Closing in on Another Renal Cancer Suppressor Locus Near Chromosome 3p14

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A powerful method to isolate candidate tumor suppressor genes begins with genetic linkage analysis in multigenerational cancer families and requires the collection of DNA samples from affected and unaffected family members. An implicit advantage of studying familial susceptibility to disease is the guarantee that, given sufficient perseverance and luck, a susceptibility locus will ultimately be identified. Such was the case in 1993 with the isolation of the von Hippel–Lindau (VHL) gene at chromosome 3p25 (1) and in 1997 with the identification of alterations within the Met gene in hereditary papillary renal carcinoma (2,3).

In the case of the VHL gene, this work culminated several years of intense research efforts on families who are characterized by a susceptibility to a range of benign and malignant tumors, including renal cell carcinoma (RCC). Subsequent analysis of the VHL gene showed that both alleles were targeted for mutational inactivation or silenced epigenetically in both VHL-associated renal cancers and many sporadic clear cell renal carcinomas (4,5). Although biochemical properties of the VHL gene product are still under investigation (6), these observations confirmed its role as a key tumor suppressor gene underlying the development of nonpapillary renal cancers.

An alternate strategy for isolating cancer genes relies on the identification of patterns of tumor-specific chromosomal loss, often scored as loss of DNA heterozygosity (LOH), which point to a chromosomal region that may harbor a locus with tumor suppressor activity. This approach, however, has been limited by the large, heterogeneous deletions that are frequently detected and by the presence of background levels of allele loss throughout the genome of cancer cells.

Despite these limitations, the short arm of chromosome 3 (3p) has stimulated an intense search for cancer genes for more than a decade because of the wide range of human cancers, including nonpapillary RCC, that show allele loss in this region (7). In the case of RCC, two regions near chromosomal bands 3p21.3 and 3p12–14 have been consistently identified in addition to the VHL locus at 3p25 (1). In the case of RCC, two regions near chromosomal bands 3p21.3 and 3p12–14 have been consistently identified in addition to the VHL locus at 3p25 [reviewed in (8)]. Without the benefit of linkage analysis, however, the task of isolating all of the genes within these regions of LOH has been daunting, given the limitations of mapping and sequencing technology of the early to mid-1990s. In addition, the isolation of expressed genes within these deleted regions only begins the arduous mutational analyses required to exclude each gene as a candidate tumor suppressor. For example, a three-generation family with multifocal RCC was identified where all affected members carried a t(3;8) translocation (9). Although the breakpoint of this translocation has been cloned, there is still uncertainty as to whether the causal RCC susceptibility gene in this family is 1) the FHIT gene, which is interrupted by the rearrangement (10); 2) the TRC8 gene, which is involved as a fusion transcript with untranslated regions of FHIT (11); or 3) simply a mutant VHL allele, which has been identified in the tumors of some of these patients and is unmasked when the rearranged chromosome is subsequently deleted (5,8).

In this issue of the Journal, Julicher et al. (12) employ a common genetic complementation assay to screen adjacent chromosomal fragments from the 3p14 region for growth suppressor or tumor suppressor activity in an RCC tumor cell line. The expectation of this strategy is that a functional screen for growth or tumor suppression will allow investigators to focus their search for RCC cancer genes on a smaller, well-defined stretch of genomic DNA. In this study, Julicher et al. observed that a 530-kilobase genomic fragment, designated yeast artificial chromosome 145F7 (YAC 145F7), encoded the ability to induce cellular senescence in vitro and tumor suppression in vivo when transferred by spheroplast fusion into the parental tumor cell line called RCC-1. The authors conclude that this discrete YAC clone contains at least one RCC gene locus, provisionally called NRC-2. They predict that the subsequent isolation and characterization of all of the expressed genes on this clone will ultimately uncover the elusive 3p tumor suppressor gene involved in the pathogenesis of RCC and, perhaps, other solid tumors that are also associated with frequent 3p deletions.

To put these data in perspective, however, it is worth noting that several investigators have conducted similar chromosomal transfer experiments in RCC over the past decade. In brief, these studies have identified noncontiguous regions with growth inhibition, senescence, and/or tumor suppression activity near chromosomal bands 3p21–22 and 3p12–14 (13–17); however, a gene with RCC suppressor activity has yet to be defined by these assays. In addition, recent studies in breast cancer (18) and in human uroepithelial cells immortalized with human papilloma virus 16 (19) have refined the position of these functional loci to bands 3p21.3–p22 and 3p13–14.2 and have suggested the presence of a “telomerase repressor” sequence at these regions [reviewed in (20)].

Julicher et al. (12) argue that the YAC 145F7 clone used in their genomic transfer study represents a novel gene activity because it does not restore “telomerase repressor” activity in recipient RCC cells and does not overlap with the open-reading frames of previously known candidate genes at 3p14, such as FHIT or WNT5A, or with an RCC tumor suppressor locus at 3p12 (designated NRC-1) that was identified by the use of a similar experimental strategy (17). To strengthen the validity of this type of genetic complementation assay, it is common to include controls for the specificity of the tumor suppressor phenotype by testing sporadic revertants and by transferring chromosomal DNA from other regions of the genome. Julicher et al. report that a revertant cell line with a tumorigenic phenotype similar to the parental cells had lost the transferred YAC DNA. In addition, the authors imply that adjacent YAC clones from the chromosomal 3p14 region had no tumor suppressor activity.

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when similarly transferred to the RCC-1 line but give little detailed information on these clones in this report.

An interesting question not addressed in this study, however, would be an analysis of the effect of the transfer of the YAC 145F7 clone into parental tumor cell lines that do not commonly show evidence for chromosome 3p deletions. For example, a recent study with the NRC-1 locus at chromosome 3p12 (17) reported the suppression of papillary RCC lines, which rarely shows evidence for 3p deletions. Although these data suggest that these recipient tumor lines contain cryptic 3p deletions and that this locus regulates all cases of RCC by expressing, perhaps, a "telomerase repressor" sequence, they might also reflect a nonspecific effect from ectopic expression that raises questions of its role in the pathogenesis of these specific tumors. Ultimately, the challenge for the authors will be to isolate a gene from YAC 145F7, which spans from markers D3S1383 to D3S1384 (19), that fulfills a minimalist definition for a tumor suppressor through the identification of tumor-specific genetic or epigenetic alterations (21).

These are exciting times in the study of the molecular biology of RCC. The hope is that the identification of the elusive 3p gene(s) in RCC will also have broad implications for the wide range of common adult tumors that share a consistent allele loss on chromosome 3p.

REFERENCES


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