Abstract

Clear and unambiguous associations have been established between therapeutic exposures and specific complications. However, considerable interindividual variability is observed in the risk of developing an outcome for a given therapeutic exposure. Genetic predisposition and especially its interaction with therapeutic exposures can potentially exacerbate the toxic effect of treatment on normal tissues and organ systems, and can possibly explain the interindividual variability. This article provides a brief overview of the current knowledge about the role of genomic variation in the development of therapy-related complications. Relatively common outcomes with strong associations with therapeutic exposures, including cardiomyopathy, obesity, osteonecrosis, ototoxicity, and subsequent malignancies are discussed here. To develop a deeper understanding of the molecular underpinnings of therapy-related complications, comprehensive and near-complete collection of clinically annotated samples is critical. Methodologic issues such as study design, definition of the endpoints or phenotypes, identification of appropriate and adequately sized study population together with a reliable plan for collecting and maintaining high-quality DNA, and selection of an appropriate approach or platform for genotyping are also discussed. Understanding the etiopathogenetic pathways that lead to the morbidity is critical to developing targeted prevention and intervention strategies, optimizing risk-based health care of cancer survivors, thus minimizing chronic morbidities and improving quality of life.

Introduction

The 5-year overall survival rate for all invasive cancer types has increased from 50% in 1975–1977 to 67% in 1999–2005 and exceeds 80% for many of the common cancer types, including breast, prostate, testicular, thyroid, bladder, and endometrial cancers, melanoma, and Hodgkin lymphoma (HL; ref. 1). As a result of this improvement in survival, the number of cancer survivors has quadrupled in the past 4 decades and now exceeds 12 million, of which 8 million have survived 5 years or more. This population is growing at a rate of almost 2% per year (2).

The increasing number of long-term survivors is attended by a growing awareness that many will develop health conditions as a direct or an indirect consequence of their cancer therapy (3–6). Although some of these conditions occur during therapy and persist well after the therapy has been completed (such as steroid-induced osteonecrosis or alkylator/radiation-induced hypogonadism), many outcomes are not evident until 10 to 20 years later, such as subsequent malignant neoplasms (SMN) and anthracycline-related late onset congestive heart failure (CHF).

Individuals exposed to radiation and chemotherapy are vulnerable to long-lasting organ toxicity; the very young because their organs are developing and the elderly because of organ senescence. In addition, genetic predisposition and its interaction with therapeutic exposures can potentially exacerbate the toxic effect of treatment on normal tissues and organ systems. Thus, it becomes imperative to understand the individual variability in (i) the internal dose of the therapeutic agent; (ii) the biologically effective dose; (iii) the alterations in structure or function of the tissue or organ; and (iv) the consequent development of preclinical disease (Fig. 1) to understand the pathogenesis of therapy-related complications, and also to develop a better idea of the individual susceptibility.

The following sections describe a brief overview of the current knowledge about the role of genomic variation in the development of therapy-related complications. The focus is on outcomes that are relatively common, have clearly established associations with therapeutic exposures, and are associated with significant long-term morbidity and hence carry the potential to negatively impact the quality of life of the survivors. These outcomes include cardiomyopathy, obesity, osteonecrosis, ototoxicity, and SMNs. An attempt has been made to present scientifically robust studies that have used a variety of methodologies.
Thus, the studies presented here included (i) GWAS studies using large populations with successful validation of the findings in independent cohorts or extension of the findings with some functional data; (ii) candidate gene studies using large carefully and comprehensively curated sets of genes that were biologically plausible; and (iii) single gene studies, where there was ample preclinical (in vitro and/or in vivo) data that provided a compelling rationale for examination of the association in a single gene setting. The fact that there are not enough scientifically or methodologically robust studies highlights the issue that there are methodologic challenges to conduct such studies, and these are detailed here. These challenges notwithstanding, understanding the etiopathogenetic pathways that lead to long-term morbidity is critical to developing targeted prevention and intervention strategies, optimizing risk-based health care of cancer survivors, and improving quality of life.

Methodologic Issues

To develop a deeper understanding of the molecular underpinnings of therapy-related complications, careful attention needs to be devoted to develop the appropriate study design with an appropriate and adequately sized study population and a precise definition of endpoints or phenotypes and a reliable plan for collecting high-quality DNA. It is critical to have a comprehensive and near-complete collection of clinically annotated samples. Ideally, this should be in the form of blood, collected to allow subsequent extraction of DNA and RNA, as well as to establish lymphoblastoid cell lines. The study design, study question, and the available sample size should help in the selection of an appropriate approach or platform for genotyping.

If the intent is to analyze a single endpoint, a case-control study design is most efficient; however, a cohort design is more appropriate for the study of complex or multiple phenotypes. Furthermore, consideration should be given to issues related to survival bias when designing prevalent case-control studies, especially where the endpoint is associated with high early lethality. Study design must include rigorous power estimations to determine the number of subjects necessary to meet statistical objectives. Another efficient and cost-effective methodology is the use of a nested case-control study design, especially when the samples have been banked on the entire cohort and a comprehensive longitudinal follow-up of the cohort has resulted in a near-complete ascertainment of the outcome of interest. Finally, use of appropriate comparison groups is critical. Previous studies have either used healthy individuals or used individuals with histologically identical de novo cancer as comparison groups [e.g., de novo AML as a reference group for therapy-related myelodysplasia/acute myeloid leukemia (t-MDS/AML)]. This strategy could be problematic because of the possibility of shared genotoxic insults (e.g., benzene), driving the association toward null. The ideal comparison group should consist of individuals identical to the cases with respect to primary cancer but who do not develop the outcome of interest. It is also important to ensure that the controls have been followed for at least as long as the cases, from the time of diagnosis and preferably for a longer duration. This is done to ensure that the “controls” have had ample opportunity to develop the outcome of interest. Ideally, the outcome of interest should be clinically validated to avoid misclassification. It is also important that the controls be subjected to similar validation as the cases to confirm the absence of disease.

There are 2 approaches to the study of genetic variation in disease: (i) candidate gene studies based on the selection of a limited number of biologically relevant genes and pathways and (ii) genome-wide association studies (GWAS) using DNA arrays capable of detecting a million or more SNPs. The candidate gene approach is guided by a specific hypothesis whereas a genome-wide approach is necessary for comprehensive discovery analysis. Both strategies have their own strengths and limitations. A GWAS approach offers the ability to study complex pathways, allowing for an assessment of the action/interaction of many genes; importantly, it allows for new genes to be identified. It has gained significant favor, because of the fact that several studies that have used a candidate gene approach have failed replication. However, a GWAS approach requires a large sample size, to account for false discovery, and is accordingly an expensive endeavor. In addition, there is the need for a replication cohort so that the genes identified in the discovery set can be validated in the test set. The GWAS approach does not have an a priori hypothesis, and is considered to be hypothesis-generating and more suited for complex disorders where a clear etiologic lead is not established. However, this is not true for outcomes observed in survivors, where for the most part there is a clearly established etiologic association between the exposure and outcome (e.g., radiation and SMNs; anthracyclines and CHF). In such a case, an argument could be made for a comprehensively selected (and
biologically plausible) list of genes identified along the path of the action of therapeutic exposure on the target organ. The need for a large sample size due to issues related to multiple testing is less of an issue with a candidate gene approach if limited to a few genetic variants, and the cost is accordingly less than that of a GWAS approach. Finally, although gene–gene interactions would require prohibitively large samples in a GWAS setting, they are logistically feasible when conducting a candidate gene study.

**Anthracrycline-Related Cardiomyopathy**

Anthracryclines are among the most widely used chemotherapeutic agents (7). However, cardiomyopathy is a dose-limiting complication. The incidence of CHF is less than 10% in patients exposed to cumulative dose of anthracrycline of less than 500 mg/m², and approaches 36% for doses exceeding 600 mg/m² (8). The risk of anthracrycline-related cardiomyopathy is higher among those exposed at a younger age (<5 years), among girls, among those with preexisting heart disease, and among those who received chest irradiation (8, 9). Extended follow-up of childhood cancer survivors has made it clear that lower cumulative doses of anthracryclines may place children at risk for cardiac compromise; exposure to 250 mg/m² increased the relative hazard of CHF by 5-fold when compared with children with cancer who were not exposed to anthracryclines (10). CHF is associated with a poor prognosis; 5-year overall survival rates are reported to be less than 50% (11). Utilization of noninvasive cardiac imaging has identified a growing population of survivors with asymptomatic left ventricular dysfunction who may be at risk for late CHF (12, 13).

**Pathogenesis of anthracrycline-related cardiomyopathy**

Anthracryclines cause direct myocardial injury due to free radical formation. Myocardial injury results in thinning of the left ventricular wall, increased myocardial stress, and decreased contractility (14). A 1- or 2-electron reductive activation of anthracryclines leads to cardiotoxicity. A 1-electron reduction of the quinone moiety of doxorubicin results in formation of a semiquinone free radical, which in turn regenerates its parent quinone by reducing molecular oxygen to superoxide anion and hydrogen peroxide—members of the family of reactive oxygen species (ROS). The ROS then cause oxidative stress and energy depletion in cardiomyocytes. A 2-electron reduction of the side-chain carbonyl moiety converts anthracryclines to secondary alcohol metabolites that dysregulate calcium and iron homeostasis. Oxidative stress and ion dysregulation eventually combine to induce cardiomyopathy (Fig. 2; ref. 15).

Studies using transgenic and knockout mice showed altered sensitivity to anthracryclines, suggesting a role for genetic susceptibility in the development of anthracrycline-induced CHF in humans. Thus, transgenic overex-
Table 1. Role of genetic susceptibility in the development of treatment-related adverse events

<table>
<thead>
<tr>
<th>Study</th>
<th>GWAS vs. candidate gene</th>
<th>Study design</th>
<th>Replication study</th>
<th>Sample size</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthracycline-related cardiomyopathy</td>
<td>Candidate gene</td>
<td>Prospective cohort study</td>
<td>No; instead,</td>
<td>1,697 patients (54 with chronic anthracycline-related cardiomyopathy)</td>
<td></td>
</tr>
<tr>
<td>Wojnowski et al (24)</td>
<td>(82 genes with conceivable relevance to anthracycline-related cardiomyopathy)</td>
<td>follow-up with mice studies</td>
<td></td>
<td>Anthracycline-related cardiomyopathy was associated with a variant of the NAD(P)H oxidase subunit NCF4 (rs1883112: OR = 2.5; 95% CI, 1.3–5.0). Mice deficient in NAD(P)H oxidase activity were resistant to chronic doxorubicin exposure, unlike the wild-type mice.</td>
<td></td>
</tr>
<tr>
<td>Blanco et al (23)</td>
<td>Candidate gene</td>
<td>Nested case–control study</td>
<td>No; instead,</td>
<td>30 cases with CHF; 115 matched cancer survivors with no CHF</td>
<td></td>
</tr>
<tr>
<td>(CBR3, NQO1)</td>
<td>(CBR3, NQO1)</td>
<td>follow-up with functional studies</td>
<td></td>
<td>A trend toward an association between the CBR V244M polymorphism and risk of CHF (OR = 8.16, $P = 0.056$ for G/G vs. A/A; OR = 5.44, $P = 0.09$ for GA vs. AA). Recombinant CBR3 V244 (G allele) synthesized 2.6-fold more cardiotoxic doxorubicin per unit of time than CBR3 M244 (A allele; CBR V244, $P = 0.01$)</td>
<td></td>
</tr>
<tr>
<td>Osteonecrosis</td>
<td>GWAS</td>
<td>Prospective cohort study</td>
<td>No</td>
<td>364 patients (69 with symptomatic osteonecrosis)</td>
<td></td>
</tr>
<tr>
<td>Kawedia et al (52)</td>
<td></td>
<td></td>
<td></td>
<td>Polymorphisms of ACP1 (e.g., rs12714403: OR = 5.6; 95% CI, 2.7–11.3) were associated with symptomatic osteonecrosis; ACP1 regulates lipid levels and osteoblast differentiation.</td>
<td></td>
</tr>
<tr>
<td>French et al (47)</td>
<td>Candidate gene</td>
<td>Prospective cohort study</td>
<td>No</td>
<td>64 (25 patients with osteonecrosis)</td>
<td></td>
</tr>
<tr>
<td>(47)</td>
<td>(TYMS, MTHFR, ABCB1, BLGAP, ACP5, LRP5)</td>
<td></td>
<td></td>
<td>Vitamin D receptor FokI start site CC genotype (OR = 4.5, $P = 0.045$) and thymidylate synthase low activity 2/2 enhancer repeat genotype (OR = 7.4, $P = 0.049$)</td>
<td></td>
</tr>
<tr>
<td>(Continued on the following page)</td>
<td></td>
<td></td>
<td></td>
<td>PAI-1 polymorphism (rs6092) was associated with risk of osteonecrosis (OR = 2.89, $P = 0.002$), PAI-1</td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Role of genetic susceptibility in the development of treatment-related adverse events (Cont’d)

<table>
<thead>
<tr>
<th>Study</th>
<th>GWAS vs. candidate gene</th>
<th>Study design</th>
<th>Replication study</th>
<th>Sample size</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity</td>
<td>ESR1, PAI-1, VDR, PTH, PTHR – 12 polymorphisms – chosen on the basis of putative mechanisms underlying osteonecrosis risk</td>
<td>Retrospective cohort study</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ross et al (84)</td>
<td>Candidate gene (polymorphism in LEPR gene, Gln223Arg)</td>
<td></td>
<td></td>
<td>600 patients (278 with BMI ≥ 25 kg/m²)</td>
<td>polymorphisms and PAI-1 serum levels have been associated with thrombosis.</td>
</tr>
<tr>
<td>Ototoxicity</td>
<td>Candidate gene (220 drug metabolism genes; 1,949 polymorphisms)</td>
<td>Case–control</td>
<td>Yes</td>
<td>Discovery set: 33 cases, 20 controls; replication set: 73 cases, 36 controls; controls treated with cisplatin but with no ototoxicity</td>
<td>Genetic variants in TPMT (rs12201199: OR = 17.0; 95% CI, 2.3–125.9) and COMT (rs9332377: OR = 5.5; 95% CI, 1.9–15.9) were associated with cisplatin-induced hearing loss in children</td>
</tr>
<tr>
<td>Oldenburg et al (67)</td>
<td>Candidate gene [known functional polymorphisms in GSTT1 and GSTM1 and codon 105 A/G (Ile/Val) in GSTP1]</td>
<td>Prospective cohort study</td>
<td>No</td>
<td>173 patients; 89 with hearing impairment</td>
<td>Risk of inferior audiometric result was higher in testicular cancer survivors with 105Ile/105Ile-GSTP1 or 105Val/105Ile-GSTP1 compared with 105Val-GSTP1 (OR = 4.21; 95% CI, 1.99–8.88). GSTM1-positivity increased risk of hearing loss. Two combined genotypes were associated with hearing ability. Presence of pattern 1 (GSTT1-positive, GSTM1-positive,</td>
</tr>
<tr>
<td>Study</td>
<td>GWAS vs. candidate gene</td>
<td>Study design</td>
<td>Replication study</td>
<td>Sample size</td>
<td>Results</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>-------------------------</td>
<td>--------------</td>
<td>-------------------</td>
<td>-------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Riedemann et al (69)</td>
<td>Candidate gene (polymorphisms in megalin gene (rs2075252 and rs146681233)); megalin is a member of the low-density lipoprotein receptor family, and is highly expressed in the marginal cells of the stria vascularis of the inner ear – resulting in high accumulation of platinum-DNA adducts</td>
<td>Case–control</td>
<td>No</td>
<td>25 cases; 25 controls (cancer patients exposed to cisplatin with no hearing loss)</td>
<td>An association was found between the A allele of rs2075252 and hearing impairment (OR = 3.45; 95% CI, 1.11–11.2), indicating that SNPs at the megalin gene may impact the individual susceptibility against cisplatin-induced toxicity.</td>
</tr>
<tr>
<td>Therapy-related leukemia</td>
<td>GWAS</td>
<td>Case–control (healthy controls)</td>
<td>Yes</td>
<td>Discovery set: 80 cases, 150 controls; replication set: 70 cases, 95 controls</td>
<td>Among patients with acquired abnormalities of chromosomes 5 or 7; 3 SNPs [rs1394384 (OR = 0.29; 95% CI, 0.15–0.56), rs1381392 (OR = 2.08; 95% CI, 1.29–3.35), and rs1199098 (OR = 0.46; 95% CI, 0.27–0.79)] were associated with t-MDS/AML; rs1394384 is intronic to ACCN1, a gene encoding an amiloride-sensitive cation channel that is a member of the degenerin/epithelial sodium channel; rs1199098 is in LD with IPMK, which encodes a multikinase that positively regulates the prosurvival AKT kinase and may modulate Wnt/b-catenin signaling;</td>
</tr>
<tr>
<td>Study</td>
<td>GWAS vs. candidate gene</td>
<td>Study design</td>
<td>Replication study</td>
<td>Sample size</td>
<td>Results</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------------------</td>
<td>--------------------</td>
<td>-------------------</td>
<td>-------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Ellis et al (173)</td>
<td>Candidate gene (2 common functional p53-pathway variants, the MDM2 SNP309 and the tIPS3 codon 72)</td>
<td>Case–control (healthy controls)</td>
<td>Yes</td>
<td>Discovery set: 80 cases; replication set: 91 cases</td>
<td>rs1381392 is not near any known genes, miRNAs, or regulatory elements, although it lies in a region recurrently deleted in lung cancer. Neither polymorphism alone influenced the risk of t-MDS/AML; however, an interactive effect was detected such that MDM2 TT TP53 Arg/Arg double homozygotes, and individuals carrying both a MDM2 G allele and a TP53 Pro allele, were at increased risk of t-MDS/AML (OR = 2.04; 95% CI, 1.20–3.48; $P_{interaction} = 0.009$).</td>
</tr>
<tr>
<td>Allan et al (141)</td>
<td>Candidate gene approach (polymorphisms in GSTM1, GSTT1, GSTP1)</td>
<td>Case–control</td>
<td>No</td>
<td>89 cases; 420 patients with de novo AML; 1,022 healthy controls</td>
<td>Individuals with at least one GSTP1 codon 105 Val allele were significantly overrepresented in t-AML cases compared with de novo AML cases (OR = 1.81; 95% CI, 1.11–2.94). Also, relative to de novo AML, the GSTP1 codon 105 allele occurred more often among t-AML patients with prior exposure to chemotherapy (OR = 2.66; 95% CI, 1.39–5.09), particularly among those with prior exposure to known GSTP1 substrates (OR = 4.34; 95% CI, 1.43–13.20) and not among t-AML patients with exposure to radiation alone. The variant (C) hMSH2 allele was significantly overrepresented in t-AML cases that had previously been treated with O6-guanine alkylating agents, including cyclophosphamide and procarbazine, compared with controls (OR = 4.02; 95% CI, 1.40–11.37); 38% of the patients were MSI-positive; hMSH2 -6 exon 13 (C) allele confers a</td>
</tr>
<tr>
<td>Worrellow et al (134)</td>
<td>Candidate gene (hMSH2-6 exon 13 polymorphism); evaluation of MSI</td>
<td>Case–control</td>
<td>No; verification done by direct sequencing</td>
<td>91 cases; 420 patients with de novo AML, 837 healthy controls</td>
<td></td>
</tr>
</tbody>
</table>

(Continued on the following page)
Table 1. Role of genetic susceptibility in the development of treatment-related adverse events (Cont’d)

<table>
<thead>
<tr>
<th>Study</th>
<th>GWAS vs. candidate gene</th>
<th>Study design</th>
<th>Replication study</th>
<th>Sample size</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worrillow et al (137)</td>
<td>Candidate gene [polymorphism of MLH1 (position -93, rs1800734)]</td>
<td>Case-control</td>
<td>No</td>
<td>133 cases: 420 patients with de novo AML, 242 patients with primary HL, 117 healthy controls</td>
<td>Carrier frequency of MLH1 -93 variant allele was higher in patients who developed AML or secondary breast cancer after alkylating agent exposure, compared with patients without exposure. The MLH1-93 variant allele was more common in cases with higher grade MSI than in controls. The relative risk of developing t-AML among patients exposed to alkylating agents was 5.31 (95% CI, 1.40–20.15) for the MLH1 -93 variant allele.</td>
</tr>
<tr>
<td>Seedhouse et al (166)</td>
<td>Candidate gene [polymorphisms in XRCC1, XRCC3, XPD, NQO1]</td>
<td>Case-control</td>
<td>No</td>
<td>34 cases: 134 patients with de novo AML, 118 healthy controls</td>
<td>Presence of at least one XRCC1 399Gln allele indicated a protective effect for the allele in controls compared with patients with t-AML (OR = 0.44; 95% CI, 0.20–0.93).</td>
</tr>
<tr>
<td>Jawad et al (154)</td>
<td>Candidate gene [C/T-3 untranslated region (UTR) polymorphism in HLX; polymorphism in RAD51 (135G/C-5 UTR)]</td>
<td>Case-control</td>
<td>No</td>
<td>42 cases: 166 patients with de novo AML, 189 healthy controls</td>
<td>Presence of the variant HLX1 allele significantly increased the risk of t-AML (OR = 3.36; 95% CI, 1.65–6.84). Polymorphism in RAD51 (135G/C-5 UTR) also increased the risk of t-AML. Combined analysis revealed a synergistic 9.5-fold increase (95% CI, 2.22–40.64) in risk for t-AML.</td>
</tr>
</tbody>
</table>

(Continued on the following page)
Table 1. Role of genetic susceptibility in the development of treatment-related adverse events (Cont’d)

<table>
<thead>
<tr>
<th>Study</th>
<th>GWAS vs. candidate gene</th>
<th>Study design</th>
<th>Replication study</th>
<th>Sample size</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subsequent solid malignancies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Best et al (175)</td>
<td>GWAS</td>
<td>Case—control (controls: cancer survivors with no SMNs)</td>
<td>Yes</td>
<td>Discovery set: 100 cases, 89 controls; replication set: 62 cases, 71 controls</td>
<td>Two variants at chromosome 6q21 [rs4946728 (OR = 11.4; 95% CI, 3.23–40.25), rs1040411 (OR = 6.57; 95% CI, 3.19–13.52)] were associated with SMNs in childhood HL survivors, but not in adult-onset HL survivors. The variants comprise a risk locus associated with decreased basal expression of PRDM1 and impaired induction of PRDM1 protein after radiation exposure. PRDM1 encodes a zinc finger transcriptional repressor involved in cellular processes such as proliferation, differentiation, and apoptosis.</td>
</tr>
<tr>
<td>Mertens et al. (136)</td>
<td>Candidate gene (polymorphisms in GSTM1, GSTT1, XRCC1)</td>
<td>Cohort study</td>
<td>No</td>
<td>650 patients with HL (178 with subsequent malignancy)</td>
<td>Individuals lacking GSTM1 were at increased risk of any subsequent malignancy (OR = 1.5; 95% CI, 1.0–2.3). A non-significant increased risk for thyroid cancer was observed in individuals lacking either GSTM1 (OR = 2.9; 95% CI, 0.8–10.9) or GSTT1 (OR = 3.7; 95% CI, 0.6–23.5). Individuals with the genotype of the arginine/glutamine polymorphism at codon 399 in the XRCC1 gene (R399) showed a non-significant increased risk of breast cancer (OR = 1.4; 95% CI, 0.7–2.7).</td>
</tr>
</tbody>
</table>
biological relevance to anthracycline-related cardiotoxicity. CHF was associated with a variant of the NAD(P)H oxidase subunit NCF4 (rs1883112: OR = 2.5; 95% CI, 1.3–3.9). In agreement with these findings, mice deficient in NAD(P)H oxidase activity were resistant to chronic doxorubicin treatment. Thus, this report suggests that genetic variants in free radical metabolism may modulate the individual risk to develop anthracycline-related cardiotoxicity. However, these studies used small samples (30 and 54 cases, respectively), and the findings need to be validated in larger independent replication cohorts.

Osteonecrosis

Osteonecrosis is the death of bone that results in collapse of the bone architecture, leading to progressive joint damage, pain, limited range of motion, articular collapse, arthritis, and loss of function (25, 26). Weight-bearing joints are commonly affected, and the destruction is often severe enough to require total joint replacement surgery for functional rehabilitation and symptomatic and chronic pain. Osteonecrosis accounts for 10% of the 500,000 total joint replacement procedures carried out annually in the United States (25).

Osteonecrosis is a well-recognized complication of corticosteroid use (25, 27–29). Corticosteroids are integral to the management of acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma (NHL), and HL. Mattano and colleagues described the incidence of symptomatic osteonecrosis and associated risk factors in children with ALL treated with intensive chemotherapy including multiple, prolonged courses of corticosteroids (28). The cumulative incidence of osteonecrosis was 9.3% ± 0.9% at 3 years. The incidence was higher among older children (>10 years: 14.2% ± 1.3% vs. <10 years: 9.9% ± 0.4%, P < 0.001). Among 10- to 20-year-olds, the incidence was higher for females (17.4% ± 2.1% vs. 11.7% ± 1.6%, P = 0.03). White children had the highest incidence of osteonecrosis (16.7% ± 1.4%) and black children the lowest (3.3% ± 2.3%). However, although the incidence of osteonecrosis was higher for patients randomized to receive two 21-day dexamethasone courses versus one course (23.2% ± 4.8% vs. 16.4% ± 4.3%), the difference did not reach statistical significance (P = 0.27). Mattano and colleagues extended these findings to patients treated on high-risk ALL study Children’s Oncology Group (COG) AALL0232 (30). Induction rapid early responders receive single delayed intensification (DI) whereas slow responders received double DI; all patients receive monthly 5-day dexamethasone pulses during maintenance. To limit the risk of osteonecrosis in adolescents, children ages 13 years or more received discontinuous dexamethasone during single or double DI, and those younger than 13 years received continuous dexamethasone. The incidence of osteonecrosis was 10.4%, and was higher for those ages 10 years or more vs. less than 10 years (15.2 vs. 2.6%, P < 0.0001, HR = 6.38). Among all patients, the incidence of osteonecrosis incidence was higher in the dexamethasone regimen (11.6 vs. 8.7%, P = 0.014, HR 1.64). Among patients ages 13 years or more, the incidence of osteonecrosis was higher in the dexamethasone regimen (18.9 vs. 9.9%, P = 0.02, RHR 1.97). There was no difference between regimens for children younger than 10 years. These findings inform the utilization of dexamethasone in the future trials.

The contribution of dexamethasone versus prednisone during induction to the development of osteonecrosis has been explored systematically by Teuffel and colleagues (31). This review included all randomized controlled trials comparing dexamethasone with prednisone during induction in children with ALL. These studies included CALGB 7111, CCG-1922, MRC ALL-97/99, TCCSG L95-14, EORTC 58951, COG AALL0232, ALL-BFM 2000, and AIEOP-BFM ALL 2000. The authors concluded that there was no statistically significant difference in the risk of osteonecrosis between the patients treated with dexamethasone or prednisone.

Increased susceptibility to osteonecrosis between 10 and 20 years of age (32–35) is possibly related to puberty-related hormonal and physiologic changes combined with the maturing phase of the adolescent bone.

Certain nonglucocorticoids have also been linked with osteonecrosis, including methotrexate and asparaginase (36, 37), bleomycin/vinblastine (38), melphalan/doxorubicin/cyclophosphamide/5-fluorouracil (39), cyclophosphamide/doxorubicin (40), and daunomycin/cytarabine/all-transretinoic acid (41). However, these observations are in the form of case reports; a systematic effort is needed to explore the role of noncorticosteroid exposures in the development of osteonecrosis.

Pathogenesis of osteonecrosis

Vascular compromise to the bone underlies the development of osteonecrosis. This could be due to vascular occlusion resulting in ischemia, altered fat metabolism and fat emboli, intravascular coagulation, increased intracortical pressure, inhibition of angiogenesis, and mechanical stress, and finally because of direct death of the osteocytes. Glucocorticoids may induce osteonecrosis because of their propensity to cause hyperlipidemia, hypercoagulation, and hypofibrinolysis (42, 43) resulting in intravascular thrombotic occlusion and extravascular lipid deposition. Glucocorticoids also increase the size of intrasosseous lipocytes, leading to increased intracortical pressure, vascular compromise, marrow ischemia, and apoptosis followed by necrosis (44, 45). Glucocorticoids also cause direct injury to the osteocytes (46).

Previous studies have identified polymorphisms in genes putatively related to the development of osteonecrosis, including SERPINE1 (47), VDR (32), and CYP3A4 (48), but with conflicting results (refs. 47, 49; Table 1). Kawedia and colleagues prospectively screened children with ALL and identified older age (>10 years), more intensive treatment, lower albumin, and elevated cholesterol to be associated with symptomatic osteonecrosis, including SERPINE1 (47), VDR (32), and CYP3A4 (48), but with conflicting results (refs. 47, 49; Table 1). Kawedia and colleagues prospectively screened children with ALL and identified older age (>10 years), more intensive treatment, lower albumin, and elevated cholesterol to be associated with symptomatic osteonecrosis.
osteonecrosis; severe osteonecrosis was linked to poor dexamethasone clearance (50). After adjusting for clinical features, polymorphisms in ACP1 were associated with osteonecrosis risk, as well as with low albumin and high cholesterol. Thus, polymorphisms of ACP1 (e.g., rs12714403: OR = 5.6; 95% CI, 2.7–11.3) were associated with symptomatic osteonecrosis. ACP1 is associated with serum cholesterol and triglyceride levels (51), and regulates osteoblast differentiation via Src kinase (52). These findings suggest that ACP1 might act via multiple mechanisms to affect bone homeostasis and dexamethasone-induced osteonecrosis (50). The strengths of this study include the use of clinically validated internal controls (i.e., ALL patients screened with MRI to confirm absence of osteonecrosis), inclusion of clinical risk factors, and markers of bone metabolism as well as markers involved in the proposed pathogenetic pathway for development of osteonecrosis. Although there seems to be biological plausibility to this association, the findings need to be validated in an independent replication cohort.

Ototoxicity

Several potentially ototoxic agents including platinum-based chemotherapy, aminoglycoside antibiotics, loop diuretics, and radiation therapy are commonly used in management of patients with cancer, and can result in sensorineural hearing loss (53). Platinum-containing chemotherapy, particularly cisplatin and myeloablative doses of carboplatin, are well-established risk factors for therapy-related hearing loss. Cisplatin causes serious, permanent, bilateral hearing loss in 10% to 25% of adults and in 41% to 61% of children (54–60) receiving the drug. Risk of ototoxicity increases with cumulative exposure to platinum agents. Radiation-related hearing loss is infrequent (<3%) when cochlear radiation dose is less than 35 Gy (61). Mild to moderate hearing loss occurs in as many as 37% of children exposed to doses exceeding 60 Gy (61). Similar to platinum-related ototoxicity, radiation-induced injury is caused by dose-dependent injury to the cochlea, and specifically affects high frequency hearing. Concomitant use of platinum agents with radiation may have synergistic sensorineural ototoxicity (62). Use of proton beam radiation has made it possible to limit cochlear radiation doses to nearly zero (63). Risk of platinum-related hearing loss is modified by treatment involving multiple ototoxic agents; and young age (less than 4 years) at exposure to ototoxic agents (53).

Pathogenesis of ototoxicity

Platinum-related ototoxicity results from destruction of cochlear hair cells of the organ of Corti, probably as a result of oxidative stress (64). Because of the tonotypical arrangement (in order of pitch) of these specialized hair cells, the initial hearing loss is generally in frequency ranges that exceed 2,000 Hz. As cumulative exposure to platinum chemotherapy increase, so does the progression of injury toward the cochlear apex where frequencies between 500 and 2,000 Hz are affected (65). High-dose radiation may also result in tympanosclerosis, otosclerosis, and Eustachian tube dysfunction (66).

Interindividual variations in ototoxicity among patients receiving similar cumulative cisplatin exposure, suggests the role for SNPs in genes encoding drug metabolizing enzymes (results of extant literature summarized in Table 1). Oldenburg and colleagues examined the association between functional polymorphisms in cisplatinum-detoxifying enzymes—especially glutathione S-transferases (GST) in cisplatin-treated testicular cancer survivors (67). Known functional polymorphisms in GSTTI and GSTM1 and codon 105 A/G (Ile/Val) in GSTP1 were analyzed. The risk of an inferior audiometric test result was 4 times higher in testicular cancer survivors with 105Ile/105Ile-GSTP1 or 105Val/105Val-GSTP1 compared with 105Val/105Val-GSTP1. Presence of 105Val-GSTP1 offered protection against cisplatin-induced hearing impairment. Other candidate gene studies have also reported associations of cisplatin ototoxicity with genetic variants in genes encoding GSTs and megalin (68, 69). Ross and colleagues reported association analyses for 220 candidate drug-metabolism genes in genetic susceptibility to cisplatin-induced hearing loss in children (70). A total of 1,949 SNPs were genotyped in an initial cohort of 54 children with a replication cohort of 112 children. Genetic variants in TPMT (rs1220119) and COMT (rs9332377) were identified to be associated with cisplatin-induced hearing loss in children. TPMT and COMT are methyltransferases and are dependent on the S-adenosyl-methionine (SAM) methyl donor substrate in the methionine pathway. Reduced TPMT and COMT activity result in increased levels of SAM. Administration of SAM and cisplatin together has been shown to increase cisplatin toxicity substantially, whereas the administration of SAM alone was not ototoxic, and cisplatin alone resulted in a moderate increase the ototoxicity (71). These results suggest that cisplatin-induced ototoxicity could be related to increased levels of SAM because of reduced TPMT or COMT activity. Although the samples sizes were small, the investigators were successful in confirming the findings in an independent replication set. An additional strength of the study was the utilization of an appropriate comparison group, that is, cancer patients exposed to cisplatin but with no hearing loss.

Obesity

Several studies suggest a role for therapeutic exposures in the development of obesity in cancer survivors (72–74). Using a cross-sectional approach, Oeffinger and colleagues (73) identified cranial radiotherapy in doses of 20 Gy or more as the primary risk factors for the increased prevalence of obesity noted among ALL survivors, with the highest risk observed in survivors exposed to radiation at the age of 4 years or less. Recently, they extended their findings to describe the rate of increase in body mass
index (BMI) after final height attainment in childhood ALL survivors, over a mean interval of 7.8 years of observation (75). ALL survivors treated with cranial radiation had a significantly greater increase in BMI (women, 0.41 units/y; men, 0.29 units/y) compared with sibling comparison group (women, 0.25 units/y; men, 0.23 units/y). Patients exposed to chemotherapy alone did not differ from the siblings with respect to the change in BMI. Younger age at cranial radiation significantly modified the rate of increase.

Pathogenesis of obesity

Leptin insensitivity possibly plays a role in the development of cranial radiation-related obesity. Brennan and colleagues reported significantly higher levels of leptin in 32 survivors of childhood ALL who had been treated with high doses of cranial radiation compared with age- and BMI-matched controls (76). Leptin is an adipocyte-derived hormone that binds to the biologically active receptor in the hypothalamus (77). It has been proposed that radiation-induced damage to the pituitary–hypothalamus axis disrupts leptin signal, resulting in obesity (76). However, the individual variability is possibly related to genetic susceptibility.

Polygenic variants presumably account for most of the genetic variation relevant to body weight regulation in the general population. Obesity results via the interaction of several polygenic variants and their interaction in turn with environmental factors. Two gene variants with small but replicable effects on body weight have been identified so far: melanocortin-4 receptor genes (MC4R; refs. 78–80) and fat mass and obesity associated genes (FTO; refs. 81, 82). Overall, the mechanism of action of most obesity genes is not well understood; adipose tissue and skeletal muscle are sites for storage, release, and metabolism of fatty acids, and therefore may be involved. The observed genetic variation in obesity explains only a minor fraction (2%-4%) of the total genetic variation explained to be present in the general population (83).

Given that the magnitude of risk for obesity is much higher among cancer survivors than in the general population, it is possible that there is a role for gene–environment (therapy) interactions that influence obesity in the survivors. Ross and colleagues evaluated the potential association between polymorphism Gln223Arg, in the leptin receptor (LEPR) gene and risk of obesity in ALL survivors enrolled in the CCSS (ref. 84; Table 1). Overweight female ALL survivors were significantly more likely to possess a homozygous Arg genotype compared with female survivors with BMI less than 25 kg/m² (24% vs. 12%, \( P = 0.007 \)). In contrast, no significant difference was observed for male survivors (25% vs. 21%, \( P = 0.37 \)). Further, for females, there was a statistically significant (\( P = 0.04 \)) interaction with higher levels of radiation treatment; survivors treated with more than 20 Gy who were homozygous for the Arg allele were greater than 6 times more likely to be overweight/obese compared with those who possessed at least one Gln allele. The risk was considerably lower for females treated with less than 20 Gy radiation or with chemotherapy alone. This study supported the role for LEPR polymorphism as a potential mechanism for obesity in female survivors of childhood ALL exposed to cranial radiation. However, there is a paucity of data in the literature examining the role of genetic susceptibility in the development of obesity in cancer survivors. There is a need to examine the role of genes implicated in obesity in the general population in the cancer survivor population, with careful attention to exploration of gene–environment interactions.

Subsequent Malignant Neoplasms

The cumulative incidence of SMNs exceeds 20% at 30 years after diagnosis of childhood cancer, representing a 4- to 6-fold increased risk for cancer survivors, compared with the general population (85, 86). SMNs are a leading cause of nonrelapse late mortality (87, 88). Unique associations with specific therapeutic exposures have resulted in the classification of SMNs into 2 distinct groups: t-MDS/AML and radiation-related solid SMNs. Characteristics of t-MDS/AML include a short latency (<3 years from primary cancer diagnosis) and association with alkylating agents and/or topoisomerase II inhibitors. Solid SMNs have a strong and well-defined association with radiation, and are characterized by a latency that exceeds 10 years (85, 89–91).

t-MDS/AML has been reported after conventional treatment of HL, NHL, ALL, sarcomas, and breast, ovarian, and testicular cancers (89, 92–97), and after autologous hematopoietic cell transplantation (HCT) for HL or NHL, where it is the major cause of nonrelapse mortality (98–103). The cumulative incidence of t-MDS/AML ranges from 2% at 15 years after conventional therapy (89) to 8.6% at 6 years after autologous HCT (98).

Radiation induces solid SMNs within the radiation field (89, 90, 93, 104, 105). The latency for radiation-related solid SMNs usually exceeds 10 years (89, 90, 93, 105). The risk is highest when radiation exposure occurs at a younger age (89, 91, 105–112), and increases with increasing doses of radiation (Fig. 3) and with increasing time since radiation (90, 93). Eighty percent of the entire burden of subsequent malignancies is accounted for by radiation-related solid SMNs. Some of the well-established radiation-related solid SMNs include breast cancer, thyroid cancer, brain tumors, sarcomas, and basal cell carcinomas (BCC; refs. 85, 89–91, 93, 110, 113). Breast cancer is the most common solid SMN after HL, largely due to high-dose chest radiation for HL (standardized incidence ratio = 25 to 55; refs. 89, 93, 113). For female HL patients treated with chest radiation at less than 16 years of age, the cumulative incidence of breast cancer approaches 20% by the age of 45 years (93). Thyroid cancer develops after neck irradiation for HL or ALL (89, 91, 93, 94, 105). Brain tumors develop after cranial radiation for histologically distinct brain tumors (90), or for management of ALL or NHL (91, 94, 105). Sarcomas develop within the radiation field.
after a latency period of approximately 10 years. More than 90% of BCCs develop within radiation field (89–91, 94, 95, 105).

**Pathogenesis of subsequent malignant neoplasms**

$t$-MDS/AML is a clonal disorder characterized by distinct chromosomal changes (114–116). Two types are recognized by the WHO classification: alkylating agent-related type, and topoisomerase II inhibitor-related type (117). Alkylating agents associated with $t$-MDS/AML include cyclophosphamide, ifosfamide, mechlorethamine, melphalan, busulfan, nitrosoureas, chlorambucil, dacarbazine (118), and platinum compounds (96). Mutagenicity is related to the ability of alkylating agents to form cross-links and/or transfer alkyl groups to form DNA monoadducts. Alkylation results in inaccurate base pairing during replication and single- and double-strand breaks in the double helix, as the alkylated bases are repaired. The risk of alkylating agent-related $t$-MDS/AML is dose dependent, with a latency of 3 to 5 years after exposure; it is associated with abnormalities involving chromosomes 5 ($-5/\text{del}[5q]$) and 7 ($-7/\text{del}[7q]$), and a high frequency of multidrug-resistance phenotype (119).

The magnitude of association between specific chemotherapeutic agents and radiation and SMNs are moderate to large (OR = 3.1 to 15.9), with a clear dose–response relationship adding further biological credibility to that association. The risk of second breast cancer after chest radiation increases in a linear fashion with radiation dose ($P_{\text{trend}} < 0.001$; refs. 113, 120), so does the risk for second brain tumors (ref. 90; Fig. 3) and second sarcomas (121). Literature clearly supports the role of chemotherapy and radiation in the development of SMNs (118), but interindividual variability suggests a role for genetic variation in susceptibility to genotoxic exposures. The risk of SMNs could potentially be modified by mutations in high-penetrance genes that lead to serious genetic diseases, for example, Li–Fraumeni syndrome (122) and Fanconi anemia (123–126). However, the attributable risk is expected to be very small because of their extremely low prevalence. The interindividual variability in risk of therapy-related SMNs is more likely related to common polymorphisms in low-penetrance genes that regulate the availability of active drug metabolite, or those responsible for DNA repair. Genetic variation contributes 20% to 95% of the variability in cytotoxic drug disposition (127). Polymorphisms in genes involved in drug metabolism and transport are relevant in determining disease-free survival and drug toxicity (128). Variation in DNA repair plays a role in susceptibility to de novo cancer (129-133) and likely modifies SMN risk after exposure to DNA-damaging agents, such as radiation and chemotherapy. Gene–environment interactions may magnify subtle functional differences resulting from genetic variations (134–137). Results from studies examining genetic susceptibility in the development of SMNs are summarized in Table 1.

**Drug metabolism.** Metabolism of genotoxic agents occurs in 2 phases. Phase I involves activation of substrates into highly reactive electrophilic intermediates that can damage DNA—a reaction principally performed by the cytochrome p450 (CYP) family of enzymes. Phase II enzymes (conjugation) function to inactivate genotoxic substrates. The phase II proteins comprise the GST, NAD (P)H:quinone oxidoreductase-1 (NQO1), and others. The balance between the 2 sets of enzymes is critical to the cellular response to xenobiotics; for example, high activity of phase I enzyme and low activity of a phase II enzyme can result in DNA damage from the excess of harmful substrates. Given that these enzymes activate/detoxify chemotherapeutic agents, their role in the development of SMNs applies mainly to $t$-MDS/AML. The xenobiotic substrates of CYP proteins include cyclophosphamide, ifosfamide, thiotapec, doxorubicin, and dacarbazine (138). The CYPs transfer singlet oxygen onto their substrates creating highly reactive intermediates which, unless detoxified by phase II enzymes, have a strong ability to damage DNA (139). The expression of these enzymes is highly variable among individuals because of several

---

**Figure 3.** Radiation-related risk of subsequent malignant neoplasms. 
functionally relevant genetic polymorphisms. GSTs detoxify reactive electrophiles via conjugation to reduced glutathione, preventing damage to DNA. Polymorphisms exist in cytosolic subfamilies: μ [M], π [P], θ [T], and others. GSTs detoxify doxorubicin, lomustine, busulfan, chlorambucil, cisplatin, cyclophosphamide, melphalan, and others (140). Quinone oxidoreductase NQO1 uses the cofactors NADH and NADPH to catalyze the electron reduction of its substrates, produces less reactive hydroquinones, and therefore prevents generation of ROS and free radicals which may subsequently lead to oxidative damage of cellular components. Allan and colleagues used a candidate gene approach to examine associations between polymorphisms in the GST genes (GSTM1, GSTT1, and GSTP1) and t-MDS/AML (141). A case–control study design was used (80 cases, 420 patients with de novo AML, 1,022 healthy controls). Individuals with at least one GSTP1 codon 105 Val allele were significantly overrepresented in t-AML cases compared with de novo AML cases (OR = 1.81; 95% CI, 1.11–2.94). Also, relative to de novo AML, the GSTP1 codon 105 allele occurred more often among t-AML patients with prior exposure to chemotherapy (OR = 2.66; 95% CI, 1.39–5.09), particularly among those with prior exposure to known GSTP1 substrates (OR = 4.34; 95% CI, 1.43–13.20) and not among t-AML patients with exposure to radiation alone. Although the findings were biologically plausible, they were not replicated in this study. Furthermore, the comparison groups consisting of healthy controls and patients with de novo AML could possibly compromise the findings.

Drug transport. P-glycoprotein (encoded by MDR1) traps hydrophobic drugs in the plasma membrane of cells and effuxes them by using an ATP-dependent process; many chemotherapeutic drugs are substrates of this protein. A number of polymorphisms exist in the MDR1 gene, some proposed to be functional and evaluated as risk factors for t-MDS/AML (142).

DNA repair. DNA repair mechanisms protect somatic cells from mutations in tumor suppressor genes and oncogenes that can lead to cancer initiation and progression. An individual’s DNA repair capacity seems to be genetically determined (143). A number of DNA repair genes contain polymorphic variants, resulting in large interindividual variations in DNA repair capacity (143). Even subtle differences in an individual’s DNA repair capacity may be important in the presence of large external influences such as chemotherapy or radiotherapy. Individuals with altered DNA repair mechanisms are likely susceptible to the development of genetic instability that drives the process of carcinogenesis as it relates to both chemotherapy-related t-MDS/AML as well as radiation-related solid SMNs.

Mismatch repair (MMR) functions to correct mismatched DNA base pairs that arise as a result of misincorporation errors that have avoided polymerase proofreading during DNA replication (144). Defects in the MMR pathway result in genetic instability or a mutator phenotype, manifested by an elevated rate of spontaneous mutations characterized as multiple replication errors in simple repetitive DNA sequences (microsatellites)—functionally identified as microsatellite instability (MSI). Approximately 50% of t-MDS/AML patients have MSI, associated with methylation of the MMR family member MLH1 (145, 146), low expression of MSH2 (147), or polymorphisms in MSH2 (134, 148–150). The appearance of MMR-deficient, drug-resistant clones during genotoxic treatment for a primary cancer could be a vital factor in SMN susceptibility, particularly because the mutator phenotype (inherent of MMR-deficient cells) would be expected to accelerate the accumulation of further mutations and eventually SMN initiation. In addition, loss of MMR may result in deregulation of homologous recombination repair and consequent chromosomal instability (151).

Double-strand breaks (DSB) in DNA may lead to loss of genetic material, resulting in chromosomal aberrations. High levels of DSBs arise following ionizing radiation and chemotherapy exposures. Cellular pathways available to repair DSBs include homologous recombination (HR), nonhomologous end-joining (NHEJ), and single-strand annealing (152). HR uses the second, intact copy of the chromosome as a template to copy the information lost at the DSB site on the damaged chromosome—a high-fidelity process. RAD51 is one of the central proteins in the HR pathway, functioning to bind to DNA and promote ATP-dependent homologous pairing and strand transfer reactions (153, 154). RAD51-G135C polymorphism is significantly overrepresented in patients with t-MDS/AML compared with controls (C allele: OR = 2.7; ref. 155). XRCC3 also functions in the HR DSB repair pathway by directly interacting with, and stabilizing RAD51 (156, 157). XRCC3 is a paralog of RAD51, also essential for genetic stability (158, 159). A polymorphism at codon 241 in the XRCC3 gene results in a Thr→Met amino acid substitution (160). The variant XRCC3-241Met allele has been associated with a higher level of DNA adducts compared with cells with the wild-type allele, implying aberrant repair (161) and has also been associated with increased levels of chromosome deletions in lymphocytes after exposure to radiation (162). Although XRCC3-Thr241Met was not associated with t-MDS/AML (OR = 1.4; 95% CI, 0.7–2.9), a synergistic effect resulting in an 8-fold increased risk of t-MDS/AML (OR = 8.1; 95% CI, 2.2–29.7) was observed in the presence of XRCC3-241Met and RAD51-135C allele in patients with t-MDS/AML compared with controls (159). NHEJ pathway joins broken DNA ends containing very little homology. This process is not always precise and can result in small regions of nontemplate nucleotides around the site of the DNA break, potentially relevant in MLL-translocation associated with t-MDS/AML. Many of the translocation junctions have been cloned and sequenced and found to contain regions of microhomology consistent with the operation of the NHEJ pathway and an impairment of this pathway has been hypothesized to modulate t-MDS/AML risk (163).
Base excision repair (BER) pathway corrects individually damaged bases occurring as a result of ionizing radiation and exogenous xenobiotic exposure. The XRCC1 protein plays a central role in the BER pathway and also in the repair of single strand breaks, by acting as a scaffold and recruiting other DNA repair proteins (164, 165). The protein also has a BRC A1 C-terminal domain—a characteristic of proteins involved in DNA damage recognition and response. The presence of variant XRCC1-399Gln has been shown to be protective for t-MDS/AML (166) and BCC (167).

Nucleotide excision repair (NER) removes structurally unrelated bulky damage induced by radiation and chemotherapy. The NER pathway is linked to transcription, and components of the pathway comprise the basal transcription factor IIH complex (TFIIH), which is required for transcription initiation by RNA polymerase II. One of the genes involved in the NER pathway (ERCC2) is a member of the TFIIH complex. The polymorphic Gln variant (ERCC2 Lys751Gln) is associated with t-MDS/AML (135).

Association of variants in XPD, XRCC1, XRCC2, XRCC3, ERCC1, and APE1 with several primary cancers has been reported (130), usually with the risk increased 4- to 5-fold (129). Furthermore, individuals with a reduced capacity in 2 different pathways exhibit an even higher risk (168). Adaptive response to low-dose radiation in human lymphocytes displays heterogeneity with respect to chromosomal aberrations (169). Chromosomal assays, micronucleus tests (163), hprt gene mutation, and comet assays for DNA damage show significant interindividual variation (170, 171). More than 80 DNA repair genes have been screened and show evidence of extensive polymorphic variation (172).

Ellis and colleagues used a case–control study design (171 cases) and examined the association between patients with t-MDS/AML and 2 common functional p53-pathway variants—the MDM2 SNP309 and the TP53 codon 72 polymorphism (173). Neither polymorphism showed a significant association. However, an interactive effect was detected such that MDM2 TT TP53 Arg/Arg double homozygotes, and individuals carrying both a MDM2 G allele and a TP53 Pro allele were at increased risk of chemotherapy-related t-MDS/AML. The strengths of the study included the utilization of a discover set (n = 80 case) and replication set (n = 91 cases). However, the investigators used healthy controls as a comparison group, thus precluding the ability to assess whether the case–control differences reflected differences in susceptibility to primary disease or t-MDS/AML.

Knight and colleagues used a case–control study design to conduct a GWAS in patients who had developed therapy-related leukemia (cases) and healthy controls (174). The discovery set included 80 cases and 150 controls. The relevant findings were replicated in an independent set of 70 cases and 95 controls. The investigators identified 3 SNPs (rs1394384 (OR = 0.29; 95% CI, 0.15–0.56), rs1381392 (OR = 2.08; 95% CI, 1.29–3.35), and rs1199098 (OR = 0.46; 95% CI, 0.27–0.79]) to be associated with t-MDS/AML with chromosome 5 or 7 abnormalities. rs1394384 is intronic to ACCN1, a gene encoding an amiloride-sensitive cation channel that is a member of the degenerin/epithelial sodium channel; rs1199098 is in linkage disequilibrium (LD) with IPMK, which encodes a multikinase that positively regulates the prosurvival AKT kinase and may modulate Wnt/β-catenin signaling; rs1381392 is not near any known genes, miRNAs, or regulatory elements, although it lies in a region recurrently deleted in lung cancer. Although the investigators were able to confirm findings in an independent replication cohort, the utilization of a noncancer healthy control group raises concerns about the case–control differences being generated by the genetics of the primary cancer vs. t-MDS/AML.

Best and colleagues conducted a GWAS to identify variants associated with radiation-related solid malignancies in survivors of HL (175). They identified 2 variants at chromosome 6q21 associated with second malignancies. The variants comprise a risk locus associated with decreased basal expression of PRDM1 and impaired induction of the PRDM1 protein after radiation exposure. These data suggest new gene–exposure interaction that may implicate PRDM1 in the etiology of radiation therapy-induced second malignancies.

Summary and Future Directions

Several studies have examined single gene polymorphisms in small heterogeneous samples, contributing to the largely inconclusive results. Functional redundancy often results in the availability of more than one gene product to detoxify the same substrate or repair the same damage type. Hence, a variant in 1 gene may have minimal consequences, whereas the combination of variants in 2 or more genes could have more serious consequences resulting in the emergence of a malignant phenotype. Furthermore, previous studies have generally failed to systematically examine gene–therapy interactions, because of the absence of detailed therapeutic exposure data collected by the previous studies and the small sample sizes. A systematic assessment of the role of drug-metabolizing enzymes, DNA repair genes and drug transport in the development of SMNs is currently under way in a Children’s Oncology Group–wide study, funded by the National Cancer Institute. This study uses a case–control study design to assess the role of genetic susceptibility in the development of key treatment-related adverse outcomes, including SMNs.

The current report has focused on the current state of the knowledge related to the pathogenesis of adverse events that have clearly defined associations with therapeutic exposures, and that result in significant long-term morbidity. There are several other adverse outcomes with clearly defined associations with therapeutic exposures that were not included in this report, largely because of paucity of published literature with respect to the mechanistic relationship between therapy and outcome. These
outcomes include endocrine dysfunction, pulmonary compromise, stroke, and peripheral neuropathy.

This review is not meant to serve as a comprehensive overview of all published studies; rather it provides examples of some of the more scientifically and methodologically robust studies and shows that genetic variation could possibly interact with therapeutic exposure and increase the risk of adverse outcomes. However, there remains a critical need to replicate these findings in large independent cohorts before these findings can be incorporated into the clinical management of the patients.

The discovery of functional genetic variants associated with key outcomes will have significant implications for future research aimed at improving risk assessment and conducting mechanistic research. Identification of new and informative genetic markers have utility for developing objective pretherapy risk assessment and patient counseling, and serving as rational tools for clinical management and treatment planning. The ultimate goal is to identify those at highest risk, such that targeted prevention and intervention strategies can be instituted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work is supported in part by NCI grants U10 CA98543 (P. Adamson), U24 55727 (L.L. Robison), and ROI CA 139633 (S. Bhatia), and the Leukemia and Lymphoma Society: 6903-08 (S. Bhatia).

Received July 13, 2011; revised August 16, 2011; accepted August 16, 2011; published online October 6, 2011.

References


Role of Genetic Susceptibility in Development of Treatment-Related Adverse Outcomes in Cancer Survivors

Smita Bhatia

_Cancer Epidemiol Biomarkers Prev_ 2011;20:2048-2067.

Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/20/10/2048

Cited articles
This article cites 164 articles, 75 of which you can access for free at:
http://cebp.aacrjournals.org/content/20/10/2048.full.html#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
/content/20/10/2048.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.