Short communication

c-Ki-ras amplification in human lung cancer

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Amplification of cellular oncogenes, leading to enhanced expression, has been implicated as a causative factor in a range of human tumours (Little et al., 1983; Lee et al., 1984; Pelicci et al., 1984) and studies on transforming retroviruses in animals have indicated that aberrant expression of certain sequences related to cellular oncogenes is responsible for tumour induction (Aaronson, 1983).

Human lung cancer can be divided into three common histopathological classes (a) small cell carcinoma (SCCL), (b) squamous cell carcinoma (SQCL) and (c) adenocarcinoma (ADCL). Nau et al. (1984) reported elevated levels of c-myc or N-myc oncogenes in 13 out of 25 cell lines derived from SCCL tumours and they found a positive correlation between aggressiveness of the tumour and oncogene amplification: McCoy et al. (1983) identified a threefold amplification of c-Ki-ras in a SCCL cell line and Zech et al. (1985) in a cytogenetic study identified a SQCL cell line with a numerical over-representation of chromosome 12, which carries this oncogene. Involvement of c-Ki-ras oncogene activation, by point mutation, in human lung and other tumour material has also been demonstrated (Capon et al., 1983; Santos et al., 1984). As previous studies have involved mainly SCCL it was decided to examine the DNA extracted from freshly-excised lung tumour samples of various histological types, for amplification of either the c-Ki-ras or c-myc oncogenes.

Tumours were removed from patients before chemotherapy or radiotherapy was given. Mechanical disaggregation of the fresh tissue using scissors was carried out in a solution of 75 mm NaCl, 25 mm EDTA, 200 μg ml⁻¹ proteinase K and the cells lysed by the addition of SDS to 1% (w/v). The DNA was purified by phenol extraction, ethanol precipitation, ribonuclease digestion followed by a second phenol extraction and ethanol precipitation and dialysis of the DNA for 4 hours against distilled water. The isolated tumour DNA (10 μg) was digested with restriction endonuclease Sac I (for c-myc probe) or EcoR I (for Ki-ras probe), electrophoresed and transferred to nitrocellulose by the method of Southern (1975). Plasmid DNA was purified and nick translated using 32P to a specific activity of ~1 x 10⁸ c.p.m. μg⁻¹ (Rigby et al., 1977). Hybridisation of the probes, washing and autoradiography was carried out as described (Maniatis et al., 1982). Fragment sizes after autoradiography were determined by comparison to a λ phage DNA marker digested with Hind III, 32P labelled and co-electrophoresed with the genomic samples.

DNA was isolated from 25 lung tumours, comprising 18 primary SQCL, 1 lymph node metastasis from a bronchial SQCL, 3 ACL and 3 SCCL. The tumour DNA was screened initially for amplification of c-myc. The pSVc-myc-1 probe (Land et al., 1983) detected two Sac I fragments of 1.7 and 2.8 Kilobases (Kb) in all samples and no amplification was observed (data not shown). The isolates were further screened for amplification of the c-Ki-ras gene using pHiHi3 (Ellis et al., 1981). This probe detected two Ki-ras hybridising fragments in EcoR I digests, of 3.0, and 6.3 Kb, in all samples. None of the primary tumours showed amplification of the cellular sequences. However the DNA isolated from the lymph node metastasis showed a considerably elevated Ki-ras copy number (Figure 1). A peripheral blood sample was obtained from this patient and DNA extracted by the method of Kunkel et al. (1977).

This sample showed no amplification of Ki-ras (data not shown). The degree of amplification in the tumour sample was estimated by dilution of the tumour DNA and comparison with the Ki-ras level in the patient's peripheral blood DNA (Figure 2). From these results it can be shown that there has been an ~30-fold increase in gene copies over normal cellular levels. Further digestion of the tumour and the other DNA samples from normal individuals with the restriction endonucleases Pvu II, Sac I, Kpn I and Pst I followed by Southern hybridisation with pHiHi3, suggested that there had been no major rearrangement or truncation of the gene during amplification.

There was no detectable amplification of the c-myc gene in any of the tumours studied. These data and that of Nau et al. (1984) suggests that c-myc amplification in human lung cancer may be mainly restricted to SCCL. Additionally c-Ki-ras amplifica-

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tion was detected only in a lymph node metastasis from a SQCCL and none of the primary samples. This result suggests that amplification of the oncogene is unlikely to be an important causal factor in lung cancer but does not preclude the possibility that amplification of this gene is linked to progression of the disease.

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References

AARONSON, S.A. (1983). Unique aspects of the interactions of retroviruses with vertebrate cells. Cancer Res., 43, 1.

CAPON, D.J., SEEBURG, P.H., McGrath, J.P. & 4 others. (1983). Activation of Ki-ras 2 gene in human colon and lung carcinomas by two different point mutations. Nature, 304, 507.

ELLIS, R.W., DEFEO, D., SHIH, T.V. & 5 others. (1981). The p21src genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes. Nature, 292, 506.

KUNKEL, L.M., SMITH, K.D., BOYER, S. H. & 6 others. (1977). Analysis of Y-chromosome reiterated DNA in chromosome variants. Proc. Natl Acad. Sci., 74, 1245.

LAND, H., PARADA, L.F. & WEINBERG, R.A. (1983). Tumorigenic conversion of primary embryo fibroblasts requires at least two co-operating oncogenes. Nature, 304, 596.

LEE, W.H., MURPHREE, A.L. & BENEDICT, W.F. (1984). Expression and amplification of the N-myc gene in primary retinoblastoma. Nature, 309, 458.

LITTLE, C.D., NAU, M.M., CARNEY, D.N., GAZDAR, A.F. & MINNA, J.D. (1983). Amplification and expression of the c-myc oncogene in human lung cancer cell lines. Nature, 306, 194.

MAINIATIS, T., FRITSCH, E.F. & SAMBROOK, J. (1982). Molecular cloning: A laboratory manual. p. 387 Cold Spring Harbour Laboratory, New York.

McCOY, M.S., TOOLE, J.J., CUNNINGHAM, J.M., CHANG, E.H., LOWY, D.R. & WEINBERG, R.A. (1983). Characterization of a human colon/lung carcinoma oncogene. Nature, 302, 79.

NAU, M.M., CARNEY, D.N., BATTLEY, J. & 4 others. (1984). Amplification, expression and rearrangement of c-myc and N-myc oncogenes in human lung cancer. Curr. Topics Microbiol. Immunol., 113, 172.
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PELICCI, P.G., LANFRANCOME, L., BRAITHWAITE, M.D., WOLMAN, S.R. & DALLA-FAVERA, R. (1984). Amplification of the c-myc oncogene in a case of human acute myelogenous leukaemia. Science, 224, 1117.

RIGBY, P.W.J., DIEKMANN, M., RHODES, C. & BERG, P. (1977). Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol., 113, 237.

SANTOS, E., MARTIN-ZANCA, D., REDDY, E.P., PIEROTTI, M.A., PORTA, G.D. & BARBACID, M. (1984). Malignant activation of a Ki-ras oncogene in lung carcinoma but not in normal tissue of the same patient. Science, 223, 661.

SOUTHERN, E. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol., 98, 503.

ZECH, L., BERGH, J. & NILSSON, K. (1985). Karyotypic characterization of established cell lines and short term cultures of human lung cancers. Can. Genet. Cyto., 15, 335.