A Comparison of Gene Expression Profiles between Primary Human AML Cells and Therapy-related AML Cells

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Abstract – To identify genes whose expression correlated with biological features of therapy-related AML (t-AML), we analyzed the expression profiles of de novo AML t(9;11) and t-AML t(9;11) bone marrow samples using previously published SAGE data. Three-hundred twenty-nine transcripts that satisfied statistical (P<0.05) and magnitude-of-change (≥ 4-fold) criteria were identified as differentially expressed between de novo AML t(9;11) and t-AML t(9;11) cells. Of these transcripts, 301 (91%) matched known genes or ESTs and were classified according to functional categories (http://david.abcc.ncifcrf.gov/). The majority of differentially expressed genes in t-AML t(9;11) were involved in the regulation of biological and metabolic processes. Especially prominent among these were genes related to immune and drug responses. These results establish a framework for developing new drugs for the treatment of t-AML.

Keywords : Therapy-related AML, Gene expression, SAGE

INTRODUCTION

Therapy-related acute myeloid leukemia (t-AML) can develop in de novo AML or other malignant diseases after exposure to cytotoxic agents used to treat the disease, including radiation, drugs and other chemicals (Seedhouse and Russell, 2007). t-AML, which constitutes approximately 10%–15% of all AML, is induced mainly by chromosomal aberrations, such as translocations, deletions and inversions (Rowley, 1999; Lee et al., 2006). These include the t(15;17) and t(8;21) translocations, the inv(16) inversion, and rearrangements at the 11q23 locus, such as the t(9;11) translocation.

Compared with de novo AML, t-AML has a high frequency of atypical karyotypes (Kantarjian et al., 1986; Pedersen-Bjaergaard et al., 1990), which occur as a direct result of the action of alkylating agents, topoisomerase II inhibitors and radiation used as treatment modalities (Pedersen-Bjaergaard et al., 2002). Alkylating agents generate various chromosomal abnormalities by inducing DNA interstrand cross-linking (Hemminki and Kallama, 1986; Povirk and Shuker, 1994), and topoisomerase II inhibitors produce aberrant chromosomes by interfering with the repair of damaged chromosomes (Seedhouse and Russell, 2007; Liu, 1989). These karyotypic abnormalities have ramifications for whole-gene expression patterns, and the resulting disturbance in the normal function of myeloid cells is responsible for t-AML.

The death rate from t-AML is higher than of de novo AML, thus heightening the importance of accurately diagnosing and treating t-AML (Schuch et al., 2004). This urgency is particularly acute with respect to leukemias of the 11q23 rearrangement type, which are an unfavorable group with a poor prognosis and high death rate relative to t(15;17), t(8;21) and inv(16) karyotypic leukemias (Schuch et al., 2004). Currently, the diagnosis of leukemia is based on cytological and morphological properties of leukemic cells (Harris et al., 1999). However, this process is time consuming and requires pathology expertise. To overcome this problem, recent efforts have focused on finding...
molecular markers that can be used to distinguish various types of leukemia from one another (Valk et al., 2004; Haferlach et al., 2005). Of the approaches available for identifying candidate molecular markers for leukemia, microarray techniques have been among the most widely used. In this study, we compared the gene expression profiles of de novo AML t(9;11) and t-AML t(9;11) cells using previously published AML SAGE data (Lee et al., 2006). Our focus was on identifying factors that distinguish these two AML types with the ultimate goal of providing new insight into the etiology of AML.

MATERIALS AND METHODS

Acquisition of de novo AML t(9;11) and t-AML t(9;11) cell SAGE data

De novo AML t(9;11) and t-AML t(9;11) cells were compared using SAGE data obtained from the recent report by Lee et al. (Lee et al., 2006) (http://www.ncbi.nlm.nih.gov/projects/geo/, GEO accession #: GSE3255). The data from three individual de novo AML t(9;11) cells and three individual t-AML t(9;11) cells were integrated to create a profile of each cell type.

Bioinformatics and Statistical Analysis

SAGE tags were matched to a SAGEmap database updated on February 19, 2008 (http://www.ncbi.nlm.nih.gov/SAGE/). For SAGE tags shared by multiple genes, only single-matched SAGE tags were selected. Genes that were differentially expressed between the de novo AML t(9;11) and t-AML t(9;11) cells were determined by analyzing SAGE data using IDEG6(http://telethon.bio.unipd.it/bioinfo/IDEG6_form/). A general Chi-squared test (significance threshold = 0.05) was used to determine significance. Differentially expressed genes were defined as those for which P values were < 0.05 and ≥ 4-fold differences in expression. These 291 matched genes were classified using the DAVID functional annotation tool (http://david.abcc.ncifcrf.gov/).

RESULTS AND DISCUSSION

A comparison of gene expression profiles between de novo AML t(9;11) and t-AML t(9;11) cells

A total of 65,307 and 61,709 individual SAGE tags were selected from the de novo AML t(9;11) and t-AML t(9;11) cells, respectively, from which 36,465 and 28,723 unique SAGE tags were identified (Table. I). A comparison of the SAGE tags from t-AML t(9;11) cells with those from de novo AML t(9;11) cells showed that the patterns of gene expression in these two cell types were different, identifying 329 unique SAGE tags with P values < 0.05 and ≥ 4-fold differences in expression. Of these tags, 187 (57%) were increased and 142 (43%) were decreased in the t-AML t(9;11) cells; 301 (91%) were matched to the SAGE map database and 28 (9%) had no match (Table. II). Sixteen of the genes in the “increased” group had more than two SAGE tags and four in the “decreased” group had more than two tags. These 291 matched genes were classified using the DAVID functional annotation tool (http://david.abcc.ncifcrf.gov/).

Identification of genes differentially expressed in de novo AML t(9;11) and t-AML t(9;11) cells

Examples of genes that were differentially expressed between t-AML t(9;11) cells and de novo AML t(9;11) cells are presented in Table III. Among the over-expressed genes, NFKBIA is known to be a major oncogene in various malignant tumors and diseases (Srivastava and Anderson, 2007; Hatta et al., 2003); MPO protects DNA from oxygen radical-mediated damage during the maturation and differentiation of myeloid cells.

Table I. Number of total and unique SAGE tags in de novo AML t(9;11) and t-AML t(9;11) cells

<table>
<thead>
<tr>
<th>ITEM</th>
<th>de novo AML</th>
<th>t-AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unique tags</td>
<td>36,465</td>
<td>28,723</td>
</tr>
<tr>
<td>Total tags</td>
<td>65,307</td>
<td>61,709</td>
</tr>
<tr>
<td>Unique tags /total tags</td>
<td>55.8 %</td>
<td>46.5 %</td>
</tr>
</tbody>
</table>

Table II. Total SAGE tags classified by expression level (p < 0.05)

<table>
<thead>
<tr>
<th>Group</th>
<th>Increase</th>
<th>Decrease</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identified UNIGENE</td>
<td>169</td>
<td>132</td>
<td>301</td>
</tr>
<tr>
<td>Novel tags</td>
<td>18</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>187</td>
<td>142</td>
<td>329</td>
</tr>
</tbody>
</table>

The score means tag count number
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(Murao et al., 1988); RNF7 exerts an anti-apoptotic function against reactive oxygen species (ROS) generated by redox-reagents (Duan et al., 1999); TPT1 inhibits apoptosis induced by the antineoplastic agent, etoposide, used in the treatment of leukemia (Zhang et al., 2002); SIVA induces apoptosis through the caspase-dependent mitochondrial pathway (Py et al., 2004); PBEF1 delays neutrophil apoptosis by inhibiting the activity of caspase-8 and caspase-3 (Jia et al., 2004); and SNRPF, SNRPD2 and SNRPC have important roles in RNA splicing.

Genes under-expressed in t-AML t(9;11) cells include EGR1, which is involved in cell growth and differentiation (Liu et al., 1996); HSPA1A, which is a member of the heat shock protein 70 family that regulates the cell cycle and proliferation (Rohde et al., 2005); JUND, which inhibits tumor angiogenesis by inhibiting RAS-associated ROS generation (Gerald et al., 2004); TSC22D3, which is a glucocorticoid-induced leucine zipper (GILZ) protein that controls the negative regulation of RAS- and RAF-induced cell proliferation (Ayrolldi et al., 2007); and SH3GLB1, which regulates apoptosis induced by the pro-apoptotic gene, BAX (Cuddeback et al., 2001). One of the most striking differences in expression between t-AML t(9;11) cells and de novo AML t(9;11) cells was MHC class I B, which was 100-fold over-expressed in t-AML cells. MHC class I and class II serve similar functions, but details of their functions are different. MHC class I regulates the activity of CD8+ T cells and natural killer cells, and MHC class II activates various immune cells by bind-

<table>
<thead>
<tr>
<th>Tag sequence</th>
<th>Symbol</th>
<th>tAML†</th>
<th>De novo‡</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase</td>
<td></td>
<td></td>
<td></td>
<td>Major histocompatibility complex, class I, B</td>
</tr>
<tr>
<td>CTGATCTGTG</td>
<td>HLA-B</td>
<td>100</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TAACAGCAG</td>
<td>NFKBIA</td>
<td>43</td>
<td>2</td>
<td>Nuclear factor of kappa light polypeptide gene</td>
</tr>
<tr>
<td>GACTTGTATA</td>
<td></td>
<td>22</td>
<td>2</td>
<td>enhancer in B-cells inhibitor.</td>
</tr>
<tr>
<td>GCTCCCTTT</td>
<td>MPO</td>
<td>61</td>
<td>3</td>
<td>Myeloperoxidase</td>
</tr>
<tr>
<td>CTTATGGTG</td>
<td>RNF7</td>
<td>7</td>
<td>1</td>
<td>Ring finger protein 7</td>
</tr>
<tr>
<td>GTGCTCTAT</td>
<td>TPT1</td>
<td>17</td>
<td>1</td>
<td>Tumor protein, translationally-controlled 1</td>
</tr>
<tr>
<td>TTCTATTTTG</td>
<td>SIVA1</td>
<td>6</td>
<td>1</td>
<td>SIVA1, apoptosis-inducing factor</td>
</tr>
<tr>
<td>GCCTTAACAA</td>
<td>PBEF1</td>
<td>6</td>
<td>1</td>
<td>Pre-B-cell colony enhancing factor</td>
</tr>
<tr>
<td>CTGCTGTGAT</td>
<td>SNRPC</td>
<td>10</td>
<td>1</td>
<td>Small nuclear ribonucleoprotein polypeptide C</td>
</tr>
<tr>
<td>CAGCTTGCAA</td>
<td>SNRPD2</td>
<td>5</td>
<td>1</td>
<td>Small nuclear ribonucleoprotein polypeptide D2 polypeptide 16.5kDa</td>
</tr>
</tbody>
</table>

| Decrease     |            |       |          | CD74 molecule, MHC class II invariant chain                           |
| CTGACTGTCC   | CD74       | 1     | 5        |                                                                        |
| GAGTTAAAAA   | HLA-DRB4   | 1     | 5        | MHC class II, DR beta 4                                              |
| TTCCCTCTCT   | HLA-DPB1   | 4     | 50       | MHC class II, DP beta 1                                              |
| TCAAGCCATC   | EGR1       | 1     | 8        | Early growth response 1                                              |
| GGATATGTTG   |            | 1     | 22       |                                                                        |
| TAGAAACCGG   | HSPA1A     | 1     | 7        | Heat shock 70kDa protein 1A                                           |
| CAGAGATGAA   |            | 3     | 21       |                                                                        |
| ACCCCCCGCC   | JUND       | 0     | 5        | Jun D proto-oncogene                                                 |
| TTGAAGGCCC   | TSC22D3    | 1     | 7        | TSC22 domain family, member 3                                        |
| TTAGAGACTT   | SH3GLB1    | 1     | 7        | SH3-domain GRB2-like endophilin B1                                   |

†-AML† and de novo‡ values indicate tag count number
According to the T cell receptor of CD4+ T cells (Bierer and Burakoff, 1988). Thus, by distinctly disrupting CD4+ and CD8+ T cell, and the natural killer cell functions and associated immune responses, differential expression of MHC class I and MHC class II between the de novo AML t(9;11) cells and t-AML t(9;11) cells can be expected to contribute to the differences in the symptoms between the two types of t(9;11) AML. These results indicate that, although de novo AML t(9;11) and t-AML t(9;11) have the same aberrant chromosome, their whole-gene expression patterns, and thus their symptoms, are different.

Functional clustering of the genes and differences in expression levels between t-AML t(9;11) de novo AML t(9;11)

The 291 differentially expressed genes were classified using the DAVID functional annotation tool. Because one gene can have multiple functions, the gene count based on function is higher than the original number of genes. The genes in the “increased” group were mainly clustered into regulation of biological processes, macromolecule metabolic processes and primary metabolic processes, whereas most genes in the decreased group were related to primary metabolic processes and macromolecule metabolic processes (Table IV).

Table IV. Functional annotation of differentially expressed genes.

<table>
<thead>
<tr>
<th>Annotation group</th>
<th>Gene count</th>
<th>Annotation group</th>
<th>Gene count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase</td>
<td></td>
<td>Decrease</td>
<td></td>
</tr>
<tr>
<td>Translation</td>
<td>16</td>
<td>Cell proliferation</td>
<td>11</td>
</tr>
<tr>
<td>Biosynthetic process</td>
<td>21</td>
<td>Translation</td>
<td>9</td>
</tr>
<tr>
<td>RNA processing</td>
<td>8</td>
<td>Cellular component organization and biogenesis</td>
<td>22</td>
</tr>
<tr>
<td>Maintenance of localization</td>
<td>3</td>
<td>Biosynthetic process</td>
<td>15</td>
</tr>
<tr>
<td>Response to chemical stimulus</td>
<td>9</td>
<td>Cellular localization</td>
<td>10</td>
</tr>
<tr>
<td>Regulation of biological process</td>
<td>38</td>
<td>Secretion</td>
<td>6</td>
</tr>
<tr>
<td>Sequestering of metal ion</td>
<td>2</td>
<td>Primary metabolic process</td>
<td>47</td>
</tr>
<tr>
<td>Macromolecule metabolic process</td>
<td>53</td>
<td>Macromolecule metabolic process</td>
<td>42</td>
</tr>
<tr>
<td>Regulation of developmental process</td>
<td>5</td>
<td>Establishment of cellular localization</td>
<td>9</td>
</tr>
<tr>
<td>Primary metabolic process</td>
<td>59</td>
<td>Establishment of protein localization</td>
<td>8</td>
</tr>
<tr>
<td>Cellular component organization and biogenesis</td>
<td>23</td>
<td>Catabolic process</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Response to chemical stimulus</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immune response</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leukocyte activation</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immune system development</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Macromolecule localization</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immune defector process</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cell activation</td>
<td>4</td>
</tr>
</tbody>
</table>
ations are manifested in several different aspects of cell function could be expected change various biological pro-
cesses of the cells.

In conclusion, several genes with distinctive expres-
sion profiles were found in the de novo AML t(9;11) and t-
AML t(9;11) SAGE datasets. Among these genes, MHC
class I and MHC class II expression levels were signifi-
cantly and substantially altered between de novo AML
t(9;11) and t-AML t(9;11) cells. These distinct differences
in MHC class I and MHC class II expression and their
effects on biological pathways can be thus used to dis-
tinguish de novo AML t(9;11) from t-AML t(9;11). In addi-
tion, this data provides basic information that may help to
eucidate the etiology of de novo AML t(9;11) and t-AML
t(9;11) and contribute to the development of new treat-
ments.

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