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Loss of Heterozygosity or: How I Learned to Stop Worrying and Love Mitotic Recombination

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Since Knudson's (1993) so-called two-hit hypothesis has been shown to explain the origin of many different cancers with a dominant pattern of inheritance, the term "loss of heterozygosity" ("LOH") has acquired new and increased significance with regard to malignancy. In this article, however, I discuss the implications of recent findings regarding the frequency, nature, and consequences of LOH in normal somatic cells in vivo. I also will propose a broad operational definition of LOH, since the term has been used inconsistently in the literature, mostly in connection with cancers, and often interchangeably with terms such as "allelic imbalance" and "reduction to homozygosity."

The Genetics of Somatic Cells Mandates a Broad Definition of LOH

In strict molecular terms, a locus is heterozygous whenever each allele has a different DNA sequence. Classic germ-line transmission genetics defines dominant and recessive alleles by the phenotypic consequences of each (e.g., the expression of a dominant, a recessive, or a codominant phenotype) and zygosity by the various pairings of alleles. However, with regard to so-called heritable cancers, tumor-suppressor genes (TSGs), and the two-hit hypothesis, LOH has been defined most frequently in functional terms. In the somatic cells of an individual at high risk for an inherited cancer, there is one functionally inactive tumor-suppressor allele of germ-line origin (the first so-called hit) and one normal allele encoding a functional gene product. Most often, the functionally inactive familial allele originates as a consequence of intragenic point mutation, but other mutational mechanisms may apply. The second hit is a subsequent somatic alteration (or mutation, in the broadest sense) in the remaining normal allele, and its absolute certainty of occurrence causes the trait (the germ-line mutation) to appear to exhibit dominant familial inheritance. Actually, the apparent dominance results from the fact that the number of cells at risk in a particular tissue (e.g., retinoblast cells in retinoblastoma) is sufficient to ensure that at least one cell will undergo a mutation inactivating the second allele, given the known rate of allelic mutation (Knudson 1993). This so-called LOH results in the cellular absence of functional TSG product and in the growth of that cell into a tumor, as a consequence of cell-cycle deregulation (Levine 1997), defective DNA repair, or altered cell-to-cell communication (Kinzler and Vogelstein 1996), which are phenotypes that are actually cellular recessives (e.g., requiring both alleles to be inactivated). Interestingly, the stochastic nature of somatic mutation predicts the intrafamilial variation in the age at which a primary tumor occurs, despite the fact that all individuals within a family carry the same germ-line mutation.

LOH, or loss of the normal, functional allele at a heterozygous locus, may arise by any of several mechanisms. It may be a consequence of a multilocus chromosomal event, such as deletion, mitotic recombination (MR), or nondisjunctional chromosome loss with or without reduplication; a locus-restricted event, such as gene conversion or point mutation; or even an epigenetic allelic inactivation (Cappione et al. 1997). Thus, the locus may become (1) homozygous through MR, gene conversion, or chromosome loss with reduplication, (2) hemizygous through deletion or chromosome loss without reduplication, or (3) compound heterozygous through the introduction of a different point mutation in the second allele or (4) may remain heterozygous, in terms of the DNA nucleotide sequence, if one allele is inactivated epigenetically. In this review, I will use the term "LOH" in a broad sense, to indicate the loss of the functional gene product encoded by a particular locus. Likewise, I will use the term "mutation" in the broadest sense, to describe any of the above events that result in the expression of a trait that is a cellular re-

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cessive. One should bear in mind, however, that LOH at a heterozygous locus also can lead to a homozygous dominant genotype if the recessive allele is lost, although this outcome is usually undetectable because it produces no obvious change in phenotype. The differences between the genetics of germ-line and of somatic cells mandate this broader, more functionally based definition of LOH.

LOH in Normal Somatic Cells In Vivo

LOH at loci such as *RB1, WT1,* or *TP53* clearly has been demonstrated to be a key event in retinoblastoma, Wilms tumor, or Li-Fraumeni syndrome, respectively (Knudson 1993). In general, however, LOH is observed for as many as one-half the chromosomes in a tumor cell, some of which are not known to bear TSGs (Kinzler and Vogelstein 1997). Although these so-called secondary regions of LOH may incidentally harbor TSGs that contribute to malignancy, it is most likely that they arise as a consequence of general chromosome instability in advanced tumors (Kinzler and Vogelstein 1997). It may be the case, for example, that absence of the normal *TP53* gene product, which is observed in more than one-half of tumors, produces significant genomic instability (Levine 1997). In any case, by the time that LOH is observed in a tumor, its cells have undergone selection for greater growth deregulation, as well as for other traits that increase tumorigenicity (Kinzler and Vogelstein 1996). Clearly, if one is to observe primary LOH under circumstances in which secondary genetic changes favoring increased genomic instability have yet to occur, one must examine LOH in completely normal cells. Thus, one should define a human system in which LOH can be studied in detail at both the phenotypic and the molecular levels in normal cells of individuals without premalignant conditions. Having satisfied these conditions for a human model (Gupta et al. 1997*a*), we established a similar mouse model that ultimately would allow the introduction of genetic and of environmental variables, in order to test their effects on LOH (Stambrook et al. 1996; C. Shao and J. A. Tischfield, unpublished data).

The adenine phosphoribosyltransferase (APRT) locus is an ideal surrogate marker for LOH studies (Stambrook et al. 1996). Profound autosomal recessive APRT deficiency frequently manifests as 2,8-dihydroxyadenine nephrolithiasis, in humans (Simmonds et al. 1997) and in knockout mice (Engle et al. 1996). Human and mouse APRT genes are located near the telomeres of human chromosome 16 and of mouse chromosome 8, respectively (Ceci 1996; Simmonds et al. 1997). Thus, proximal chromosomal events such as MR or translocation may produce changes in the linkage relationships between syntenic markers and specific *APRT* alleles. APRT is expressed in all tissues, and the human and mouse APRT genes are both well studied and similar. Furthermore, germ-line or somatic mutations in a large number of codons have been shown to inactivate APRT (Simmonds et al. 1997). Most importantly, loss of cellular APRT activity has little or no direct effect on cellular viability and produces a selectable phenotype that is manifested as resistance to toxic adenine analogs such as 2,6-diaminopurine (DAP) (Tischfield and Ruddle 1974). Thus, somatic LOH at a heterozygous *APRT* locus, where one allele is inactivated as a consequence of germ-line mutation, may produce cells without APRT activity. These can be selected and clonally expanded by growth in a medium containing DAP.

Studies of human APRT deficiency identified obligate heterozygotes with known germ-line mutations. Peripheral blood T cells from four such heterozygotes were cultured in DAP medium, immediately following venipuncture. The half-life of cellular APRT activity is long enough so that only cells that had undergone LOH in vivo at a much earlier time would grow in DAP. The observed frequency of DAP-resistant T-cell clones, corrected for cloning efficiency, was from 2×10^{-5} to 15×10^{-5}, for the four heterozgotes. Eighty clones from two heterozygotes were analyzed, of which at least 75 were determined to be of independent origin, by T cell–receptor restriction-fragment patterns. A total of 61 clones (76%) exhibited LOH for *APRT,* as well as variable LOH for flanking polymorphic simple-sequence repeats (SSRs) spaced along the entire length of 16q. In all the clones from informative individuals, LOH appeared to begin at some variable point proximal to *APRT* and to extend through *APRT* and an even more telomeric SSR marker. Conventional and FISH analyses of 10 of these clones showed a normal diploid karyotype, whereas 9 of the 10 clones showed two copies of *APRT,* with one clone the apparent result of an interstitial deletion of the normal allele. With the exception of the data for the latter clone and for one other, these data are consistent only with MR as the mechanism of LOH in this largest group of clones. In one other clone, LOH extended through SSRs on 16p, suggesting chromosome loss or major translocation. Somatic point mutation in *APRT* was demonstrated definitively in 13 of 19 clones in which there were two distinct *APRT* alleles (Gupta et al. 1997*a*). The revised definition of LOH based on the loss of functional gene product includes this latter group.

To obviate the possibility that T cells but not most other cell types exhibit high rates of MR due to the existence of enzyme systems for T cell–specific recombination processes (Jackson and Jeggo 1995), we developed a heterozygous, primary-fibroblast, mouse LOH model based on our *Aprt* knockout mouse (Engle et al. 1996). Heterozygous ($Aprt^{neo}/Aprt^{+}$), dissociated, mouse

ear fibroblasts from strain 129 × C3H hybrid mice were plated immediately into culture medium containing DAP. The incidence of DAP-resistant cell colonies was ~10^{-4} (corrected for cloning efficiency), and 90% of the colonies showed loss of the wild-type *Aprt* allele. Cells with *Aprt*$^+$ loss also exhibited variable loss of *cis* chromosomal SSRs, which extended uninterrupted through the most telomeric SSR. Analyses of fibroblast chromosomes indicated euploid karyotypes (C. Shao and J. A. Tischfield, unpublished data). These data are most compatible with MR as the major mechanism for high-frequency LOH in mouse primary fibroblasts and are quantitatively and qualitatively quite similar to the data from human T cells. More recently, we also have made similar preliminary observations for mouse T cells (L. Liang and J. A. Tischfield, unpublished data).

Thus, both the mouse and the human data determined by use of APRT as a marker support the notion that LOH is relatively frequent (from 10^{-4} to 10^{-5}) in normal cells in vivo and that it mainly is due to MR. Earlier indirect evidence for MR in vivo has been reviewed by Morley (1991), although none of the cell/marker systems previously used allowed for the unambiguous phenotypic, molecular, and cytogenetic analyses that are possible with *APRT*. For example, LOH frequency at the heterozygous *HLA-A2* locus has been reported to be between 2.7×10^{-5} (Morley 1991) and 10^{-4} (Kushiro et al. 1992), and at least 30% of the LOH appears to be due to MR. The frequency of LOH in vivo and the extent to which it is a consequence of MR or of point mutation depend on a number of variables, such as the size of the mutational target and its distance from the centromere. Perhaps some of the differences between the data from the *HLA-A2* and *APRT* loci stem from their different sizes and from the fact that *HLA-A2* is much closer to its centromere than *APRT* is to its centromere.

LOH in Cell Lines, Tumors, and Other Diseases

LOH at *HLA-A2* in lymphoblastoid permanent cell lines was observed at a frequency similar to that observed for normal cells in vivo, but the majority of events, rather than the minority, appeared to involve recombination (de Nooij–van Dalen et al. 1997). DAP selection of cultured human lymphoblasts showed either (1) LOH beginning primarily at one point or chromosome translocations (Smith and Grosovsky 1993) or (2) high frequencies of *APRT* deletion (Fujimori et al. 1992). DAP selection of cultured fibrosarcoma cells showed a broad range of molecular and chromosomal mechanisms for LOH that were not observed in vivo, such as chromosome loss and translocation (Shao et al. 1996; Gupta et al. 1997*b*). Recently, a comparison of mismatch-repair-competent and -incompetent human colorectal carcinoma cell lines revealed profound quantitative and qualitative differences in *APRT* LOH (broad definition), highlighting the notion that the nature of LOH is dependent on the cellular genotype (Phear et al. 1996). Perhaps LOH in some cell lines more closely resembles that observed in tumors and should be interpreted with caution, since it is not possible to know the status of all mismatch-repair and similar types of genes in any particular cell line or tumor. Thus, much of the literature on LOH in cell lines and in tumors may not directly speak to events of primary LOH in normal cells in vivo. Rather, the literature is likely to reflect the events in tumor progression that produce increasing genomic instability (Kinzler and Vogelstein 1996), which in turn alters the nature of LOH and may include the evolution of mutator phenotypes that accelerate the rate of further genetic change (Loeb 1991).

In addition to the well-studied inherited cancers, LOH also has been implicated in several diseases manifesting hyperplasia, as discussed by Qian and Germino (1997 [in this issue]). For each disease, it remains to be determined whether normal cellular mechanisms of LOH apply or whether the genotype predisposes to LOH. Alternatively, in some diseases LOH merely may be an incidental finding, perhaps associated with hyperplasia.

The Role of MR in Vertebrates

Homologous recombination (HR), manifested as either MR or gene conversion, is well established as the major mechanism for the repair of double-strand breaks (DSBs) in yeast (Shinohara and Ogawa 1995). In contrast, it was thought that DSBs in vertebrates were repaired by the direct joining of DNA ends, mediated by DNA-dependent protein kinase, without reliance on sequence homology (Jackson and Jeggo 1995). Recent evidence indicates, however, that HR also plays a highly significant role in vertebrate DNA repair. Mouse and chicken cells with defects in *RAD54*, a member of the *RAD52* epistasis group, which mediates HR in yeast, show reduced HR and sensitivity to ionizing radiation and to certain mutagens. These data highlight the contributions of HR to normal repair in these species (Bezzubova et al. 1997; Essers et al. 1997). Also, there is evidence that certain point mutagens stimulate the repair of mutations by inducing HR (Sengstag 1997), and HR in mouse embryonic stem cells is increased by at least two orders of magnitude, after induction of a specific chromosomal DSB (Moynahan and Jasin 1997). Cumulatively, these data indicate that HR is an inducible process that mediates some vertebrate DNA repair, perhaps in a manner similar to that of HR in yeast. It is not clear whether HR is distinct from or complementary to the other repair process, which allows direct joining of the ends of DSBs.

HR in mammals has been viewed as a process with

mainly negative consequences, because of its role in the production of homozygosity for mutant TSGs—an event that, for over a decade, has been known to cause cancer (Knudson 1993). Recent speculation is that vertebrate DNA repair by direct joining of DSB ends evolved because it bypassed the negative effects of LOH that were caused by HR-mediated repair (Moynahan and Jasin 1997). However, direct joining of DSB ends also can have occasional negative consequences. Direct joining of DSB ends is impaired in radiosensitive cell mutants (Jackson and Jeggo 1995), and errors in the process produce various chromosomal aberrations, the frequencies of which are increased by ionizing radiation (Lloyd and Edwards 1983).

Although HR in mammals may be, on one hand, merely a reflection of ongoing repair processes, one can speculate about additional beneficial roles, such as the correction of mutant phenotypes in heterozygotes (De Sepulveda et al. 1995; Jonkman et al. 1997) or the promotion of homozygosity for certain normal alleles. With regard to the latter, embryonic ontogeny may be more demanding than adult homeostasis, in requiring the functional product of both alleles of certain genes (e.g., a morphogen or a receptor whose proper action depends on its concentration). Thus, heterozygosity at a particular locus in an embryonic cell might reduce its fitness. One also could postulate that embryonic cells might have rates of HR higher than those of adult cells. Thus, HR and MR in particular could establish homozygosity for key normal alleles, in a significant fraction of embryonic cells. This subset of cells, which are homozygous at certain loci, might have a selective advantage that would allow them to make a disproportionate contribution to the development of the embryo. However, LOH of large chromosomal segments, owing to MR, also may unmask a recessive phenotype(s) that is lethal to cells. This is not likely to be critical because, after the death of a fraction of the cells in early embryos, compensatory growth of the remaining viable cells allows for normal development. Thus, the benefits of homozygosity at some points in ontogeny may outweigh the risks of LOH in the adult, in terms of species survival.

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