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بعض الوثائق الأصلية تالفة

بالرسالة صفحات لم ترد بالأصل

Hepatitis C Virus Serological Status Among Cases Of Hepatocellular Carcinoma.

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CHAPTER 1

INTRODUCTION

Introduction

Discovery of Hepatitis C Virus

In the 1970s hepatitis C (HCV) has emerged from obscurity as an agent of hepatitis transmitted by blood and blood products, distinct from the hepatitis A and hepatitis B viruses (HAV, HBV). [1-3]

Bradley and colleagues [4] had demonstrated that hepatitis could be transmitted to chimpanzees by inoculation of factor VIII known to have been contaminated with an agent that had caused NANBH in haemophiliacs. The size of the virion had been estimated to be less than 80 nm by ultracentrifugation (Bradley et al,1985), [5] while filtration studies suggested 30-60 nm (He et al.,1987). [6] It was inactivated by lipid solvents such as chloroform, suggesting a small enveloped virus. Plasma from chimpanzees, that had developed chronic hepatitis following inoculation with factor VIII, was reasonably considered a good source of the putative virus, and that virions could be concentrated from the plasma by ultra-centrifugation. Following these descriptive assumptions HCV remained frustratingly elusive. Nevertheless, in 1989, a team of molecular biologist in the United States succeeded in an ambitious assignment to identify the viral genome. The ingenious protocol they devised can serve as a prototype which could be applied in the future to the discovery not only of additional non-A, non-B hepatitis viruses (NANBH) but also of many other currently unknown noncultivable infectious agents.

In their experiment they utilized the NANBH chimpanzee plasma as a potential source of viral nucleic acids, they attempted to synthesize cDNA from all nucleic acid sequences extracted through utilizing random primers of reverse transcriptase. The resulting complementary DNA was cloned into bacteriophage

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λgt11 expression vector. Screening about 10^6 of the resulting recombinant λ gt11 phage led to the identification of positive cDNA clone 5-1-1, that hybridized to total RNA extracted from infectious chimpanzee liver. This cDNA did not hybridize either to control human or chimpanzees DNA nor to total RNA derived from control, uninfected chimpanzees livers. This proved that clone 5-1-1 is not derived from the host genome. Furthermore, total nucleic acid extracted from ultracentrifuged pellets of the high-titer NANBH chimpanzee plasma hybridized to this clone, but this hybridization signal was lost after treatment with ribonuclease but not deoxyribonuclease. Hence, it appears that these clones are derived from an exogenous RNA molecule associated with NANBH infection. (Choo et al .1989). [7]

This clone (clone 5-1-1) showed no similarity with any human or E.coli genomic sequences and produced a protein that was consistently recognized by sera from patients with NANBH, but not by sera from control individuals or those with hepatitis of other aetiologies (Kuo et al . 1989).[8] The 5-1-1 clone was then used as a hybridization probe to derive a larger overlapping clone from cDNA library, which in turn was used to identify the full length plus sense ssRNA hepatitis C viral genome. (Choo et al. 1991). [9] Subsequently a variety of reliable diagnostic assays were developed, allowing detailed study of epidemiology and disease associations of HCV .

Classification

Recent, accumulating information on HCV morphology and comparative analysis of the nucleotide (nt) sequences have led to recognition of considerable genetic and biological analogies to the members of the family *Flaviviridae* and especially to those grouped in the genus pestivirus.

Similarties included: *First*, similarity in the size of the polyproteins encoded by the positive-stranded RNA genomes of the hepatitis C virus, flaviviruses,and pestiviruses. (3010, 3400, and ± 4000 aa., respectively). [9,10] *Second*, the existence of several small genomic regions in HCV which showed significant homology to the 5'untranslated region (5' UTR) of the pestiviruses. This homology is colinear, i.e. occurring at the same relative position in HCV and pestiviruses. [11] Two studies[12,13], have revealed the presence of a large stem–loop structure in the proximal part of the 5`UTR of these viruses that serves as a putative internal ribosomal entry site (IRES), which directs translation from an internal methionine (AUG) codon. This contrast strongly with translation of other flavivirus genomes, which resembles that of cellular mRNAs, in which ribosomal binding occurs at the 5` end of the RNA, followed by scanning of the sequence in the 5` to 3` direction with translation commencing at the first AUG codon.[14,15] *Third*, the presence of a number of small dispersed aa. sequence homologies in nonstructural proteins 3 and 5 of HCV and that of the flaviviruses, the first (aa. 1230-1500) has residues in common with putative NTP-binding helicase encoded by flaviviruses, pestiviruses, and plant potyviruses, but the greatest similarity was between HCV and pestiviruses.[9,10] The second region is upstream of the first and shares similar features with the putative chymotrypsin-