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# Oncogene and onco-suppressor gene lesions in lung cancer

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Recent advances in molecular genetics have made it possible to understand the molecular mechanisms of human cancer progression. The results indicate that clinically evident tumor cells already carry multiple genetic alterations and further accumulation of genetic alteration occurs during tumor progression. The process of carcinogenesis can be divided into the three stages of initiation, promotion and progression based on evidence mainly from experimental models. However, such processes have been studied indirectly in humans by measurements of age-dependent cancer incidence. The development of a human cancer probably requires at least 5 to 10 different genetic alterations, each assumed to consist of the activation of an oncogene or the inactivation of a tumor-suppressor gene that are able to disrupt strictly controlled cellular processes such as differentiation and growth (1,2).

Lung cancer, although second to prostate cancer in terms of frequency, is clearly the number one cause of cancer deaths among both males and females. Prognosis is poor with fewer than 13% of all lung cancer patients surviving more than 5 years after diagnosis. Lung tumors are carcinomas originating from the respiratory epithelium and are classified on the basis of histological type. The

two main types are small cell lung cancer (SCLC) with few subtypes and non-small cell lung cancer (NSCLC) with a large number of subtypes. Small cell lung cancer (SCLC) most probably arises from pulmonary neuroendocrine (NE) cells and accounts for about 25% of all lung tumors. It is an extremely aggressive neoplasm, is frequently associated with distant metastases and has the poorest prognosis of all lung tumors. The remaining 75% are non-small cell lung cancers (NSCLC) which usually lack the neuroendocrine features that characterises the SCLC tumors and are classified according to their cell of origin. The NSCLCs include: squamous cell carcinomas which may originate from a metastatic squamous epithelium; adenocarcinomas which arise in the major bronchi; and large cell carcinomas which are predominantly undifferentiated tumors some of which may represent their basal or stem cell origin. NSCLC are treated primarily with surgery and radiotherapy while SCLC with chemotherapy (3).

The conversion of normal lung stem cells to malignant ones involves a series of changes in different set of genes (oncogenes and onco-suppressor genes). Such a series of events has been demonstrated in colorectal carcinoma by Fearon and Vogelstein (4).

Although in lung cancer the sequential events are not yet established, data from different laboratories have focused on the following chromosomes, oncogenes and onco-suppressor genes:

## 1. CHROMOSOMES

Several studies have shown alterations in chromosomes 1, 3, 6, 7, 8, 11, 13, 15, 17 and 19 by RFLP analysis. The most frequent changes are deletions in the region p21.3 of chromosome 3 in both NSCLCs and SCLCs. This region is believed to encode a yet unknown onco-suppressor gene (5,6).

## 2. ONCOGENES

2a Type I growth factor receptors. This group of molecules include three transmembrane glycoprotein (c-*erb*B-1, c-erbB-2 and c-*erb*B-3) related sequences and intrinsic tyrosine kinase activity. It has been shown that c-*erb*B-1 and c-*erb*B-2 are commonly overexpressed in NSCLCs. Overexpression of c-*erb*B-1 (EGF-r) is frequently a result of c-*erb*B-1 gene amplification (7-9).

2b *ras* gene family. Members of the *ras* family of proto-oncogenes, comprised of K-*ras*, H-*ras* and N-*ras*, are inner plasma membrane associated GTPases that bind GTP to cleave it to GDP. They are involved in signal transduction. Activated *ras* oncogenes, by point mutations in codons 12, 13 and 61 have frequently been detected in NSCLCs. There is evidence linking mutations in each member of the *ras* family with poor prognosis. K-*ras* is mutated in 30% of adenocarcinomas and there is a correlation with smoking history with about 30% of smokers compared with 2% of non-smokers having G-T

transversions at codon 12. This type of mutation is consistent with exposure of the lung to carcinogens in tobacco smoke such as benzo[a]pyrene (10,11).

2c *myc.* The *myc* gene family encodes at least three proteins c-MYC, n-MYC and l-MYC of MW 62-68 kDa. Gene amplification and overexpession of the *myc* family has been shown to be an important feature of SCLCs. It has been postulated that *myc* amplification occurs more frequently in patients who have undergone chemotherapy. Amplification of the c-*myc* gene is found in about 10% and increased expression in about 50% of NSCLCs of all types (12-14).

2d c-*raf*-1. The c-*raf* proto-oncogene located in chromosome 3p14-25 encodes a serine/ threonine specific protein kinase, p74 which is located on the internal side of the plasma membrane. Molecular analyses have shown 45% loss of heterozygocity in SCLCs (1).

2e c-*fos*, c-*jun*. c-*fos* and c-*jun* oncogenes form a complex, AP-1, which acts as a transcriptional factor. Recent reports show an increased AP-1 activity in NSCLCs. As there is evidence that AP-1 may be involved in signal transduction, elevated levels may be oncogenic.

2f c-*myb*. This proto-oncogene is located on chromosome 6q24 and encodes a nuclear transactivator. Recent studies in NSCLCs show that aberrant c-*myb* expression, either deletions or defect RNA transcription, may play a role in lung carcinogenesis (1).

## 3. ONCO-SUPPRESSOR GENES

3a p53. The p53 is located in 17q13 chromosome and encodes a 393bp protein which structurally resembles a transcriptional activator factor. p53 functions as a negative regulator of cell growth and may play an important role in genomic stability and DNA repair. Loss of wild type p53 functions either by mutation, complex formation with viral products or cellular negative regulator(s) such as the *mdm2* gene product, or alteration in subcellular localisation removes an important tumor suppressor mechanism and promotes tumorigenesis.
Mutations in p53 are the commonest genetic changes detected in several different types of cancers and are a common feature of NSCLCs. The frequency varies with the type of pulmonary cancer with about 67% of 5q CLCs and 37% of adenocarcinomas. p53 mutations carrying G:C---T:A transversions are found in about 50% of NSCLCs. This is a type of mutation that may be caused by benzo[a]pyrene a potent mutagen found in tobacco known to cause this kind of transversions. SCLCs mutations in p53 are present in over 75% of cases. p53 alterations have been observed in primary tumors as well as in metastases suggesting its early role in the

pathogenesis of SCLC (15-17).

3b RB. The Rb-1 protein product, pRB, is a DNA binding protein of 110 kDa that is thought to be related to events crucial to cell division. The cloning and characterisation of the Rb1 gene showed that the loss of the gene or its protein product by homozygous deletion or mutation was the event that resulted in development.

There is evidence that the survivors of hereditary retinoblastoma are at higher risk for developing lung tumors as adults and to develop them at an earlier age than the general population. Relatives of retinoblastoma patients who are carriers of an RB-1 mutation have a 15-fold increased risk of lung cancer the general population (18).

## CONCLUDING REMARKS

The development of a cancer cell from a normal cell involves a series of changes that contribute to a loss of normal growth control mechanisms. As far as lung cancer is concerned we are still far from understanding the nature of these events.

Furthermore, detailed studies are required in order to correlate mutations and factors such as diagnosis, clinical parameters, genetic predisposition and prognosis.

## REFERENCES

1. ANDERSON MLM AND SPANDIDOS DA. Oncogenes and onco-suppressor genes in lung cancer. Resp Med 87: 413-420, 1993.
2. SPANDIDOS DA, ANDERSON MLM. Oncogenes and onco-suppressor genes. Their involvement in cancer. J Pathol 157: 1-10, 1989.
3. MINNA JD, HIGGINS GA, GLATSTERN FJ. Cancers of the lung. In: Cancer: Principles and Practice of Oncology. De Vits VT Jr, Hellman S, Rosenberg SA (eds). Second ed. Vol. 1 Philadelphia: JB Lipponcott, pp507-597, 1985.
4. FEARON F, VOGELSTEIN B. A genetic model for colorectal tumorigenesis. Cell 61: 759-767, 1990.
5. TESTA JR, SIEGFRIED JM. Chromosome abnormalities in human non-small cell lung cancer. Cancer Res 52: 2702s-2706s, 1992.
6. KOK K, OSINGA J, CARRIT B *et al.* Deletion of a DNA sequence at the chromosomal region 3p21 in all types of lung cancer. Nature 330: 578-581, 1987.
7. CERNY T, BARNES DM, HASLETON P *et al* : Expression of epidermal growth factor receptor (EGF-R) in human lung tumors. Br J Cancer 54: 265-269, 1986.
8. VEALE D, ARHCROFT T, MARXH G, GIBSON GJ, HARRIS AL. Epidermal growth factor receptors in non-small cell lung cancer. Br J Cancer 55: 513-526, 1987.
9. KERN JA, SCHWARTZ DA, NORDERG JE *et al.* $p185^{neu}$ expression in human lung adenocarcinomas predicts shortened survival. Cancer Res 50: 5184-5191, 1990.

10. SLEBOS RJC, EVERS SG, WAGENAAR SS, RODENHUIS S. Cellular protooncogenes are infrequently amplified in untreated non-small cell lung cancer (NSCLC). Br J Cancer 59: 76-80, 1988.
11. REYNOLDS S, ANNA CK, BROWN KC *et al.* Activated oncogenes in human lung tumors from smokers. Proc Natl Acad Sci USA 88: 1085-1089, 1991.
12. KAWASHIMA K, NOMURA S, HIRAI H *et al.* Correlation of L-*myc* RFLP with metastasis, prognosis and multiple cancer in lung-cancer patients. Int J Cancer 50: 557-561, 1992.
13. WONG AJ, RUPPERT JM, EGGLETON J, HAMILTON SR, BAYLIN SB, VOGELSTEIN B. Gene amplification of c-*myc* and N-*myc* in small cell carcinoma of the lung. Science 23: 461-464, 1986.
14. JOHNSON BE, MAKUCH RW, SIMMONS AD, GAZDER AF, BURCH D, CASHELL AW. *myc* family DNA amplification in small cell lung cancer patients tumors and corresponding cell lines. Cancer Res 48: 5163-5166, 1988.
15. CHIBA I, TAKAHASHI T, NAU MM *et al.* Mutations in the p53 gene are frequent in primary resected, non-small cell lung cancer. Oncogene 5: 1603-1610, 1990.
16. MILLER CW, SIMON K, ASLO A *et al.* p53 mutations in human lung tumors. Cancer Res 52: 1695-1698, 1992.
17. TAKAHASHI T, NAU MM, CHIBA I *et al.* p53: a frequent target for genetic abnormalities in lung cancer. Science 246: 491-494, 1989.
18. LEONARD RCF, MACKAY T, BROWN A *et al.* Small cell lung cancer after retinoblastoma. Lancet 2: 1503, 1988.