Copy Number Alterations in DNA Repair/Repair-Related Genes Identify Disease-Specific Biomarkers with Potential Clinical Relevance to Hematologic Malignancies

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DNA repair and repair-related genes encode molecules that function as critical guardians of the genome, both in terms of chromosome stability and single base integrity. Defects in such functions, both inherited and acquired, represent key factors that contribute to the etiology of cancer (Ref 1, 2). Most mutations and large genomic alterations (deletions, translocations, loss of heterozygosity, and amplifications) that are relevant to cancer originate from DNA injury or

aberrant genome maintenance. In addition to the catalytic repair of damage, maintaining the genome requires repair-related functions such as alterations in the components of chromatin and safeguards against precancerous clonal expansion. Thus, in addition to initiating carcinogenesis, defects in DNA repair drive the evolution of disease from benign to malignant by allowing additional genetic and epigenetic changes to accumulate facilitating progression to a more aggressive state (Ref 2). Furthermore, since most cancer therapies are based on the deliberate introduction of damage into DNA, DNA repair is also important for a therapeutic response and resistance to therapy is often facilitated through the loss of the mechanisms that mediate cell death. Given the multitude of aforementioned roles, it is not surprising that evidence of defective DNA damage repair is detected in essentially all tumors (e.g., *RB1*, *TP53* and *ATM*). Importantly, such weakened DNA repair mechanisms associated with cancers may also serve as the targets for selected therapy (Ref 2, 4, 5).

To gain a fuller appreciation of the specific DNA repair/repair-related functions defective in unique hematologic malignancies, we analyzed 155 DNA repair/repair-related genes for copy number alterations (CNAs) in 425 cases of hematologic malignancy using microarray analysis. Cases represented nine distinct clinical entities with at least 30 cases per entity. Disorders represented include myelodysplastic syndrome (MDS), chronic lymphocytic leukemia (CLL), myeloproliferative neoplasm (MPN), chronic myeloid leukemia (CML), B-cell and T-cell acute lymphoblastic leukemia (B-ALL, T-ALL), acute myeloid leukemia (AML), non-Hodgkin lymphoma (NHL), and multiple myeloma (MM).

OligonucleotideCGH-based copy number analysis was performed using a 135K-feature whole-genome oligonucleotide microarray (aCGH) (Signature OncoChip®, designed by Signature Genomics, Spokane, WA; manufactured by Roche NimbleGen, Madison, WI). When compared to probe coverage over the rest of the genome, this microarray has denser oligonucleotide coverage over 1,893 cancer features, including 155 DNA repair/repair-related genes. The microarray has an average oligonucleotide coverage of one oligonucleotide per 0.2–7.0 kb for the targeted cancer features and genomic backbone coverage of one oligonucleotide per 35 kb. Labeling, hybridization, and washing were performed using previously published methods. Data

were analyzed and displayed using custom oligonucleotide aCGH data analysis and visualization software (Oncoglyphix®; Signature Genomics).

Results were assessed relative to multiple databases of known benign variation. A proof of principal analysis included *RB1*, *TP53* and *ATM*, for which genomic alterations and disease preference have been well established. Analysis of *RB1* revealed CNAs in 74 cases (4 gains and 70 losses) (17.4% of total) with copy gains uniformly attributable to trisomy 13 in cases of B-ALL. As expected, loss of *RB1* typically reflected either monosomy 13 (31/70 seen predominately in MM) or smaller deletions (~1 Mb, seen in CLL). CNAs in *TP53* included 2 gains and 42 losses (10.4% of total), with gains again related to trisomy (exclusively hyperdiploid B-ALL), whereas deletions varied in size and disease association. CNAs in the *ATM* included 28 gains and 19 losses (11.1% of total). Gains reflecting trisomy correlated with diverse diagnoses, while more focal gains (~10 Mb) were seen exclusively in B-ALL (3 cases). Not surprisingly, small losses of *ATM* (<10 Mb) occurred almost exclusively in CLL. Thus, microarray findings for these classic tumor suppressor genes correlated well with disease specificity for alterations submicroscopic in size (<10 Mb).

An additional 152 DNA repair/ repair-related genes were analyzed. Alterations in a significant portion of these genes reflected only large non-specific gains or losses (e.g., monosomy, trisomy, whole arm gains/losses). However, several genes showed disease-specific correlations for submicroscopic CNAs. For example, deletion of *ERCC5* was seen in B-ALL (2 cases); however, co-deletion of *ERCC5* and *LIG4* was confined to NHL (3 cases). Smaller deletions involving a number of other genes also occurred specifically in NHL (*GTF2H4*, *POLQ*, *REV1L*, *FANCL*, *BRCA2*, and *NUDT1*). While abnormalities in *TERT* were infrequent (21 gains and 4 losses) and nearly always associated with aneuploidy, an intragenic duplication of three exons was seen in a single case of diffuse large B-cell lymphoma with a history of failed therapy. Regarding AML, deletion of UBE2V2 was seen in a case exhibiting numerous multi-megabase amplifications, a potential consequence of dysregulated DNA replication (Ref 7). AML-specific CNAs include deletions of *RPA2* that always associated with translocation-positive cases (*PML*/*RARA*; *MLL*/*MLLT3*; *MLL*/*MLLT4*), and deletions of *APTX* (reduced expression correlates with favorable therapy response)(Ref 8), as well as an intronic duplication within *MGMT* (therapy

resistance associates with overexpression). Smaller CNAs involving *ALKBH3* were seen specifically in MM (3 cases), while deletions of *ALKBH5* impacting nearby *TOP3A* were seen in MDS (2 cases). Additional genes in which recurrent CNAs were associated with multiple disorders included *NEIL1*, *PMS2*, *ENDOV*, *CHEK2*, and *HELQ*.

The data reveal CNAs in DNA repair and repair related genes; many are recurrent abnormalities that exhibit disease specificity. The findings are consistent with previous data addressing biological function, disease progression and response to therapy. Further study is warranted to validate these alterations as diagnostic/prognostic biomarkers or as predictive biomarkers useful in directing more personalized treatment decisions. They may also represent potential new targets for therapy.

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