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COAMPLIFICATION OF THE *hst-1* AND *int-2* GENES IN HUMAM CANCERS

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The *hst-1* gene, previously designated the *hst* gene, was identified as a transforming gene by transfection assays of genomic DNAs from various types of human cancers. We analyzed for alterations of the *hst-1* gene in 18 bladder cancers, 23 renal cell carcinomas and 5 esophageal cancers. Although rearrangement of the gene was not detected in any of these samples, amplification of the *hst-1* gene was found in 4 samples of tumors, including an invasive bladder cancer, both primary esophageal cancer and its lymph node metastasis, and kidney metastasis of an esophageal cancer. The same degree of amplification of the *int-2* gene, the product of which has significant homology with the *hst-1*-encoded protein, was also observed in all of these DNA samples with amplified *hst-1* gene. This result indicates close chromosomal localization of the two genes, which were amplified as one amplification unit.

Key words: Coamplification — *hst-1* — *int-2* — Bladder cancer — Esophageal cancer

The *hst* gene was initially identified as a transforming gene in DNAs from human stomach cancers and a noncancerous portion of stomach mucosa by NIH3T3 cell transfection assay.[1,2] The human genome contains two types of DNA sequences hybridized to the *hst-1* cDNA. One is the *hst-1* gene, which was previously designated the *hst* gene, and the other is the *hst-2* gene. The *hst-2* gene might be a pseudogene or a gene closely related to the *hst-1* gene. Both the *hst-1* cDNA clone inserted into an expression vector[2] and the genomic *hst-1* clones obtained from leukocyte DNAs from a leukemic patient[3] and a normal healthy individual (unpublished data) were shown to have transforming activity on NIH3T3 cells. The *hst-1*-encoding protein with 206 amino acid residues had significant homology to basic and acidic fibroblast growth factors (FGFs) and *int-2*-encoded protein,[4,5] suggesting that the *hst-1*-encoded protein is a novel growth factor. To learn whether the structural changes of the *hst-1* gene occur in several types of tumors *in vivo*, we analyzed bladder cancers, renal cell carcinomas and esophageal cancers by Southern blot hybridization. We also analyzed the changes of the *int-2* gene, the product of which has a significant homology with the *hst-1*-enocoded protein. Eighteen primary tumors and one bone metastasis of bladder cancers, 23 primary tumors and one lung metastasis of renal cell carcinomas and 4 primary tumors, one lymph node metastasis and one kidney metastasis of esophageal cancers were obtained at the time of surgery. When it was available, a noncancerous portion adjacent to the tumor was also subjected to examination. Histologically, all bladder cancers were transitional cell carcinomas, all renal cell carcinomas were adenocarcinomas, and all esophageal cancers were squamous cell carcinomas. Genomic DNA was extracted from the tissues and digested with restriction enzymes, fractionated on 0.8% agarose gel and transferred to nitrocellulose filters as described previously.[6] The filters were hybridized with ^{32}P-labeled DNA probes: Probe AA, a 0.6-kilobase-pair (kbp) *Ava*II-*Ava*II fragment of the human *hst-1* cDNA[2] and SS6, a 0.9-kbp *Sac*I-*Sac*I fragment of the human *int-2* gene.[7] The *hst-1* cDNA probe AA hybridized to four fragments of 8.0, 5.8, 2.8 and 0.8 kbp, when normal genomic DNA was digested with

*Eco*RI. The 5.8, 2.8 and 0.8 kbp bands were derived from the *hst-1* gene and the 8.0 kbp band from the *hst-2* gene (unpublished data). The *int-2* probe SS6, showing restriction fragment length polymorphisms (RFLPs) with *Bam*HI digestion, hybridized to an 8.4 kbp

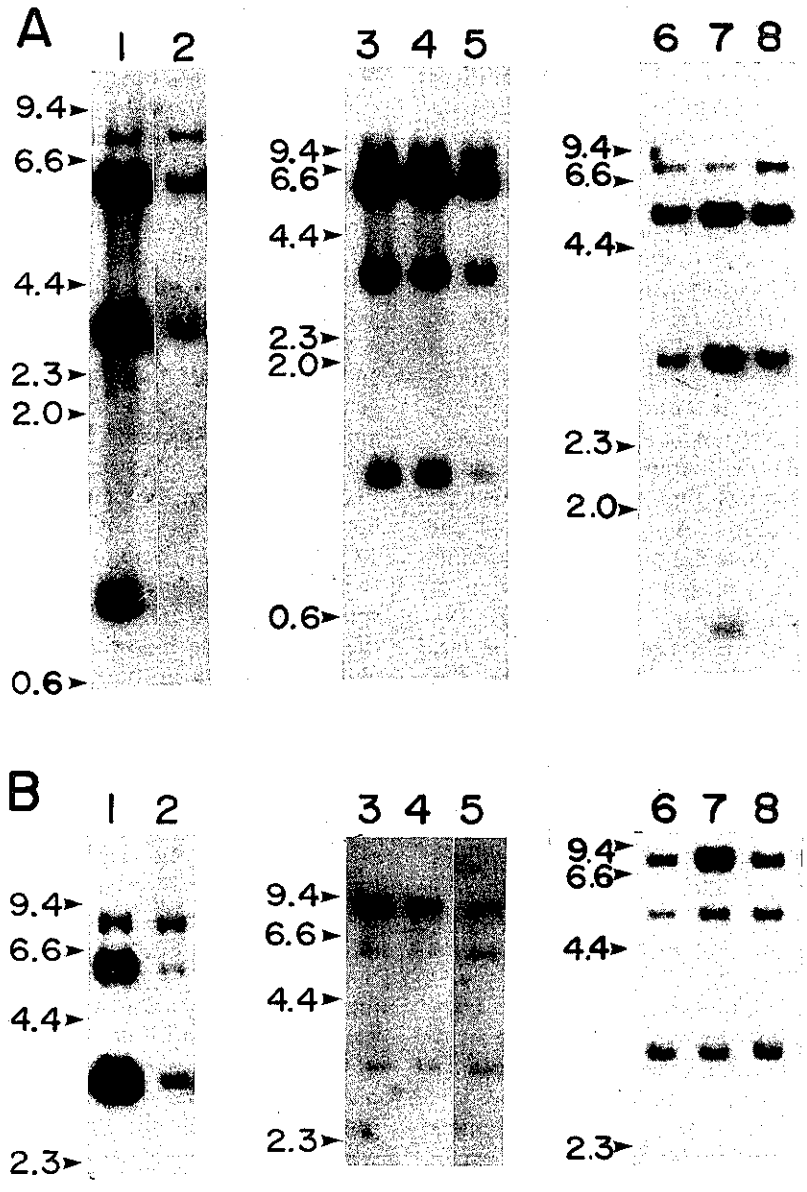

Fig. 1. Amplification of the *hst-1* and *int-2* genes in bladder cancer and esophageal cancer. Lane 1; bladder cancer of patient B-151. Lane 2; noncancerous portion of patient B-151. Lane 3; primary esophageal cancer of patient E-41. Lane 4; metastatic lymph node of patient E-41. Lane 5; noncancerous portion of patient E-41. Lane 6; primary renal cell carcinoma of patient E-71. Lane 7; metastatic esophageal cancer to the same kidney of patient E-71. Lane 8; noncancerous portion of patient E-71. *Hin*dIII-digested λ DNA was used as a size marker, and the numbers on the left indicate the molecular size in kilobase pairs (kbp). A; All DNA samples were digested with *Eco*RI and were probed with radiolabeled probe AA. B; All DNA samples were digested with *Bam*HI and were probed with radiolabeled SS6.

fragment in one allele and to 5.6 and 2.8 kbp fragments in another one.

With probe AA or probe SS6, all samples tested showed no gross rearrangement of the *hst-1* gene or the *int-2* gene. However, the signals corresponding to the *hst-1* gene-specific fragments of 5.8, 2.8 and 0.8 kbp were amplified in four samples obtained from three patients (Fig. 1A). The first case was an invasive bladder cancer of patient B-151 (Lane 1), whose degree of amplification of the *hst-1* gene was about twenty-fold. The second case was primary esophageal cancer of patient E-41 and its metastasis to lymph node (Lanes 3 and 4), with five-fold amplification in both tumors. The third case was a metastatic esophageal cancer to the right kidney of patient E-71 (Lane 7), with four-fold amplification. The primary esophageal cancer of patient E-71 was not available for analysis. However, renal cell carcinoma also found in the same kidney of this patient revealed no amplification (Fig. 1A). Strikingly, all the DNA samples containing amplified *hst-1* gene showed the same degree of amplification of the *int-2* gene as the *hst-1* gene (Fig. 1B). No amplification of *int-2*-specific fragments was detected in the other samples of DNAs. The SS6 probe can detect RFLPs of *Bam*HI-*Bam*HI fragments of the *int-2* gene: One is 8.4 kbp fragment and the others are 5.6 kbp and 2.8 kbp fragments. Since amplification occurred in either 8.4 kbp or in 5.6 kbp and 2.8 kbp fragments, it is concluded that the *int-2* gene in one allele was amplified in all these DNA samples. It is likely that *hst-1* was also amplified in one allele which contained amplified *int-2* gene.

Only a limited number of reports are available on protooncogene amplification in bladder cancer and esophageal cancer. Amplification of c-Ha-*ras*-1 gene in bladder cancer and the EGF receptor gene in esophageal cancer has been reported.[8,9] After identification of the *hst-1* gene as a transforming gene, the gene was also identified in DNAs from several tumors, including those from three stomach cancers and a colon cancer,[10] two hepatocellular carcinomas,[11] and a Kaposi sarcoma.[5,12] Recently, the deduced amino acid sequence of the *hst-1*-encoded protein was reported to be 40%, 43% and 38% homologous to *int-2* protein, basic FGF and acidic FGF, respectively.[4] Thus, the *hst-1* gene is likely to be a member of a new gene family encoding growth factors.

The *int-2* gene was identified as a mouse genomic sequence adjacent to integrated provirus of mouse mammary tumor virus (MMTV), and its protein is considered to play an important role in the development of mammary tumors induced by MMTV.[13] It was also found to be related to human breast cancers.[14] The FGFs are potent mitogens for a variety of cell lineages, including those of mesodermal, neuroectodermal and epithelial origins.[15] They may play an important role in tumor development[16] and in normal angiogenic processes such as tissue repair.[17]

Several reports have indicated the presence of coamplification of two proto-oncogenes.[18–22] We previously reported the coamplification of c-*erbB-2* and cellular v-*erbA* homologue in human stomach cancers.[23] The two genes were in the same amplification unit because of their close linkage on chromosome 17 at band q21-22. Using *in situ* hybridization, the human *int-2* gene was mapped to chromosome 11 at band q13.[7] We recently mapped the chromosome locus of the *hst-1* gene to chromosome 11 at band q13 (unpublished data). Therefore, these two genes appear to be closely linked and both are amplified in the same amplification unit. It can be speculated that the genes encoding growth factors related to the *hst-1* and *int-2* gene products are clustered in this region of chromosome 11 at band q13. The biological significance of the coamplification is unclear, but coamplification of the *hst-1* and *int-2* genes may have important implications. In fact, all three cases showing the coamplification were aggressive types or in stage IV, and therefore, coamplification of these genes may produce a more malignant phenotype *in vivo*. It is also possible that the other genes on chromosome 11 at band q13, which were closely linked to the *hst-1* and *int-2* genes, are amplified in the same amplification unit. These genes other than the *hst-1* and *int-2* genes might have important roles in the malignant phenotypes of the tumors.

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