A novel system to detect loss of heterozygosity (LOH)

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Abstract

LOH occurs when a tumor exhibits loss of one of the two alleles at a loci. LOH is an important component of tumor progression. Loss of heterozygosity (LOH) is a frequent event in many types of cancer. The process whereby LOH occurs is not fully understood. Current estimates are that the incidence of LOH is a random consequence of chromosome nondisjunction during mitosis, of chromosome deletion or loss, or by gene conversion. The monosomic and disomic states are specified by the presence or absence of a particular chromosomal region from either the maternal or paternal allele. LOH represents the loss of one or both copies of a particular chromosomal region from either the maternal or paternal allele. LOH is an important component of tumor progression.

Background

Types of somatic recombination

- Chromosome nondisjunction during mitosis
- Chromosome deletion
- Gene conversion

Results of microsatellite marker analysis of 50 clones of NCI-H292

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>Allele sizes (bp)</th>
<th>Clone(s) with LOH</th>
<th>Total number of clones with LOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>D11S2000</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>417,18,20,24</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>D11S4464</td>
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<tr>
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<td>0</td>
<td>0</td>
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<td>000D11S2000</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>008</td>
<td>0</td>
<td>0</td>
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Conclusion

The problem with this approach is that isolation of clones is very labor intensive, and so the numbers of clones that can be examined is very small. By examining this small number, we are able to detect events that are seen at an incidence of 1%. This has been useful in the above study where we are identifying LOH distal as a "hot spot" for chromosome breakage, such as 11q23. In order to make conclusions about changes in the incidence of LOH in general throughout the genome after treatment with apoptotic inducing agents, we sought to find a method that would allow the interrogation of millions of cells.

Rationale for use of dual fluorescence cell line

In order to evaluate millions of cells for LOH, we took advantage of the fluorescent protein markers which have been developed by Larson et al3. This cell line (31SV) was derived from mice that carry two different fluorescent protein genes as described by Larson et al3. This cell line has a yellow fluorescent protein (YFP) and the other for yellow fluorescent protein (CFP). Use of one of these alleles as a measure of LOH that may occur through gene deletion.

Method

1) 50 cells were treated with 0.25 µg/ml anti-CD95 antibody to induce apoptosis. PCR analysis using primers for microsatellite markers was performed. 2) A novel system to detect loss of heterozygosity (LOH) in cultured mouse embryonic fibroblasts and stem cells carrying allelic fluorescent protein genes. BMC Molecular Biology 2006;7(1):36.

REFERENCE

1Larson J, Yin M, Fischer J, Stringer S, Stringer J. Expression and loss of alleles in a mouse embryonic fibroblast cell line that carries two different fluorescent protein genes as alleles in the ROSA26 locus on chromosome 6. Loss of either one of the alleles can be detected using fluorescent detection system.

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