The antigens of lung cancer

R L Souhami

The growth of interest in the biology of lung cancer is a welcome product of frustration with the results of current treatment. The last 15 years have seen a considerable increase in our knowledge of the disease, and we can but hope that therapeutic advances will result. It is the most common cause of death from cancer in men, and in the United States in the last two years it has overtaken breast cancer as the most common cause of fatal cancer in women. An additional spur to biological investigation has been the recognition of four pathological types of the disease. There is growing acceptance of the hypothesis that lung cancer starts in a cell capable of differentiating into various pathological forms (fig 1). This is supported by the occurrence of mixed pathological types (for example, mixed squamous and small cell). In cell lines and in biopsy material it has been shown that neuroendocrine features, typical of small cell lung cancer, can be found in adenocarcinoma and that cytokeratins, which are uniformly found in squamous cell carcinomas, are expressed in small cell lung cancer.

Small cell lung cancer shows the typical pathological features of neuroendocrine differentiation: neurosecretory dense core granules, chromogranin A, cytoplasmic l dopa decarboxylase, the glycolytic enzyme neurone specific enolase, and production of hormones and neuropeptides. There is some evidence that when some of these features are detected in any adenocarcinoma this is more likely to respond to chemotherapy, though more recent studies have indicated that the effect is small.

These considerations indicate that the antigens detected in lung cancer are likely to be expressed, in some degree, in all pathological types and will be representative of antigens found in neuroendocrine, squamous, and glandular tissues, and in carcinomas at other sites. Some of these antigens are potential targets for antibody directed treatment (see below). They are molecules that are associated with a wide range of functions of normal fetal and malignant cells. Strictly speaking, they are not "tumour antigens" (though they are sometimes referred to as such) because they are not tumour specific in the sense of appearing on tumour cells only. Monoclonal antibodies, produced by somatic cell hybridisation, have led to the definition of a wide range of cellular antigens, many of them proteins whose function has now been well characterised and whose amino acid sequences have been determined.

Types of cellular antigens

CELL-CELL ATTACHMENT MOLECULES

Carcinoembryonic antigens are a family of proteins closely related to the immunoglobulin gene "superfamily." Carcinoembryonic antigens are expressed during fetal life and re-expressed in various neoplasms, typically in adenocarcinomas. Other members of this "superfamily" include the neural cell adhesion molecule, and recent workshops on lung cancer antigens (see below) have shown that this is expressed in most small cell lung cancer lines and biopsy specimens, and appears to be an important marker of the neuroendocrine phenotype. Carcinoembryonic antigens are also expressed in small cell lung cancer and other forms of lung cancer and may be associated with an adverse prognosis. The neural cell adhesion molecule is a cell-cell attachment protein that is heavily glycosylated. It exists in several major isoforms. There are two transmembrane linked forms, a truncated form attached to membrane phospholipid (via glycosylphosphatidylinositol) and a soluble form in muscle and brain. The genetic basis for this variation lies in alternative splicing of the gene for the neural cell adhesion molecule. The molecule has the property of homophilic binding (fig 2). The degree of binding is inversely related to the amount of glycosylation. A heavily glycosylated form has been described in Wilms' tumour. The forms of the neural cell adhesion molecule expressed in small cell lung cancer have not yet been systematically analysed. Many monoclonal antibodies have been made to the neural cell adhesion molecule.

Figure 1  Hypothesis for the development of histological types of lung cancer.

Small cell carcinoma

Oncogenic events

PLURIPOTENT CELL

Adenocarcinoma

Large cell carcinoma

Squamous carcinoma

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A linked lung molecule is also synthesized that is detached from the cytoplasmic membrane.

**Figure 2** Major isoforms of neural cell adhesion molecule showing homophilic binding. The dark regions indicate repetitive domains. PI—phosphatidylinositol linked form. A form of neural cell adhesion molecule is also found in the extracellular matrix proteins.

**Figure 3** Binding of integrins to extracellular matrix proteins.

**Figure 4** Some membrane receptor molecules, recognised by monoclonal antibodies, on the lung cancer cell. GRP—gastrin releasing peptide; ADH—antidiuretic hormone; EGF—epidermal growth factor; PDGF—platelet derived growth factor.

and many appear to recognise a relatively restricted region of the peptide backbone.

Other carcinoembryonic antigen like molecules are myelin associated glycoprotein and an antigen found on melanoma cells. A surface glycoprotein of molecular weight 40 Kd was found in the lung cancer workshops (see below) to be expressed on lung cancers and a wide range of epithelia and epithelial malignancies. The gene has been cloned and appears to be closely related to an extracellular matrix protein, nidogen. Other cell attachment molecules, such as the intercellular adhesion molecule, are expressed on melanoma and other cancer cells. The class I histocompatibility antigens are found on all forms of lung cancer, but class II is poorly expressed on small cell lung cancers, though its presence can be induced by treatment with interferon α.

**Antigens that bind to extracellular matrix proteins**

Many tumour cells both secrete and have receptors for extracellular matrix proteins (fig 3). Proteins such as collagen, fibronectin, laminin, and tenascin are essential components of the extracellular matrix. Integrins are heterodimeric transmembrane receptor proteins that bind to laminin, collagen, and fibronectin. In doing so they link the cytoskeleton of the cell with the matrix. The integrin family is a complex in which the α chain varies and the β peptide defines the class. The variety of integrin expression is apparently reduced in cancer. Integrins appear in a later stage of cellular differentiation and are found on squamous cancers. Glycolipid antigens (GD₂ and GD₃) are also expressed in lung cancer and may be important in cell invasion in this and other tumours.

**Antigens that are receptors concerned with transmembrane signal induction**

The cell membrane contains a range of receptors for growth factors, cytokines, and toxins as well as proteins concerned with drug attachment and efflux. Figure 4 shows some of these proteins and indicates some of the diversity of function. The epidermal growth factor receptor is expressed on non-small cell lung cancers and receptors for insulin like growth factors on small cell lung cancers. These receptors and others, such as those for platelet derived growth factor, are widely distributed on tumours and normal cells. Of particular interest in small cell lung cancer is the expression of receptors for growth inducing peptides, such as gastrin releasing peptide (bombesin), which are secreted by the tumour and which, on becoming bound to the tumour cell, promote cell division—that is, autocrine growth stimulation.

Other membrane associated proteins concern cellular functions of detoxification and cation transport. The p170 glycoprotein is associated with cellular resistance to the effects of naturally occurring antitumour substances, such as doxorubicin, epipodophyllotoxin, and colchicine. The protein acts as a drug efflux mechanism and methods to circumvent its action may yet give useful therapeutic results. MBr1 is a glycosphingolipid expressed on breast and lung cancer cells whose function is not clear.

**Blood group antigens**

Carbohydrate antigens on glycolipids are strongly expressed on many epithelial
tumours.\textsuperscript{11} Many of these are blood group antigens such as Lewis\(^a\) and Lewis\(^b\), and some are expressed less (or not at all) on the normal epithelium from which the tumour is derived. In this sense they sometimes represent oncofetal antigens, which may be expressed transiently during tissue development and are re-expressed in neoplasia.

Some of these carbohydrate antigens are also found on glycoproteins, such as mucins or adhesion molecules, and the patterns of glycosylation in these high molecular weight structures may be different in normal and malignant cells. Blood group and glycolipid antigens are expressed on both small cell and non-small cell lung cancers.\textsuperscript{12}

Membrane receptors taking part in the transport of calcium, iron, copper, and other cations are found on the surface of lung cancer cells (and other tumour cells) as on normal cells and other tumours. Up regulation or altered expression of these receptors has not yet been described in lung cancer.

\textbf{Lung cancer antigen workshops} Two recent workshops\textsuperscript{13,14} have been undertaken to attempt to introduce a taxonomic description of the antigens being recognised by the numerous monoclonal antibodies to lung cancer cells. A summary of the major clusters of reactivity is given in the table. Cluster 1 is the neural cell adhesion molecule\textsuperscript{3} and there are now numerous examples of monoclonal antibodies that react with this molecule. Many of them cross block, indicating recognition of a common epitope, but some do not. They appear to recognise protein rather than sugar residues. Cluster 2 is the gp40 glycoprotein previously described. Clusters W6 and W8 are blood group haptons, and W7 is a high molecular weight mucin. Clusters 4 and 5 are extremely interesting glycoproteins with both neural and epithelial reactivity, found on small cell lung cancer cells and of unknown function. The workshops showed that neuroendocrine differentiation was a much more pronounced feature of small cell lung cancer than of non-small cell lung cancer, but that overlap of neuroendocrine and epithelial features occurred.

\textbf{Clinical approaches using cell surface antigens} Clinical interest in these antigens comes from their potential use as targets for antibody directed treatment (fig 5). Such treatment might be with radiolabelled antibody, where the cell and its neighbours might be killed by radiation, or by toxins (such as the ricin A chain) linked to antibody (gp40 may be especially effective at internalising bound antibody), or by more complex methods, such as the use of a prodrug administered with an antibody coupled to an enzyme, which converts the prodrug to a cytotoxic form only at the site at which the antibody–enzyme conjugate is bound (that is, the tumour). Other approaches use antibodies that lyse cells in the presence of complement or allow cell killing by effector cells such as activated cytotoxic T cells. Phase I and II clinical trials using these approaches are now beginning.

Some of the antigens are more promising targets than others. Ideally, the antigen should be expressed on most cells in all cases of a given

\textbf{Lung cancer antigen classification based on the analysis of the Second Lung Cancer Antigen Workshop*}

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Monoclonal antibodies</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RNLI, MOC-1, MOC-191, NCC-LU-243, NCC-LU-246, SEN 6, SEN 36, NE 150, S-L 11-14, NE 25 (also UJ13A, ERIC-1 from other sources)</td>
<td>Neural cell adhesion molecule.\textsuperscript{16} Evidence for different epitopes. Form of molecule on SCLC not known. Distribution: SCLC, carcinoid, renal carcinoma, neuroblastoma, nerve, muscle, thyroid epithelium</td>
</tr>
<tr>
<td>2</td>
<td>MOC-31, MOC-38, MOC-151, MOC-181, AUA 1, S-L 2-21, probably PE-35 and S-L 4-20</td>
<td>40 kDa transmembrane glycoprotein gene is cloned.\textsuperscript{17} Efficient in immunotoxin mediated cytotoxicity. Distribution: SCLC, carcinoid, normal and malignant epithelium</td>
</tr>
<tr>
<td>w4</td>
<td>SWA 21, SWA 22, probably SWA 11</td>
<td>Glycosylated protein 45 kDa. Distribution: SCLC, neuroblastoma, carcinoid, adenocarcinoma and squamous carcinoma, renal tubules, granulocytes</td>
</tr>
<tr>
<td>5</td>
<td>SWA 4, SWA 23, LAM 8</td>
<td>Antigens of 90-135 and 200 kDa, probably sialoglycoproteins. Distribution: SCLC, carcinoid, adenocarcinomas, weakly with squamous carcinomas, nerve, renal tubules</td>
</tr>
<tr>
<td>5A</td>
<td>SWA 20, probably SEN 3 and SEN 31</td>
<td>Antigens of 40, 100, 180 kDa sialoglycoproteins. Distribution: similar to cluster 5</td>
</tr>
<tr>
<td>w6</td>
<td>MOV 15, NCC-ST-433, probably NCC-LU-152</td>
<td>Le(^b) hapten. Distribution: broad epithelial reactivity</td>
</tr>
<tr>
<td>w7</td>
<td>NCC-ST-439, NCC-CO-450</td>
<td>High molecular weight mucins. Distribution: broad epithelial reactivity</td>
</tr>
<tr>
<td>w8</td>
<td>A-80, NCC-LU-35, NCC-LU-81</td>
<td>Blood group A trisaccharide. Distribution: broad epithelial reactivity</td>
</tr>
</tbody>
</table>

\*Fifty one of 87 monoclonal antibodies were not assigned to a cluster, so this is in no sense a complete description of the antigens to which monoclonal antibodies have been made. SCLC—small cell lung cancer.

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure5.png}
\caption{Potential mechanism for tumour cell killing by monoclonal reagents. 1: a toxin is coupled to antibody that is internalised, resulting in toxin mediated cell death; 2: the antibody acts as a bridge to a cytolytic effector cell; 3: the antibody induces complement mediated lysis; 4: the antibody is conjugated to a radionuclide that irradiates the tumour; 5: the antibody is conjugated to an enzyme that activates an inert prodrug in the region of the tumour (or normal tissue).}
\end{figure}
type of tumour. In practice some degree of heterogeneity is always found. The antigen should be expressed on as small a number of normal tissues as possible, and especially not on those that are likely to be dose limiting (bone marrow precursor cells, for example). So far there has been little evidence that antigens unique to particular tumours (and not found in normal tissues) are likely to be found, but this may not invalidate the approach. Of course, the finding on tumour cells of altered epitopes of an antigen found on normal tissues would be a step towards increased specificity, but so far there is little indication that this is likely. New methods of production of monoclonal antibodies by techniques of molecular biology may make the rather “hit or miss” approach based on hybridomas obsolete. Progress in this approach to lung cancer treatment is highly probable.

New perspectives in lung cancer. 3. The antigens of lung cancer.

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