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Benzene and the hemopoietic stem cell

Gareth J. Morgan *, Caroline L. Alvares

Royal Marsden Hospital, Downs Road, Sutton, Surrey SM2 5PT, UK

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Abstract

The emerging understanding of the biology of the hemopoietic stem cell is beginning to shed light on the mechanisms by which benzene gives rise to acute myeloid leukaemia. These mechanisms are complex, affecting not only the DNA, but also the complex intercellular interactions present in the bone marrow microenvironment. The toxic effects of benzene are mediated within the bone marrow and we are beginning to understand the contributions of inter-individual variation in xenobiotic metabolisms and DNA repair to the definition of risk following exposure to benzene in the environment. This process is likely to be accelerated by recent advances in high throughput genotyping. Until now, research has focussed directly on mutation and chromosomal translocations, but we are beginning to understand more how environmental exposures can modify chromatin structure giving rise to heritable changes not affecting DNA. These epigenetic studies are likely to give important further insights into the mode of action of benzene as are studies of its effect on the immune system.

Keywords: Acute myeloid leukemia; Bone marrow stem cell; CD34; Cytogenetics; Genomic instability

1. Introduction

The epidemiology of acute myeloid leukemia is very well described with an age specific incidence rate of around 1–2 per 100,000 up to the age of 40 years, from when the incidence increases to approximately 6 per 100,000 in the 60 year old population [1]. There is an interesting inflection point at the age of 50 where the male to female ratio, which is equal until that age point, changes such that there is an excess of male cases of approximately 2 per 100,000 per year.

The cellular origins of AML are pertinent to a discussion of the role of benzene based leukemogenesis. Hematopoiesis is based upon an ordered cellular differentiation pathway, where a pleuripotent hemopoietic stem cell matures to either a committed lymphoid or myeloid precursor. The myeloid precursor cell gives rise to monocytes, neutrophils, eosinophils and basophils, together with erythrocytes and platelets. AML results from clonal proliferation of a hemopoietic stem cell, which undergoes a limited degree of differentiation which is easily recognised morphologically. This differentiation is described by the FAB classification system, which recognises types M1 to M7, based on the degree and nature of differentiation of the blast cells [2,3]. For all of the acute myeloid leukemias, except

* Corresponding author.
E-mail address: gareth.morgan@rmh.nthames.nhs.uk (G.J. Morgan).

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possibly for M3, the primary leukemic precursor cell is thought to lie within the stem cell compartment defined by CD34 expression [4]. Thus, if one is to consider the impact of benzene in leukemogenesis, it is important to consider the biology of this stem cell in its microenvironment within the bone marrow. The bone marrow is an ordered environment with the hemopoietic stem cell being in close proximity to protective stromal cells. Ordered maturation of myeloid progenitors can be seen in relation to the normal hemopoietic stem cells and one of the diagnostic features of myelodysplasia is abnormal location of these immature precursor cells [5]. In addition to these myeloid components, mature B and T cells are present which may exert significant effects on the stem cell compartment. Thus, hemopoietic stem cells are found in a relatively protected environment within the bone marrow. Oxygen and toxins are delivered by the vascular system, and the differentiating myeloid precursors, which are rich in myeloperoxidase, provide an environment which easily generates oxidative stress. Environmentally ingested toxins such as benzene will have encountered hepatic first pass metabolism leading to metabolic activation or inactivation before they reach the bone marrow [6]. Consequently, before any consideration of the effects of environmental toxins on the hemopoietic stem cells can be considered, this prior metabolism must be taken into account.

For a hemopoietic stem cell to become malignant, it must develop genomic instability in order for it to acquire sufficient mutations in a short enough time span. There are a number of factors which predispose to this genomic instability, these include: error prone DNA repair, imbalance in the nucleotide precursor pool, generation of reactive oxygen free radicals, alkylation of the DNA and environmentally encountered xenobiotic agents delivered to the bone marrow. Of course, the majority of people do not go on to develop AML, as there are a number of factors, which have evolved to prevent DNA instability, including maintenance of the primary DNA sequence by base selection, proof reading and mismatch correction. In addition, there are also well defined DNA repair pathways, which can repair a range of damage together with cell cycle checkpoints, which can induce apoptosis in the presence of DNA damage which cannot be repaired [7,8]. The genetic material within the cell is also organized in such a way as to prevent the development of heritable DNA damage. Thus, for a cell to become malignant these different pathways need to be bypassed. In the context of inherited predisposition to AML, small changes in these DNA repair pathways may as a consequence of inherited DNA variants lead to the development of more penetrant changes later in the disease course with more rapid progress to AML.

From the study of AML blasts, we have a good understanding of the molecular lesions which give rise to AML. These include balanced translocations, interstitial deletions, mutations and DNA methylation [9,10]. From an etiological perspective, it is important to distinguish changes involved in the initiation of AML from changes associated with disease progression because by understanding the factors involved in the generation of initiating lesions, we can understand more about the etiological mechanisms giving rise to AML. There are clearly different types of mutation, which can collaborate, including lesions which largely affect apoptosis and those that affect differentiation. The acquisition of lesions affecting both of these pathways is more likely to lead to the development of AML.

Descriptively, there are three common balanced translocations which are associated with a good prognosis, including the t(8;21) seen in AML M2 [11], the t(15;17) seen in M3 [12] and the inversion (16) associated with M4 EO [13]. Poor prognostic cytogenetics constitute another large group and are associated with MDS and secondary leukemia, and include the group characterised by interstitial deletions of 5q and 7q, and are associated with a poor prognosis [14]. It is interesting to look at the age distribution of these cytogenetic subtypes. While the number of cases with the balanced translocations remains stable with age, the number of cases carrying 5/7q− increases, suggesting there are distinct etiological mechanisms underlying these two types of leukemia.

For the balanced translocations to arise, it is essential for the DNA to have undergone double stranded DNA breaks at two sites, with exchange of genetic material between the two different chromosomal regions. Attempts at understanding the causation of leukemia by characterising the structure of these break point regions has been largely unsuccessful with no common motifs identified. However, a number of environmentally encountered agents are recognised to cause double stranded breaks, including the topoisomerase II inhibitors [15] such as etoposide, the anthracyclines, radiation [16], the repair of alkylation damage and oxida-

tive DNA damage, all of which have been associated with leukemia. The precise molecular lesions leading to interstitial deletion are less well defined, but this group of cases seems to be associated with environmental exposure and exposure to previous chemotherapy. Thus, we have two broad groups of AML, those characterised by balanced translocations, in which the translocation is necessary to develop the disease, and the rate of acquisition seems to be relatively constant throughout life, and a group characterised by 5q7q−, which requires multiple genetic hits as shown by the increasing incidence with age and its association with environmental factors. Further characterisation of the balanced translocation group has illustrated a number of oncogenes that are deregulated. In particular, disruption of CBFβ/AML 1 complex is a molecular feature which is well characterised and affects hemopoietic cell differentiation. Genes affected include TEL/AML 1 in the t(12;21), EVI 1/AML 1 in the t(3;21) and AML 1/ETO in the t(8;21) [17]. The inversion (16) leads to the generation of a CBFβ/MYH 11 gene, a binding partner of AML 1. Further characterisation of the mechanism underlying the pathogenesis of AML resulting from these translocations, as well as the t(15;17) have identified that they are dominant negative inhibitors of transcription. The translocations alter the chromatin status at the binding site of the complex implicating epigenetic factors in the control of gene expression in AML. This raises a possible role for environmental factors in mediating chromatin structure and gene expression without altering the DNA itself. Further studies of methylation patterns of CPG islands in AML suggest that P15 is hyper-methylated in up to 80% of AML cases, MGMT a DNA repair protein is hyper-methylated in 5% and cadherin in up to 28% of AMLs [18].

Mutations of CCAT enhancer binding protein (C/EBPα) have recently been identified as being frequent in AML [19]. This is a transcription factor which can affect both proliferation and differentiation of AML blast cells. These lesions provide further evidence for the importance of deregulation of transcription factors. They also provide some support for the idea of selection of mutations, which collaborate to give an AML phenotype. A further frequent mutation, which seems to be associated with disease progression in AML, is mutation and internal tandem duplication of the flt-3 tyrosine kinase, which confer a poor prognosis and are a potential target for treatment [20]. However, it seems unlikely that these are the etiological mechanism of AML, but rather are acquired during the disease course.

There are a number of well recognised inherited genetic abnormalities that can contribute to the risk of AML. There are two distinct types of genes, which can be identified, those which are of high penetrance but of low prevalence, and are associated with familial cancer syndromes, and those which are of low penetrance but of high prevalence. These contribute to the population risk of developing leukemia. There are a number of lessons to be learned from the study of familial leukemic syndromes. The inherited low platelet disorder, associated with mutations in AML 1, the gene deregulated by the t(8;21) highlights the role of leukemogenesis genes in familial predisposition to AML [21]. Other interesting associations with AML include Kostmann syndrome, associated with mutations within the G CSF receptor [22]. Down syndrome associated with trisomy 21 has an increased incidence of AML, (acute megakaryoblastic) and is associated with GATA 1 mutations [23]. Children with neurofibromatosis and mutations of the NF1 gene have a 2–500-fold increased risk of AML, and haplo-insufficient mouse models of this lesion, when treated with alkylators tend to develop AML [24]. The other large group of familial diseases associated with leukemias and lymphomas are those with chromosomal/DNA instability syndromes including xeroderma pigmentosum, ataxia telangiectasia, Bloom syndrome and Fanconi anemia [25]. The characteristic feature underlying these conditions is genetic instability within a hemopoietic precursor. This increases the likelihood that a cell will acquire sufficient genetic lesions to transform to a leukemic phenotype. These syndromes, in particular Fanconi anemia and ataxia telangiectasia, suggest that DNA double strand break repair in a hemopoietic precursor is an important mechanism preventing leukemic transformation. Double strand break repair can be broken down into two distinct pathways—non-homologous end joining (NHEJ), which is associated with inaccurate repair, and homologous recombination repair (HRR), which is associated with accurate repair of the DNA breaks. Both of these mechanisms if deregulated can give rise to chromosomal translocations and loss of heterozygosity, two of the characteristic features seen in AML. RAD 50 (a central gene in double strand DNA repair) knockout mice have normal VDJ recombination in their lymphoid system, but have progressive germ cell

failure, hematopoietic failure, and can develop AML [26].

There are a number of established risk factors for AML including radiation exposure, cancer chemotherapy and benzene exposure. It is worth comparing and contrasting the relationship of therapy related AML with AML arising following benzene exposure. There are two distinct groups of therapy related AML: those occurring after topoisomerase II inhibitors, which are characterised by 11q23 abnormalities, and occur 2–3 years after exposure; the other main group occurs following alkylating agent exposure and is normally associated with a prior MDS phase, tends to occur 5–8 years following exposure, and is associated with interstitial deletions of chromosomes 5q and 7q. Similar to alkylating agent-associated AML, hematotoxicity and MDS often precede AML associated with benzene exposure. The latency period between exposure and disease lies between 4 months and 10 years. Metabolism of benzene seems to be necessary for the toxicity [27]. Cytogenetic changes seen include monosomy of chromosomes 5 and 7 together with interstitial deletions of chromosomes 5 and 7 together with translocation into 21q22.

Inherited genetic variants can impact on the risk of AML. These can be grouped into different subtypes including variants affecting xenobiotic metabolism such as GSTT l and NQ01, those which effect apoptosis such as the Fas ligand, ERCC2, and those that bring about repair of DNA, examples of which include ERCC2, MSH2 and MLH1 [28,29]. There is a developing literature using association studies to examine these risks [30]. Often the risks are low, in the order of 1.5–2. Most studies have not looked at the interaction of gene environment changes because of the difficulty in obtaining adequate sized samples. Both AML and benzene-associated leukemia provide model systems in which inherited genetic variants within the pathway known to metabolize the leukemogenic agent can be examined in more detail. Benzene is an established cause of AML and aplastic anemia with relative risks up to 4 being described [31]. The metabolism of benzene is well understood with key enzymes being cytochrome P450 2E1, epoxide hydrolase, myeloperoxidase and NQ01. The most widely studied of these enzymes is NQ01, which is located on chromosome 16q23, and has inactivating mutations at position 607. This enzyme exerts a major role in protecting cells from the genotoxic stress associated with exposure to quinones. These products occur naturally as well as being the break down products of benzene. Thus, this gene can be considered to protect cells from the deleterious effects of quinones, and under active metabolites would be expected to increase the risk of AML. Studies of these under active variants have shown that there is a 2.5-fold increased risk of hematotoxicity in homozygotes. There is an association with therapy related AML as well as an association with infant leukemia and de novo AML in adults. The risks seem to be evenly distributed between different cytogenetic groups, but there was a slightly increased risk in the group with the inversion 16. The effect of these variants has been extensively investigated by David Ross who has shown that in the basal state NQ01 is not expressed in the hemopoietic stem cell, but in an induced state NQ01 can be detectable [32]. In the presence of the genetic variant, this inducibility of NQ01 is not seen thus providing a biological rationale for the association of these agents with increased leukemogenic risks.

Another important feature of benzene-associated AML is the development of bone marrow hypoplasia. This association has been discussed above and is also seen with inherited mutations affecting genomic stability, which also have increased risk of AML. Another AML predisposition entity also associated with hypoplasia is paroxysmal nocturnal hemoglobinuria (PNH). PNH is a deficiency of cell membrane molecules held in place by GPI anchors. As part of the disease course, there is emergence of clones lacking these GPI anchored cell membrane protein on a background of bone marrow hypoplasia. This has been suggested to be due to immune mediated hypoplasia directed against GPI linked proteins on the cell surface, and that the loss of these proteins allows clones to escape from the immunological suppression [33]. Further evidence for an immunological effect in AML and MDS is the description of hypoplastic MDS, which can respond to anti-lymphocyte globulin [34]. Therefore, the well described association of benzene with hypoplasia and AML suggest that looking for effects of benzene on the immune system would be worthwhile. However, there are a number of better described mechanisms by which benzene exerts its effects. It produces oxidative stress, it can lead to the development of translocations, and it can cause mutation. The effects of benzene on chromatin structure are currently unknown

and this subject is worth pursuing. Studies looking at the impact of benzene exposure on hemopoietic stem cell gene expression patterns have shown a range of changes, but at this stage it is safe to conclude that no specific molecular abnormalities have been successfully identified by this process [35]. Understanding the molecular effects which underly the impact of benzene on hemopoietic stem cell function remains an important goal.

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