DIFFERENTIATION MARKERS FOR LUNG-CANCER SUB-TYPES. A
COMPARATIVE STUDY OF THEIR EXPRESSION IN VIVO AND IN VITRO

Jos L.V. Broers1 and Frans C.S. Ramakers

Department of Molecular Cell Biology and Genetics, University of Limburg, P.O. Box 616, NL-6200 MD Maastricht,
The Netherlands.

Cell lines representing the major sub-types of lung cancer have proved to be useful tools to study the molecular and cellular biology of these malignancies, provided that they are well established and well characterized. Antibodies directed against constituents of different cellular compartments can detect the type and degree of differentiation in lung cancer and derived cell lines. Antibodies can detect cell-surface adhesion molecules, such as NCAM, cadherins and integrins. NCAM antibodies are able to differentiate between small-cell lung cancer (SCLC) and non-SCLC, both in cell lines and in tumours. In addition, a spectrum of other membrane proteins, expressed in solid tumours, such as epidermal-growth-factor receptor, IRS-1 and carcino-embryonic antigen, are retained in cell lines. Cytoplasmic intermediate filament proteins appear to be generally retained in lung-cancer cell lines, their combinations being the same as in solid SCLC, adenocarcinomas and squamous-cell carcinomas. Nuclear expression of lamin is comparable in tumours and in their corresponding cell lines and can be used to differentiate between SCLC and non-SCLC: A-type lamins, which are present in non-SCLC, are absent in most SCLC.

Initial efforts have been made to characterize cell lines, which are derived from the main sub-types of lung cancer, i.e., small-cell lung cancer (SCLC), adenocarcinomas and squamous-cell carcinomas (Carney et al., 1985; Bepler et al., 1988). Such cell lines are now being widely applied as models for studies on lung-cancer biology and to predict the in vivo behaviour of the corresponding solid tumour in patients (Stevenson et al., 1990). In studying the biology of tumours by means of cell lines, a major question is to what extent these cultures represent the behaviour and characteristics of the original tumour. As will be outlined here, a vast volume of evidence has evolved indicating that cell lines do indeed resemble their in vivo solid-tumour counterparts to a significant extent and can therefore serve as investigational tools for studying the biology of lung cancer. Although some biomarkers are lost in cell culture, many are retained and can be used to determine the type and degree of differentiation. We shall focus on several such biomarkers, which can differentiate between the major sub-types of lung cancer and which are in part used in lung-cancer diagnosis.

Cell-surface markers

NCAM. The neural-cell adhesion molecule (NCAM) is a major cell-cell-contact protein in neuronal tissues, and belongs to the immunoglobulin superfamily. As a result of the First International Workshop on Small-Cell-Lung-Cancer Antigens (Sauhany et al., 1988), several antibodies, now known to recognize NCAM (Putel et al., 1989; Modaena et al., 1989), were grouped into cluster 1, including TFS-4, MOC-1, MOC-21, MOC-52, SL-11-14, NE-25, NCC-LU243, NCC-LU246, 123C3 and 123A8. At the Second Workshop, this group was extended to include antibodies RNL-1, MOC-191, SEN6, SEN36, and NE-150 (Beverley et al., 1991). At this Third Workshop, cluster analysis added 5 more NCAM antibodies, i.e., ITK2, MAB735, MB-2, SEN36 and SEN7 to the group of cluster-1 antibodies.

Several groups have investigated the usefulness of NCAM antibodies to distinguish between SCLC and non-SCLC (Schol et al., 1987; Berendsen et al., 1988). Their results indicate that NCAM can be found in nearly 100% of all SCLC and 20 to 30% of all non-SCLC. Meanwhile, it has become clear that these non-SCLC expressing NCAM also show other neuro-endocrine characteristics. Moreover, patients with non-SCLC expressing NCAM have significantly shorter survival than patients presenting with non-SCLC that does not express this protein (Kibbelaar et al., 1991). These findings indicate that antibodies to NCAM are useful for detecting a distinct population of non-SCLC with poor prognosis. In addition, antibodies to NCAM are very sensitive in detecting bone-marrow metastases of SCLC (Berendsen et al., 1988; Besale et al., 1992).

The NCAM protein is expressed in nearly all SCLC cell lines, while non-SCLC cell lines in general are negative, except for non-SCLC cell lines with neuro-endocrine properties (Broers et al., 1991; Carbone et al., 1991; Rygaard et al., 1992). Such correlation between neuro-endocrine phenotype and the expression of NCAM is further supported by studies showing the highest levels of expression of NCAM in the classic sub-type of SCLC, which expresses a spectrum of neuro-endocrine differentiation markers (Broers et al., 1986). Variant SCLC cell lines, which show a transition towards non-SCLC differentiation, lose expression of the NCAM protein (Broers et al., 1988a; Doyle et al., 1991). A major clue to understanding the mechanism by which this down-regulation is accomplished may come from a study by Malby et al. (1988) showing that H-ras-transfected variant SCLC cell lines lose several neuro-endocrine properties, including NCAM expression. Therefore, in vivo studies provide deeper insight into the mechanisms by which transitions from SCLC to non-SCLC take place, as also seen in vivo.

Cadherins. Cadherins, which include E-cadherin (also called uvomorulin), P-cadherin, N-cadherin (A-CAM) and L-CAM are differentially expressed during morphogenesis, and may play a role in tumour invasion and metastasis. Of these, the expression of E-cadherin, P-cadherin and to some extent N-cadherin have been examined in lung carcinomas. Shimoyama et al. (1989) found a heterogeneous staining pattern for E-cadherin in SCLC, in non-SCLC and in lymph-node metastases. The expression of P-cadherin appeared to be reduced in well-differentiated adenocarcinomas and in the highly differentiated areas of squamous-cell carcinomas of the lung, while poorly differentiated non-SCLC as well as all SCLC were strongly positive.

Rygaard et al. (1992) have shown the expression of cadherin, most likely N-cadherin, in 18 out of 19 SCLC cell lines and their xenografts. Shimoyama et al. (1992) showed that in non-SCLC a non-functional E-cadherin protein might be expressed, which could correspond with the increased metastatic potential of tumour cells upon loss of cadherin expression.

Integrins. Integrins form a family of membrane-spanning cell-surface proteins that promote cell-cell or cell-matrix

1To whom correspondence and reprint requests should be addressed. Fax: +31-43670948.
adhesion and connect the extracellular matrix to the cytoskeleton.

Adenocarcinomas, squamous-cell carcinomas and undifferentiated carcinomas of the lung appear to express at least VLA2, VLA3 and in most cases VLA6 integrins in a heterogeneous manner (Miettinen et al., 1993).

Chen et al. (1990) have demonstrated the presence of VLA2 in human non-SCLC cell lines, while Feldman et al. (1991) have described the expression of VLA3 in human SCLC cell lines. Metcalf et al. (1993) found that non-SCLC cell lines and their xenografts heterogeneously express VLA2, VLA3 and VLA6 integrins, but no clear correlation between lung-cancer sub-type and expression pattern has been observed.

**Epidermal-growth-factor receptor (EGF-R).** The epidermal-growth-factor receptor (EGF-R) is a trans-membrane glycoprotein which serves as a target both for EGF and for transforming growth factor alpha (TGF-α, for review, see Burgess, 1989). At this Workshop, 3 antibodies to EGF-R have been described and assigned to cluster 14: EMD5590, ICR12 and ICR16.

Cerny et al. (1986) observed EGF-R expression in most non-SCLC, but not in 15 cases of SCLC. Similarly, Berger et al. (1987) found EGF-R expression in 74% of squamous-cell carcinomas, but only in 34% of adenocarcinomas and in none of the SCLCs. Similar observations were made by Kaseda et al. (1989), using a radioreceptor assay on lung-cancer specimens. These studies indicate that EGF-R expression can be used to differentiate between non-SCLC and SCLC, but no significant correlation can be found between EGF-R expression and clinical or pathological characteristics (Kaseda et al., 1989).

Sherwin et al. (1981) demonstrated the expression of EGF-R in non-SCLC cell lines, while SCLC cell lines were negative. Other groups have also identified EGF-R in non-SCLC cell lines (Bergh et al., 1981; Loh et al., 1984). In agreement with these findings, Haeder et al. (1988), using a radiolabeled EGF-R-binding assay, have shown that non-SCLC cell lines express a much higher level than SCLC cell lines, which, however, are not completely negative for EGF-R. Gamou et al. (1990) found that the expression of EGF-R was induced in a variant SCLC sub-line, isolated by 5-azacytidine treatment of a classic parental line expressing no EGF-R.

**Carcino-embryonic antigen (CEA).** CEA is a trans-membrane glycoprotein (180 kDa), belonging to the immunoglobulin superfamily. It is not a tumour-specific serum marker, but CEA serum levels can be used for monitoring lung cancer, since good-in-excellent correlation exists between changes in CEA levels and the clinical course of SCLC (Goslin et al., 1981).

A marked variation in the frequency of CEA expression was reported in lung-carcinoma patients. The highest percentage of cases positive for CEA is generally found in adenocarcinomas, followed by squamous-cell carcinomas, large-cell carcinomas and SCLC (Broekein et al., 1987). Several reports indicate that antibodies to CEA can be used to discriminate between adenocarcinomas of the lung and mesotheliomas. (see for instance Hammar et al., 1985; Jacobs et al., 1988). However, when antibodies to CEA are used, the reactivity can be partially due to recognition of non-specific cross-reacting antigen (NCA) which occurs in normal lung (Kim et al., 1992).

Kim et al. (1992) observed CEA expression both in SCLC and in non-SCLC cell lines. They found a higher level of CEA mRNA and protein expression in neuro-endocrine cell lines, with SCLC cell lines, expression of CEA mRNA and protein is restricted to classic SCLC cell lines, while variant SCLC lines are negative.

**Cytoplasmic markers**

**Keratin.** Keratin filaments occur as heteropolymers, always consisting of at least one type-I keratin combined with at least one type-II keratin. To date, 20 different human keratin sub-types have been described.

All lung carcinoma types, including SCLC, express keratins (Blobel et al., 1984, 1985; Broers et al., 1985; Hammar et al., 1985). The distribution of the different keratin polypeptide heteropolymers depends on the degree and type of differentiation. By the use of a panel of polypeptide-specific keratin antibodies it has been shown that SCLC express generally only keratins 8 and 18, and occasionally keratin 19 (Blobel et al., 1985; Broers et al., 1989). In adenocarcinomas of the lung, the keratin polypeptide profile comprises keratins 7, 8, 14, and in most cases keratin 19. Some studies propose expression of keratin 7 as a specific marker for glandular differentiation in lung cancer (Van de Molengraft et al., 1993). Lung squamous-cell carcinomas express keratin 5 (Moll et al., 1989), 10, 13, 14, and 17 (Broers et al., 1986a; Wetzels et al., 1992). However, poorly differentiated squamous-cell carcinomas express additional keratins, which are also expressed in adenocarcinomas, i.e., keratins 7, 8, 18 and 19.

Most SCLC cell lines express keratins 8, 18, and 19, and occasionally low levels of keratin 7, while many variant SCLC cell lines do not express any keratins at all (Broers et al., 1986). In adenocarcinoma cell lines, keratins 7, 8, 18 and 19 can be found. Squamous-cell carcinoma cell lines express keratins comparable to those of poorly differentiated squamous-cell carcinomas (Broers et al., 1986). However, if these cell lines are allowed to differentiate, keratins 10, 13 and 14 are expressed (data not shown). Similar observations have been made in autografts of cultured rat lung carcinomas (Kal et al., 1983).

**Vimentin.** Next to keratins, vimentin is detected in several lung carcinomas (Jasani et al., 1985; Upton et al., 1986). The highest frequency of vimentin expression is found in adenocarcinomas (about 40% of all cases tested; Jasani et al., 1985) while in other lung-carcinoma types, vimentin can be detected only occasionally. However, expression of vimentin in carcinoma is always accompanied by the expression of keratins.

The expression of vimentin in lung-cancer cell lines is a general phenomenon, and to our knowledge all non-SCLC cell lines express vimentin (Broers et al., 1986). Early passages, especially of adenocarcinoma cell lines may, however, devoid of vimentin (data not shown). These findings are in line with previous observations, namely, that expression of vimentin is induced in many cell lines upon cell culturing and adherence to the culture dish (Franke et al., 1979). In SCLC cell lines, especially in classic SCLC, vimentin expression can be absent (Broers et al., 1986).

**Neurofilaments.** Lung carcinoids express neurofilaments next to keratins (Lehto et al., 1984). In some SCLC, neurofilaments have been detected (Lehto et al., 1983), however, infrequently and in only a small number of cells (Broers et al., 1987). In non-SCLC, especially in poorly differentiated squamous-cell carcinomas and adenocarcinomas, neurofilaments can occasionally be observed (Van Muijen et al., 1984; Broers et al., 1987). These tumours probably represent neuro-endocrine non-SCLC, as defined by other neuro-endocrine markers.

As with solid tumours, neurofilaments can be observed only in a limited number of SCLC cell lines, and are most pronounced in variant SCLC cell lines (Bergh et al., 1984; Broers et al., 1985a, 1986). In non-SCLC cell lines, neurofilaments were demonstrated in only one adenocarcinoma cell line (Broers et al., 1988) and in a few large-cell carcinomas (Bergh et al., 1984). If neurofilament-positive variant...
SCLC cell lines change morphologically towards the non-SCLC phenotype, the expression of neurofilaments is lost and the expression of keratins is acquired (Broers et al., 1986).

**Nuclear markers**

Lamins. In most animal species, the nuclear lamina consists of 2 types of polypeptides, the A-type and B-type lamins. A-type lamins are represented by lamins A and C, which are different transcripts arising from the same gene (Fisher et al., 1986), and B-type lamins are represented by lamins B1 and B2 in vertebrates. In contrast to B-type lamins, A-type lamins can be differentially expressed in malignant tissues.

Expression of A-type lamins can be absent or be strongly reduced in SCLC as compared with non-SCLC (Broers et al., 1993). In addition to this general reaction pattern, we frequently found cytoplasmic localization of A-type lamins in non-SCLC. Another unexpected finding was the absence of B-type lamins in a sizable population of non-SCLC, especially adenocarcinomas (Broers et al., 1993).

In SCLC cell lines, A-type lamin levels were decreased by more than 80% in small-cell lung cancer as compared with non-SCLC cell lines (Kaufmann et al., 1991). These findings have been confirmed in a large number of SCLC and non-SCLC cell lines obtained from different institutes (Broers et al., 1993).

**CONCLUSION**

Comparison of the expression of antigens in solid carcinomas of the lung with those in cell lines derived from these tumours has shown that many cellular constituents are remarkably well preserved in most lung-cancer cell lines. However, when cell lines are cultured, changes in differentiation may occur. In general, many cell lines acquire a de-differentiated appearance after prolonged culturing. However, by changing the conditions of cell culture, differentiation can be re-induced in most cases (Taylor-Papadimitriou et al., 1989). We therefore conclude that lung-cancer cell lines are reliable and suitable models for the main types of pulmonary malignancies.

**ACKNOWLEDGMENTS**

This study was supported by the Dutch Cancer Society.

**REFERENCES**


