Report By Martin

http://www.eruptingmind.com/


'The mechanisms by which somatic genomes are altered so resulting in cancer.'

DESCRIBE THE MECHANISMS BY WHICH SOMATIC GENOMES ARE ALTERED SO RESULTING IN CANCER. CHOOSE ONE COMMON HUMAN CANCER SUCH AS LARGE BOWEL OR LUNG CANCER TO ILLUSTRATE YOUR ANSWER.

ABSTRACT –

Cancer is a result of accumulated genetic damage which results in the alteration of our somatic genomes having adverse effects on biological processes such as the cell cycle, allowing uncontrolled cellular proliferation and the development of tumours. These tumours may become malignant at a later stage resulting in mobilisation throughout the body via a process entitled metastasis, at this point treatment becomes difficult and decreases in effectiveness with increasing time. Therefore prevention remains the best means of combating cancer, and can be achieved via an understanding of the risk factors and mechanistic principles associated with the disease.

INTRODUCTION –

Cancer is defined as any malignant tumour resulting from uncontrolled cell division, it is not caused by poor diet, lack of exercise, environmental contaminants, radiation or tobacco, but rather by a series of genetic mutations in deoxyribonucleic acid (*DNA*) which may be inherited (*germline*) or acquired during an individuals life (*somatic*). However the likelihood of these mutations occurring in sufficient number to result in cancer is greatly affected by all of the preceding factors, therefore cancer could be described as a genetic disease resulting from mutational accumulations in our genome derived from exogenous and/or endogenous factors.

WHY IS CANCER A PROBLEM?

FIGURE 1(1)

Cancer is a global pandemic that affects people of all ages, sex and race (*figure 1)*; 10 million new cases are reported annually accompanied by 6.2 million deaths[2].

If cancer is identified at an early stage the likelihood of successful treatment increases greatly, however symptoms may not appear until advanced stages (*e.g. lung cancer*) making treatment less effective and not all countries are able to implement early detection through screening procedures (*e.g. for breast cancer*); these factors among many others contribute to making cancer a leading cause of death throughout the world.

WHAT CAUSES CANCER?

There are over 200 different types of cancer which may develop in almost any tissue of the body and can be divided into three main categories; carcinomas (*from epithelial tissue*), sarcomas (*from connective tissue*) and leukaemias (*from haematopoietic tissue*)[3].

SOMATIC MUTATION -

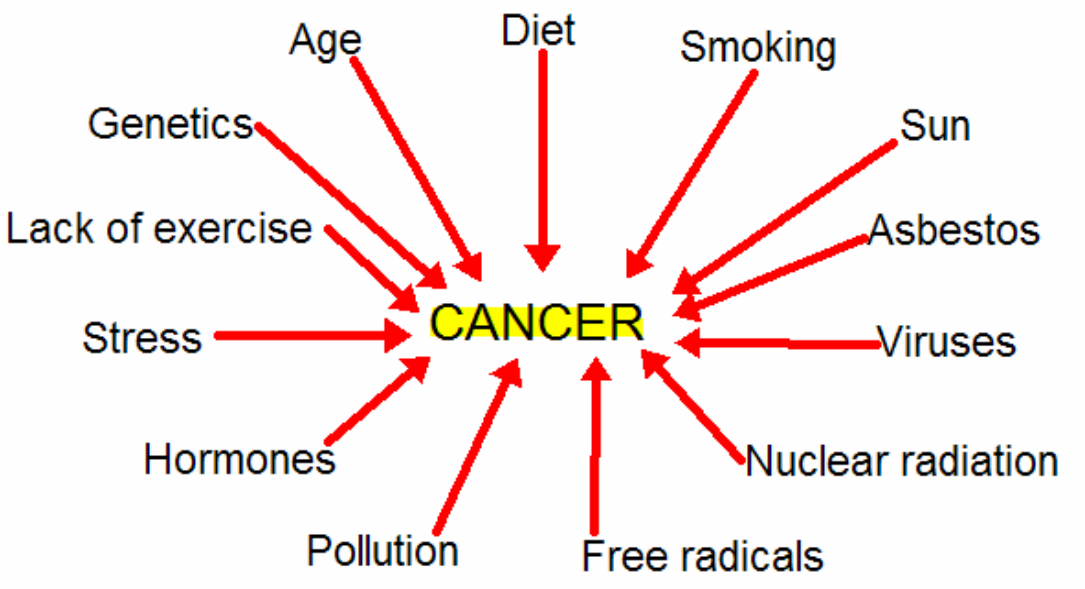
Mutations naturally occur throughout our lives and accumulate as we get older by exposure to carcinogens, replicative errors or errors during chromosomal segregation; therefore the somatic genome is dynamic and subject to change. However, the rate of these mutations typically occur at a low rate ($6x10^{-6}$ *per locus*) thereby allowing the cell to repair damage or undergo programmed cell death (*apoptosis*).

In order for a cell to undergo clonal expansion with a mutation(s) in its genome it must first evade cellular control mechanisms designed to prevent the replication of compromised genomes. Most mutations occur within inert regions of the genome thereby providing no selective advantage to the host cell, however occasionally mutations do occur in regions which provide the cell with a selective advantage over its neighbours[4].

Such mutations may increase proliferation or reduce apoptosis thereby giving the mutant cell the advantage it needs to undergo clonal expansion. Furthermore a second mutation might occur in this expanding set of clones providing an additional growth advantage thereby allowing the clone of cells to outnumber their neighbouring wild type counterparts. Ultimately the accumulation of growth promoting mutations would allow continued expansion of the clones and the development of cancer[5].

However the likelihood of one cell sustaining multiple mutations is low under normal conditions, but certain factors may increase the rate of mutation thus increasing the likelihood that these control mechanisms are evaded (*figure 2).*

FIGURE 2 – FACTORS ATTRIBUTABLE TO CANCER



PREVENTION IS BETTER THAN CURE –

Understanding the factors which may lead to an increased risk of developing cancer is essential in combating the disease as reducing exposure to risk factors can reduce the risk of developing certain types of cancer (*e.g. prolonged exposure to the sun and the risk of skin melanoma*).

FIGURE 3 (6)

However the battle against cancer is not simply a means of information warfare as many individuals may know the risk(s) but ultimately are unwilling to change their lifestyle.

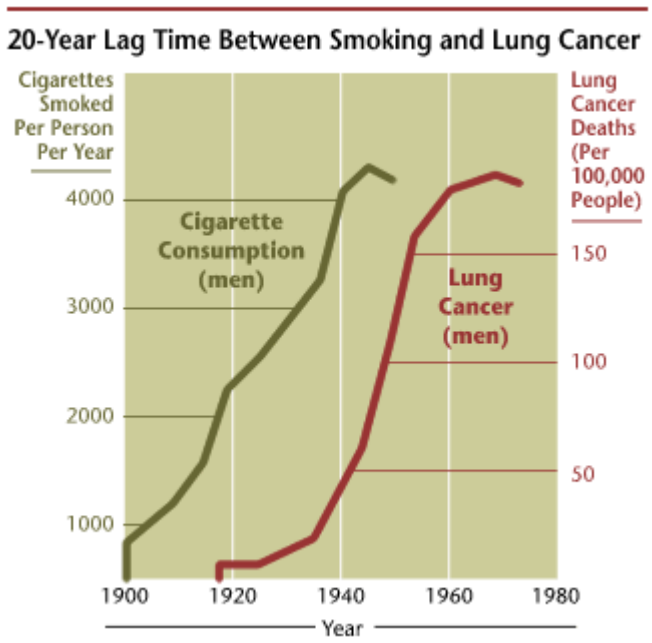
Smoking cigarettes and the risk of lung cancer exemplifies this point throughout many successive generations making this particular type of cancer not only the most deadly but frustratingly the most preventable (*figure 3).*

UK Mortality 2002: Cancers which contribute one per cent or more to total cancer mortality

Lung	33,600	(22%)
Bowel	16,220	(10%)
Breast	12,930	(8%)
Prostate	9,940	(6%)
Oesophagus	7,250	(5%)
Pancreas	6,880	(4%)
Stomach	6,360	(4%)
Bladder	4,910	(3%)
Non-Hodgkin's lymphoma	4,750	(3%)
Ovary	4,690	(3%)
Leukaemia	4,310	(3%)
Brain and CNS	3,370	(2%)
Kidney	3,360	(2%)
Head and neck	3,000	(2%)
Multiple myeloma	2,600	(2%)
Liver	2,510	(2%)
Mesothelioma	1,760	(1%)
Malignant melanoma	1,640	(1%)
Cervix	1,120	(1%)
Body of Uterus	1,070	(1%)
Other	22,910	(15%)
Persons: all malignant neoplasms	155,180	(100%)

LUNG CANCER – A PREVENTABLE DISEASE

FIGURE 4 (7)



During the nineteenth century coinciding with the increasing popularity of cigarettes, medical professionals began to notice a rise in a previously rare form of cancer which was soon attributed to the inhalation of tobacco smoke (*figure 4).*

Today we know that smoking accounts for 90% of all lung cancer cases, whereby ~20% of those who smoke will eventually develop the disease[8].

THE CIGARETTE –

Tobacco smoke not only contains over 4000 undesirable chemical compounds (*figure 5*) but is also host to 55 agents capable of causing cancer (*carcinogens*) of which 20 are related to lung cancer (*Table 1*).

FIGURE 5 - CHEMICALS FOUND IN A CIGARETTE (9)

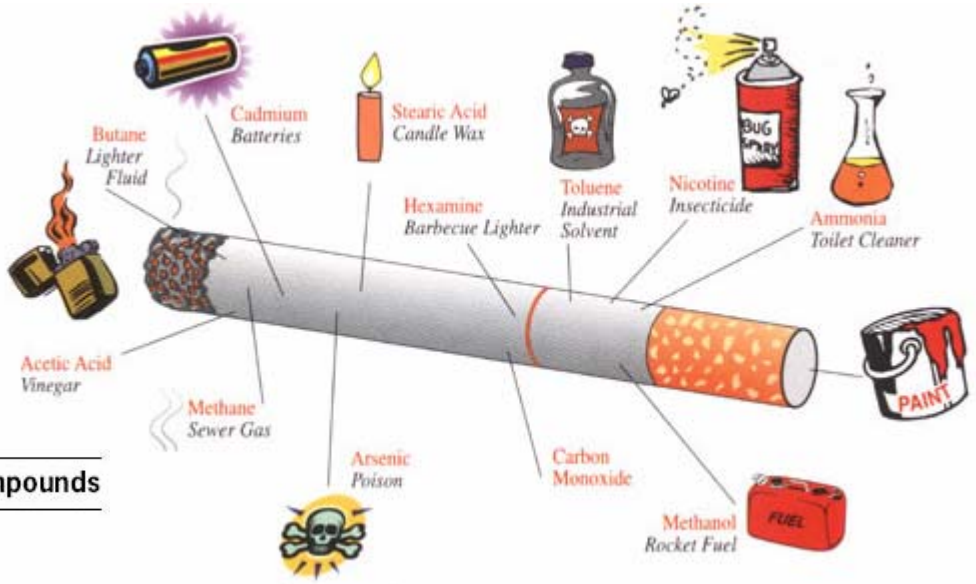


TABLE 1– CARCINOGENS IN TOBACCO(10)

Type	No. of compounds
Polycyclic aromatic hydrocarbons (PAHs)	10
Azaarenes	3
N-Nitrosamines	7
Aromatic amines	3
Heterocyclic aromatic amines	8
Aldehydes	2
Miscellaneous organic compounds	15
Inorganic compounds	7
Total	55

CARCINOGENS AND CARCINOGENESIS -

Cancer is a disease of cells and in general arises from a single somatic cell via a process entitled carcinogenesis. This multi-step process is driven by carcinogens which may either promote the process or as with the polycyclic aromatic hydrocarbons (*PAHs*) initiate it.

INITIATION –

Benzo(*a*)pyrene (*BaP*) is a member of the PAHs and is formed during the combustion of tobacco, once metabolically activated by cytochrome P450 (*figure 6*) the reactive intermediate benzo(*a*)pyrene diol epoxide (*BPDE*)[12] is able to exert its genotoxic effects via the formation of DNA adducts. Therefore the genotoxin BaP or more precisely it's activated intermediate BPDE, is said to initiate the process of carcinogenesis via the formation of DNA adducts which unless repaired lead to irreversible changes in the cellular DNA as a result of its direct interaction or binding with the DNA molecule. The significance of this event is that the first step in the multi-stage model of carcinogenesis has taken place and has thus resulted in an initiated cell, however the severity of this event will largely be determined by other factors; for now an overview is provided in figure 7.

FIGURE 6- BAP ACTIVATION (11)

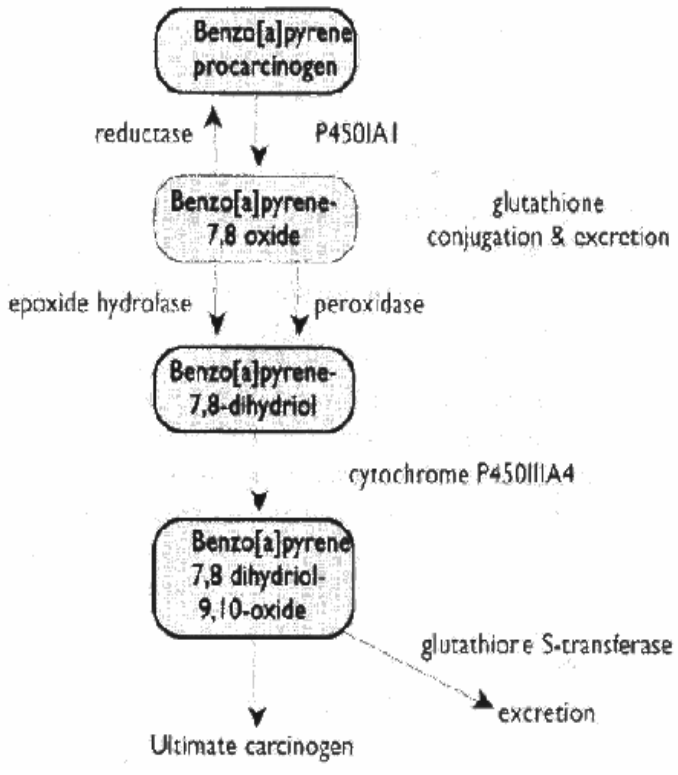
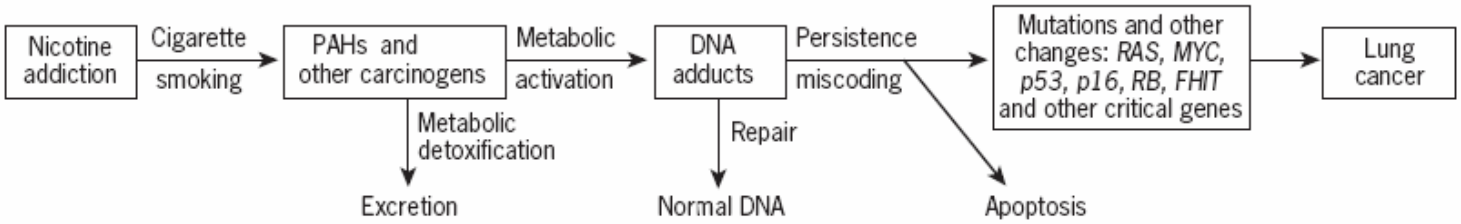


FIGURE 7 – FROM CIGARETTES TO CANCER (13)



A 'WINDOW OF OPPORTUNITY' -

As previously stated mutations occur naturally and therefore the cell is adequately equipped to deal with such events, in essence a window of opportunity exists for the cell to turn a potentially irreversible event to a reversible event before genome replication begins thus preventing initiation of carcinogenesis. This means of reversion exists in the form of DNA polymerases and repair genes which have the ability to repair errors in DNA, however should they be unable to do so apoptosis may be induced by regulators of the cell cycle.

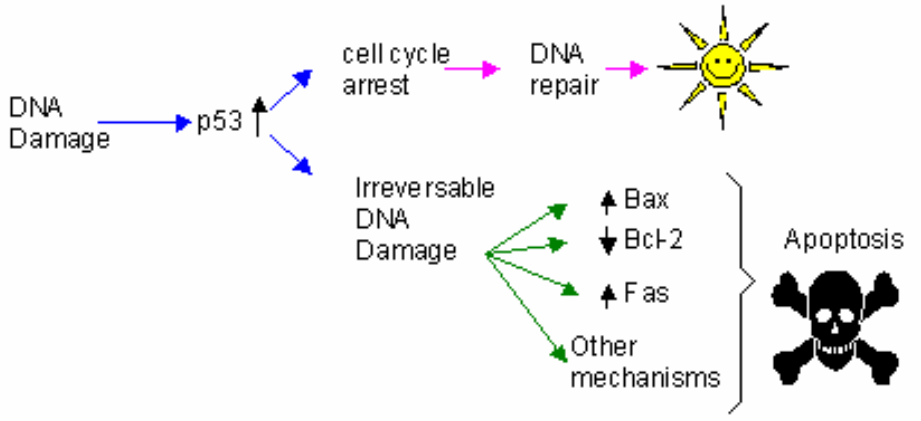
THE CELL CYCLE –

At this stage it is worth stressing the importance of the cell cycle and its associated components as whilst carcinogens may cause errors in DNA it is not until the cell undergoes a full mitotic cycle that these errors will be converted to true mutations; therefore targeting components critical to the cycle can enhance the formation of mutations.

p53-

p53 is a tumour suppressor gene (TSG) capable of arresting the cell cycle via the actions of its p53 protein and therefore provides the cell with extra time to repair damage before genome replication commences, or later induce apoptosis if the damage cannot be repaired (*figure 8*).

FIGURE 8 – p53 ACTING IN RESPONSE TO DNA DAMAGE (14)



THE MUTATION OF p53-

If BPDE induced adducts are not repaired G → T transversions may occur due to the DNA polymerase reading the adducted DNA incorrectly ultimately resulting in a mutated p53 gene; other mechanisms may also result in the mutation of p53 causing a multitude of effects (*figure 9*).

FIGURE 9 – (15)

Mechanism of inactivating p53	Typical tumours	Effect of inactivation
Amino-acid-changing mutation in the DNA-binding domain	Colon, breast, lung, bladder, brain, pancreas, stomach, oesophagus and many others	Prevents p53 from binding to specific DNA sequences and activating the adjacent genes
Deletion of the carboxy-terminal domain	Occasional tumours at many different sites	Prevents the formation of tetramers of p53
Multiplication of the MDM2 gene in the genome	Sarcomas, brain	Extra MDM2 stimulates the degradation of p53
Viral infection	Cervix, liver, lymphomas	Products of viral oncogenes bind to and inactivate p53 in the cell, in some cases stimulating p53 degradation
Deletion of the $p14^{ARF}$ gene	Breast, brain, lung and others, expecially when p53 itself is not mutated	Failure to inhibit MDM2 and keep p53 degradation under control
Mislocalization of p53 to the cytoplasm, outside the nucleus	Breast, neuroblastomas	Lack of p53 function (p53 functions only in the nucleus)

Reduced activity of p53 will occur if one allele is lost through mutation(s) (*loss of heterozygosity (LOH)*) and complete inactivation if the other allele is also lost through chromosomal deletions; thereby making inactivation recessive. This may be contrasted to oncogene activation which requires mutations in only one allele and is therefore a dominant process[16].

p53 AND CELL CYCLE CHECKPOINTS –

Ultimately a mutated p53 protein will be unable to activate p21 as it can no longer bind to the p21 promoter (*figure 10*) resulting in a bypass of first the G_1/S checkpoint (*thus allowing replication of damaged DNA*) and later the G_2/M transition (*thus allowing entry into mitosis with damaged DNA and ultimately chromosomal segregation to each daughter cell) (figure 10a).*

FIGURE 10A – THE CELL CYCLE (18)

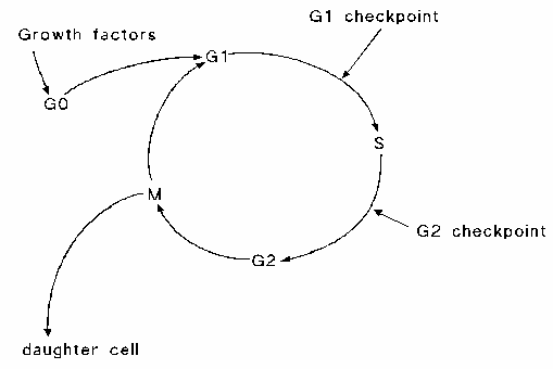
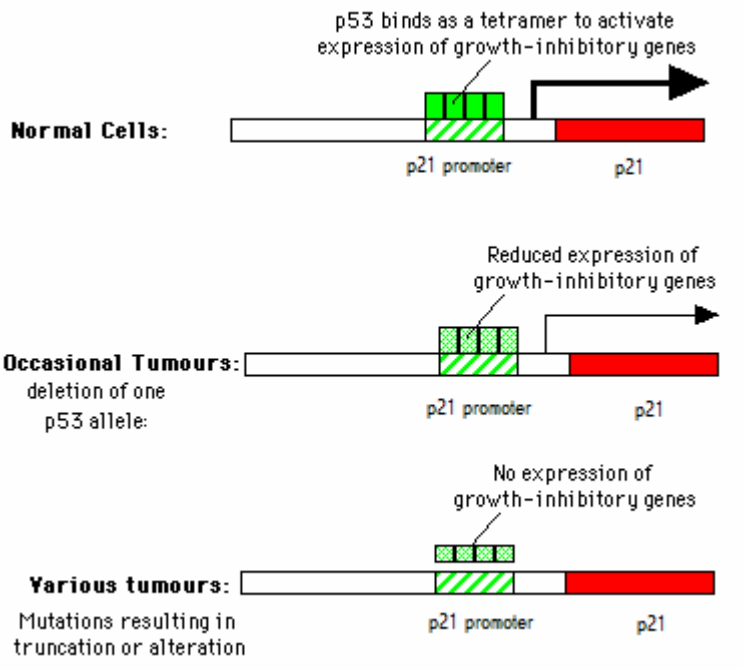
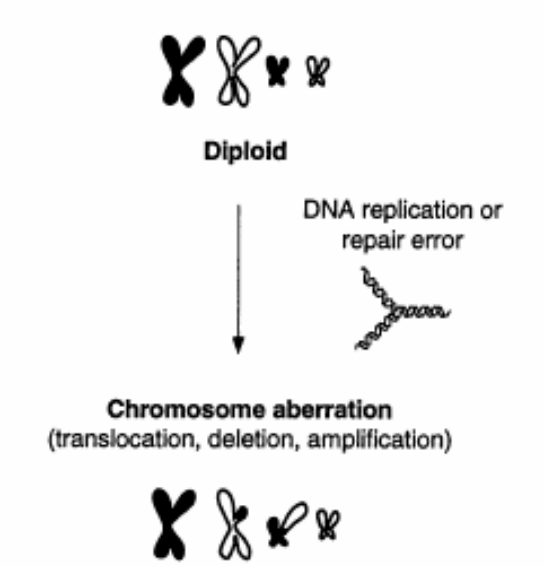


FIGURE 10- p53 AND p21 (17)



Therefore by mutating a key regulator of the cell cycle (*p53*) a mutated genome is allowed to replicate and propagate by bypassing two checkpoints of the cell cycle which would otherwise have prevented this event from occurring in an otherwise healthy genome. The wider implication of this stems from the fact that the cell cycle checkpoints are designed detect DNA damage and prevent its replication as errors in DNA replication may lead to chromosomes loosing their defined appearance (*aberrations*) (*figure 11*). However these changes are not only cosmetic but can have genome wide implications such as when deletions occur in regions containing TSGs resulting in inactivation by their deletion from the nucleus.

FIGURE 11 - CHROMOSOMAL ABERRATIONS (19)



Chromosomal aberrations may also affect other genes involved in regulating the cell cycle thus further altering the somatic genome (*table 2*).

TABLE 2 – MUTATIONS CAN AFFECT MANY GENES (20)

GENE	SITE OF MUTATION	TYPE OF MUTATION	FUNCTION
MYC	Chromosome 8	Translocation (8:14, 8:2, 8:22) / amplification	Oncogene transcription factor
p16	Chromosome 9 (short arm), 9p21	LOH / deletions (<500 kb)	Tumour suppressor gene
k-Ras	Chromosome 12 (short arm), 12p	Point mutations	Oncogene p21. GTPase
Retinoblastoma *(Rb)*	Chromosome 13 (long arm), 13q	Point mutations	Tumour suppressor gene
Bcl-2	Chromosome 14-18 translocation	Chromosomal translocation	Oncogene, inhibits apoptosis
p53	Chromosome 17 (short arm), 17p13	Deletion / LOH	Tumour suppressor gene

APOPTOSIS -

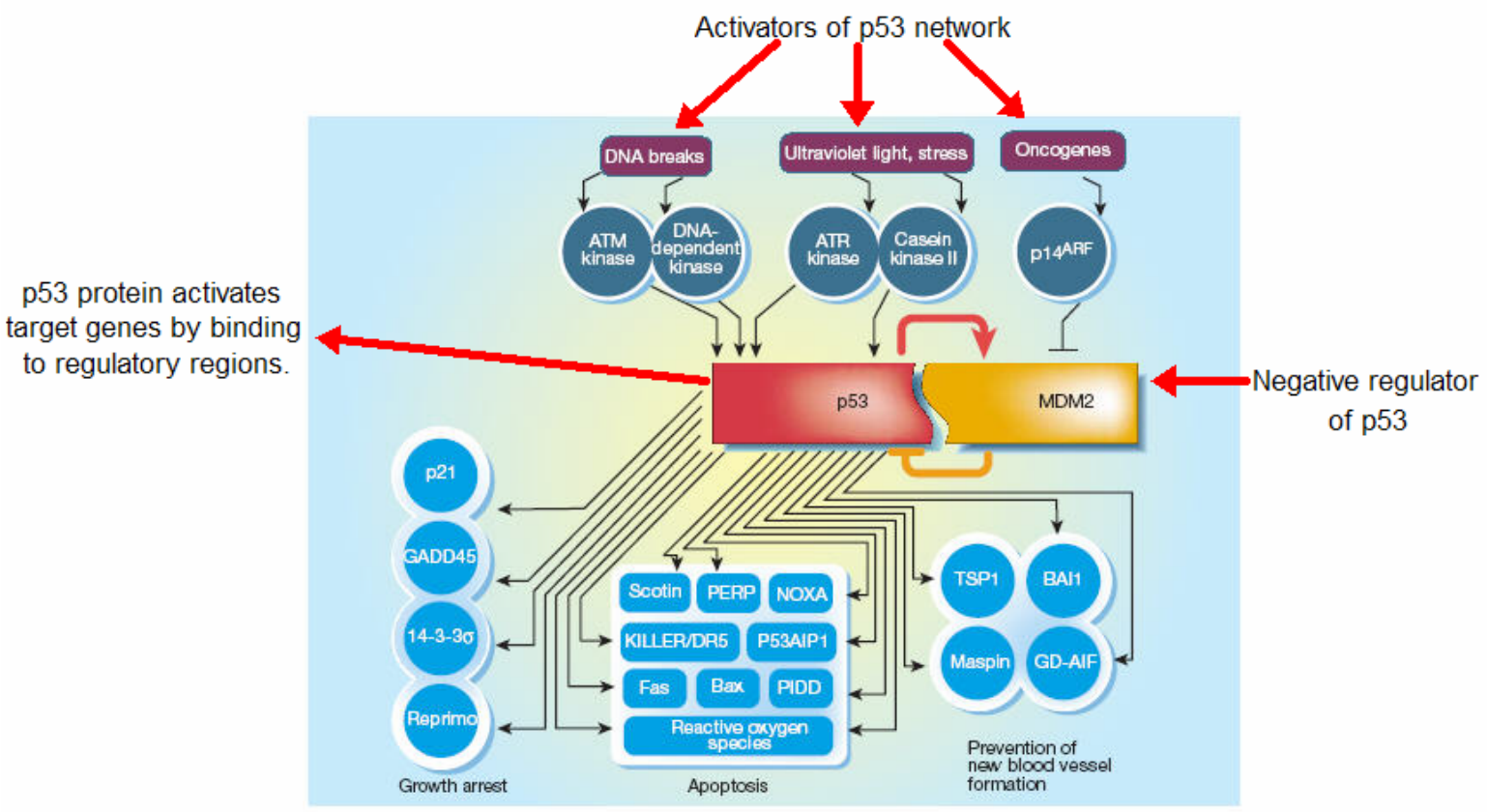
Mutations in the p53 gene may also reduce or remove the effectiveness of p53 to activate transcription of the *bax* gene, therefore generating genomic instability due to the loss of another control mechanism (*apoptosis*) designed to control cell numbers and prevent replication of mutant cells.

GENOMIC STABILITY –

p53 is also responsible for activation of the growth arrest and DNA damage gene (*Gadd 45*), this repair gene is involved in directing DNA nucleotide excision repair; a cellular repair mechanism. Therefore by mutating the p53 gene genomic instability occurs as an indirect mechanism of the inactivation of these repair genes, this ultimately allows an accumulation of errors in all genes on a genomic wide scale.

Figure 12 displays a summary of some components involved in the p53 network.

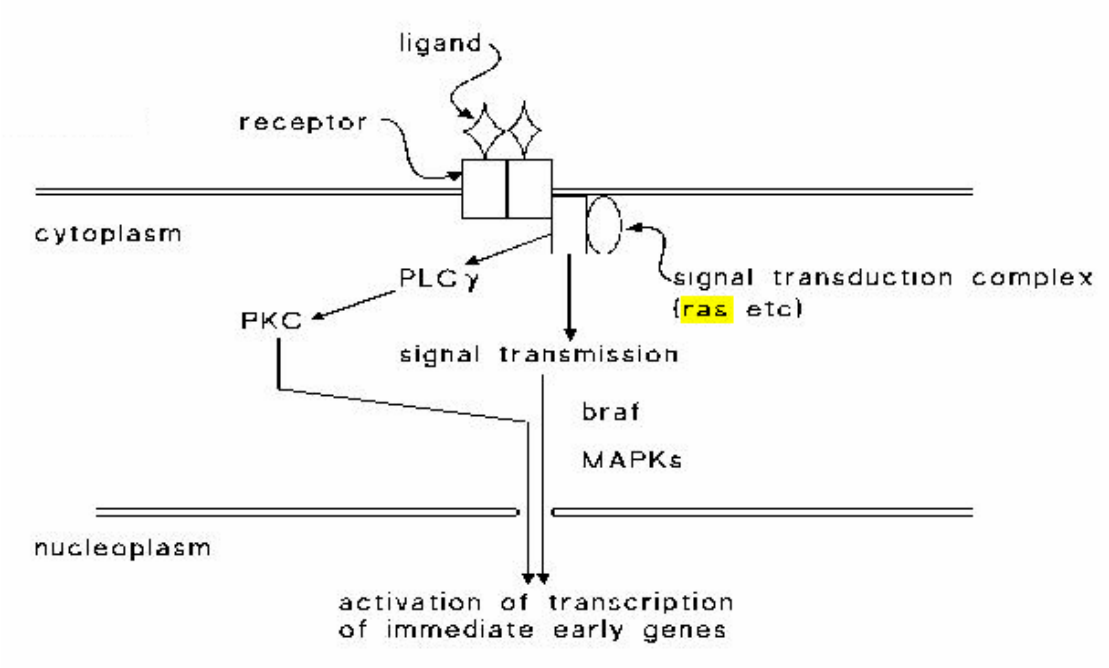
FIGURE 12 – THE p53 NETWORK (21)



ONCOGENES –

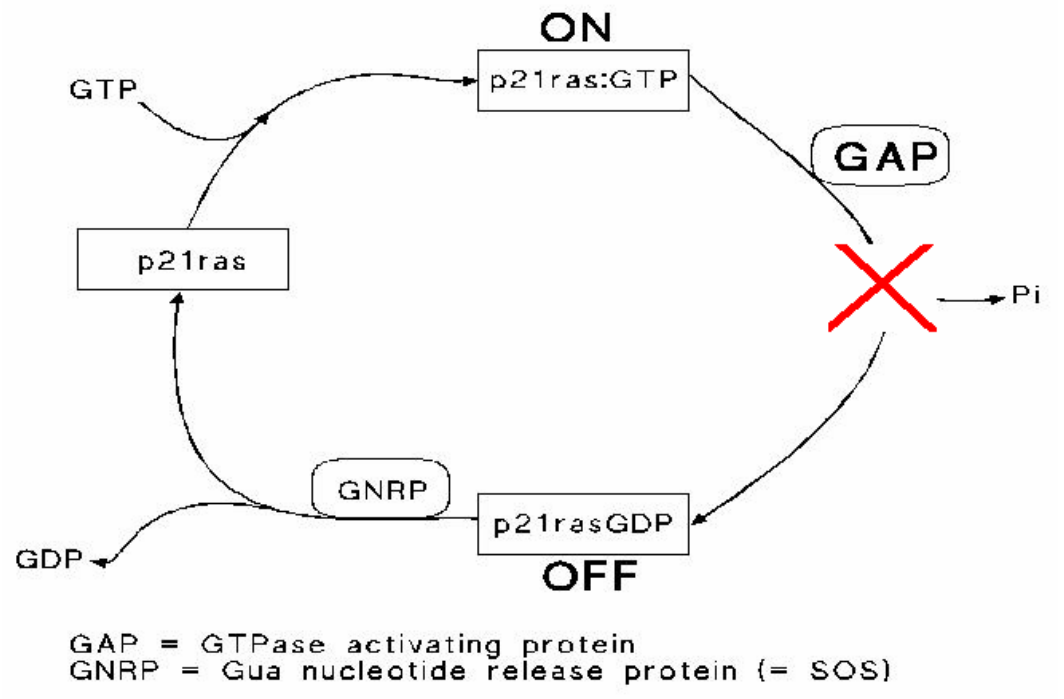
Proto-oncogenes are normal cellular genes whose protein products such as ras are involved in the growth factor signalling pathway, functioning as signal transduction complexes by assisting in signal transmission across the membrane once activated by the binding of growth factors to the transmembrane receptor (*figure 13).*

FIGURE 13 – RAS AND THE GROWTH FACTOR SIGNALLING PATHWAY (22)



However should ras become mutated (*e.g. via a point mutation)* to the oncogene k-ras its GTPase activity becomes compromised resulting in the continual transmission of the growth signal as the protein is unable to release GTP, leaving the molecular switch permanently on and thus driving cellular proliferation (*figure 14).*

FIGURE 14 – ONCOGENES CAN INCREASE CELLULAR PROLIFERATION (23)



TELOMERASE –

Another factor which may influence cellular proliferation is the presence of telomerase in cancer cells which helps maintain telomere integrity after cell division, most normal cells by comparison do not possess this enzyme and so have a reduced lifespan. The significance of this is that cancer cells have an increased life in which they may accumulate mutations whilst concurrently implementing a blockade on senescence (*a control of maximum proliferation*)[24].

Events discussed so far have provided a mutated genome with a selective advantage via the acquisition of growth promoting mutations acquired by simple point mutations through to chromosomal translocations. Tumour initiation has been successful and exerted irreversible changes in the cellular DNA thus altering gene expression, the initiated cell is now ready to progress to the next round of selection, promotion.

PROMOTION –

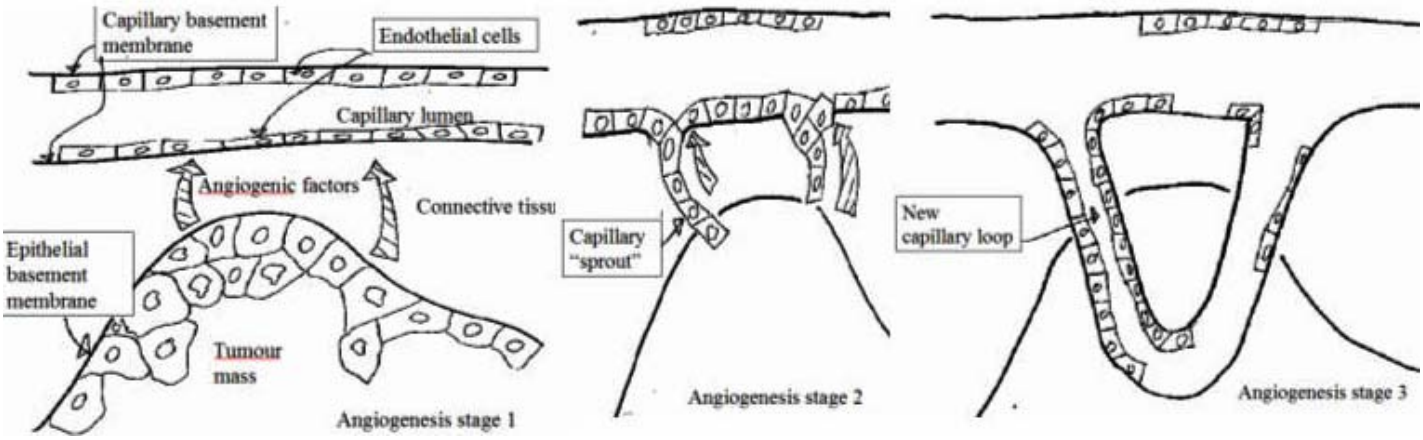
Tumour promotion is a gradual and reversible process requiring prolonged exposure to agents which promote clonal expansion of the initiated cell and may include carcinogens, co-carcinogens (*e.g. benzo(e)pyrene, which act synergistically with carcinogens*) and toxic substances.

Irritants such as the phorbol esters found in tobacco smoke do not cause cancer directly and are therefore not mutagenic, however they do result in increased cellular proliferation (*by activating protein kinase C in the growth factor signalling pathway)* and are thus said to be mitogenic, promoting carcinogenesis.

These external growth factors which drive cellular proliferation are significant as they help contribute to the establishment of genetic mutations by not only increasing cellular numbers (*therefore providing an expanded target population in which mutations may occur)* but also by decreasing the amount of time available for DNA repair processes to function, resulting in a decrease of repair fidelity. Therefore whilst carcinogens are responsible for initiating genetic changes necessary for carcinogenesis, promoters serve to amplify these changes[25].

However in order for a set of expanding clones to proliferate beyond the size of 1mm and form a benign tumour, an exclusive supply of blood is required. This is achieved primarily by the secretion of vascular endothelial growth factor (VEGF) by the tumour cells resulting in the development of new blood vessels from pre-existing capillaries and thereby fulfilling the requirements necessary for increased growth (*figure 15*).

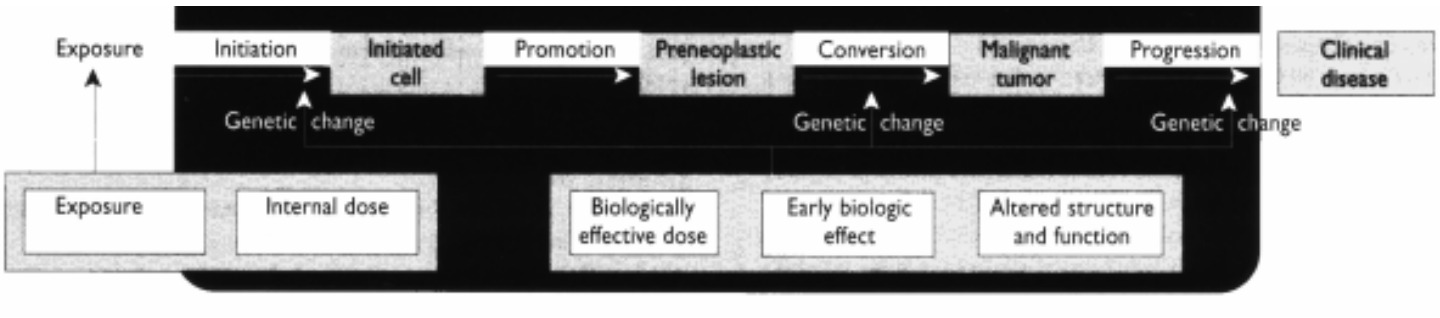
FIGURE 15 – TUMOUR ANGIOGENESIS (26)



PROGRESSION –

Progression is the last stage of the multi-step model of carcinogenesis (*figure 16*) whereby the benign tumour that arose during the previous stage begins to accumulate further genetic damage, changing it from a state of dormancy to one of malignancy whereby spread throughout the body occurs via metastasis.

FIGURE 16– THE MULTI-STEP MODEL OF CARCINOGENESIS (27)

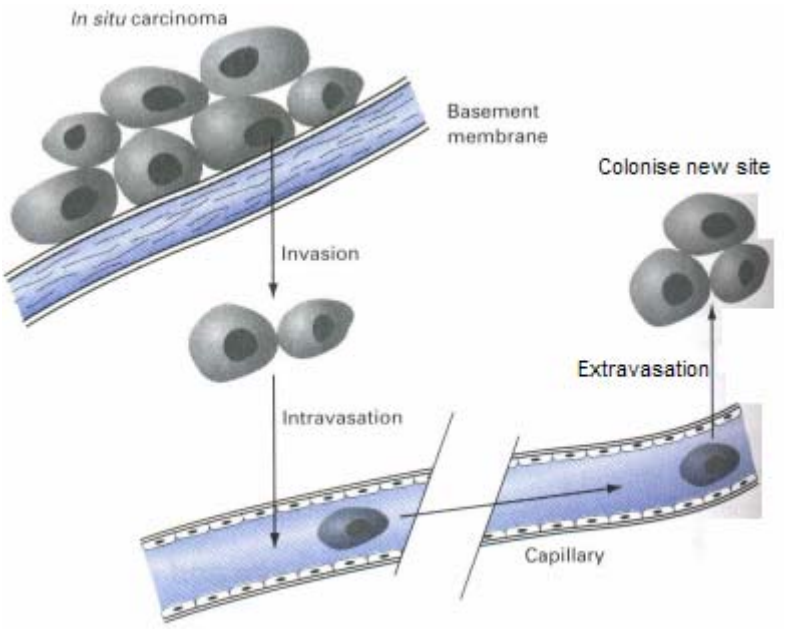


METASTASIS –

Metastasis allows a previously static tumour (*benign)* to mobilise and spread via the circulatory or lymphatic systems, however in order to do so it must first break away from neighbouring cells via a process of detachment. This may occur by mutations in the extracellular domain of the cell adhesion molecule (*CAM*) E-cadherin, essentially causing a loss of attachment between the tumour and its neighbours.

Furthermore as in angiogenesis, the extracellular matrix (*ECM*) and basement membrane (*BM*) are digested via the secretion of plasminogen activators and matrix metalloproteases thereby facilitating invasion through the stroma and intravasation into the blood capillary thus allowing escape from one location and migration to another (*figure 17).*

FIGURE 17 – INTRAVASATION (28)



Intravasation into the blood stream does not guarantee the cancerous cell(s) a safe voyage as it will be subject to cells of the immune system (*e.g. lymphocytes*) which have the potential to destroy it; those which survive will leave the blood vessel by extravasation (*figure 18*).

FIGURE 18- EXTRAVASATION (29)

Following extravasation proteases are again secreted to digest the ECM, afterwards migration occurs via attachment of the leading edge of the cell to matrix proteins and detachment at the rear edge. Movement is ultimately achieved by the interaction of intergrins with the external matrix resulting in contraction and relaxation of the internal actin cytoskeleton.

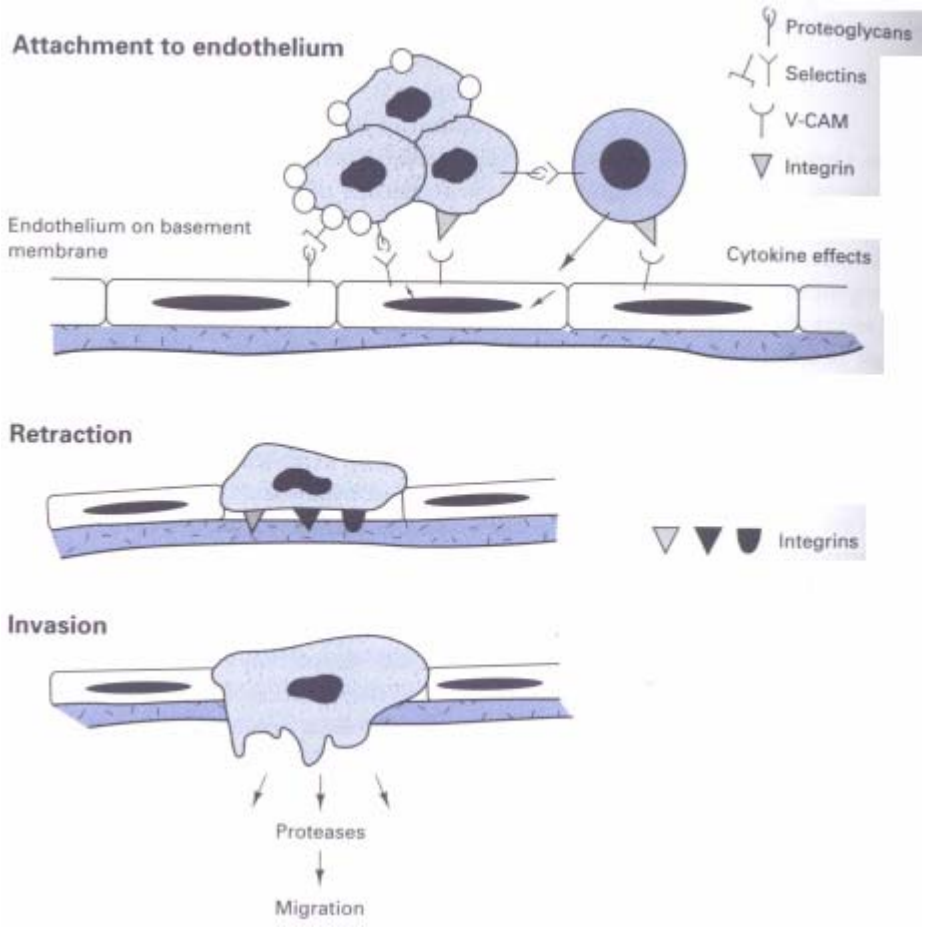
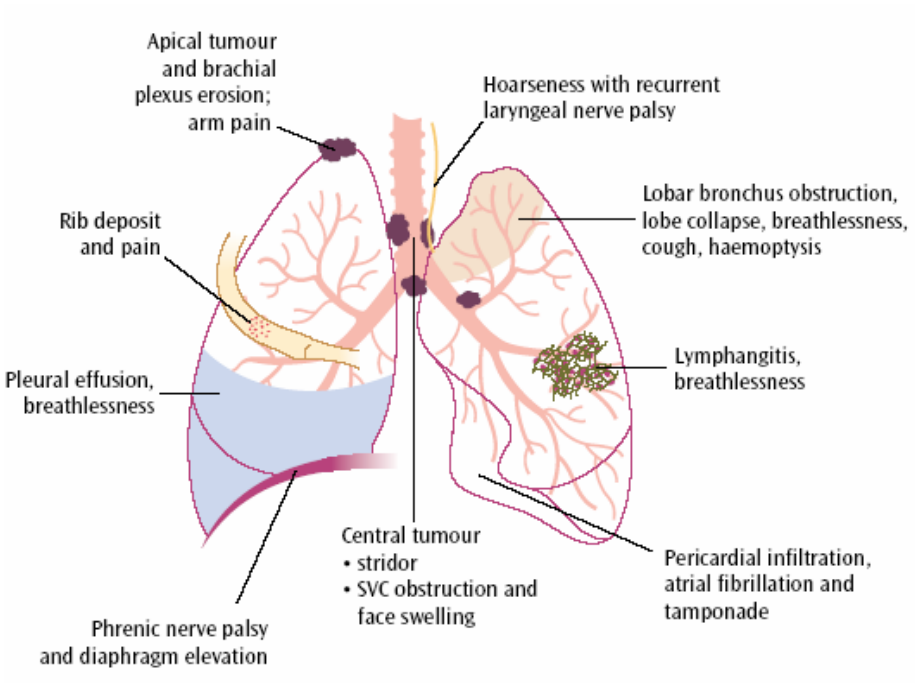


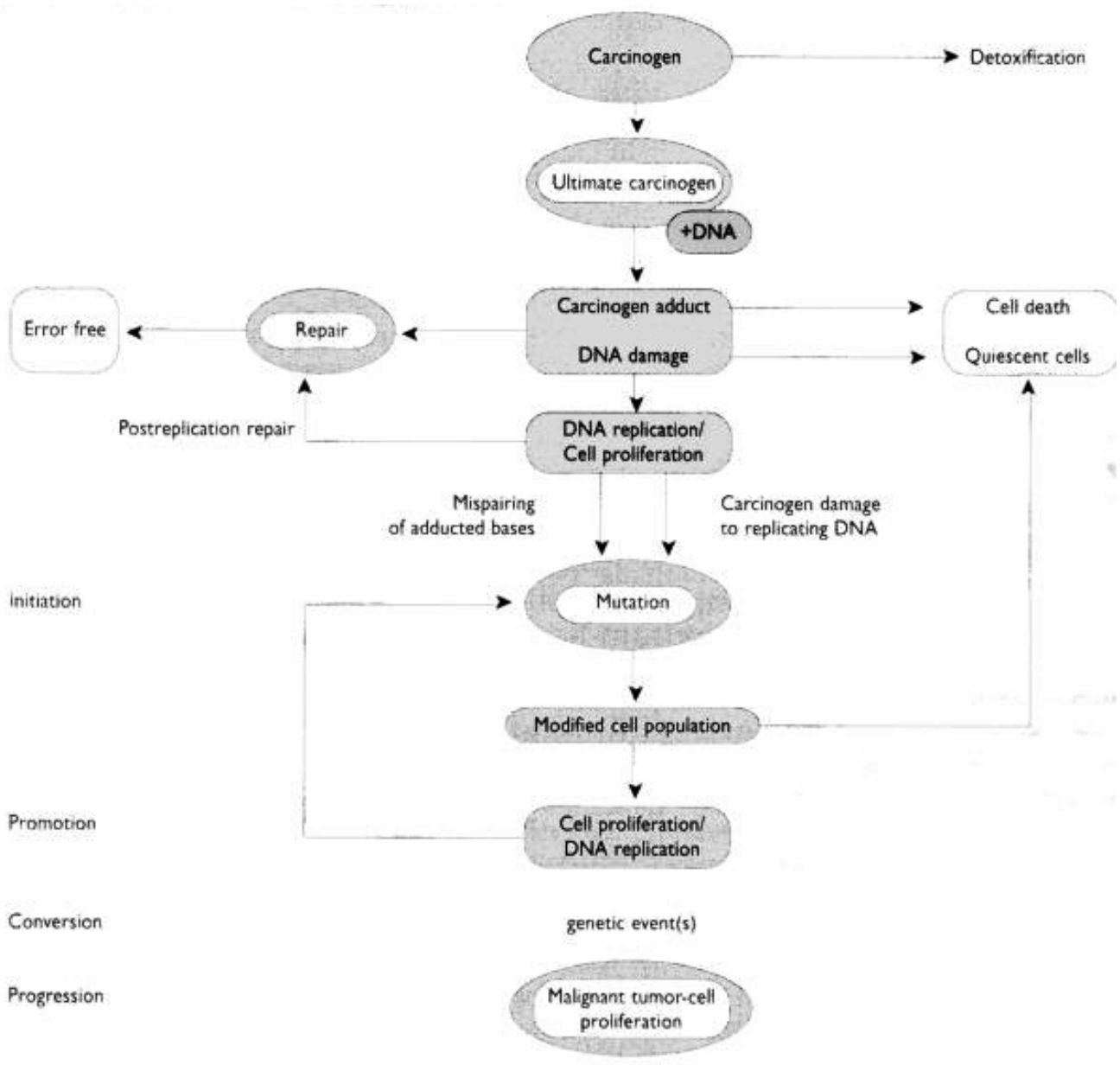
FIGURE 19 – SECONDARY TUMOURS (30)



The successful migration to a new location provides the opportunity for the generation of another tumour (*secondary tumour*) derived from the primary tumour (*figure 19)*.

This process may occur multiple times leading to lung cancer and thereby completing the multi-step model of carcinogenesis from carcinogen to cancer (*figure 20)*.

FIGURE 20 – THE MULTI-STEP MODEL OF CARCINOGENESIS (31)



LUNG CANCER –

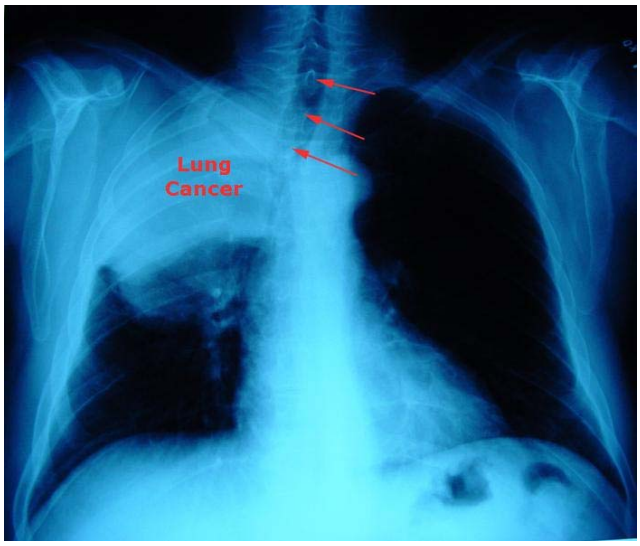
Lung cancer may take many years to develop before the individual begins to experience a reduced quality of life and experience signs and symptoms indicative of the disease (*table 3*).

TABLE 3 –SYMPTOMS ASSOCIATED WITH LUNG CANCER (32)

- a nagging, persistent cough
- wheezing and shortness of breath
- recurrent chest infections such as pneumonia and bronchitis
- blood in the sputum (phlegm)
- chest, shoulder or back pain unrelated to pain from coughing
- neck and facial swelling
- hoarseness (a 'husky' voice)
- unexplained weight loss
- loss of appetite
- unsteady walk and occasional memory lapses
- bone pain or fracture not caused by injury.

SCREENING, TESTING, CLASSIFICATION-

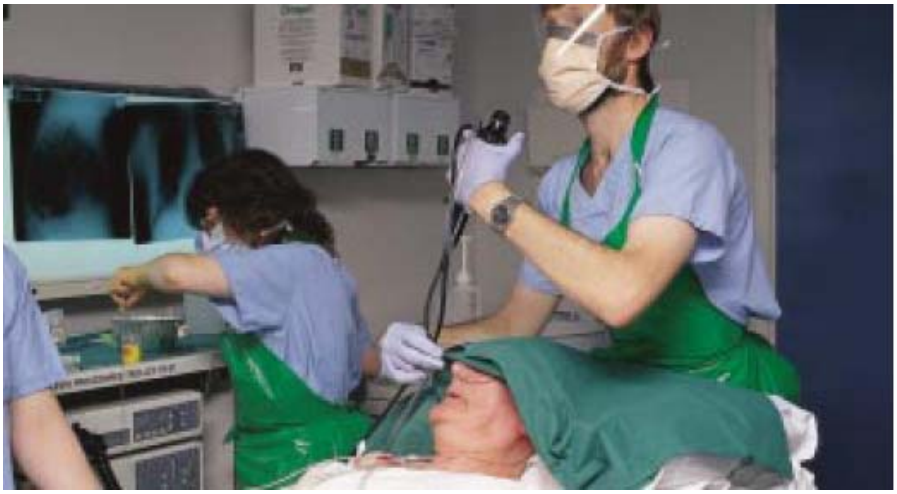
FIGURE 21 – CHEST X-RAY (33)

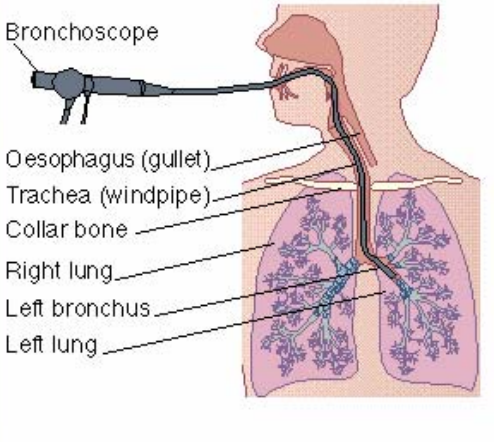


However these symptoms may not always be due to lung cancer and therefore a means of screening is necessary in order to provide a correct diagnosis and rule out other possible causes such as pneumonia; this usually occurs via a chest x-ray (*figure 21)*.

An x-ray provides a quick means of determining whether an individual has lung cancer (*although at this stage any indication of lung cancer will likely be in its advanced stages)* as shown by clouding over the lung region(s) and may indicate metastasis or lymphatic spread within the lungs. Should the x-ray indicate lung cancer is present procedures such as a bronchoscopy (*figure 22*) are then preformed on the patient.

FIGURE 22– BRONCHOSCOPY (34)

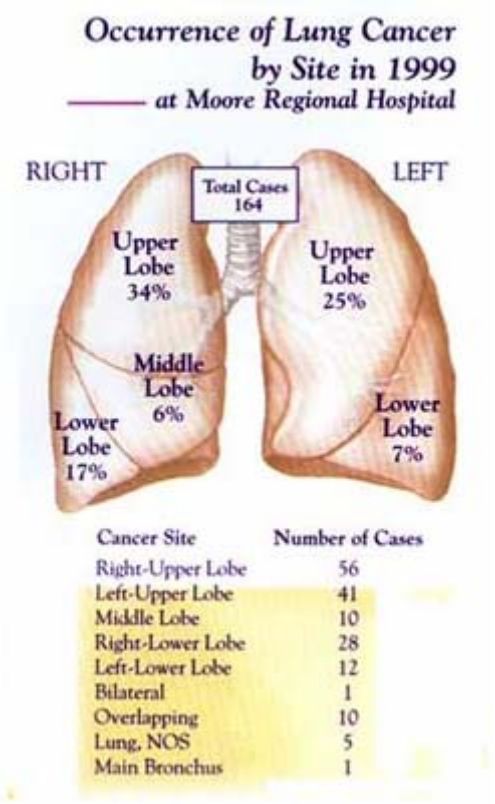




A bronchoscopy allows medical professionals to see directly into the patient's throat and lungs by use of a flexible tube with an attached camera. This is useful for performing detailed examinations of localised regions whilst concurrently allowing tissue samples to be taken[35].

At this stage classification and location (*figure 23*) of the cancer may occur with small cell lung cancers (SCLC) generally accounting for 20% of cases and non-small cell lung cancers (NSCLC) accounting for the rest; a patients chance of survival is now less than 10% and treatment if possible may begin.

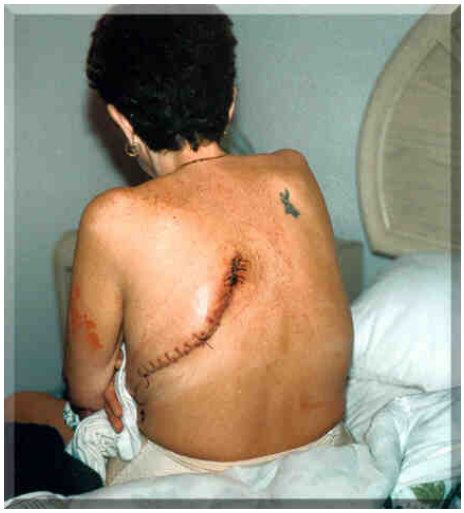
FIGURE 23 (36)



TREATMENT –

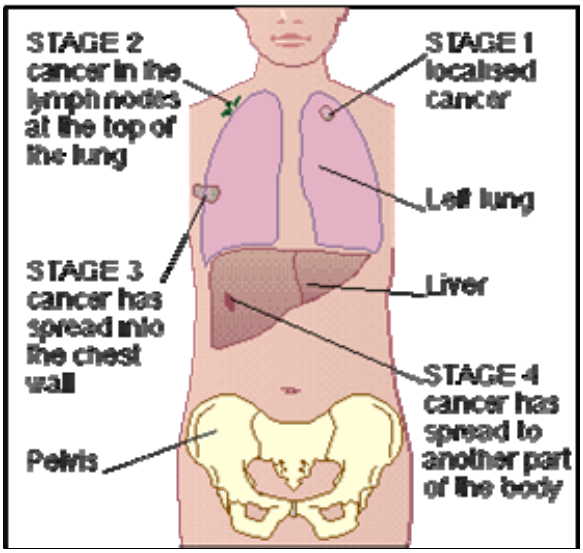
Depending on the type of lung cancer diagnosed various treatments are possible; for SCLC chemotherapy is most commonly used and if possible surgery may be preformed for those with NSCLC whereby either a small section, a lob or entire lung (*figure 24*) are removed providing an effective treatment during early stages of the disease.

FIGURE 24 – POST OPERATION (LUNG REMOVED) (37)



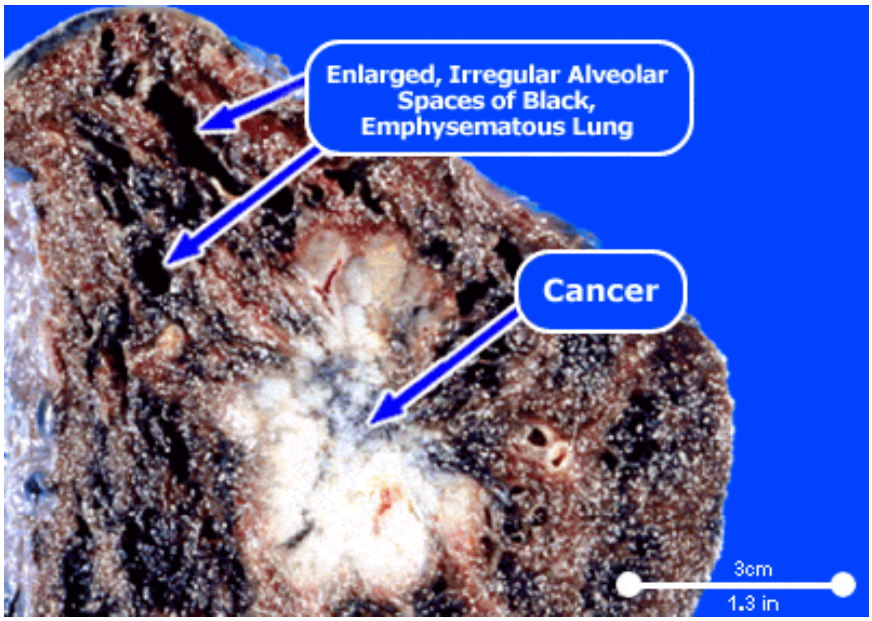
Following surgery the likelihood a patient will survive past 5 years is ~50% but only if this treatment occurred during stage 1 of the disease (*figure 25*) and surgery was a viable option.

FIGURE 25 – STAGES OF LUNG CANCER (38)



Ultimately death of lung cancer patients occur due to respiratory collapse and associated organ failure if treatment(s) have been unsuccessful (*figure 26*).

FIGURE 26 – LUNG CANCER (39)



CONCLUSION –

Cancer is an extremely complex yet interesting area of research as it demonstrates just how resilient our biological systems are and the numerous mechanisms which must be overcome before we ourselves succumb to disease. Even in response to continuous daily abuse such as smoking cigarettes some individuals may experience no symptoms of disease and live to the age of 100, whilst others may die prematurely. This goes to show that whilst carcinogens can cause cancer ultimately it is our genetic makeup which decides our molecular fate, however until the secrets of the human genome are unravelled we do not know the resilience of our own individual genomes when encountering these adversities. Therefore it is best to take precautionary measures and avoid known risk factors which we know can cause cancer.

The multi-step model discussed in this report conveniently breaks down the mechanisms of carcinogenesis into a series of flowing stages, however in reality the mechanistic principles involved may not occur in such fashion. For example promotion is assumed to be non-mutational, yet mutational changes may lead to promotional behaviour when deregulating growth control mechanisms. Therefore rather than looking at the model as a true portrayal of cancer, it is more useful to view it as exemplifying biological processes targeted during cancer.

In conclusion cancer is a genetic disease resulting from an accumulated corruption of our genetic code, ultimately distorting the blueprint from which we are created. Whether there is a threshold for a tolerable level of corruption remains to be seen, and if so why does this threshold appear to vary between individuals? Could it be that those who develop cancer are simply unfortunate in the sense that mutations have occurred in essential coding regions within their genome? Is this why we have so much ‘junk DNA’ to safely harbour these accumulated mutations? Regardless, one fact remains, cancer kills, as long as it continues to do so we must put up a fight and the secrets of the human genome may be our best means of doing so, but as always only time will tell…

REFERENCES

Title page – http://spore.swmed.edu/cancer_information.htm

1) http://www.who.int/cancer/en/

2) http://www.who.int/mediacentre/news/releases/2003/pr27/en/

3) Dr. A. G. Morris, Oncology lecture handout

4) G. P. Holmquist, S. Gao, *Somatic mutation theory, DNA repair rates, and the molecular epidemiology of p53 mutations,* Reviews In Mutational Research, 1997, 386: 69-101

5) M. Roland, R. M. Rudd, *Somatic mutations in the development of lung cancer,* Thorax, 1998, 53: 979-983

6) http://www.cancerresearchuk.org/aboutcancer/statistics/incidence

7) http://www.health-care-technology-llc.com/frbsmokecancerno.htm

8) http://www.cancerresearchuk.org/aboutcancer/statistics/

9) http://www.tuberose.com/Cigarette_Smoking.html

10) http://www.mrw.interscience.wiley.com/chb/articles/chap29/frame.html (cancer handbook)

11) S. S. Wang, J. M. Samet, *Tobacco smoking and cancer: The promise of molecular epidemiology,* Salud Publica De Mexico, 1997, 39: 331-345

12) S. S. Hecht, *Metabolically activated carcinogens and mutations in the p53 tumour suppressor gene in lung cancer,* Journal Of The National Cancer Institute, 2000, 92: 782-783

13) http://www.mrw.interscience.wiley.com/chb/articles/chap29/frame.html (cancer handbook)

14) http://www.portfolio.mvm.ed.ac.uk/studentwebs/session2/group28/p53.html

15) B. Vogeistein, D. Lane, A. J. Levine, *Surfing the p53 network,* Science, 2000, 408: 307-310

16) S. N. Rodin, A. S. Rodin, *Human lung cancer and p53: The interplay between mutagenesis and selection,* Proceedings Of The National Academy Of Sciences Of The United States Of America, 2000, 97: 12244-12249

17) http://www-micro.msb.le.ac.uk/3035/Trans4.html

18) Dr. A. G. Morris, Oncology lecture handout

19) L. H. Hartwell, M. B. Kastan, *Cell cycle control and cancer,* Science, 1994, 266: 1821-1828

20) M. Roland, R. M. Rudd, *Somatic mutations in the development of lung cancer,* Thorax, 1998, 53: 979-983

21) B. Vogeistein, D. Lane, A. J. Levine, *Surfing the p53 network,* Science, 2000, 408: 307-310

22) Dr. A. G. Morris, Oncology lecture handout

23) Dr. A. G. Morris, Oncology lecture handout

24) K. H. Vähäkangas, W. P. Bennett, K. Castrén, J. A. Welsh, M. A. Khan, B. Blömeke, M. C. R. Alavanja, C. C. Harris, *p53 and K-ras Mutations in Lung Cancers from Former and Never-Smoking Women,* American Association For Cancer Research, 2001, 61: 4350-4356

25) S. S. Wang, J. M. Samet, *Tobacco smoking and cancer: The promise of molecular epidemiology,* Salud Publica De Mexico, 1997, 39: 331-345

26) Dr. A. G. Morris, Oncology lecture handout

27) S. S. Wang, J. M. Samet, *Tobacco smoking and cancer: The promise of molecular epidemiology,* Salud Publica De Mexico, 1997, 39: 331-345

28) Roger J. B. King, *Cancer Biology 2nd edt*, Pearson Prentice Hall, 2000

29) Roger J. B. King, *Cancer Biology 2nd edt*, Pearson Prentice Hall, 2000

30) http://www.fleshandbones.com/readingroom/pdf/266.pdf

31) S. S. Wang, J. M. Samet, *Tobacco smoking and cancer: The promise of molecular epidemiology,* Salud Publica De Mexico, 1997, 39: 331-345

32) http://www.cancerresearchuk.org/aboutcancer/specificcancers/lungcancer

33) http://www.tobacco-facts.info/

34) http://www.fleshandbones.com/readingroom/pdf/266.pdf

35) S Lam, T Kennedy, M Unger, YE Miller, D Gelmont, V Rusch, B Gipe, D Howard, JC LeRiche, A Coldman, AF Gazdar, *Localization of bronchial intraepithelial neoplastic lesions by fluorescence bronchoscopy,* The Cardiopulmonary And Critical Care Journal, 1998, 113: 696-702

36)http://www.firsthealth.org/services/oncology/annual_report_2000/majorsitereport.htm

37) http://www.whyquit.com/whyquit/A_Kim.html

38) http://www.cancerhelp.org.uk/help/default.asp?page=2968

39) http://www.medicinenet.com/smokers_lung_pathology_photo_essay/article.htm

D. M. Parkin, *The global burden of cancer,* Seminars In Cancer Biology, 1998, 8: 219-235

http://www.healthandage.com/html/well_connected/pdf/doc72.pdf

C. Brambilla, F. Fievet, M. Jeanmart, F. de Fraipont, S. Lantuejoul, V. Frappat, G. Ferretti, P.Y. Brichon and D. Moro-Sibilot, *Early detection of lung cancer: role of biomarkers*, European Respiratory Journal, 2003, 21: 26-44

K. L. Braithwaite, P. H. Rabbitts, *Multi-step evolution of lung cancer,* Seminars In Cancer Biology, 1999, 9: 255-265

J.K. Field, J.H. Youngson, *The Liverpool lung cancer project: a molecular epidemiological study of early lung cancer detection,* European Respiratory Journal, 2002, 20: 464-479

G. Bepler, A. Gautam, L. M. McIntyre, A. F. Beck, D. S. Chervinsky, Y. C. Kim, D. M. Pitterle, A. Hyland, *Prognostic Significance of Molecular Genetic Aberrations on Chromosome Segment 11p15.5 in Non–Small-Cell Lung Cancer,* Journal of Clinical Oncology, 2002, 20: 1353-1360

M. P. Wong, W. K. Lam, E. Wang, S. W. Chiu, C. L. Lam, L. P. Chung, *Primary Adenocarcinomas of the Lung in Nonsmokers Show a Distinct Pattern of Allelic Imbalance,* American Association For Cancer Research, 2002, 62: 4464-4468

W. N. Rom, J. G. Hay, T. C. Lee, Y. Jiang, K. T. Wong, *Molecular and Genetic Aspects of Lung Cancer,* American Journal Of Respiratory And Critical Care Medicine, 2000, 161: 1355-1367

K. M. Fong, Y. Sekido, A. F. Gazdar, J. D. Minna, *Lung cancer • 9: Molecular biology of lung cancer: clinical implications,* Thorax, 2003, 58: 892-900

T. Kohno, J. Yokota1, *How many tumor suppressor genes are involved in human lung carcinogenesis?,* Carcinogenesis, 1999, 20: 1403-1410

S. Z. Müller, J. D. Minna, A. F. Gazdar, *Aberrant DNA Methylation in Lung Cancer: Biological and Clinical Implications,* The Oncologist, 2002, 7: 451-457