D. Y., Fujii-Kuriyama, Y., and Inoue, T. (2002) Aryl Hydrocarbon Receptor Mediates Benzene-Induced Hematotoxicity. *Toxicol. Sci. 70*, 150−156.
(73) Hirabayashi, Y., and Inoue, T. (2010) Benzene-Induced Bone-Marrow Toxicity: A Hematopoietic Stem-Cell-Specific, Aryl Hydrocarbon Receptor-Mediated Adverse Effect. *Chem.-Biol. Interact. 184*, 252−258.
(74) Badham, H. J., and Winn, L. M. (2007) Investigating the Role of the Aryl Hydrocarbon Receptor in Benzene-Initiated Toxicity in Vitro. *Toxicology 229*, 177−185.
(75) Renstrom, J., Kroger, M., Peschel, C., and Oostendorp, R. A. (2010) How the Niche Regulates Hematopoietic Stem Cells. *Chem.-Biol. Interact. 184*, 7−15.
(76) Rivedal, E., Witz, G., and Leithe, E. (2010) Gap Junction Intercellular Communication and Benzene Toxicity. *Chem.-Biol. Interact. 184*, 229−232.
(77) Zhou, H., Kepa, J. K., Siegel, D., Miura, S., Hiraki, Y., and Ross, D. (2009) Benzene Metabolite Hydroquinone up-Regulates Chondromodulin-I and Inhibits Tube Formation in Human Bone Marrow Endothelial Cells. *Mol. Pharmacol. 76*, 579−587.

(78) Zhou, H., Dehn, D., Kepa, J. K., Siegel, D., Scott, D. E., Tan, W., and Ross, D. (2010) Nad(P)H:Quinone Oxidoreductase 1-Compromised Human Bone Marrow Endothelial Cells Exhibit Decreased Adhesion Molecule Expression and Cd34+ Hematopoietic Cell Adhesion. *J. Pharmacol. Exp. Ther. 334*, 260−268.
(79) Gross, S. A., Zheng, J. H., Le, A. T., Kerzic, P. J., and Irons, R. D. (2006) Pu.1 Phosphorylation Correlates with Hydroquinone-Induced Alterations in Myeloid Differentiation and Cytokine-Dependent Clonogenic Response in Human Cd34(+) Hematopoietic Progenitor Cells. *Cell Biol. Toxicol. 22*, 229−241.
(80) Zhao, Z., He, X., Bi, Y., Xia, Y., Tao, N., Li, L., and Ma, Q. (2009) Induction of Cyp4f3 by Benzene Metabolites in Human White Blood Cells in Vivo in Human Promyelocytic Leukemic Cell Lines and Ex Vivo in Human Blood Neutrophils. *Drug Metab. Dispos. 37*, 282−291.
(81) Zhang, L., Rothman, N., Wang, Y., Hayes, R. B., Li, G., Dosemeci, M., Yin, S., Kolachana, P., Titenko-Holland, N., and Smith, M. T. (1998) Increased Aneusomy and Long Arm Deletion of Chromosomes 5 and 7 in the Lymphocytes of Chinese Workers Exposed to Benzene. *Carcinogenesis 19*, 1955−1961.
(82) Smith, M. T., Zhang, L., Wang, Y., Hayes, R. B., Li, G., Wiemels, J., Dosemeci, M., Titenko-Holland, N., Xi, L., Kolachana, P., Yin, S., and Rothman, N. (1998) Increased Translocations and Aneusomy in Chromosomes 8 and 21 among Workers Exposed to Benzene. *Cancer Res. 58*, 2176−2181.
(83) Zhang, L., Eastmond, D. A., and Smith, M. T. (2002) The Nature of Chromosomal Aberrations Detected in Humans Exposed to Benzene. *Crit. Rev. Toxicol. 32*, 1−42.
(84) Stillman, W. S., Varella-Garcia, M., and Irons, R. D. (2000) The Benzene Metabolite, Hydroquinone, Selectively Induces 5q31- and -7 in Human Cd34+Cd19- Bone Marrow Cells. *Exp. Hematol. 28*, 169−176.
(85) Zhang, L., Lan, Q., Guo, W., Li, G., Yang, W., Hubbard, A. E., Vermeulen, R., Rappaport, S. M., Yin, S., Rothman, N., and Smith, M. T. (2005) Use of Octochrome Fluorescence in Situ Hybridization to Detect Specific Aneuploidy among All 24 Chromosomes in Benzene-Exposed Workers. *Chem.-Biol. Interact. 153−154*, 117−122.
(86) Zhang, L., Lan, Q., Guo, W., Hubbard, A. E., Li, G., Rappaport, S. M., McHale, C. M., Shen, M., Ji, Z., Vermeulen, R., Yin, S., Rothman, N., and Smith, M. T. (2011) Chromosome-Wide Aneuploidy Study (Cwas) in Workers Exposed to an Established Leukemogen, Benzene. *Carcinogenesis 32*, 605−612.
(87) McHale, C. M., Zhang, L., and Smith, M. T. (2012) Current Understanding of the Mechanism of Benzene-Induced Leukemia in Humans: Implications for Risk Assessment. *Carcinogenesis 33*, 240−252.
(88) Zhang, L., Venkatesh, P., Creek, M. L., and Smith, M. T. (1994) Detection of 1,2,4-Benzenetriol Induced Aneuploidy and Microtubule Disruption by Fluorescence in Situ Hybridization and Immunocytochemistry. *Mutat. Res. 320*, 315−327.
(89) Snyder, R., Lee, E. W., and Kocsis, J. J. (1978) Binding of Labeled Benzene Metabolites to Mouse Liver and Bone Marrow. *Res. Commun. Chem. Pathol. Pharmacol. 20*, 191−194.
(90) Longacre, S. L., Kocsis, J. J., and Snyder, R. (1981) Influence of Strain Differences in Mice on the Metabolism and Toxicity of Benzene. *Toxicol. Appl. Pharmacol. 60*, 398−409.
(91) Longacre, S. L., Kocsis, J. J., Witmer, C. M., Lee, E. W., Sammett, D., and Snyder, R. (1981) Toxicological and Biochemical Effects of Repeated Administration of Benzene in Mice. *J. Toxicol. Environ. Health 7*, 223−237.
(92) Bechtold, W. E., Sun, J. D., Birnbaum, L. S., Yin, S. N., Li, G. L., Kasicki, S., Lucier, G., and Henderson, R. F. (1992) S-Phenylcysteine Formation in Hemoglobin as a Biological Exposure Index to Benzene. *Arch. Toxicol. 66*, 303−309.
(93) McDonald, T. A., Yeowell-O'Connell, K., and Rappaport, S. M. (1994) Comparison of Protein Adducts of Benzene Oxide and Benzoquinone in the Blood and Bone Marrow of Rats and Mice Exposed to [14c/13c6]Benzene. *Cancer Res. 54*, 4907−4914.
(94) Yeowell-O'Connell, K., Rothman, N., Smith, M. T., Hayes, R. B., Li, G., Waidyanatha, S., Dosemeci, M., Zhang, L., Yin, S., Titenko-Holland, N., and Rappaport, S. M. (1998) Hemoglobin and Albumin Adducts of Benzene Oxide among Workers Exposed to High Levels of Benzene. *Carcinogenesis 19*, 1565−1571.
(95) Lutz, W. K., and Schlatter, C. (1977) Mechanism of the Carcinogenic Action of Benzene: Irreversible Binding to Rat Liver DNA. *Chem.-Biol. Interact. 18*, 241−245.
(96) Arfellini, G., Grilli, S., Colacci, A., Mazzullo, M., and Prodi, G. (1985) In Vivo and in Vitro Binding of Benzene to Nucleic Acids and Proteins of Various Rat and Mouse Organs. *Cancer Letters 28*, 159−168.
(97) Snyder, R., Jowa, L., Witz, G., Kalf, G., and Rushmore, T. (1987) Formation of Reactive Metabolites from Benzene. *Arch. Toxicol. 60*, 61−64.
(98) Chenna, A., Hang, B., Rydberg, B., Kim, E., Pongracz, K., Bodell, W. J., and Singer, B. (1995) The Benzene Metabolite P-Benzoquinone Forms Adducts with DNA Bases That Are Excised by a Repair Activity from Human Cells That Differs from an Ethenoadenine Glycosylase. *Proc. Natl. Acad. Sci. U.S.A. 92*, 5890−5894.
(99) Bodell, W. J., Levay, G., and Pongracz, K. (1993) Investigation of Benzene-DNA Adducts and Their Detection in Human Bone Marrow. *Environ. Health Perspect. 99*, 241−244.
(100) Snyder, R. (2002) Benzene and Leukemia. *Crit. Rev. Toxicol. 32*, 155−210.
(101) Rothman, N., Haas, R., Hayes, R. B., Li, G. L., Wiemels, J., Campleman, S., Quintana, P. J., Xi, L. J., Dosemeci, M., Titenko-Holland, N., et al. (1995) Benzene Induces Gene-Duplicating but Not Gene-Inactivating Mutations at the Glycophorin a Locus in Exposed Humans. *Proc. Natl. Acad. Sci. U.S.A. 92*, 4069−4073.
(102) Billet, S., Paget, V., Garcon, G., Heutte, N., Andre, V., Shirali, P., and Sichel, F. (2010) Benzene-Induced Mutational Pattern in the Tumour Suppressor Gene Tp53 Analysed by Use of a Functional Assay, the Functional Analysis of Separated Alleles in Yeast, in Human Lung Cells. *Arch. Toxicol. 84*, 99−107.
(103) Mullin, A. H., Nataraj, D., Ren, J. J., and Mullin, D. A. (1998) Inhaled Benzene Increases the Frequency and Length of Laci Deletion Mutations in Lung Tissues of Mice. *Carcinogenesis 19*, 1723−1733.
(104) Forrest, M. S., Lan, Q., Hubbard, A. E., Zhang, L., Vermeulen, R., Zhao, X., Li, G., Wu, Y. Y., Shen, M., Yin, S., Chanock, S. J., Rothman, N., and Smith, M. T. (2005) Discovery of Novel Biomarkers by Microarray Analysis of Peripheral Blood Mononuclear Cell Gene Expression in Benzene-Exposed Workers. *Environ. Health Perspect. 113*, 801−807.
(105) McHale, C. M., Zhang, L., Lan, Q., Li, G., Hubbard, A. E., Forrest, M. S., Vermeulen, R., Chen, J., Shen, M., Rappaport, S. M., Yin, S., Smith, M. T., and Rothman, N. (2009) Changes in the Peripheral Blood Transcriptome Associated with Occupational Benzene Exposure Identified by Cross-Comparison on Two Microarray Platforms. *Genomics 93*, 343−349.
(106) McHale, C. M., Zhang, L., Lan, Q., Vermeulen, R., Li, G., Hubbard, A. E., Porter, K. E., Thomas, R., Portier, C. J., Shen, M., Rappaport, S. M., Yin, S., Smith, M. T., and Rothman, N. (2011) Global Gene Expression Profiling of a Population Exposed to a Range of Benzene Levels. *Environ. Health Perspect. 119*, 628−634.
(107) Bi, Y., Li, Y., Kong, M., Xiao, X., Zhao, Z., He, X., and Ma, Q. (2010) Gene Expression in Benzene-Exposed Workers by Microarray Analysis of Peripheral Mononuclear Blood Cells: Induction and Silencing of Cyp4f3a and Regulation of DNA-Dependent Protein Kinase Catalytic Subunit in DNA Double Strand Break Repair. *Chem.-Biol. Interact. 184*, 207−211.
(108) Kolachana, P., Subrahmanyam, V. V., Meyer, K. B., Zhang, L., and Smith, M. T. (1993) Benzene and Its Phenolic Metabolites Produce Oxidative DNA Damage in Hl60 Cells in Vitro and in the Bone Marrow in Vivo. *Cancer Res. 53*, 1023−1026.
(109) Ho, T. Y., and Witz, G. (1997) Increased Gene Expression in Human Promyeloid Leukemia Cells Exposed to Trans,Trans-Muconaldehyde, a Hematotoxic Benzene Metabolite. *Carcinogenesis 18*, 739−744.

(110) Wiemels, J., and Smith, M. T. (1999) Enhancement of Myeloid Cell Growth by Benzene Metabolites Via the Production of Active Oxygen Species. *Free Radical Res. 30*, 93−103.
(111) Shen, M., Zhang, L., Bonner, M. R., Liu, C. S., Li, G., Vermeulen, R., Dosemeci, M., Yin, S., and Lan, Q. (2008) Association between Mitochondrial DNA Copy Number, Blood Cell Counts, and Occupational Benzene Exposure. *Environ. Mol. Mutagen. 49*, 453−457.
(112) Zhao, Z., Mao, Z., Bi, Y., Xia, Y., Tao, N., Li, L., He, X., and Ma, Q. (2008) Induction of Cyp4f3 in White Blood Cells from Benzene-Poisoning Patients and Human Hl60 Cells, in *The Proceedings of the 17th International Symposium on Microsomes and Drug Oxidation* (Kaminsky, L. S., Ed.) pp 103−107, Medimond S.r.l., Bologna, Spain.
(113) Xiao, X., Song, W., and Bi, Y. (2010) Role of DNA-Pkcs in the Biological Effect of a Benzene Metabolite: Phenol Toxicity to Human K562 Cells in Vitro. *Chem.-Biol. Interact. 184*, 302−305.
(114) Moran, J. L., Siegel, D., Sun, X. M., and Ross, D. (1996) Induction of Apoptosis by Benzene Metabolites in Hl60 and Cd34+ Human Bone Marrow Progenitor Cells. *Mol. Pharmacol. 50*, 610−615.
(115) Martinez-Velazquez, M., Maldonado, V., Ortega, A., Melendez-Zajgla, J., and Albores, A. (2006) Benzene Metabolites Induce Apoptosis in Lymphocytes. *Exp. Toxicol. Pathol. 58*, 65−70.
(116) Qu, Q., Shore, R., Li, G., Jin, X., Chen, L. C., Cohen, B., Melikian, A. A., Eastmond, D., Rappaport, S. M., Yin, S., Li, H., Waidyanatha, S., Li, Y., Mu, R., Zhang, X., and Li, K. (2002) Hematological Changes among Chinese Workers with a Broad Range of Benzene Exposures. *Am. J. Ind. Med. 42*, 275−285.
(117) Collis, S. J., DeWeese, T. L., Jeggo, P. A., and Parker, A. R. (2005) The Life and Death of DNA-Pk. *Oncogene 24*, 949−961.
(118) Ishihama, M., Toyooka, T., and Ibuki, Y. (2008) Generation of Phosphorylated Histone H2ax by Benzene Metabolites. *Toxicol. in Vitro 22*, 1861−1868.
(119) Mohrin, M., Bourke, E., Alexander, D., Warr, M. R., Barry-Holson, K., Le Beau, M. M., Morrison, C. G., and Passegue, E. (2010) Hematopoietic Stem Cell Quiescence Promotes Error-Prone DNA Repair and Mutagenesis. *Cell Stem Cell 7*, 174−185.
(120) Shames, D. S., Minna, J. D., and Gazdar, A. F. (2007) DNA Methylation in Health, Disease, and Cancer. *Curr. Mol. Med. 7*, 85−102.
(121) Esteller, M., and Herman, J. G. (2002) Cancer as an Epigenetic Disease: DNA Methylation and Chromatin Alterations in Human Tumours. *J. Pathol. 196*, 1−7.
(122) Bibikova, M., Chudin, E., Wu, B., Zhou, L., Garcia, E. W., Liu, Y., Shin, S., Plaia, T. W., Auerbach, J. M., Arking, D. E., Gonzalez, R., Crook, J., Davidson, B., Schulz, T. C., Robins, A., Khanna, A., Sartipy, P., Hyllner, J., Vanguri, P., Savant-Bhonsale, S., Smith, A. K., Chakravarti, A., Maitra, A., Rao, M., Barker, D. L., Loring, J. F., and Fan, J. B. (2006) Human Embryonic Stem Cells Have a Unique Epigenetic Signature. *Genome Res. 16*, 1075−1083.
(123) Baccarelli, A., and Bollati, V. (2009) Epigenetics and Environmental Chemicals. *Curr. Opin. Pediatr. 21*, 243−251.
(124) Bollati, V., Baccarelli, A., Hou, L., Bonzini, M., Fustinoni, S., Cavallo, D., Byun, H. M., Jiang, J., Marinelli, B., Pesatori, A. C., Bertazzi, P. A., and Yang, A. S. (2007) Changes in DNA Methylation Patterns in Subjects Exposed to Low-Dose Benzene. *Cancer Res. 67*, 876−880.
(125) Xing, C., Wang, Q. F., Li, B., Tian, H., Ni, Y., Yin, S., and Li, G. (2010) Methylation and Expression Analysis of Tumor Suppressor Genes P15 and P16 in Benzene Poisoning. *Chem.-Biol. Interact. 184*, 306−309.
(126) Zhang, L., McHale, C. M., Rothman, N., Li, G., Ji, Z., Vermeulen, R., Hubbard, A. E., Ren, X., Shen, M., Rappaport, S. M., North, M., Skibola, C. F., Yin, S., Vulpe, C., Chanock, S. J., Smith, M. T., and Lan, Q. (2010) Systems Biology of Human Benzene Exposure. *Chem.-Biol. Interact. 184*, 86−93.
(127) Ji, Z., Zhang, L., Peng, V., Ren, X., McHale, C. M., and Smith, M. T. (2010) A Comparison of the Cytogenetic Alterations and Global DNA Hypomethylation Induced by the Benzene Metabolite, Hydroquinone, with Those Induced by Melphalan and Etoposide. *Leukemia 24*, 986−991.
(128) Hirschfelder, A. D., and Winternitz, M. C. (1913) Studies Upon Experimental Pneumonia in Rabbits. *J. Exp. Med. 17*, 657−665.
(129) White, W. C., and Gammon, A. M. (1914) The Influence of Benzol Inhalations on Experimental Tuberculosis in Rabbits. *Trans. Assoc. Am. Phys. 29*, 332.
(130) Laskin, J. D., Rao, N. R., Punjabi, C. J., Laskin, D. L., and Synder, R. (1995) Distinct Actions of Benzene and Its Metabolites on Nitric Oxide Production by Bone Marrow Leukocytes. *J. Leukocyte Biol. 57*, 422−426.
(131) Dougherty, D., Garte, S., Barchowsky, A., Zmuda, J., and Taioli, E. (2008) Nqo1, Mpo, Cyp2e1, Gstt1 and Gstm1 Polymorphisms and Biological Effects of Benzene Exposure: A Literature Review. *Toxicol. Lett. 182*, 7−17.
(132) Lan, Q., Zhang, L., Shen, M., Jo, W. J., Vermeulen, R., Li, G., Vulpe, C., Lim, S., Ren, X., Rappaport, S. M., Berndt, S. I., Yeager, M., Yuenger, J., Hayes, R. B., Linet, M., Yin, S., Chanock, S., Smith, M. T., and Rothman, N. (2009) Large-Scale Evaluation of Candidate Genes Identifies Associations between DNA Repair and Genomic Maintenance and Development of Benzene Hematotoxicity. *Carcinogenesis 30*, 50−58.
(133) Lan, Q., Zhang, L., Shen, M., Smith, M. T., Li, G., Vermeulen, R., Rappaport, S. M., Forrest, M. S., Hayes, R. B., Linet, M., Dosemeci, M., Alter, B. P., Weinberg, R. S., Yin, S., Yeager, M., Welch, R., Waidyanatha, S., Kim, S., Chanock, S., and Rothman, N. (2005) Polymorphisms in Cytokine and Cellular Adhesion Molecule Genes and Susceptibility to Hematotoxicity among Workers Exposed to Benzene. *Cancer Res. 65*, 9574−9581.
(134) Lv, L., Kerzic, P., Lin, G., Schnatter, A. R., Bao, L., Yang, Y., Zou, H., Fu, H., Ye, X., Gross, S. A., Armstrong, T. W., and Irons, R. D. (2007) The Tnf-Alpha 238a Polymorphism Is Associated with Susceptibility to Persistent Bone Marrow Dysplasia Following Chronic Exposure to Benzene. *Leuk. Res. 31*, 1479−1485.