deduced mass of the heptamer peptide is 797·94 Da, which differed no more than 1 Da from the one detected by mass spectrometry. We searched the GenBank with a BLAST search using the National Center for Biotechnology Information Website (www.ncbi.nlm.nih.gov accessed on Jan 16, 2001). The peptides were derived from aminoacid residues 88–94 of the core protein of HBV. No other matches were obtained in the BLAST search. It is noteworthy that a similar peptide, P2 (Tyr-Val-Asn-Val-Asn-Met-Gly-Leu-Lys), was reported to be the ligand of HLA-A11 and recognised by HLA-A11 restricted $CD8^+$ cytotoxic T lymphocytes.[5] The peptide we isolated is only two aminoacids shorter than the P2 peptide and could also be a ligand of HLA-A11. One of the patients was HLA-A11 positive (the other two patients were positive for A1/A2, A3/A26; both heterozygous). Though rare, a heptamer can be a ligand of MHC class I. Despite the possible association of this peptide with HLA-A11, gp96-peptide complexes can prime cytotoxic T lymphocytes regardless of HLA haplotypes.[1]

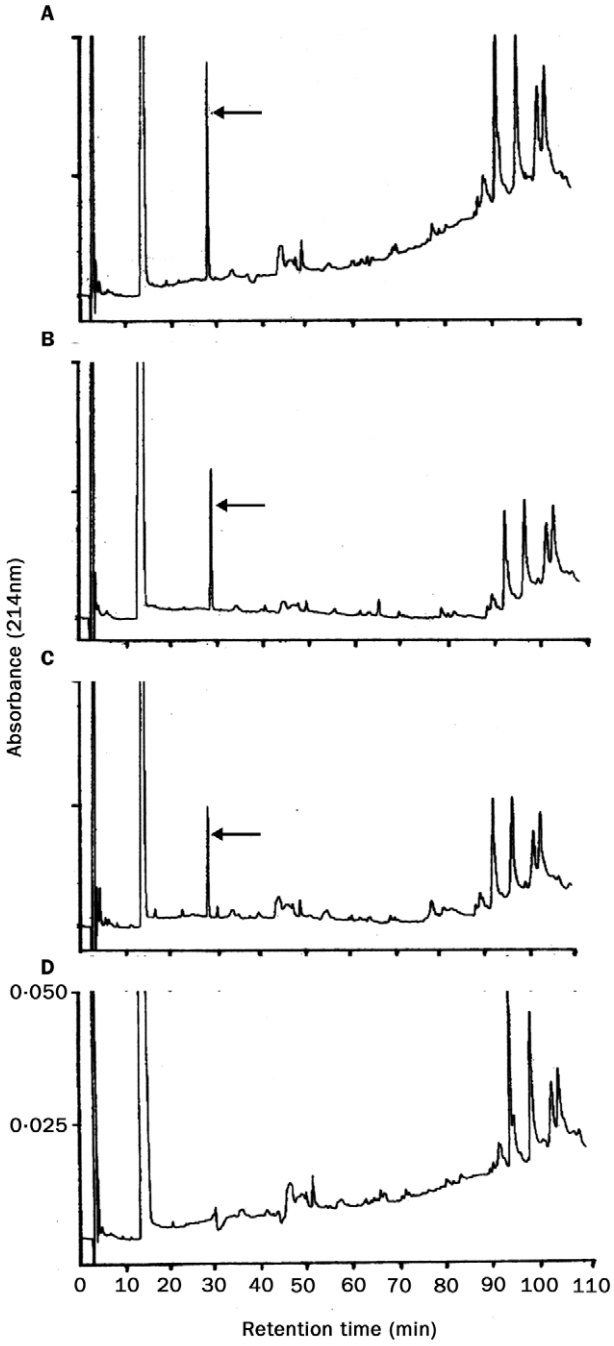

Figure 2: **Analysis of gp96-associated peptides by linear gradient of acetonitrile on a C18 reverse phase HPLC column**
Low molecular mass peptides (<10 kD) extracted from the three HBV-infected tumour tissues (chromatogram A, B, C, representing patient 1, 2, and 3 respectively) and one uninfected tissue as control (chromatogram D) were done on HPLC fractionation. The peak seen in the gp96 preparations of all three patients (arrow) had a retention time of 27 min.

Complexes of heat-shock protein and peptides have been investigated for use as tumour vaccines. Our findings suggest a possible association of the isolated HBV-specific peptide with gp96 in vivo. The potential of this gp96-peptide complex as a therapeutic vaccine for HBV-induced hepatocellular carcinoma should be investigated. A similar approach should also be taken for other virus-induced tumours.

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Development of spectral colour banding in cytogenetic analysis

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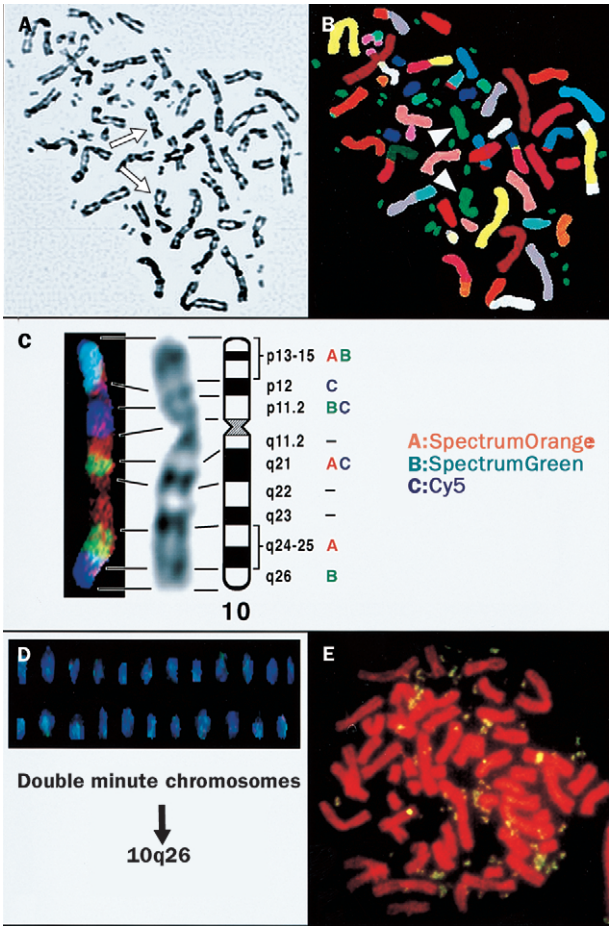
We developed a novel chromosome banding technique—spectral colour banding (SCAN). With this technique we displayed a multicolour banding pattern that almost entirely correlated with the corresponding G-banding pattern. With SCAN analysis we could identify the chromosome-band origin of double minute chromosomes in gastric cancer. Our preliminary use of this technique suggests that it has significant clinical applications for cytogenetic analysis.

The multicolour fluorescence in-situ hybridisation technique spectral karyotyping (SKY) has been introduced as a method of cytogenetic analysis.[1] SKY is a



Identification of chromosome-band origin of double minute chromosomes in gastric cancer

A: G-band image with DAPI counterstaining, shows large number of double minute chromosomes (arrows show normal homologues of chromosome 10); B: spectra-based classification colour image of the same metaphase as in panel A (arrows show normal homologues of chromosome 10); C: SCAN analysis of normal chromosome 10 (left to right: spectral colour image, G-band image, schematic G-banded ideogram, and degenerate oligonucleotide primed PCR labelling scheme); D: spectral colour image of double minute chromosomes; E: fluoresence in-situ hybridisation analysis with K-sam probe. Signals (green) were detected on double minute chromosomes.

single hybridisation technique with 24 differentially labelled human chromosome-specific probes, which allows simultaneous detection of human chromosomes in different colours during the same metaphase. Many studies have assessed the advantages and limitations of SKY.[2,3] It is difficult to detect intrachromosomal changes such as inversions, small deletions, or duplications, and the method does not identify the chromosome-band origin of small chromosome segments. To overcome these limitations, we developed a novel chromosome banding technique—spectral colour banding (SCAN). This technique is based on SKY, combined with simultaneous hybridisation of labelled chromosome band-specific painting probes. SCAN analysis simultaneously identifies the origin of chromosome bands by a unique spectrum for each band.

Band-specific genomic DNA was prepared from the microdissected specific band of an individual chromosome.[4] For chromosome ten, six band-specific genomic DNAs were obtained and used as probes (Research Genetics, Huntsville, AL, USA). We labelled these with one or a combination of three different fluorochromes: SpectrumOrange; SpectrumGreen; and Cy5 by degenerate oligonucleotide primed PCR.[5] We mixed the labelled probes, which were ethanol precipitated, with an excess of human Cot-1 DNA. We used the SKY protocol for hybridisation and detection. Hybridisation signals were converted to spectral colour images with an SD200 spectral imaging system SpectraCube (Applied Spectral Imaging, Migdal Ha'Emek, Israel).

A man aged 62 years presented with poorly differentiated gastric cancer (signet-ring cell type) with bone marrow metastases. G-banded karyotypes were complex with double minute chromosomes for the tumour cells in the bone marrow metastases. We identified the chromosomal origin of the double minute chromosomes as chromosome 10 using SKY analysis (figure). We used SCAN analysis for chromosome 10 to identify the chromosome-band origin of the double minute chromosomes.

We displayed a multicolour banding pattern of normal chromosome 10, which almost entirely correlated with the corresponding G-banding pattern (figure). The spectra of the double minute chromosomes were identical only to that on the 10q26 band of normal chromosome 10 in the same metaphase. The double minute chromosomes were, therefore, derived from the 10q26 region (figure), which led us to presume that the K-sam gene, located at 10q26, was amplified. The double minute chromosomes are a cytogenetic hallmark of gene amplification. In gastric cancers, especially poorly differentiated types, the amplification of the K-sam gene has been reported. As expected, we saw multiple signals on double minute chromosomes using fluorescence in-situ hybridisation analysis with a bacterial artificial chromosome probe containing the K-sam sequence (figure).

Our preliminary use of SCAN in combination with SKY suggests that this new technique can accurately identify chromosome-band origin for each of the 24 human chromosomes, and that it might be able to identify intrachromosomal changes not previously detected by G-banding or SKY.

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