Smoking, oncogenes and human lung cancer

A review of recent laboratory evidence linking smoking and lung cancer

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Summary

Recent molecular genetic experiments conducted in the USA and The Netherlands have isolated specific mutations in human lung cancer cells. These data implicate the activation of the K-ras oncogene in the pathogenesis of adenocarcinoma of the lungs of heavy smokers and the deletion of DNA from the short arm of chromosome 3 in small-cell and non-small-cell carcinoma of the lung. The implications of these results are discussed and explained in the light of current DNA-manipulative technology which is now available in RSA.

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Early history

The association between environmental chemicals and cancer was made 213 years ago when Percivall Pott recorded the link between cancer of the scrotum in chimney sweeps and cancer of the nose in snuff-takers.[1]

It was not until 1915, however, that Yamagiwa and Ichikawa[2] induced cancer by repeatedly applying tar to the ears of rabbits over a period of many months. After this major discovery a concerted effort was made to identify the components of tar that were responsible for causing the cancer. At the time there was great enthusiasm among research workers who were optimistic that identification of the chemicals involved would rapidly lead to finding out how they made cells cancerous. In retrospect it is clear that these expectations were highly premature because the chemistry of the substances, which turned out to be polycyclic aromatic hydrocarbons,[3] specifically dibenz(a,h)anthracene and benz(a)pyrene, did not yield the necessary information.

The next major step was the discovery by Brookes and Lawley[4] in 1964 that the carcinogenicity of six different polycyclic aromatic hydrocarbons correlated with their ability to bind to cellular DNA when they were painted onto the skin of mice. These results and others lead workers to conclude that specific genes in the cellular DNA were responsible for the induction or maintenance of cancer. It seemed obvious that these putative cancer genes could be involved in the control of cell division and differentiation, but the identification, analysis, isolation and possible manipulation of them was not yet possible. As recently as 1978, Cairns[5] commented '. . . at present so little is known about the control of cell growth that there is no way of guessing when we will arrive at the necessary understanding — it could be in the next 10 or 20 years, or not for another century'.

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Discovery of oncogenes

During the last decade molecular biology has been revolutionised by DNA manipulative technology. It is now possible to identify, isolate, analyse, and modify specific genes, and also to insert those from one organism into the genome of another. An early result of this technological triumph was the discovery of at least 20 genes out of the total human complement of about 100 000, that could induce cancer. These were called oncogenes and given names such as ras, myc, erb, myb, src, fes and fms.[6]

It has been shown that in normal healthy cells oncogenes exist as so-called proto-oncogenes and are involved in the self-regulating systems of cell growth control and differentiation; for example, the sis proto-oncogene codes for the natural platelet-derived growth factor that is released in fresh wounds and stimulates cell growth in order to facilitate healing.[7] Other proto-oncogenes code for growth factor receptors in the cell membrane or for growth signal-transducing molecules in the cytoplasm or nucleus.

The fundamental understanding of the molecular basis of cancer is that normal proto-oncogenes, which are present in all cells, can be converted into deadly cancer-causing oncogenes by carcinogens. In the case of the ras-gene family (K-ras, N-ras and Ha-ras), it has been found that a single nucleotide substitution (point mutation), in codons 12, 13 or 61, can convert the normal gene into an oncogene.[8] Indeed, there is evidence that known carcinogens can cause point mutations in ras genes of animal tumours.[9] Point-mutated N-ras genes have been isolated and spliced into bacterial plasmid DNA. When this DNA is added to non-transformed mouse fibroblasts in tissue culture as a DNA-calcium-phosphate complex, some of the fibroblasts incorporate the oncogene and transform into rapidly dividing, disorganised cells that can form tumours in nude mice. We have verified these findings and representative results are shown in Figs 1 and 2.

Oncogenes, lung cancer and smoking

From the present evidence it can be predicted that human lung cancer cells should contain oncogenes — that is, structurally abnormal proto-oncogenes — and that the incidence of these genes should correlate with the exposure of the patient to carcinogens, especially tobacco smoke.

The first evidence supporting these general predictions has recently been presented by Rodenhuis *et al.*[10] They found that the K-ras gene was activated by point mutation in codon 12 in

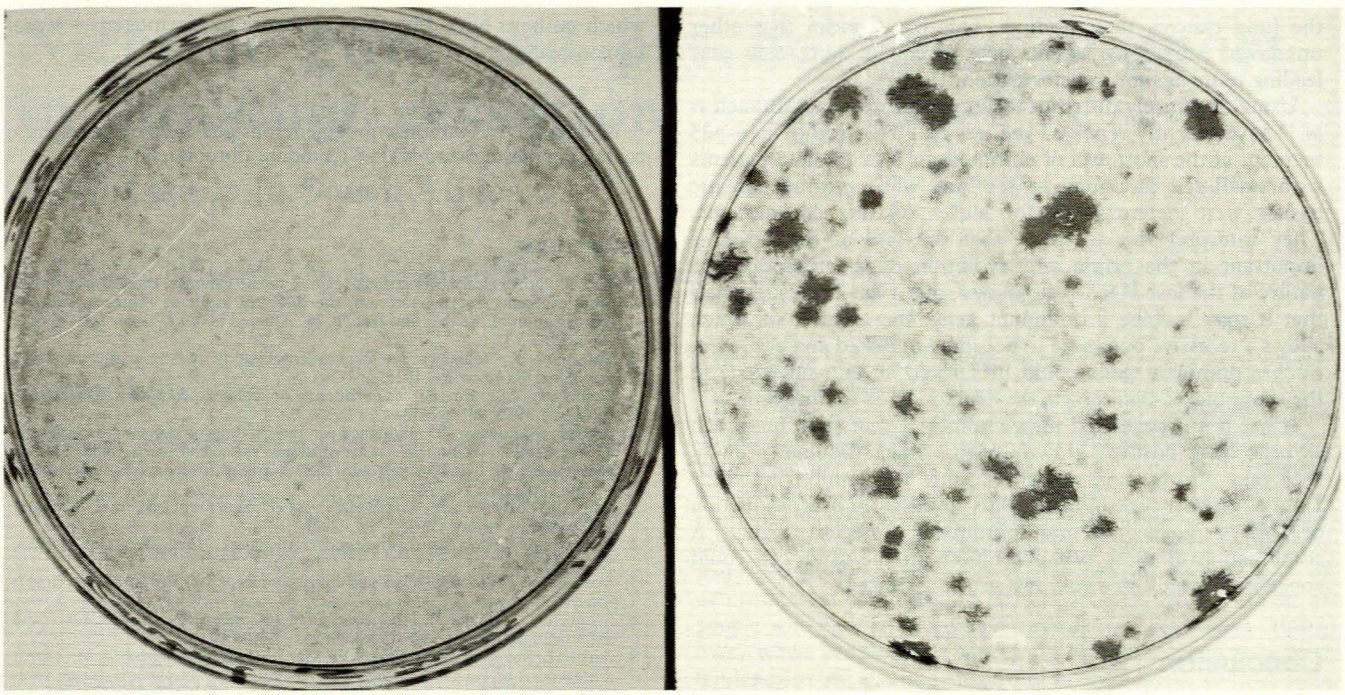

Fig. 1. Demonstration of the transforming potential of oncogenes. Petri dishes were seeded with NIH 3T3 mouse fibroblasts and exposed to 20 μg control human DNA (left) **and control DNA plus 10 ng of pBR322 bacterial plasmid containing the N-ras oncogene** (right). **After 12 days of incubation, numerous fibroblasts were transformed by the oncogenic DNA and formed multilayered colonies** (right). **The calcium phosphate technique for gene transfer was used.**[15]

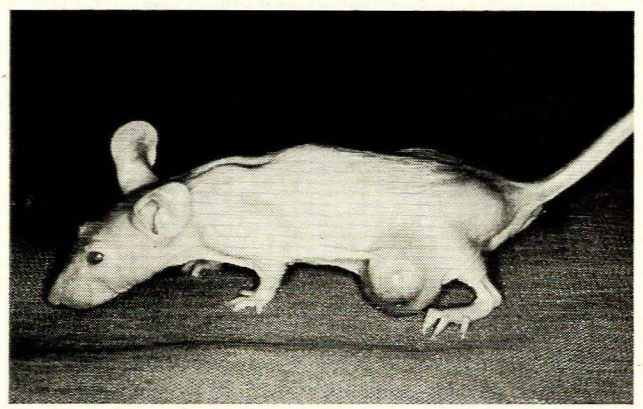

Fig. 2. Demonstration of the tumorigenic potential of N-ras oncogene-transformed NIH 3T3 mouse fibroblasts. One of the transformed colonies shown in Fig. 1. was sub-cloned and 10^7 cells injected subcutaneously into nude (nu/nu) mice. Tumours measuring about 1 cm in diameter developed within 30 days.

5 of 10 adenocarcinomas of the lung. All five patients carrying the activated K-ras gene were 'heavy smokers', whereas two of the patients with K-ras-negative adenocarcinoma, had never smoked. The authors admit that their subseries of only 10 patients is too small for statistical analysis but their sophisticated oncogene-detecting technology will surely stimulate much larger cohort studies in the near future.

Chain of molecular events

What is of importance is that a chain of molecular events has now been uncovered which links smoking to fundamental genetic lesions known to cause cancer. This chain of events can be itemised as follows: (*i*) tobacco is lit and inhaled; (*ii*) carcinogens in the smoke enter lung cells; (*iii*) some of the molecules are chemically activated and bind to DNA; and (*iv*) during cell division, coding mistakes occur due to steric hindrance caused by the bound carcinogen. Most of these mistakes are irrelevant, but if by chance a nucleotide (such as guanine) is incorrectly replaced with thymine in codon 12 of the K-ras proto-oncogene, this gene becomes an uncontrolled oncogene causing permanent growth stimulation of the lung cell.

Every step in this chain of events has now been verified. It should be pointed out, however, that a number of studies have shown that complete transformation of a normal cell into a malignant cell involves the activation of more than one oncogene.[11] It was found that an activated ras gene together with an activated myc gene was a potent combination causing malignant transformation. Amplification of at least three myc genes (c-myc,N-myc, and L-myc) have been shown to occur in small-cell carcinoma cell lines.[12] It is thus quite likely that a number of improbable mutational events need to occur in the same lung cell before a tumour can develop and metastasise. This could explain the 20-year lag period between the advent of smoking and the eventual diagnosis of lung cancer. Bearing in mind that the average smoker inhales smoke about 400 times a day (20 cigarettes x 20 puffs), it follows that the lung cells are exposed to about 3 million carcinogenic insults over a period of 20 years. If the chances of a critical oncogenic transformation occurring are 1/1 000 puffs it follows that the chances of a similar event in the same cell are $10^3 \times 10^3 = 10^6$. In other words, it may take a million puffs before the critical combination of oncogenes is activated. Of course by chance it could occur earlier or later. Nevertheless it makes good sense to stop smoking at any time because the next puff could be the fatal one.

Discovery of the deletion of DNA in lung cancer cells

One of the difficulties with the oncogene theory of lung cancer is that Rodenhuis *et al.*[10] did not find activated ras genes in all

the lung cancers they studied. This could mean that other unknown, mutational events may occur in lung cells also leading to malignant transformation.

Using an entirely different technological approach Brauch *et al.*[13] have recently reported the loss of DNA in the 3p14-p23 segment of the short arm of chromosome 3 in 13 of 13 patients with small-cell carcinoma of the lung whose normal somatic tissues were heterozygous for alleles on this chromosome. They interpret this to mean that the loss of the DNA is important in the origin and evolution of the tumour. The nature of the lost DNA is unknown, but it has been suggested that it may involve a dominant gene, the absence of which allows a recessive oncogene to become unmasked and activated. Such a dominant gene could be termed an anti-oncogene in the sense that it counteracts the expression of an oncogene.

It has been speculated for a number of years that inasmuch as cells have positive growth factors that stimulate growth, they may also contain negative factors that inhibit growth.[14] Loss of growth control could thus result from overproduction of positive signals or an underproduction of negative signals. A combination of these two aberrations in the same cell could conceivably result in a very fast growing cancer.

Conclusion

The fundamental molecular mechanisms of chemical carcinogenesis are no longer a total mystery. The advent of DNA-manipulative technologies has made it possible to identify, analyse and isolate oncogenes. A chain of molecular events leading from the inhalation of tobacco smoke to cancer cells has been established and is most likely to be elaborated in the near future. The unravelling of the mechanisms of chemical carcinogenesis over the last 213 years is a tribute to human intellect and ingenuity that is unfortunately marred by the fact that in the case of smoking the 'experimental animals' — of which millions have died from exposure to carcinogens —were human beings.

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