HEPATOLOGY *Elsewhere*

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General Reviews

Lewin B. Commitment and activation at Poll II promoters: a tail of protein-protein interactions. Cell 1990;61:1161-1164.
Ptashne M, Gann AF. Activators and targets. Nature 1990;346:329-331.

These two reviews analyze present concepts on gene regulation. Specifically, models of DNA-protein and protein-protein interactions are discussed.

Sigel E. Use of *Xenopus oocytes* for the functional expression of plasma membrane proteins. J Membrane Biol 1990;117:201-221.

This article reviews the use of this method for the expression/study of plasma membrane proteins.

Sell S. Is there a liver stem cell? Cancer Res 1990;50: 3811-3815.

This article discusses the problem of the origin of hepatocytes and bile duct cells. It proposes that this is a periportal stem cell from which transitional cells, and later oval cells, originate.

Elsewhere Reviews

DNA INTEGRATION SITES AND HEPATOCELLULAR CARCINOMA

Fourel G, Trepo C, Bougueleret L, Henglein B, Ponzetto A, Tiollais P, Buendia MA. Frequent activation of N-myc genes by hepadnavirus insertion in woodchuck liver tumours. Nature 1990;347:294-298.

EDITOR'S ABSTRACT

This study is part of an ongoing analysis of woodchuck hepatitis virus integration sites in the host genome of hepatocellular carcinomas. The study of woodchuck hepatitis virus–DNA integration sites may shed light on the oncogenic mechanisms involved in cellular transformation and tumor formation. Viral integration enhancing cellular proto-oncogene expression is one such mechanism and has been well documented for oncogenic retroviruses such as mouse mammary tumor virus and interleukin-1. By cloning a woodchuck hepatitis virus integration site from a woodchuck hepatocellular carcinoma the authors were able to identify a new member of the myc gene family, N-*myc*-2. Examination of 30 additional woodchuck hepatomas revealed viral integration commonly occurred near N-*myc* loci with an additional five woodchuck hepatitis virus integrants near the N-*myc*-2 gene and one viral integrant near N-*myc*-1. Three of these N-*myc*-2 viral integrations were further evaluated and found to be localized within 200 bp of the translation stop codon. This 3′ noncoding region has recently been identified as a common site of murine leukemia virus integration in virally induced T-cell lymphomas and results in increased expression of the N-*myc* gene. Similar mechanisms can be proposed for hepatocellular carcinoma formation. Woodchuck hepatitis virus integration near cell–growth related proto-oncogenes, such as N-*myc*, can juxtapose viral enhancer elements and growth-regulatory genes. Virally induced overexpression of proto-oncogene messenger RNA could result from enhanced transcription or increased messenger RNA stability. To search for such effects the authors analyzed N-*myc*-2 RNA levels in 30 woodchuck hepatitis virus–related hepatomas. Increased levels of N-*myc*-2 RNA were found in 18 of 30 tumors, whereas nontumorous portions of the same livers had no detectable N-*myc*-2 RNA. Taken together these findings suggest that woodchuck hepatitis virus integration can result in altered N-*myc*-2 gene expression in a significant proportion of woodchuck hepatocellular carcinomas. The deregulation of N-*myc* gene expression could result in cellular transformation and ultimately tumor formation. Such examples of hepadnavirus-specific oncogenic mechanisms lend credence to theories of hepatitis B virus-induced tumorigenesis and provide models to design molecular investigations of human hepatocellular carcinoma formation.

COMMENTS

The association between chronic HBV infection and human HCC has been established by epidemiological studies. The risk of developing primary HCC is 100-fold greater in chronic HBV carriers than in age-matched noncarriers (1). Chronic hepatitis B is estimated to affect 5% of the world's population (2), making it a major oncogenic risk factor. Although this association has been known for several years, a mechanism linking chronic HBV infection to the initiation of tumor formation has not been defined (3). Current theories of virus-associated HCC tumorigenesis fall into two categories: (a) nonspecific mechanisms, where HBV initiates a chronic inflammatory state that leads to unregulated hepatocyte proliferation and regeneration, random mutations and ultimately a transformed phenotype and (b) specific virally induced mechanisms such as expression of viral oncogenes or transcriptional transactivating factors, inactivation of host tumor suppressor genes, activation of host proto-oncogenes or mutations of host chromosomes such as translocations and gene re-

arrangements. Murine and avian oncogenic retroviruses induce tumor formation by several of these mechanisms and provide models for examining potential molecular mechanisms of hepadnavirus tumorigenesis. Previous studies have demonstrated that none of the hepadnaviruses contain oncogenes. Of great interest are the reports of viral proteins encoded by the X gene and the pre S2/S region that contain transactivating activities. These genes can be integrated in a truncated form and produce viral-host chimeric RNA transcripts and proteins that can transcriptionally activate other cellular genes (4, 5). One member of the hepadnavirus family, woodchuck hepatitis virus (WHV), serves as a particularly useful model of hepatocarcinogenesis. This virus acts as a potent inducer of HCC formation after neonatal infection and provides a convenient animal model for studying hepadnavirus oncogenesis. The complex replication and integration life cycle of hepadnaviruses results in random chromosomal insertions of full-length and partial viral sequences. Viral integration in the host genome is observed almost invariably in hepatoma cells and is proposed to be critical to HCC development. This report examines WHV integration sites in the host genome to search for mechanisms such as proto-oncogene activation or tumor suppressor gene inactivation.

This paper describes three significant findings regarding N-*myc* gene expression in WHV-associated hepatomas. The authors initially cloned a WHV integration site from a single hepatoma that developed in a chronically infected woodchuck. Sequencing of the viral-host DNA junction revealed the woodchuck genomic target sequence had homology with the N-*myc* proto-oncogene. N-*myc* is a member of the *myc* oncogene family of DNA-binding proteins that includes c-*myc*, N-*myc*, L-*myc* and s-*myc*. These proteins appear to be transcriptional activators and play a role in cell growth, differentiation and neoplastic transformation. Using this woodchuck target sequence as a probe, they screened the tumor's genomic DNA and found two subsets of N-*myc*–related clones. The first subset revealed an N-*myc* locus that was organized into three exons and was the woodchuck homolog of the human and murine N-*myc* genes. This gene was designated N-*myc*-1. The second locus found (named N-*myc*-2) was an N-*myc* gene lacking both introns – a structure typical of a processed gene or retroposon. The presumed mechanism for formation of such "cDNA genes" involves reverse transcription and genomic integration of a fully spliced intronless mRNA at some distant time point during the evolution of the woodchuck genome. There are numerous examples of such retroposons scattered throughout mammalian genomes. Usually these processed genes are mutated so they are unable to express functional polypeptides. However, the deduced amino acid sequence of N-*myc*-2 contained 454 amino acids with 80% homology to N-*myc*-1 and 78% with the human N-*myc* protein. Functional domains were highly conserved between these N-*myc* proteins, as well suggesting that N-*myc*-2 could encode a functional proto-oncogene product. Turning to a *myc* functional assay involving cotransfection of primary embryo fibroblasts with an activated *ras* oncogene, it was found that N-*myc*-2 resulted in tumorigenic conversion of these primary cells with the same efficiency as c-*myc*. This is the first major finding of this study – identification of an intronless "retroposon" *myc* gene, the woodchuck N-*myc*-2, that encodes a functional oncogene protein. It is this gene locus that contained their original WHV integration. In this tumor a portion of viral DNA containing the WHV enhancer had integrated in the 3′ untranslated region of N-*myc*-2, just 6 bp beyond the translational stop codon. This finding suggests a possible oncogenic event wherein the WHV enhancer element integrates in proximity to N-*myc* and enhances expression of the proto-oncogene product.

To define the frequency of WHV enhancer insertion near N-*myc* genes the authors surveyed 30 additional independent woodchuck hepatomas. Analysis of genomic DNA revealed six additional tumors with DNA rearrangements of N-*myc* alleles. Five of these six rearrangements were of N-*myc*-2 and one tumor had a rearranged N-*myc*-1. Further characterization of these N-*myc* rearrangements revealed the presence of WHV sequences in five of six tumors, suggesting viral integration sites near the N-*myc* gene. The viral sequences hybridized to a 600 bp WHV probe containing the enhancer element. In addition, these integration sites were localized in the 3′ untranslated region of N-*myc*-1 or N-*myc*-2 analogous to the original tumor. Two tumors were selected for polymerase chain reaction amplification of the N-*myc*-2–WHV junction sequence. Just as the initial tumor had WHV sequences integrated 6 bp after the N-*myc* translation stop signal, these tumors had integration occur 18 bp and 176 bp beyond the stop codon, respectively. This high frequency (20%) of viral integration near N-*myc* genes led to the second major finding of this report. All six tumors containing N-*myc*–associated viral sequences expressed N-*myc* WHV RNA cotranscripts. Four of six tumors had elevated levels of these N-*myc*–related transcripts, suggesting a relationship between WHV enhancer integration and increased expression of N-*myc* RNAs.

In addition to DNA rearrangements of N-*myc* genes in WHV-associated hepatomas the authors analyzed N-*myc* RNA expression. The third observation of this study showed that 18 of 30 tumors expressed varying levels of the nonrearranged 2.3 kb N-*myc*-2 messenger RNA (mRNA), whereas no detectable N-*myc*-2 mRNA was found in nontumorous portions of the same livers. Livers of uninfected or WHV-infected woodchucks without tumors also contained no N-*myc*-2 transcripts by Northern hybridization. This finding suggests that enhanced expression of N-*myc*-2 RNA is mediated by transacting factors produced in a majority of WHV-associated hepatomas. In sum, these investigators have identified a novel intronless N-*myc* gene that is functional in transformation assays and whose expression can be induced by WHV infection. Viral infection can increase N-*myc*-2 gene expression by either downstream

enhancer insertion or in the absence of N-*myc*-2 genetic rearrangements.

The initial studies of woodchuck HCC by this group involved screening RNA prepared from nine separate tumors and adjacent nontumorous liver for overexpression of a panel of 15 oncogenes (6). The only elevated oncogene RNA was c-*myc*, found in three of nine hepatomas. Further analysis of these three tumors revealed that rearrangements of the c-*myc* gene had occurred in all three tumors. One tumor had a truncated c-*myc* gene joined to a unique cellular sequence of unknown function. The two other tumors had integration of WHV sequences, including the enhancer element, near the c-*myc* gene (7). One integrant was 90 bp beyond the coding region's stop codon and the second was 621 bp upstream of c-*myc*'s exon 1. The current study was initiated simply by looking at WHV integration sites rather than by looking at tumors selected for oncogene overexpression. Amazingly, the authors discovered a common integration site in a new member of the *myc* oncogene family, N-*myc*-2. This report of a new functional retroposon oncogene is of interest not only because it defines a new member of the *myc* family but also because of its evolutionary interest as a genomic "retroelement" or processed gene (8). The authors follow this lead by analyzing whether N-*myc*-2 is frequently rearranged or overexpressed in HCC. They find several tantalizing clues including six other tumors with N-*myc*/WHV rearrangements and increased N-*myc*-2 RNA levels in 18 of 30 tumors. The latter N-*myc*-2 transcripts did not contain WHV sequences, suggesting enhancement of their expression by viral transactivating factors. Three of the tumors with N-*myc*-2 gene rearrangements resulting from WHV integration had insertions within 200 bp of the translation stop signal. Recent reports of murine retroviruses integrating into the same 3′ untranslated region of N-*myc* in T-cell lymphoma and myeloma cells (9, 10) suggest that this small region may be an "integration hot spot." A recurring theme of *myc* oncogene expression may be emerging in the woodchuck HCC studies. This enhanced expression may be caused by WHV enhancer insertion near *myc* gene loci or by virally induced transcriptional activating factors. It must be remembered that these oncogene alterations do not occur in all tumors. Presumably there are several genetic events necessary for HCC formation. Different combinations of genetic mutations could contribute to tumorigenesis—in some cases certain virally mediated changes in *myc* expression are involved in cellular transformation whereas other tumors may contain a different set of genetic lesions.

Do WHV integration events mirror HBV-induced carcinogenic events? Obviously the answer is not yet known, but recent reports of human HCC containing HBV integrations near two growth-related genes suggest similar mechanisms may be involved (11-13). Although a short time ago it appeared that hepadnavirus tumorigenesis was most likely the result of nonspecific inflammatory and immune responses, the recent spate of reports linking viral infection with alteration of growth-related genes and their expression creates newfound interest in specific viral mechanisms. As is often the case in pathophysiological events, perhaps both specific and nonspecific mechanisms may be involved in HCC formation. Specific combinations of genetic lesions may lead to selective growth advantages for certain cells. Nonspecific host responses may provide an environment for such genetic events to frequently occur, or they may prevent selective destruction of such genetically favored clones.

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THE CLONING OF CHOLESTEROL 7 α-HYDROXYLASE: WILL MOLECULAR TECHNIQUES HELP US UNDERSTAND THE PHYSIOLOGY OF BILE ACID SYNTHESIS?

Jelinek DF, Anderson S, Slaughter CA, Russell DW. Cloning and regulation of cholesterol 7 α-hydroxylase,