Symposium no. 10: Gene Alterations in Human Cancer Cells

10.013

HMG CHROMOSOMAL PROTEINS IN TRANSFORMED CELLS

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A group of proteins named HMGI was studied in murine and human transformed cells as regard its expression and primary structure. HMGI proteins, whose expression is elevated in murine cells following neoplastic transformation, were also identified in a human hepatoma cell line. The gene of a protein of the group (HMGI-C) was cloned and sequenced.

10.014

DETECTION OF MINIMAL RESIDUAL TUMOUR CELLS IN STEM CELL HARVESTS USING MOLECULAR MARKERS. Jennifer E. Hardingham, Alexander Dobrovic, Barry M. Dale, L. Terence Gooley, Gina Velissaris, Dusan Kotasek and Robert E. Sage.

Thirty three patients with chronic myeloid leukaemia (CML), or non-Hodgkin's lymphoma (NHL) have undergone peripheral blood stem cell (PBSC) harvesting for autologous transplantation.

The polymerase chain reaction (PCR) and Southern blotting hybridisation techniques were used to identify tumour specific markers in lymph node and/or bone marrow specimens at presentation. These markers were used in follow-up studies to detect tumour cells throughout the course of the disease and in stem cell collections. At presentation a specific molecular marker was found in tissue of 29/33 patients. PCR detected the 14:18 translocation in 10/25 NHL patients and 9:22 translocation in 7/7 CML patients. Immunoglobulin heavy chain (IgH) or T cell receptor (TCR) β chain rearrangments were detected by Southern blotting in a further 12 NHL patients. Fourteen autologous transplants have been performed in 10 patients with NHL and 5/10 relapsed 89–346 days post transplantation. Contaminating tumour cells were found retrospectively in 2 stem cell collections infused. In thirteen patients (7 CML, 6 NHL) awaiting transplantation residual tumour cells have been detected in the stem cell collection in all 13. The findings suggest that tumour cell contamination of peripheral blood stem cell harvests is not uncommon. Whether these cells are clonogenic and contribute to disease relapse remains to be elucidated.

10.015

No germ-line p53-mutations detected in familial and bilateral testicular cancer.

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Mutations in the p53 tumor suppressor gene are considered to be one of the most common genetic alterations in human cancers. If germ-line mutations in tumor suppressor genes are important in the pathogenesis of germ cell tumors, such mutations would be expected to be found with the highest frequency among patients with bilateral tumors, in patients with germ cell tumors in the family, and/or patients with LOH for chromosome arm 17p. We have examined leukocyte DNA from 33 patients and tumor DNA from 12 patients with testicular germ cell tumor belonging to one of these groups. We have used Constant Denaturing Gel Electrophoresis which identifies mutations in 80 per cent of the conserved regions where mutations have frequently been reported in other cancers. No mutations were identified in the germ-line or in tumors. The significance of these findings is discussed.

10.016

TRANSFORMING GROWTH FACTOR β (TGFβ) AND C-MYC ANTISENSE OLIGONUCLEOTIDES INHIBIT PROLIFERATION OF HEP3B HUMAN HEPATOMA CELLS.

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The human hepatoma cell line Hep3B expresses mRNAs of TGFβ and c-myc. Hep3B cells were growth-inhibited in a dose-dependent manner by both a 15mer antisense phosphorothioate-oligonucleotide against c-myc (50% inhibition at 20μM) and by a 25mer TGFβ antisense oligonucleotide (60% inhibition at 20μM) in the presence of 10% FBS. However, exogenous TGFβ protein also inhibited proliferation of Hep3B cells (90% inhibition by 0.3 ng/ml). The 15mer phosphorothioate-oligonucleotide was stable for 24 hrs. in culture. 4°C binding with radiolabeled antisense-oligonucleotides showed that Hep3B cells had specific cell surface binding sites for both c-myc and TGFβ antisense-oligonucleotides.

These results showed that c-myc and TGFβ genes may be important in regulating Hep3B cell growth and suggest the biological potential of exogenous antisense-oligonucleotides.

10.017

A QUANTITATIVE PCR FOR THE MEASUREMENT OF mRNA LEVELS IN CELL LINES.

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In collaboration with Dr. B. Carritt, MRC Human Biochem. Unit, London, we have cloned a candidate lung tumor suppressor gene (D8) from the chromosomal region 3p21. This gene appeared to be expressed at very low levels in SCLC-derived cell lines. We, therefore, developed a method to measure mRNA concentrations. An aberrant cDNA is constructed by ligating a small restriction fragment into a unique restriction site of the cellular cDNA. Two primers are selected on either site of this insert so that, upon PCR analysis, the aberrant cDNA gives rise to a slightly longer product than the cellular cDNA does. Next, RNA is synthesized from the cDNA-plus-insert clone using T7 RNA polymerase. The concentration of cellular mRNA in an RNA-sample is estimated by a RT-PCR analysis of mixtures of a fixed amount of cellular RNA and varying amounts of the RNA obtained from the cDNA-plus-insert. Thus, it is possible to determine the number of copies of a gene per μg of total RNA, even for mRNAs of very low abundancy. The method can easily be set up for any gene of interest.

10.018

Properties of monomer and dimer forms of purified estrogen receptor.

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The hormone binding subunit of the estrogen receptor (hbs) purified from calf uterus by immunoaffinity chromatography when analized on sucrose gradients and gel chromatography, was a mixture of monomer 65 kDa and predominant homodimer forms. The hbs was purified in absence of hormone. The addition of the hormone did not modify the sedimentation profile of purified hbs. The purified dimer could be reversible dissociated. We immobilized both the monomer and the homodimer form on an immunoaffinity resin containing a monoclonal antibody that recognizes only the NH_2 portion of the hbs. We assayed the DNA binding and other functional properties of the two resins. A radiolabeled palindromic estrogen responsive element (ERE) bound to the dimer and to the monomer form of hbs, whereas a non-palindromic ERE bound only to the monomer form. These results suggest a different role of the monomer and the homodimer forms of the estrogen receptor on regulation of genes containing palindromic and non-palindromic estrogen responsive elements. We also demonstrate that only the immobilized monomer was able to release the chromogenic group from synthetic peptides containing phenylalanine at the carboxyl terminus.

Supported by A.I.R.C.