Science, medicine, and the future

Lung cancer

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Summary

Lung cancer, the most prevalent cancer in the Western world, is mainly caused by smoking. Nevertheless, only 20% of smokers develop lung cancer and while prevention is important, environmental factors are expected to contribute to the predicted rise in the incidence of lung cancer in the next 25 years. Survival of lung cancer is still poor, and new treatments are urgently needed. This review examines potential new therapeutic developments which have arisen from a greater understanding of the molecular and cellular biology of lung cancers.

Introduction

Lung cancer is the commonest fatal malignancy in the developed world. Cancer accounts for a quarter of deaths in England and Wales, and lung cancer accounts for 1 in 3 deaths from cancer in men and 1 in 6.5 in women. Mortality from lung cancer now exceeds that from breast cancer among women in Scotland. During the past 20 years medical and surgical intervention has resulted in little change in the five year survival for lung cancer, with roughly 90% of affected patients dying within one year of diagnosis. The epidemic proportions of the disease are contrasted sharply with the general failure of conventional treatment. Nevertheless, I feel optimistic that this frustrating and dismal situation will change in the next 25 years. Firstly, there is a genuine desire to do something about lung cancer, and, secondly, with a greater understanding of the molecular and cellular biology of lung cancer the rational development of new and specific antitumour drugs is a realistic goal.

Cigarette smoking is by far the most important single factor in causing lung cancer and is directly responsible for at least 90% of lung cancers. Asbestos exposure is the main occupational cause of lung cancer, although several atmospheric pollutants and other industrial products are also important. Prevention will continue to play an important part in the strategy against lung cancer. This should take the form of increased public awareness campaigns particularly for schoolchildren, extension of smoking bans in public places, further restrictions on cigarette advertising, and increasing taxation on cigarettes. However, despite decades of antismoking campaigns the incidence of lung cancer deaths is expected to rise further over the

next 25 years. An increasing number of cases are expected to be unrelated to smoking, presumably due to environmental pollution.

Molecular events in development

The current hypothesis is that at least 10-20 genetic mutations are required to produce a lung cancer cell from the normal one. These mutations cause activation of oncogenes (dominant cellular factors which stimulate or predispose a cell to divide) and deletion of tumour suppressor genes. Figure 1 shows that these genetic changes result in pathological changes which in the early stages are reversible. However, as progressive chromosomal changes become more complex lung cancer develops.

Cancer is due not only to abnormal cell proliferation with loss of growth control but to abnormalities in the cell's intrinsic cell death programme (apoptosis). Tumour mass is a balance between proliferation and cell death (necrosis and apoptosis). Resistance to chemotherapy seems also to relate to deficits in this cell death programme, resulting in an increasing population of chemoresistant cancer cells. These processes are regulated by factors from both inside and outside the cell. The regulators include growth factors, cytokines, and hormones. These interact with specific receptors and communicate with the nucleus through intracellular signalling pathways resulting in activation and repression of genes associated with proliferation and inhibition of apoptosis.

Many oncogenes in lung cancer cells code for mutated proteins or overexpression of proteins

	Pathological state	Chromosomal changes (%)
Precancerous	Normal mucosa	Simple rearrangements (20%) (3p,7,11,17)
	Bronchial metaplasia	
	Bronchial dysplasia	Same + 17p mutations
Cancer	In situ carcinoma	
	Early invasive cancer	Complex karyotypes (60%) (3p,7,8,9,11,17)
	Metastatic cancer	
		More complex karyotypes (100%)

Fig 1 Sequence of genetic events in lung cancer

Fig 2 Pathogenesis of lung cancer

involved in the pathways signalling proliferation and apoptosis. The pathways are thus subverted, allowing the tumour to grow. For example, a growth factor receptor that is mutated so that it stimulates growth signals in the absence of the growth factor or mutated intracellular signalling molecules which continuously stimulate growth even in the absence of receptor stimulation will predispose to unrestrained cell growth. In addition, high expression of proteins that prevent cells from undergoing programmed cell death in response to drugs may lead to an expanding population of cancer cells that are resistant to chemotherapy induced cell death. Lung cancer cells can also manipulate their local environment to aid tumour growth, particularly by damping down the host immune response to the lung cancer and promoting angiogenesis (fig 2).

Tumour initiation and development of drug resistance

All lung cancer cells produce hormones and peptides which can function as growth factors and generate growth loops. These include epidermal growth factor, transforming growth factor α , platelet derived growth factor, insulin-like growth factors, gastrin releasing peptide, neurotensin, gastrin cholecystokinin, and vasopressin. Small cell lung cancer (which constitutes 25% of all lung malignancies) is known to secrete many hormones and peptides, and patients can present with clinical syndromes related to this—for example, inappropriate secretion of antidiuretic hormone and ectopic secretion of adrenocorticotrophic hormone.

As small cell lung cancer progresses it becomes resistant to chemotherapy. There is evidence that during this progression the potency and number of peptide growth loops increase; bronchoalveolar lavage fluid from smokers without evidence of cancer contains increased concentrations of these growth factors compared with that from healthy non-smokers.¹ The source of the growth factors is predicted to be the pulmonary neuroendocrine cells, which multiply abnormally in smokers. These observations suggest that hyperplasia of neuroendocrine cells and growth factor secretion may precede the induction of lung cancer and then be important in tumour progression and the development of resistance to chemotherapy.

New therapies may prevent cell division

Increased understanding of the effects of growth factors in lung cancer has made it possible to plan

rational interventions. Growth of lung cancer is driven by multiple and diverse stimulators, and so blocking the action of a single growth factor is unlikely to be effective. However, inhibition of intracellular signal transduction mechanisms that control multiple growth stimulating inputs offers a realistic potential for intervention. Growth of cancer cells seems more dependent on a smaller number of intracellular enzymes (kinases) and signal transduction pathways than growth of normal cells, making cancer cells more susceptible to inhibition of a specific kinase.

The signal transduction pathways important in lung cancer are beginning to be elucidated. Recent studies have shown that activation of tyrosine kinases (which phosphorylates tyrosine residues) is an important growth signal in all histological types of lung cancer cells.² In small cell lung cancer inhibition of tyrosine kinase activity (by tyrphostins) inhibits cell growth and also stimulates apoptosis. Thus the level of tyrosine kinase activity and phosphorylation of tyrosine residues may determine whether cells proliferate or die by apoptosis.

The structure of tyrphostins has been greatly modified, and a large number have now been tested. Some of these compounds have high specificity against individual tyrosine kinases and show promise as anticancer drugs. Thus in the future it may be possible to identify critical enzymes which regulate the balance between cell proliferation and apoptosis in lung cancer cells. These tumours could then be treated with the specific tyrphostin for that kinase. As with other inhibitors of second messenger signals such as lithium, calcium channel blockers, and digoxin, these drugs would not be as toxic as standard chemotherapeutic drugs and could therefore be prescribed to outpatients regularly for long periods.

Treatments may also trigger cell death by apoptosis

Apoptosis is an active cell death programme which is initiated by the stress kinase pathway. This pathway activates specific intracellular proteases and DNases resulting in the characteristic morphological changes of apoptosis (cell shrinkage, chromatin condensation) and DNA cleavage. Stimulation of apoptosis in lung cancer cells is an exciting potential treatment. Compound G ([Arg⁶, D-Trp^{7,9}, MetPhe⁸] substance P(6-11)) has been identified as a potent inhibitor of growth of small cell lung cancer.³ Interestingly, compound G stimulates apoptosis of lung cancer cells and can activate key enzymes in the cell death pathway from its activity in the cell membrane. Compound G is now in

Gene mutations that could be targeted for gene therapy

Oncogenes

Non-small cell

Mutant epidermal growth factor receptor/neu Ras

Small cell
myc,bcl-2

Tumour suppressor genes

Non-small cell

p53

Small cell
Retino-blastoma gene, p53, protein tyrosine phosphatases

phase I clinical trials in Edinburgh. Future work will identify the second messenger signals that activate the cell death pathway and the key effectors of apoptosis in lung cancer cells.

Immunotherapy for lung cancer

Lung cancers can both stimulate a humoral antibody response and activate cell mediated immunity. Lung cancers are heavily infiltrated by lymphoid cells, yet tumours continue to grow. Therefore some *in situ* functional deficiency in the immune response must exist within lung cancers. A greater understanding of the mechanisms by which lung cancers escape immune surveillance will make it possible to stimulate the host immune response to kill lung cancer cells. New treatments will emphasise enhancement of specific tumour cytotoxicity rather than non-specific immune stimulation (for example, with BCG, which in clinical trials was not particularly effective on its own).

The immune response can be stimulated by vaccination with tumour associated antigens. This approach has been tried in patients with non-small cell lung cancer. In a pilot study patients were immunised with autologous cryopreserved irradiated tumour cells mixed with BCG. The vaccinated patients had a delayed cutaneous reaction to autologous irradiated tumour cells showing that it is possible to stimulate an immune response to cancer cells.⁴

The cure of micrometastasis after surgery is a major goal of cancer immunotherapy. In the case of lung cancers resurrection of the primary tumour could be followed by the identification or isolation of specific tumour associated peptides. These peptides could then be used as vaccines against metastatic disease. Studies in a mouse model system suggest that peptide vaccine therapy may be effective in treatment of residual tumours.⁵ Another study showed that vaccination with lung cancer associated antigen and Freund's complete adjuvant in patients with stage 1 or stage 2 lung cancer improved five year survival from 34% in the control group to 75% for the specific active immunotherapy group.⁶ These results are encouraging and more specific and active vaccinations could be developed.

Inhibition of angiogenesis

Growth of lung cancers and their metastases depends on their blood supply and neovascularisation. Recent studies have identified critical targets for inhibiting tumour neovascularisation. The cytokine interleukin 8 is secreted by lung cancer cells and induces neovascularisation, which can be blocked by an interleukin 8 monoclonal antibody.⁷ Another study used monoclonal antibodies to the integrin $\alpha_v\beta_3$ to induce apoptosis of migrating endothelial cells.⁸ These antibodies inhibit adhesion to extracellular matrix proteins, thus preventing neovascularisation. In both of these studies inhibition of neovascularisation led to inhibition of tumour growth.

Gene therapy for lung cancer

The concept of curing disease by correcting gene mutations (gene therapy) has captured the imagination of doctors, scientists, and the public. Most of the above

treatment strategies could be achieved by using genetic manipulation to knock out, correct, or enhance the function of a critical protein.

Some genetic mutations lend themselves particularly well to gene therapy—for example, mutations in the tumour suppressor gene p53 and bcl-2, which was the first oncogene identified that prevented cells from dying. Bcl-2 concentrations are high in small cell lung cancer and seem to correlate with resistance to chemotherapy. p53 is a nuclear DNA binding protein that acts as a regulator of transcription. Concentrations of p53 increase after chromosomal damage, arresting growth of the cell and preventing it from entering mitosis. If chromosomal damage cannot be repaired, apoptosis is initiated. The p53 gene is mutated in most malignant tumours including lung cancers. The mutation seems to occur early resulting in cells which cannot arrest their growth cycle or initiate apoptosis in response to chromosomal damage. This in the long run will induce instability within the chromosome and predispose the cell to further genetic insults.

It remains unclear whether correcting a single mutation in multistage carcinogenesis can significantly affect disease progression. Despite this, recent studies suggest that correction of a single critical genetic lesion may be sufficient to abrogate tumorigenicity in human cancer cells, particularly if the correction upregulates the activity of the apoptosis pathway. The first clinical trial in which patients with non-small cell lung cancer were injected directly with a retroviral vector containing the normal p53 gene produced tumour regression in three of nine patients in whom conventional treatments had failed; in three other patients tumour growth stabilised for up to five months with no clinically important toxic effects from the vector. There was an increase in apoptosis in post-treatment biopsy specimens compared with pretreatment specimens.⁹ These initial results are exciting, and gene therapy is now being evaluated in patients with early stage disease and also in the perioperative period.

The efficiency of gene transfer needs to be high to correct the genetic abnormality present in all cells of a tumour. At present a single shot of adenovirus vectors can transfer genes to about 57% of the cells exposed. However, a proportion of non-transfected cells are also killed by a mechanism that is not understood. Unlike the case for inherited disorders, such as cystic fibrosis, transient transfection of genes may be sufficient as a cancer cell need to be killed only once. Hence it may be that genetic correction strategy does not require 100% transfection. The box shows other gene mutations that may be amenable to correction by gene therapy.

Conclusions

I see the next 25 years as being an exciting time. I have tried to show the several enticing potential strategies for the treatment of lung cancer. I believe that pharmacological approaches and gene therapy to inhibit growth and stimulate apoptosis are the two avenues most likely to succeed. Which of these approaches eventually proves the most effective depends on future technology. Though gene therapy does seem to offer the possibility of delivering genes to specific organs, local p53 gene therapy and stimulation of the stress kinase pathway and apoptosis by compound G

represent early steps in a long journey. Further improvements in the understanding of the basic biology of lung cancer and in biomedical science are sure to result in multimodal treatments of lung cancer in the future.

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Possible future treatment of lung cancer

First line treatment (aims)

Surgery
Debulk tumour
Gain biological/biochemical information

Chemotherapy and radiotherapy

Reduce tumour mass
Increase sensitivity to compound G
Gene therapy (for example antisense bcl-2)
Resensitise tumour to standard chemotherapy and radiotherapy
Transfer genes into cultured host

T lymphocytes

Enhancing specific direct killing of tumour cells
Induce effective systemic immune responses

Second line treatment

Immunisation with tumour associated antigens
Specific enzyme inhibitors—for example, tyrosinase
Compound G

Chemotherapy and radiotherapy

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Lesson of the week

Mediterranean spotted fever mimicking Kawasaki disease

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Doctors in Britain are likely to recognise that sick travellers returning from the tropics may have infections that are not transmitted in the United Kingdom. They may not be aware that travellers to neighbouring countries in Europe may also return with infections that are not encountered in the United Kingdom. These diseases may then be difficult for doctors in Britain to diagnose, especially when patients present with non-specific features such as fever and rash. Millions of British travellers to Europe are potentially exposed to Mediterranean spotted fever every year. This can present with vague features, but early and specific treatment can help to avoid serious or fatal complications.

Case report

In August 1995 a 14 year old British boy spent two weeks on holiday in a villa in the Algarve, Portugal. A week after returning to Britain he became unwell with nausea, anorexia, and fever. On the third day of illness he developed a non-pruritic rash and was given penicillin for a presumed streptococcal infection. Six days later he was admitted to hospital.

On examination his temperature was 39.4°C. He had a widespread macular erythematous rash with raised purpuric centres (fig 1 (left)). Investigations on

admission included a full blood analysis. Haemoglobin concentration was 125 g/l, white cell count $4.2 \times 10^9/l$ (neutrophils $3.2 \times 10^9/l$, lymphocytes $0.9 \times 10^9/l$, monocytes $0.1 \times 10^9/l$), platelet count $151 \times 10^9/l$, partial prothrombin time 38.8 seconds (range 23-36 seconds), and D-dimer concentration 2000 µg/l (<500 µg/l). An initial differential diagnosis included meningococcal septicaemia, and treatment with cefotaxime was started.

On re-examination on the following day mild oedema of the hands and feet, bilateral conjunctivitis, a strawberry tongue, cracked lips, inflamed oral mucosa (fig 1 (right)), and cervical lymphadenopathy were noted. These features were suggestive of Kawasaki disease, prompting treatment with high doses of intravenous immunoglobulin and aspirin and investigation by echocardiography to detect possible coronary artery aneurysms. Also included in the differential diagnosis were infections with measles and rubella viruses, parvovirus, enterovirus, adenovirus, Epstein-Barr virus, group A β haemolytic streptococcus, and *Staphylococcus aureus*; autoimmune vasculitis; and a drug reaction.

Review of the patient's history showed that he had had contact with a dog while in Portugal and, given his clinical features, Mediterranean spotted fever was also considered, although he denied having a tick bite and

Consider Mediterranean spotted fever in feverish patients who have recently returned from southern Europe or north Africa

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Fig 1 Axillary rash on admission (left). Rash, conjunctival injection, and swollen cracked lips one day later (right). Reproduced with permission

no eschar was present. Accordingly, 48 hours after admission, treatment with cefotaxime was stopped and oral ciprofloxacin 500 mg twice daily was started. After four days of treatment with this antibiotic his fever settled. He was discharged three days later and remained well.

Serum samples taken on admission and 14 days later showed a rise in spotted fever group antibodies from undetectable to >1:2560. Other serology and bacterial culture results were unremarkable.

Discussion

Mediterranean spotted fever occurs throughout the Mediterranean littoral and contiguous regions, including Portugal and northern Spain.¹ It is caused by infection with *Rickettsia conorii* and is transmitted by bites from canine brown ticks, *Rhipicephalus sanguineus*, most commonly during summer, although only 37% of patients recall contact with a tick.² People of all ages are vulnerable to infection. Organisms resembling *R. conorii* are found throughout Africa, the Middle East, and South East Asia,³ where similar spotted fevers occur. Seroprevalence studies indicate that in many endemic areas mild infection is common, underdiagnosed, and underreported. In southern Portugal 7.6% of the population have antibodies to *R. conorii*, and nationally up to 20 000 cases are estimated to occur each year, although only about 5% are reported.⁴ Our patient had features of severe disease. This is characterised by fever of greater than 39°C, an extensive maculopapular rash, and in 72% of cases an eschar (tâche noir), or necrotic area at the tick bite site.² In one series 2.5% of the patients died from a malignant form of the disease, characterised by a purpuric rash, thrombocytopenia, stupor, and multiorgan failure.² Death from severe disease has been associated with a delay in diagnosis and treatment.

When a history of travel to an endemic area and the triad of fever, rash, and eschar are present the clinical diagnosis is straightforward. A history of close

contact with a dog is a helpful pointer, especially when, as in this case, an eschar is absent. Antibodies develop late on in the disease, so serological confirmation is of use only in retrospect. Diagnosis is more quickly confirmed by the presence of *R. conorii* in skin biopsy specimens on immunofluorescence microscopy, but this test is not widely available. Early diagnosis therefore relies on clinical suspicion. Possible treatments include either tetracycline or chloramphenicol, but ciprofloxacin seems to be equally effective and is probably safer.⁵

Kawasaki disease is an acute illness of uncertain aetiology, characterised by fever lasting for more than five days, a polymorphous rash, conjunctival injection, oropharyngeal redness and cracking, peripheral oedema, and cervical lymphadenopathy. A diagnosis of Kawasaki disease requires at least five of these features and no other explanation for the illness. It occurs predominantly, but not exclusively, in children under 4 years old, but it has been reported in adults.⁶ Our patient had all the clinical features and, at the time of illness, no established alternative diagnosis. This observation has been made before in a Greek child with Mediterranean spotted fever.⁷

The infection should be included in the differential diagnosis of Kawasaki disease in countries where Mediterranean spotted fever is endemic and in other countries when the patient has recently returned from an endemic area. Because Kawasaki disease is associated with the development of coronary artery aneurysms, which can be prevented by early treatment with high dose intravenous immunoglobulin and aspirin, this treatment should not be delayed but given concurrently with appropriate antibiotics when Mediterranean spotted fever is also a possible differential diagnosis.

Rickettsia-like particles have previously been implicated in the aetiology of Kawasaki disease.⁸ It is interesting to speculate whether a proportion of cases of Kawasaki disease could be caused by *R. conorii*, perhaps acting as a superantigen.

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