Selective Integration of Rous Sarcoma Virus Genome in High- and Low-Metastatic Transformed Cell Lines

A. G. Tatosyan, M. S. Shtutman, L. Z. Topol, N. P. Kisseljova, E. A. Musatkina, and G. I. Deichman [1]

Previously, different hamster cell lines transformed in vitro by Rous sarcoma virus (RSV) (SR-D strain) were established [1]. These cells displayed different levels of spontaneous metastatic activity in syngenic animals. The structure of RSV provirus in these cells has been analyzed. All cell lines contained integrated RSV genome without any indication of provirus rearrangement. The comparative analysis of restriction maps of the integrated provirus in the genomes of these cells showed that in most of the highly metastatic cell lines the exogenous RSV proviruses were localized within structurally similar loci of host DNA. For the analysis and sequencing of the flanking region downstream from the integrated provirus, the method of inverted polymerase chain reaction (PCR) with oligonucleotide primers to certain long terminal repeats (LTR) and *src* regions of RSV was used. We analyzed the primary structure of cellular DNA for a number of different highly metastatic cell lines. It was found that the flanking sequences of cellular DNA are similar in at least three cell lines. This common fragment was used as a probe for the analysis of normal DNA and a unique region in the hamster genome was found. Homologous sequences have been also identified in the human genome. Computer sequence analysis did not show any consistent homology of this locus with any known genes.

Previously it has been shown that RSV-transformed hamster cells in vitro have two discrete characteristics essential for in vivo selection by the effectors of the host's natural resistance. These two biochemically different characteristics, i.e., resistance to hydrogen peroxide (H_2O_2) and ability to secrete prostoglandin E (PGE) in contact with natural killer (NK) cells, macrophages, and neutrophils could be utilized as cell type markers. We tried to change the biological properties of high- and low-metastatic variants of these cells using various transfection protocols with a transforming oncogene. The low-metastasis cells (HET-SR) were transfected with pSVcN-*ras* containing the N-*ras* oncogene linked with the *neo* gene [2]. As a control in these experiments the plasmid carrying only the *neo* gene was used and G-418-resistant clones were selected. We analyzed the structure of integrated sequences, showing that the integrated N-*ras* oncogene is transcribed in transfected cells. Using MAb259 against the protein $p21^{ras}$ the processing of this protein was observed in transfected cells.

In work performed recently, it was found that the same clones lost resistance to H_2O_2 and the ability to secrete PGE (Deichman et al., 1986). The loss of these characteristic properties correlated with induction of N-*ras* expression and drastically reduced expression of RSV-specific RNA. In particular, the amount of *src* mRNA decreased more than five- to sevenfold as compared with nontransfected and *neo*-transfected cells. At the same time, in these cells the highest levels of tyrosine-specific protein kinase activity was observed in an immune complex of $pp60^{src}$ with MAb327. We were also

[1] Cancer Research Center, Moscow, USSR.

Haematology and Blood Transfusion Vol. 35
Modern Trends in Human Leukemia IX
R. Neth et al. (Eds.)

able to show that the specific activity of pp60^{src} was changed. The molecular mechanism of v-*src* and N-*ras* gene interactions in these cells is rather unique and needs to be investigated in further details.

References

1. Deichman G, et al. (1986) Int J Cancer 37:401–409
2. Souyri M, et al. (1987) Virology 158: 69–78