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Abbreviations

| | |
|-----------|---|
| 1. BER | Base excision repair |
| 2. CAM | Cell adhesion molecule |
| 3. CBP | CREB- binding protein |
| 4. CDK | Cyclin dependent kinase |
| 5. CKII | Casein kinase II |
| 6. DNA-PK | DNA- protein kinase |
| 7. GADD45 | Growth arrest DNA damage-inducible protein 45 |
| 8. HAT | Histone acetyl-transferase |
| 9. HDM2 | Human double minute 2 |
| 10. ICAM | Intracellular adhesion molecule-1 |
| 11. LOH | Loss of heterozygosity |
| 12. MDM2 | Mouse double minute 2 |
| 13. MMR | Miss match repair |
| 14. MPF | Mitosis promoting factor |
| 15. NCAM | Neural cell adhesion molecule |
| 16. NER | Nucleotide excision repair |
| 17. p21 | Protein 21 (kDa) |
| 18. p53 | Protein 53(kDa) (tumour suppressor gene) |
| 19. PKC | Protein kinase C |
| 20. pRb | Retinoblastoma protein |
| 21. SPF | Synthesis phase promoting Factor |
| 22. T.S.G | Journal T.S.G |
| 23. TAF | TBP- associated factors |
| 24. TBP | TATA box binding protein |
| 25. TSG | Tumour suppressor gene |
| 26. VCAM | Vascular cell adhesion molecule-1 |

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Abstract

The study of cancer has proved to be an extremely diverse and complex area. Tumour suppressor genes (TSG) play a pivotal role in the development of carcinogenesis. There are many TSGs which have been discovered, I will be focusing on the p53 TSG. I intend to discuss the main functions of this gene, including its chemistry and the importance of its presence in preventing cancer. For the practical component my aim has been to design a scientific journal focusing mainly on TSGs. Letters to established international authors and various UK based publishers were sent as part of the market research. From a small response and through analysis of existing journals, a scientific journal was constructed. When designing this journal, the main aspects for consideration were the impact factor (IF), editorial board, and broadness of topic. Upon completing this project, a scientific journal with substantial scope and free online availability, has been produced.

SECTION A

1.0: What is cancer?

Cancer is a term used for many diseases characterised by abnormal and uncontrolled cell growth of normal cells in the body. (Lexicon Encyclobio website, 2004 & Cancer Research UK website III, 2005).

There are many forms of cancer, and these can be located in different areas of the body (approximately 200 different types of cancer have been identified) (Allrefer.com website, 2004).

Cancers can be divided into five broad categories according to which tissue they originate from; these are represented in the table below:

Table 1: Illustrates different types of cancers (Modified from CR UK website II, 2005)

| Types | Location of cancer |
|--------------|--|
| 1) Carcinoma | Cancers occurring in epithelial tissues which line organs and the body e.g. cancer of skin, lungs and breasts. |
| 2) Sarcoma | Cancer of connective tissues, cartilage and bone. |
| 3) Lymphoma | Cancers that occur in the lymphatic system i.e. the lymph. |
| 4) Leukaemia | Cancer of blood cells, usually white blood. |
| 5) Adenoma | Benign tumours arising in glandular tissues. |

Annually, 250,000 individuals in the UK are diagnosed with cancer, and of these 150,000 individuals die from the disease. Cancer is a prominent health issue in the UK where at least 1 in 3 individuals suffer from the disease each year (CR UK website I, 2005).

With age, the risk of cancer increases, and it is believed that approximately 50% of individuals over the age of 70 will be diagnosed with cancer (Anisimov, 2003). The older an individual becomes, the more time is available for accumulations of cellular events necessary for cancer development (Miller, 1980).

2.0: Cancer development as multistage process

Carcinogenesis requires several mutations before a cell is transformed into a malignant cell (Lakins & Jackson, 1999). These mutations may occur in tumour suppressor genes, proto-oncogenes and DNA repair genes, which shall be discussed later. Figure 1 addresses the cancer development process (Kleinsmith *et al* website, 2003).

2.1 The Multistep Model

Armitage and Doll first discovered the model in 1954. Carcinogenesis occurs in three main stages, i) Initiation ii) Promotion and iii) Progression (Macdonald & Ford, 1997 pg 9).

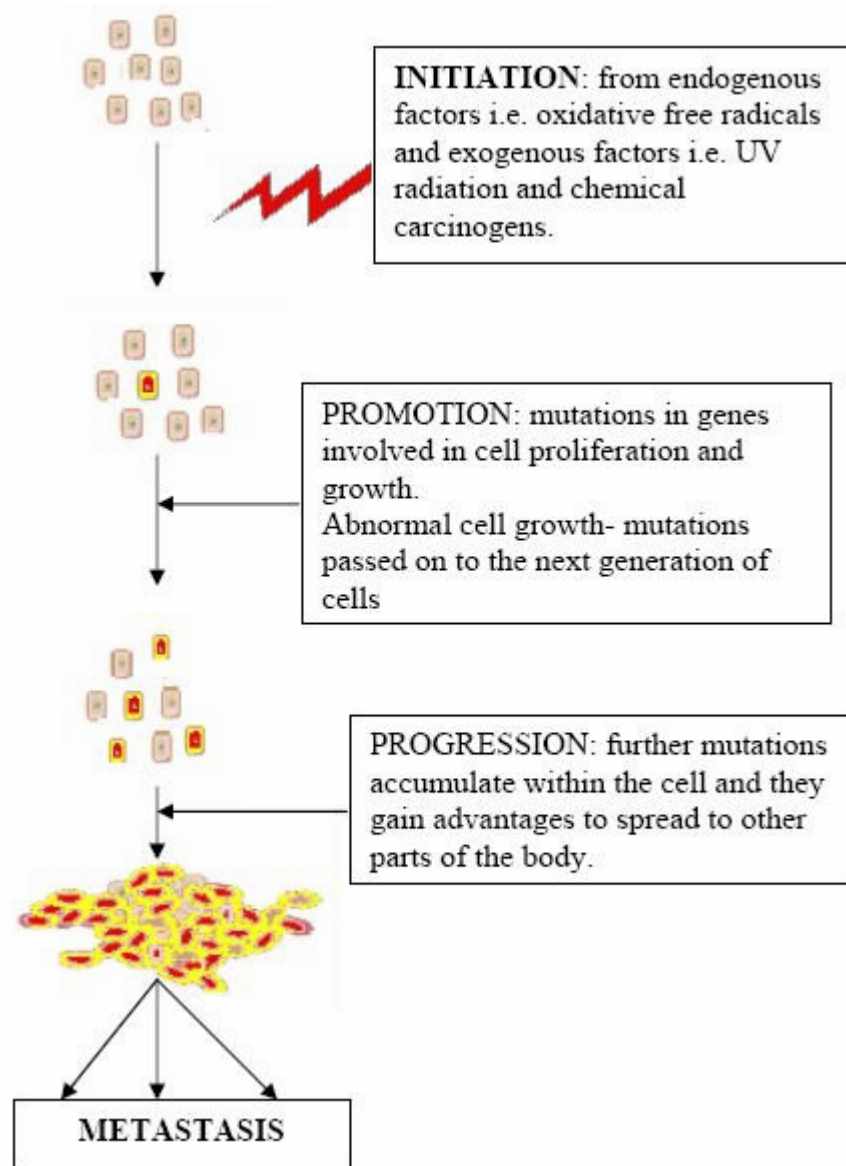


Figure 1: Illustrates the multistage development of cancer.

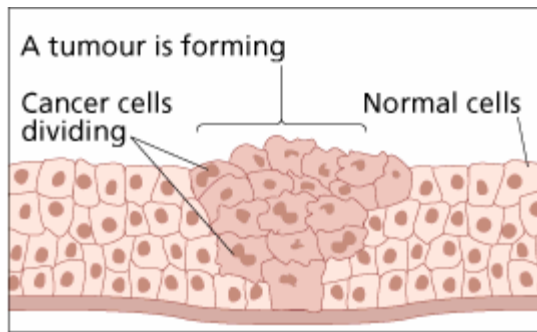
(Modified from Kleinsmith *et al* website, 2003, Macdonald & Ford, 1997 pg 9)

In the initiation stage, any form of mutation in the gene will give the cell a growth advantage. Such mutations can arise as a result of exposure to UV radiation or chemical carcinogens (Macdonald & Ford, 1997 pg 10). During the promotion stage, genes that are involved in the control of cell proliferation i.e. TSG, proto-oncogenes and DNA repair genes, undergo mutations, giving them loss of function, gain in function, and inactivation respectively. Hence, the cell loses its ability to divide normally, leading to abnormal growth and production of tumours. These mutated cells within the tumour continue to accumulate errors, thus gaining the advantages needed for metastasis. This is the progression stage (Macdonald & Ford, 1997 pg 10).

3.0: How cancer arises

Usually, genes control cell division, however, mutation in these genes may give cells advantage to grow and proliferate abnormally, giving rise to malignancy. Normal cells undergo cell division and cell proliferation in a controlled manner. However, in cancers due to mutations, control of this process is lost and new cells continue to form before the death of the old cells (WordiQ.com website, Macdonald & Ford, 1997 pg 3). This leads to the formation of structures called tumours (or neoplasms). Neoplasms are usually composed of monoclonal cells i.e. the majority of the cells in the tumour have the same genetic information as the original cell (figure 2). There are two types of tumours: benign and malignant. (WordiQ.com website, 2004 Macdonald & Ford, 1997 pg 2). Benign tumours are not malignant and usually not harmful to the body. However, they may become harmful if they grow too big and press on other organs. Their rate of growth is slow, as they grow within a fibrous capsule and are well differentiated, making it easier to remove them through surgery (CliniMed website 2004, Macdonald & Ford, 1997 pg 2).

In contrast, malignant tumours grow faster and are life threatening. These tumours are capable of invading surrounding tissues and can spread to other parts of the body (Figure 3). There are two ways in which cancers spread - one via *invasion*, which occurs through the damage and destruction of surrounding cells and tissues; the other is by a process called *metastasis* (Keleg *et al* 2003, Healthcastle.com website 2005). In metastasis the cells are capable of migrating to other parts of the body (figure 2).



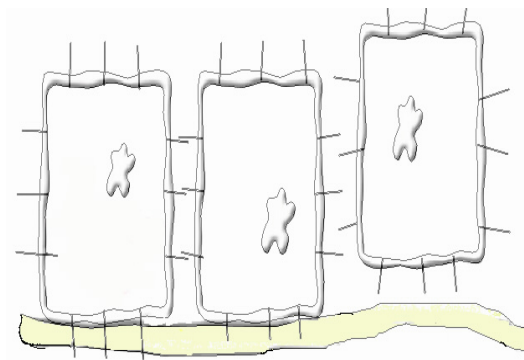
Cancer cells continue to reproduce and are unable to respond to the normal signalling system. This leads to monoclonal tumours being produced.

Figure 2 illustrates the formation of a neoplasm.

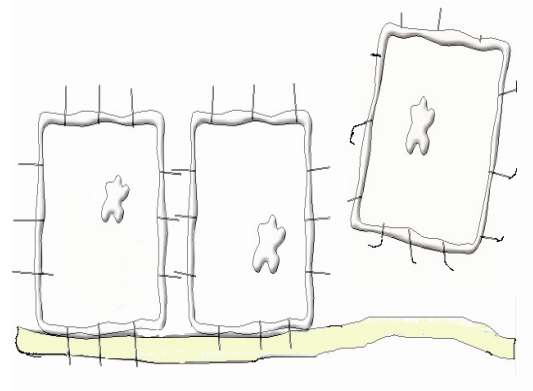
Taken from <http://cancerhelp.co.uk>

These normal cells have cell adhesion molecules, which aid cells to stick together.

These cancer cells have lost their cell adhesion molecules on their cell surface. Hence they become detached from the neighbouring cell.



a) Normal cells



b) Cancer cells

Figure 3: Illustrates the comparison of normal and cancerous cell line.

Modified from <http://cancerhelp.co.uk>

(a) Normal cells stick together as a result of their cell adhesion molecules (CAM's), which keep the cell-cell adhesion (Ikura Lab website, 2002). (b) Cancer cells lose this ability, as they will usually have lost their CAM's on the cell surface, and are therefore unable to stick together. Carcinomas can be characterised as having lost the cell-cell adhesion molecule, E-cadherin (Frixen *et al*, 1991, Navarro *et al*, 1991). E-cadherin is absent in many epithelial carcinomas and melanomas, but the ICAMS (intercellular adhesion molecule-1), VCAMS (vascular cell adhesion molecule-1), NCAM (neural cell adhesion molecule) and E-selectin are present in excess amounts (Lynch *et al*, 1997). E-cadherin is believed to act as an invasion suppressor (Navarro *et al*, 1991, Semb & Christofori).

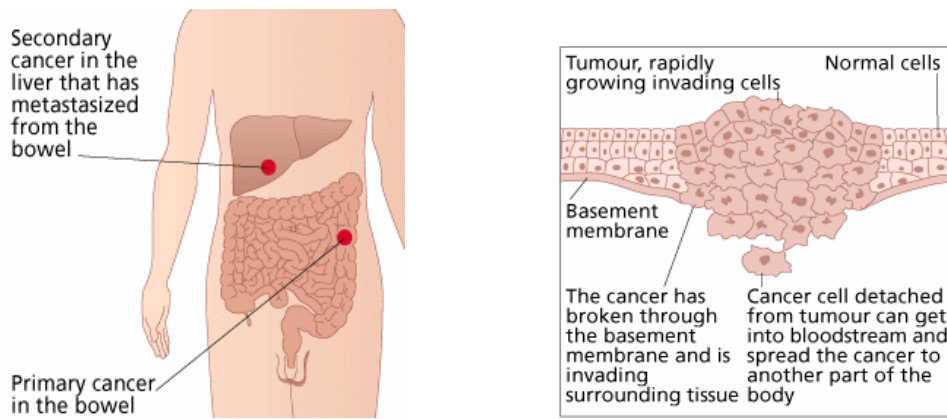


Figure 4: Illustrates how secondary cancers are formed from a Primary one

Taken from <http://cancerhelp.co.uk>

Malignant cells are capable of detaching from tumours and migrating to other areas of the body, via the blood stream or the lymphatic system, and invading other tissues.

3.1: Causes of cancer

Development of cancer is a result of DNA damage (i.e. mutations) which can be influenced by many factors. Mutations in DNA may be caused by various factors, such as living habits, diet, environment, viruses, increase in oxygen radicals within the body (known as Hypoxia) and Age. Carcinogens such as ionising radiation, UV radiation, tobacco and chemical carcinogens increase the chances of developing cancer (Ko & Prives, 1996, Lakins & Jackson, 1999).

3.2: Sporadic vs inherited cancer

Most cancers are sporadic, with a minority (approximately 5%) being inherited (familial). Sporadic cancers are usually influenced by carcinogens and radiation, and occur in somatic cells. Mutations in tumour suppressor genes are usually linked to genetically inherited cancers, and therefore occur in germline cells e.g. Li-Fraumeni syndrome disease, in which the p53 tumour suppressor gene is mutated; is inherited in an autosomal recessive manner (Schneider & Li website, 2004, Macdonald & Ford, 1997 pg 8).

4.0: The cell cycle and control of cell division

Cancerous cells usually begin to respond abnormally to the normal cell signalling. It is acknowledged that genes within tumour cells have mutations, which lead to abnormal progression through the cell cycle e.g. p53 tumour suppressor gene.

Table 2: Illustrates the stages of the cell cycle (Figure 4):

| Stage in the cell cycle | Biological activity in each stage |
|--------------------------------|---|
| G0 | Quiescence of cell |
| G1 | Preparation of cell to synthesise DNA – |
| S | DNA begins to replicate and histones are produced |
| G2 | Preparing the cell for mitosis e.g. mitotic machinery ready |
| M | Mitosis begins |

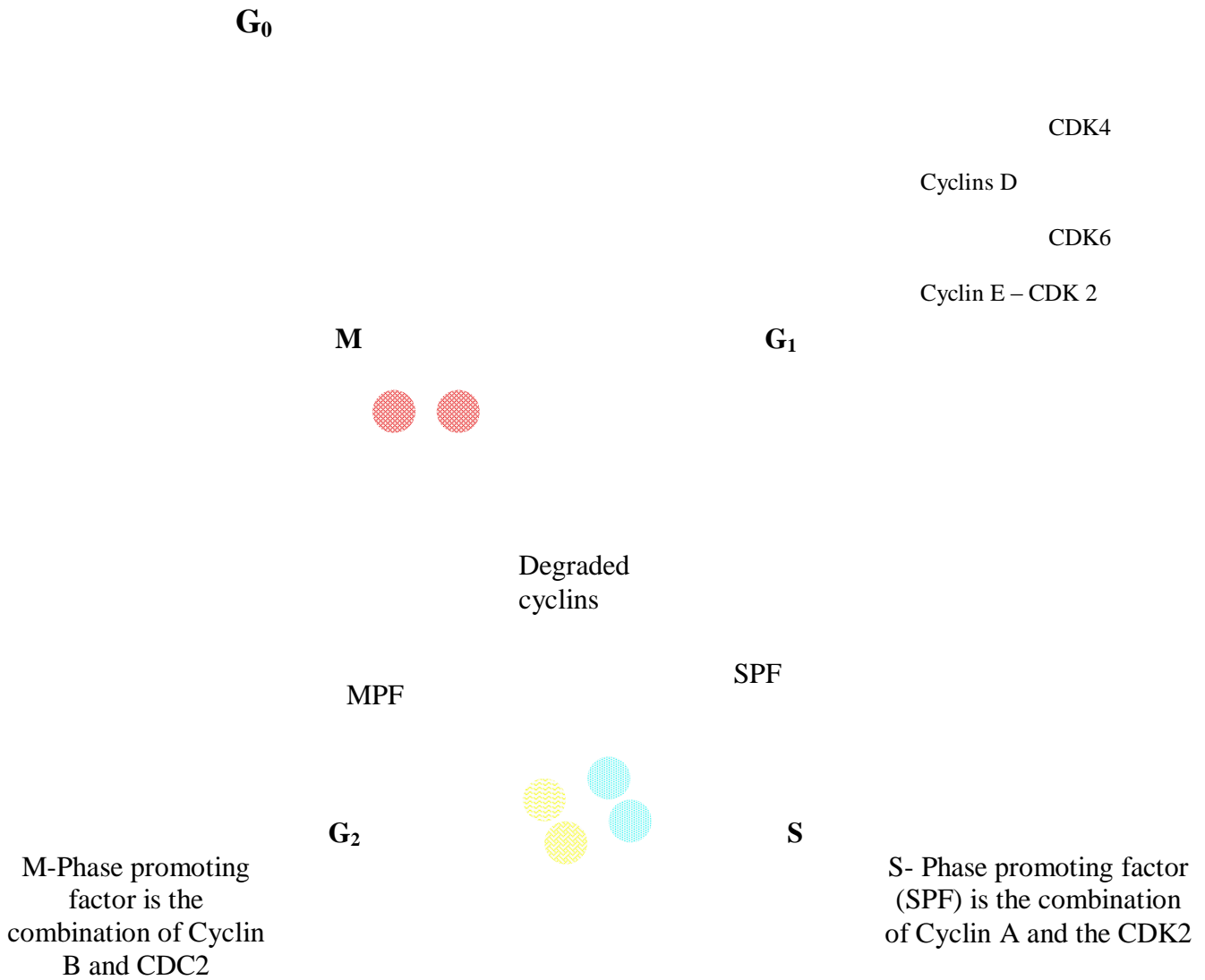


Figure 5: Illustrates the Specific cyclins and CDK molecules at different stages in the Cell cycle.

Reproduced from the Kimball website I, 2004, Hartle & Jones 2001 pg 645.

As soon as the G₁ cyclins and the CDK molecules combine, they signal the cell to prepare its DNA for replication. The SPF allows the cell to replicate the DNA. Once the G₁ and S-phase cyclins are degraded, MPF is produced. MPF leads the cell into the M- phase.

4.1: The regulation of the cell cycle

The cell cycle is governed by proteins which regulate the cell through each stage of the cell cycle. These intracellular proteins are cyclins and cyclin dependent kinases; (CDK- these are responsible for adding phosphate to proteins activating or

deactivating them, an example of protein that is phosphorylated is pRb) (University of Arizona website, 2004). pRb TSG regulates the cell cycle by inhibiting the transcription of genes that allow the cell to progress to the next stage. The pRb gene is phosphorylated by the G1 CDK-Cyclin complex which inhibits the suppressor function of this gene. pRb is an essential component for cell cycle regulation, without it there is no controlled regulation of the cycle, leading to tumourigenesis (Yamasaki & Pagano, 2004).

CDK molecules are only activated when the cyclins are bound to them. In human tumours, excess levels of cyclins D and E are frequently found, which are positive regulators in the G1 and S phase (Yamasaki & Pagano, 2004).

p27 which is the inhibitor of the CDK molecules, stops the binding of the CDK2 and E cyclin, so the cell can not progress to the next stage. However p27 is found in low levels in tumours, therefore formations of CDK2/E-cycling complexes are not prevented, leading to progression to the next stage in a short period of time (Bloom & Pagano, 2003).

4.1.2: The checkpoints in the cell cycle

The cell cycle check points are crucial for the repair of DNA damage, as they provide additional time for DNA repair and therefore maintain genetic stability (Kaufmann & Paules, 1996). There are several important check points present in the cell cycle, all of which have genes that carry out functions involved in monitoring the DNA. These are: the DNA damage check points, the S-phase check point, and the spindle check point (Kimball J website, 2004). The tumour suppressor gene (TSG) p53 plays an important role at the G1 checkpoint, as it is a checkpoint inhibitor and halts the cell cycle for DNA repair (Hartwell & Kastan, 1994, Peters & Vousden, 1997 pg 199-1). However, if damage occurs in cell cycle checkpoints, and the genes involved in cell

cycle control are attenuated, this leads to genetic instability and therefore loss of cell cycle regulation (Kaufmann & Paules, 1996, Peters & Vousden, 1997 pg 190-1).

5.0: Apoptosis

Apoptosis is also known as *programmed cell death*. This is controlled by the TSG p53, which causes the cell to commit suicide (figure 6). If there is damage to DNA, p53 halts the cell cycle and repairs the damage. However, if the damage is not repairable, p53 regulates other genes responsible for apoptosis e.g. Bax, PUMA, NOXA (figures 9 &10). P53 can be mutated, stopping it from functioning normally. Therefore, DNA repair and apoptotic cell death do not occur, resulting in tumourigenesis, leading to cancer (PSA Rising Magazine website, 1999, Macdonald & Ford, 1997 pg 5-6, Loeb &Loeb, 2000).

As Donehower *et al* (1992) demonstrated with the mouse model, in the absence of the p53 gene, the mice were more prone to develop spontaneous tumours. However the p53 - / - (meaning absence of both normal p53 alleles) mice acquired normal early development, but later developed cancers such as lymphomas and sarcomas (King, 2000, pg 14 &15, Donehower *et al*, 1992).

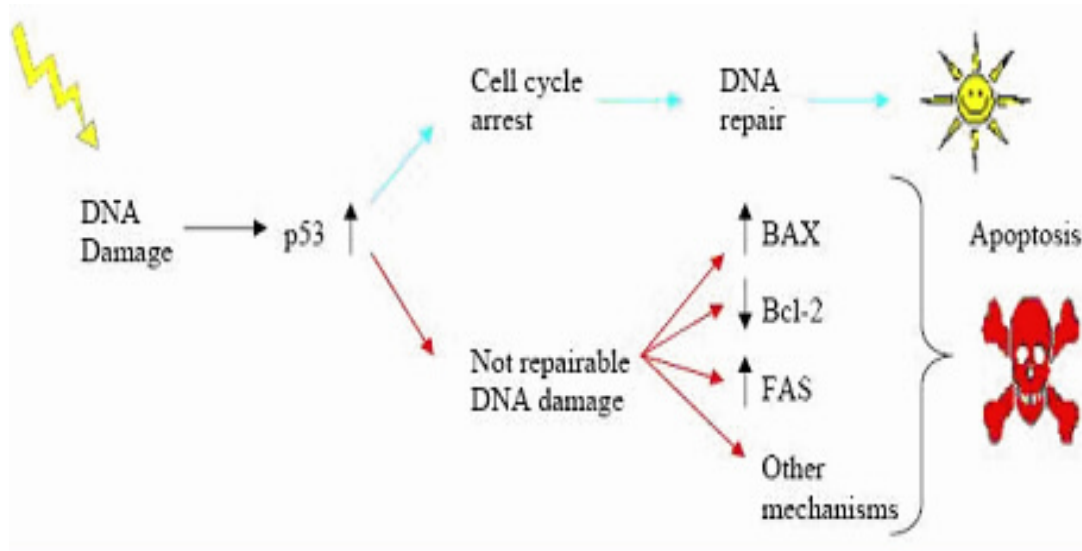


Figure 6: Illustrates how p53 TSG functions towards DNA damage

Reproduced from <http://www.portfolio.mvm.ed.ac.uk/studentwebs/session2/group28/p53.html>

p53 TSG halts the cell cycle in response to DNA damage. p53 induces the transcription of genes that are responsible for cell apoptosis when DNA is not repairable.

6.0: Genes involved in cancer

Genes are responsible for the control of cell proliferation and differentiation within the body. However, these genes can become prone to mutations, resulting in the loss of cell cycle regulation and abnormal cell growth.

- These genes are called:
- 1) Proto-oncogenes
 - 2) Tumour suppressor genes (TSG)
 - 3) DNA repair genes

6.1: How proto-oncogenes contribute to tumorigenesis

Proto-oncogenes are the dominant genes that accelerate cell growth. However, when these genes are mutated they gain an advantage in function. Mutated forms of proto-oncogenes are known as oncogenes. Oncogenes can no longer be switched off by normal cellular signals, meaning the cell continues to divide and proliferate abnormally, leading to the formation of tumours.

The discovery of oncogenes came from research into viruses, with the first being discovered in 1976, and named *SRC* oncogene (Macdonald & Ford, 1997 pg 13). This gene was discovered in normal chicken DNA, leading to the conclusion that oncogenes are not carried into cells via viruses, but are found originally within the cell (Macdonald & Ford, 1997 pg 13).

6.1.1 Mechanisms of oncogene activation

Proto-oncogenes can be converted to oncogenes by an uncomplicated point mutation in the gene. A good example of this would be the *Ras* gene on chromosome 11, converted to act as an oncogene by a point mutation of glycine to valine at position 12. However, point mutations in the *Ras* gene have been found at other positions in different tumours. The positions of point mutations in the *Ras* gene have also varied in different tumours (Cooper G.M, 1995 pg 75). In addition, oncogenes may be activated by gene amplification and chromosomal rearrangement. MDM2 is a proto-oncogene that breaks down p53 via ubiquitin. It can act as an oncogene when it is present in high levels, and keeps degrading p53 in normal cells. This result in low levels of p53, therefore damaged DNA is not corrected and is carried to the next stage of the cell cycle (Cooper G.M, 1995 pg 75).

Table 3: Illustrates some Oncogenes
(Reproduced from Pierce B.A, 2002 page 626)

| Oncogene | Cellular location product | Function of Proto-oncogene |
|--------------|---------------------------|--------------------------------|
| <i>Sis</i> | secreted | Growth factor |
| <i>erbB</i> | Cell membrane | Part of growth factor receptor |
| <i>erbA</i> | Cytoplasm | Thyroid hormone receptor |
| <i>Src</i> | Cell membrane | Protein tyrosine kinase |
| <i>Ras</i> | Cell membrane | GTP binding and GTPase |
| <i>Myc</i> | Nucleus | Transcription factor |
| <i>Fos</i> | Nucleus | Transcription factor |
| <i>Jun</i> | Nucleus | Transcription factor |
| <i>bcl-1</i> | Nucleus | Cell cycle |

6.2: How tumour suppressor genes contribute to tumorigenesis

TSG's are involved in the inhibition of abnormal cell growth, being known as the *brakes*, with oncogenes known as the *accelerators*. TSGs are much more difficult to find than the oncogenes, however they were discovered before oncogenes (Macdonald & Ford, 1997 pg 35). TSG's are recessive, therefore both alleles do not need to be normal for these genes to function correctly. If the TSG is heterozygous i.e. one mutated and one normal allele, it will still function normally. If one allele is lost, this is referred to as "loss of heterozygosity" (LOH); this may occur:

- 1) If the normal allele, or the chromosome containing this allele is missing (Kimball 2005- website II).
- 2) If the chromosome containing a normal allele is absent, the other chromosome containing a mutated allele will duplicate itself (Kimball 2005- website II)
- 3) Mitotic recombination (Kimball 2005- website II).

In order for TSG's to be inactivated, both alleles of the gene must be mutated. Knudson in 1971 proposed his "two hit" hypothesis, in which he claims that inherent

modification must take place in TSG's to transform them into cancerous cells (Knudson, 1997).

The first mutation is a germline mutation (usually a point mutation), followed by a somatic mutation of the second allele (LOH) (Knudson, 1997).

Mutations in TSG are not the only factors responsible for inactivating the gene; methylation of the tumour suppressor gene's promoter may also be a significant factor (Kimball 2005- website II, Meuller & Young, 2001).

There are a number of things that can inactivate TSGs: increased oncogene levels, inhibition of phosphorylation and acetylations; activation of genes which inhibit normal TSG functioning.

Table 4: Illustrates some TSGs

Taken from <http://web.indstate.edu/thcme/mwking/tumor-suppressors.html>

| Gene | Function | Chromosomal Location | Tumour Types Observed |
|-----------------------------|---|----------------------|--|
| P53 | Transcription factor & regulates apoptosis | 17p13.1 | brain tumours, sarcomas, leukaemia, breast cancer |
| RB1 | Transcription factor | 13q14.1-q14.2 | retinoblastoma, osteogenic sarcoma |
| WT-1 | Transcription factor | 11p13 | pediatric kidney cancer |
| NF1 | GTPase activator | 17q11.2 | neurofibromas, sarcomas, gliomas |
| NF2 | linkage of cell membrane to actin cytoskeleton | 22q12.2 | Schwann cell tumours, astrocytomas, meningiomas, ependymomas |
| APC | signalling through adhesion molecules to nucleus | 5q21-q22 | colon cancer |
| TSC1 | interacts with tuberlin, exact function unknown | 9q34 | facial angiofibromas |
| TSC2 | GTPase activation of RAP1 and RAB5 | 16p13.3 | benign growths (hamartomas) in many tissues, astrocytomas, rhabdomyosarcomas |
| DPC4 also known as Smad4 | regulation of TGF- β /BMP signal transduction | 18q21.1 | pancreatic carcinoma, colon cancer |

| | | | |
|--|---|----------|--|
| DCC | transmembrane receptor involved in axonal guidance via netrins | 18q21.3 | colorectal cancer |
| BRCA1 | repair of double strand breaks by interaction with Rad51 protein | 17q21 | breast and ovarian cancer |
| BRCA2 | similar to BRCA1 activity | 13q12.3 | breast and ovarian cancer |
| PTEN | phosphoinositide 3-phosphatase protein tyrosine phosphatase | 10q23.3 | gliomas, breast cancer, thyroid cancer, head & neck squamous carcinoma |
| LKB1 a nuclear localized kinase also called STK11 (serine-threonine kinase 11) | phosphorylates and activates AMP-activated kinase (AMPK), AMPK involved in stress responses, lipid and glucose metabolism | 19p13.3 | hyperpigmentation, multiple hamartomatous polyps, colorectal, breast and ovarian cancers |
| MSH2 | DNA mismatch repair | 2p22-p21 | colon cancer |
| MLH1 | DNA mismatch repair | 3p21.3 | colon cancer |
| CDH1 protein = E-cadherin | cell-cell adhesion protein | 16q22.1 | gastric cancer, lobular breast cancer |
| VHL | regulation of transcription elongation through activation of a ubiquitin ligase complex | 3p26-p25 | renal cancers, hemangioblastomas, pheochromocytoma, retinal angioma |
| p16 ^{INK4a} also called CDKN2A protein=cyclin-dependent kinase inhibitor 2A | cell-cycle regulation | 9p21 | melanoma, pancreatic cancer, others |
| PTCH protein = patched | transmembrane receptor for sonic hedgehog (shh), involved in early development through repression of action of smoothened | 9q22.3 | basal cell skin carcinoma |
| MEN1 | unknown | 11q13 | parathyroid and pituitary adenomas, islet cell tumours, carcinoid |

6.3: DNA repair genes

DNA is continuously under stress from endogenous and exogenous factors, and is repaired by specific DNA repair genes. DNA repair genes are also responsible for repairing other genes that are involved in regulating the cell cycle (Dixon & Kopras, 2004).

Deficits in the mismatch repair genes involved in DNA repair (approximately 130 genes have been named to be involved in the different DNA repair pathways), are associated with cancer development (Christmann *et al*, 2003). Some of the various DNA repair pathways are listed below. Each pathway has different genes involved in DNA repair:

1. Mismatch repair (MMR)
2. Base excision repair (BER)
3. Nucleotide excision repair (NER)
4. DNA double strand break repair

The inactivation of the DNA repair genes requires both the alleles of the gene to be mutated (two hit hypothesis). The malfunctioning of DNA repair genes leads to an accumulation of mutations, which are not corrected and therefore passed on to the next generation of cells. TSG's and proto-oncogenes may undergo mutations, and DNA repair genes must function coherently to repair them. Mutant phenotypes of DNA repair genes are unable to respond to DNA damage resulting in genetic instability, and leading to tumourigenesis (Stanford Cancer Centre, 2004).

SECTION B

7.0: The p53 Tumour Suppressor Gene

p53 was discovered in 1979 by Arnold Levin and David Lane, discovered bound to a T-antigen of the DNA tumour virus SV40 (simian virus 40) (Fields & Jang, 1990, Howard Hughes Medical Institute website, 1996). As p53 reacted with the *Ras* oncogene to transform a rat embryo fibroblast in cell culture, it was believed to be an oncogene for 10 years (Levine *et al*, 1991, Howard Hughes Medical Institute website, 1996). In 1989, Bert Vogelstein and Ray White concluded that it was a tumour suppressor gene. These cells act like brakes in the cell cycle when a fault in its DNA is detected (Howard Hughes Medical Institute website, 1996).

p53 is involved in several important cellular functions, for example; cell cycle arrest, activation of DNA repair genes, and apoptosis. p53 is responsible for regulating the genes which control the cell cycle, therefore, it is known as a transcriptional activator (Amundson *et al*, 1998). p53 may be activated by genotoxic stress (DNA damage), an increase in oncogenes levels or hypoxia.

In 50% of cancers, the p53 gene is inactivated by mutations, resulting in a loss of suppression function. Many tumours show high levels of inactive p53 gene (Hollstein *et al*, 1991 cited in Amundson *et el*, 1998).

There are several genes involved in activating p53, all of which are discussed in the next few sections.

7.1: Anatomy of the tumour suppressor p53

p53 is a tetrameric phosphoprotein (TP53), whose coding gene is located on the short arm of chromosome 17p13. It has a mass of 53 kDa, is comprised of 393 amino acids, and covers up to 20 kb of the DNA (Ko & Prives, 1996, University of Tokyo website, 2004).

C-terminal domain binds directly to DNA strand

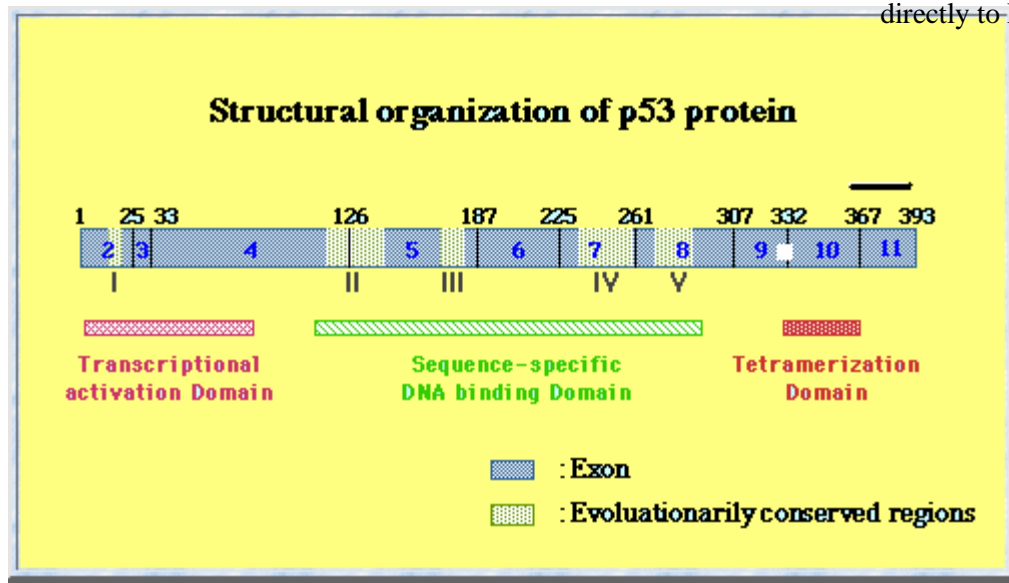


Figure 7: Illustrates a p53 protein

Adapted from http://p53.genome.ad.jp/documents/about_p53.html

The four domains of p53:

1. The N-terminal transcriptional activation domain which is acidic and very rich in proline (Fields & Jang, 1990), and lies between 1-43 amino acid residues.
2. Sequence specific DNA binding domain, is responsible for DNA binding (Pietenpol *et al*, 1994), and lies between 100-300 amino acid residues (Ko & Prives, 1996).
3. The oligomerization domain, which is important for the tetramerization of p53. This domain plays a crucial role in DNA binding, protein-protein interactions, post-translational modifications, and p53 degradation (Chene P, 2001, Oregon State University website, 2004). It lies within the C-terminal domain between 320-360 amino acid residues (Ko & Prives, 1996).
4. The C-terminal domain acts as a negative regulatory domain, inhibiting the DNA binding to the sequence specific domain (Selivonanova *et al*, 1996, Pospisilova *et al*, 2004). This domain lies between 300-393 amino acid residues in the p53 protein (Ko & Prives, 1996).

However, the C-terminal is divided further into three components:

- The flexible linker, which lies between 300-320 amino acid residues.
- The Tetramerization domain, which lies between 320-360 amino acid residues. This is the fourth domain of the p53 protein, also referred to as the Oligomerization domain.
- The final region, the carboxyl terminus which lies between the last 30 amino acid residues 363-393 (Ko & Prives, 1996).

7.2: p53 functions

p53, found in the nucleus of the cell, is a transcription activator that is present in low levels in normal cells (Pierce, 2002 pg 626). The phosphoprotein is responsible for various processes, including; the transcription of genes involved in regulating cell death, genes involved in DNA repair and genes involved in inhibiting CDK's of the cell cycle (Web-Books website, 2005). Within normal cells p53 is in its latent phase, however, if mutations are present in the DNA it is activated. This halts the cell cycle, to allow DNA repair or programmed cell death, thus it is believed to be involved in tumour suppression (Cooper, 1995 pg 314, Vogelstein *et al*, 2000).

The half life of p53 in normal cells is relatively short, as it is continuously subjected to ubiquitination and degradation by MDM2 (Meek, 1994 cited in Amundson *et al*, 1998, Haupt *et al*, 2003).

7.2.1: Growth arrest mediated by p53

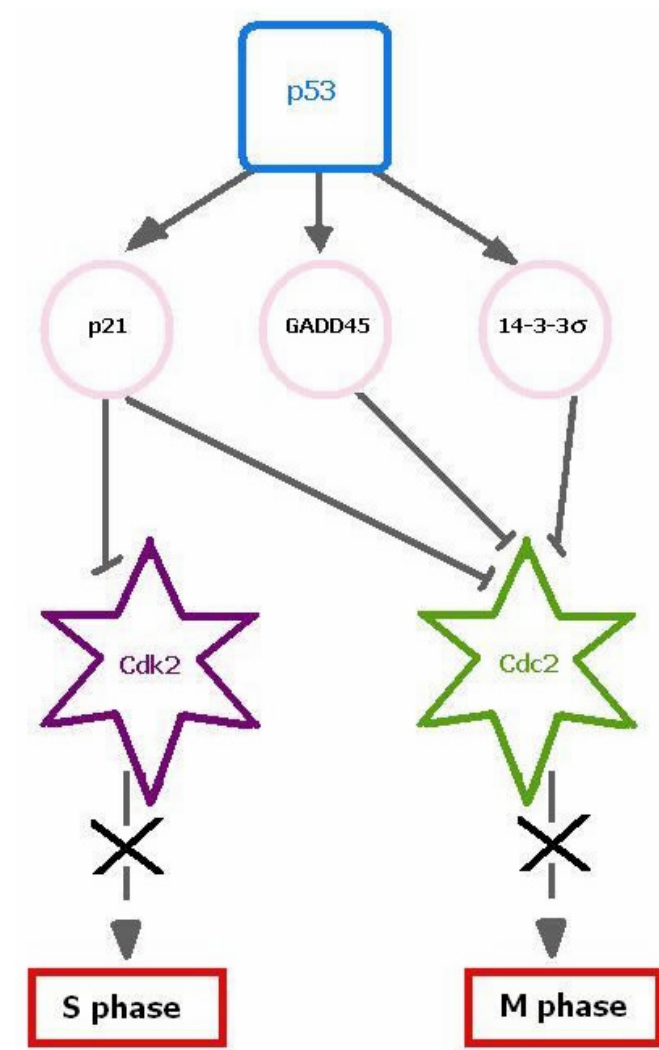


Figure 8: Illustrates the how p53 mediates growth arrest at the two checkpoints

Modified from <http://www.web-books.com/MoBio/Free/Ch4Hp53.htm>

When p53 is activated it transcribes genes that encode for proteins, which are responsible for cell growth arrest. The gene that is transcribed is called WAF1, which encodes for a 21 kDa protein called p21. P21 inhibits cyclin dependent kinase activity resulting in cell cycle arrest at the G1 check point (Cooper, 1995 pg 314, Macdonald & Ford, 1997 pg 55). CDK molecules are responsible for the phosphorylation of the retinoblastoma protein (pRb), which leads to the release of the

S phase promoting factor E2F-1. Inhibition of CDK molecules results in pRb protein not being phosphorylated, therefore there is no release of the S phase promoting factor, resulting in cell cycle arrest (Macdonald & Ford, 1997 pg 55). In order to progress to the S phase p21 activity must be inhibited. This may only occur when the p53 levels decrease by degradation and ubiquitination (Colman *et al*, 2000, Macdonald & Ford, 1997 pg 55, Askhew *et al*, 1991 cited in Amundson *et al*, 1998). P21 is regulated by p53, therefore in the absence of p53 or if the p53 protein is mutated, it can no longer carry out its normal functions in regulating this protein. If p21 is absent it can no longer inhibit CDK's, leading to uncontrolled cell growth (Cooper, 1995 pg 314). Thus p21 regulates the cell cycle by arresting it at the G1-S phase and the G2-M phase, which may give the DNA repair genes a chance to carry out their function (Cooper, 1995 pg 315).

Growth arrest DNA damage-inducible protein (GADD45) is a p53-regulated protein that controls cell cycle arrest at the G2-M checkpoint (Zhan & Univ website, 2003). GADD45 is a small acid protein, with a mass of 19 kDa, and is located in the nucleus (Carrier *et al*, 1999). GADD45 is also regulated by p53, so in the absence of p53 GADD45 would not be regulated, leading to continuous abnormal cell proliferation. Wang *et al* (1999) concluded from their experiment that GADD45 mediated cell cycle arrest at the G2-M checkpoint is dependent on the normal p53. They arrived at this conclusion as they did not observe a G2-M checkpoint arrest in the p53 null Li-Fraumeni fibroblast, nor was any observed in the normal cell actually containing the mutant form of p53 (Wang *et al*, 1999).

The 14-3-3 σ proteins are ubiquitous and are known as negative regulators of the cell cycle. This protein is regulated by p53 and is involved in cell cycle arrest at the G2-M checkpoint. Following DNA damage both p53 and 14-3-3 σ are activated, resulting

in growth arrest at the G2-M Checkpoint (Yang *et al*, 2003, Hermeking *et al*, 1997). Yang *et al* found that 14-3-3 σ and p53 cooperated together following DNA damage, resulting in p53 stabilisation. They also found that 14-3-3 σ encouraged p53 oligomerization and improved p53 transcriptional activity (Yang *et al*, 2003). 14-3-3 σ removes appropriate proteins from the cytoplasm (i.e. cyclinB1-cdc2), needed for entry into mitosis (Hermeking *et al*, 1997, Chan *et al*, 2000). The 14-3-3 σ protein is also a positive regulator of p53, as it prevents the inhibitory function that MDM2 gene has on p53 (Landes Bioscience website, 2002).

7.2.2: Apoptosis mediated by p53

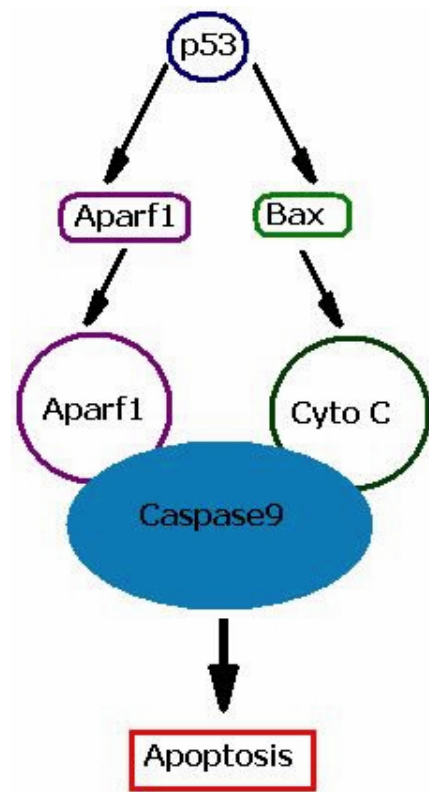


Figure 9: Illustrates how p53 mediates apoptosis

Adapted from <http://www.web-books.com/MoBio/Free/Ch4Hp53.htm>

Apoptosis is programmed cell death, and occurs when the cell can no longer function normally. Apoptosis is another function of p53; it induces the transcription of genes involved in leading the cell to apoptosis. There are several genes involved in apoptosis (figure 8 & 10).

Apoptosis mediated by p53 is usually triggered by UV radiation, hypoxia or an increase in oncogene levels. Any cells containing damaged DNA can be removed via apoptosis before that can become harmful (Ko & Prives, 1996). Cells that are deficient in p53 have been shown not to respond to apoptosis and DNA repair, leading to the uncontrolled proliferation of cells containing damaged DNA, which results in genetic instability and tumourigenesis (Kemp *et al*, 2001).

The removal of murine p53, has shown that mice are more prone to develop cancer than mice that have p53 (Donehower *et al*, 1992).

There are two pathways which may be taken by p53 to induce apoptosis: the intrinsic and extrinsic pathways (figure 11) (Haupt *et al*, 2003).

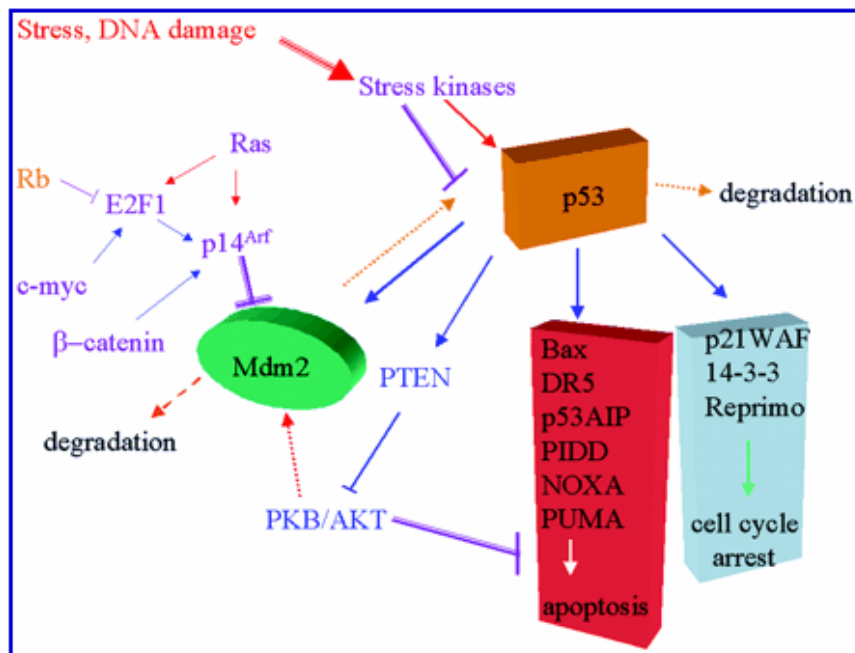


Figure 10: Illustrates the different genes activated by p53

Taken from <http://carcin.oupjournals.org/cgi/content/full/23/4/541/F5>

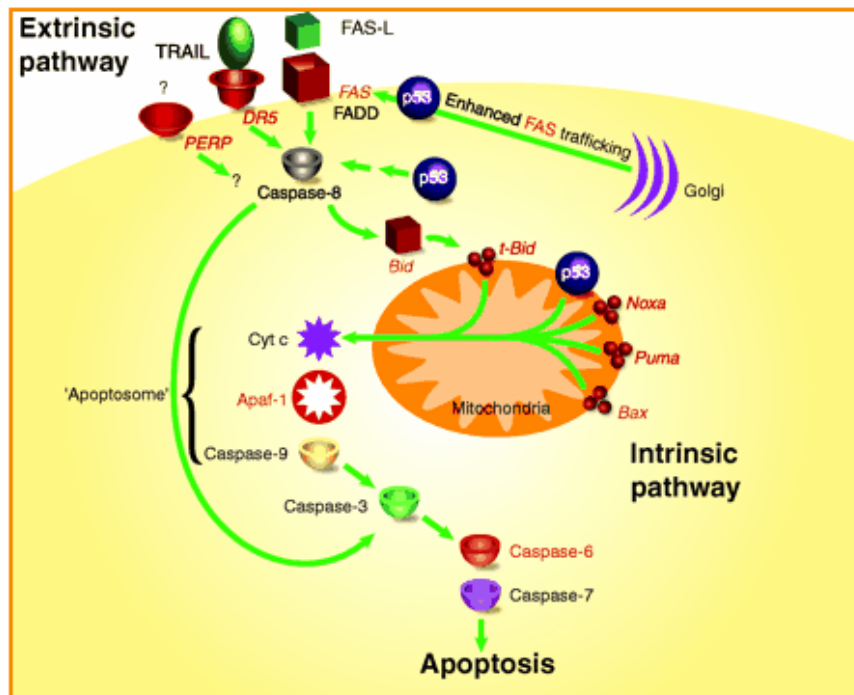


Figure 11: Illustrates the two pathways involve in apoptosis

Taken from <http://jcs.biologists.org/cgi/content/full/116/20/4077/FIG1>

The intrinsic pathway involves cytochrome C (apoptotic protease activating factor-1) which is released from mitochondria and leads to the activation of caspase cleavage. The release of cytochrome C is controlled by p53-induced pro-apoptotic Bax gene. The anti-apoptotic Bcl2 gene inhibits the release of cytochrome C (Famuboni *et al*, 2001, Henry *et al*, 2002, Shen & White, 2001). Cytochrome C combines with caspase 9 forming a complex which is known as apoptosome (Schuler & Green, 2001, Haupt *et al*, 2003). Apoptosome complexes initiate activity in other effector caspases, such as caspases 3 and 7 for apoptosis (Haupt *et al*, 2003). The extrinsic pathway involves the Fas gene, DR5 and PERP transmembrane proteins (Haupt *et al*, 2003).

7.3: Regulation of p53

p53 levels in a normal cell are very closely regulated, however, in transformed cells this control is lost leading to abnormal levels of p53 within these cells. As p53 is the main gene regulating cell growth and apoptotic cell death, it must be highly controlled.

MDM2 is a proto-oncogene that is responsible for controlling the levels of p53 (Landes Bioscience website, 2002). MDM2 negatively regulates p53 by binding to the protein and exporting it from the nucleus to the cytoplasm, where it is degraded by proteosomes (Mamand *et al*, 2000, Perry *et al*, 2000, Pan & Chen, 2003). Levels of p53 increase soon after DNA damage, inducing the transcription of MDM2 proto-oncogene. Therefore, the levels of MDM2 and p53 simultaneously increase. MDM2 then down regulates p53, forming a negative feedback loop (Freedman *et al*, 1999, Landes Bioscience website, 2002). In humans, the MDM2 proto-oncogene is known as HDM2, human double minute 2 (Haupt *et al*, 2003).

MDM2 levels are greater than normal in about 30-40% of sarcomas, and are found to be over expressed in leukaemia (Jones *et al*, 1995). After many experiments carried out on murine animals, scientists were able to conclude that the absence of the MDM2 gene in mice results in early embryonic death. However, when both the MDM2 gene and p53 protein are absent the mice develop normally (Jones *et al*, 1995, McMasters *et al*, 1996).

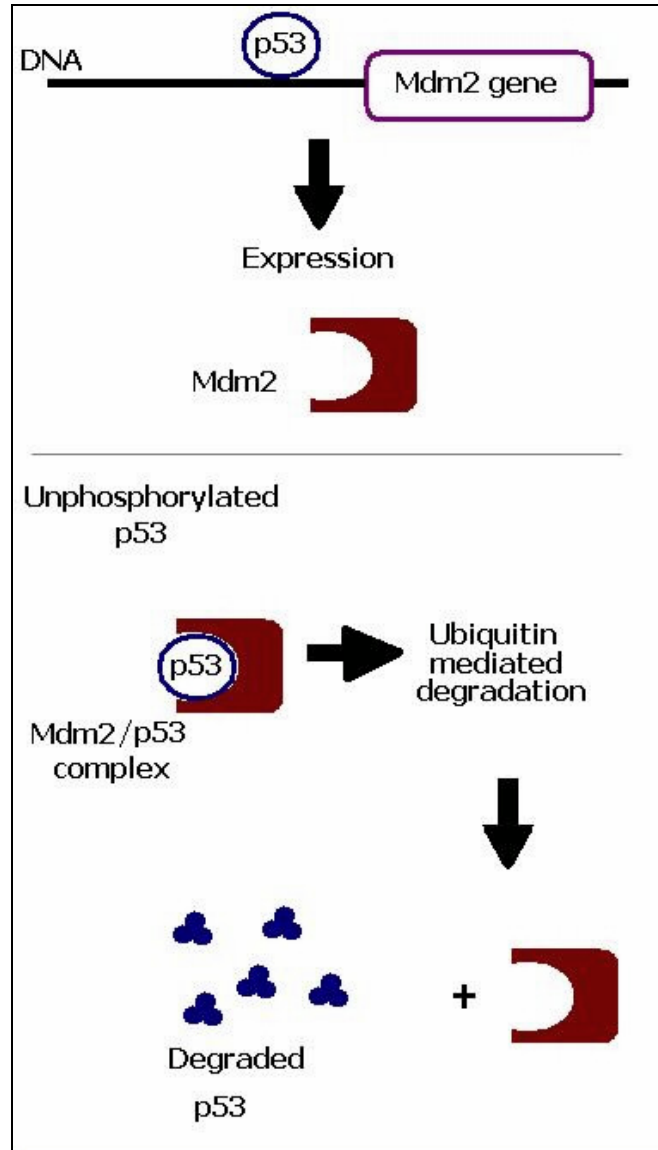


Figure 12: Illustrates how MDM causes the Ubiquitous break down of p53

Modified from <http://www.web-books.com/MoBio/Free/Ch4Hp53.htm>

When p53 is phosphorylated it becomes activated, allowing it to act as a transcriptional activator for many proteins, even those that regulate the p53 itself. MDM2 is induced by p53 soon after the DNA damage is repaired. MDM2 binds to the p53 at specific sites and degrades p53 with ubiquitin ligases, so that p53 transcriptional activity is reduced and cell arrest is not held up for long (Alarcon-Vargas & Ronai, 2002). When p53 is phosphorylated at the serine residue 15, 20 and

threonine residue 18, MDM2-p53 complex can no longer be formed, as this is the specific site where MDM2 binds (Alarcon-Vargas & Ronai, 2002). MDM2-p53 complexes are found more readily in normal cells as there is no DNA damage. Cells with damaged DNA (e.g. due to UV radiation) contain less favourable amounts of these complexes, as p53 needs to be active to repair DNA damage (Fuchs *et al*, 1998).

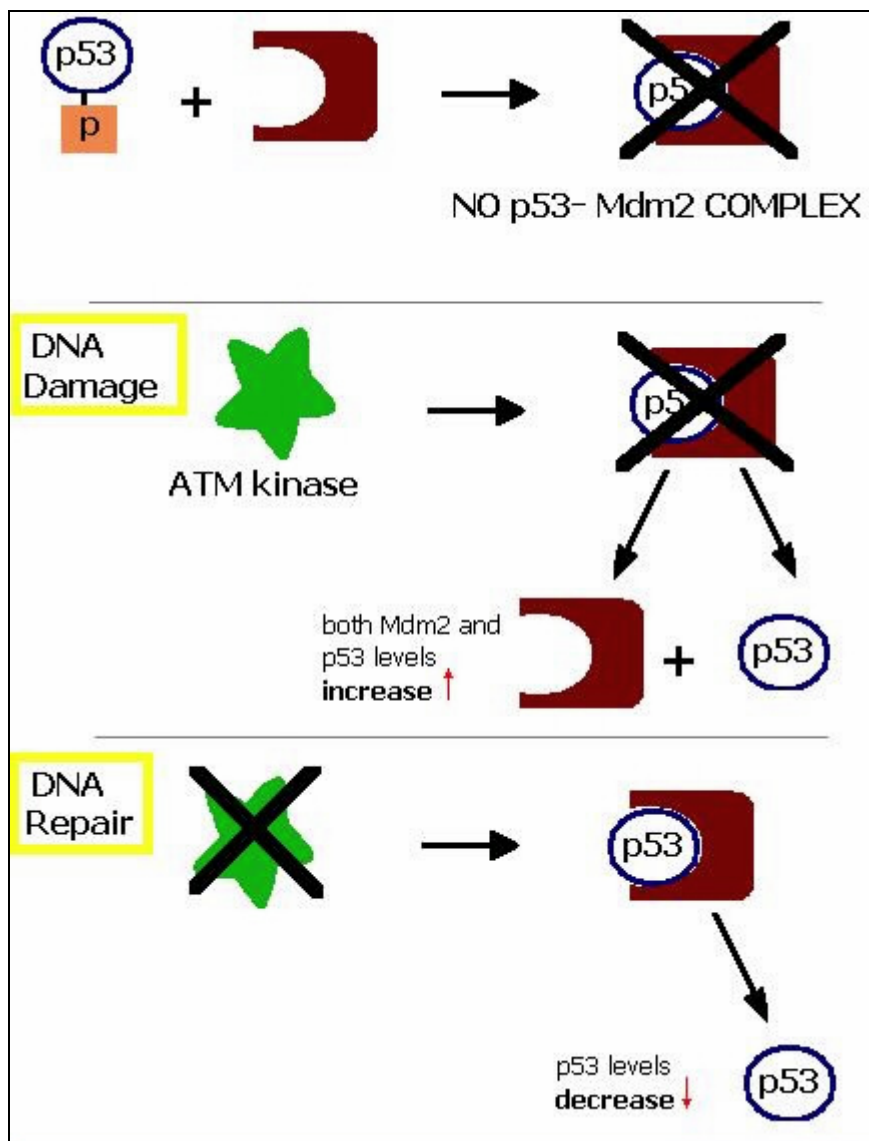


Figure 13: Illustrates how MDM2 binding may be inhibited

Modified from <http://www.web-books.com/MoBio/Free/Ch4Hp53.htm>

In order to inactivate and reduce the MDM2 gene's ability to ubiquitinate p53, p53 needs to be phosphorylated. This usually happens with the aid of protein kinases,

such as ATM, c-MYC and ART (Landes Bioscience website, 2002, Amundson *et al*, 1998). When p53 is phosphorylated by ATM its stability is increased, as the MDM2-p53 complex formations are inhibited. Once p53 is dephosphorylated the ATM is no longer active, meaning the MDM2 gene can now break down the p53 and the cell cycle can progress to the next stage (Web-Books website, 2005). MDM2 becomes inactive due to the deletion of; i) the amino terminal domain, important for the binding of p53 to MDM2 ii) the ring domain, important for its E3 ligase activity. Mutated forms of the MDM2 gene are found in human tumours, and these forms inhibit the normal MDM2 gene from functioning correctly (Alarcon-Vargas & Ronai, 2002). MDM2 is known also to have oncogenic activity if it is over-expressed. This over expression of MDM2 oncogene results in the loss of p53 regulated cell growth, leading to tumourigenesis which may result in cancer.

When p53 is mutated it can still be phosphorylated and active, but becomes prone to binding with MDM2, meaning that p53 will undergo ubiquitination. Subsequently, p53 levels decrease resulting in severe proliferation of cells that are genetically instable (Cooper 1995, pg 150).

p53 therefore regulates a number of genes that are crucial for controlling cell proliferation. Each gene that p53 transcribes has an important role in either activating the gene, or helping p53 to carry out its function by inhibiting proteins which inhibit p53 itself. If p53 were to be mutated or missing, as is the case in most human cancers, there is a very high chance the cell will become malignant. Apoptosis removes cells that have no function or are hazardous to the body, but this cannot occur in the absence of p53, leading to the formation of tumours. These tumours will eventually spread throughout the body, causing different types of secondary cancers

7.4: The activation of p53

Activation of p53 is cryptic and involves many alterations in the protein, such as phosphorylation and acetylation of domains in the p53 protein. The phosphorylation of the C-terminal domain and N-terminal serine residues are important for the activation of p53. Additions of acetyl groups to the lysine residues of the C-terminal domain are also necessary to induce activity in p53 (Oregon State University website, 2004).

7.4.1: The activation of p53 by the Phosphorylation of the N-terminal domain

The N-terminal is responsible for: interacting with the basal transcriptional machinery, transcription factors such as the TATA box binding protein (TBP) (Horikoshi *et al*, 1995 cited from Ko & Prives, 1996) and other TBP- associated factors (TAFs) (Ko & Prives, 1996). This domain is the site for the binding of MDM2, and once this domain is phosphorylated it activates the p53 protein. There have been several serine phosphorylation sites located at the N-terminal domain in murine p53, such as 7, 9, 18, and 37 (Peters & Vousden, 1997, pg 263, Wang & Eckhart, 1992). In primates, the serine residues located at positions 9, 15, 20, and/or 37 are phosphorylated to activate the p53 (Peters & Vousden, 1997, pg 263). Lee-Miller *et al* (1992) found that serine residues of human p53 and serine residues in murine p53, are phosphorylated by DNA protein Kinase (DNA-PK- a serine or threonine protein kinase) in vitro (Lees-Miller *et al*, 1992). They concluded that phosphorylation of p53 only occurs when both the p53 and DNA-PK are bound to DNA (Lees-Miller *et al*, 1992).

7.4.2: The activation of p53 by the phosphorylation of the C-terminal domain

The C-terminal is involved in controlling activity of the sequence-specific DNA binding domain of the p53 protein (Muller-Tiemann *et al*, 1998). The C-terminal domain must be modified by posttranslational modification in order to induce specific DNA binding activity of the p53 core domain (Muller-Tiemann *et al*, 1998, Pospisilova *et al*, 2004). DNA binding to p53 is an important function as it seizes cell growth and therefore inhibits tumour formation (Hupp *et al*, 1992). P53 is able to have two conformations; it can be activated, meaning it is able to bind sequence-specific DNA, or it can be in a latent phase, meaning it is present but inactive, hence not able to bind DNA (Pospisilova *et al*, 2004).

The core domain is the DNA-specific binding site, which is controlled by the C-terminal non specific-DNA binding site.

Phosphorylation of the C-terminal is essential as it neutralizes the destabilizing effect on the folding of the sequence-specific DNA binding domain, therefore the folding of the core domain is stabilized (Bell *et al*, 2002 cited from Pospisilova *et al*, 2004).

Phosphorylation of the C-terminal domain by the Casein Kinase II (CKII), protein kinase C (PKC), and an antibody called PAb421 (C-terminal monoclonal antibody) (Hupp & Lane, 1994, Kapoor & Lozano, 1998, and Pospilisova *et al*, 2000) causes this neutralising effect, which inhibits the unfolding of the sequence-specific DNA binding domain (Nicholas & Mathews, 2002 cited from Pospisilova *et al*, 2004).

The phosphorylation of the C-terminal serine residue can aid in the activation of p53 protein, as it changes the conformational structure in such a way that it can bind DNA readily (Kapoor & Lozano, 1998). There are two serine residues in murine p53: 315

and 389, and two in the human p53: 315 and 392, located at the C-terminal domains in p53 of both species (Peters & Vousden, 1997 pg 264, Milne *et al*, 1992).

Meek *et al* (1990) conducted an experiment using murine p53, in which they found Casein kinase II phosphorylates the serine residue 389 of the p53 protein. Milne *et al* (1992) carried out an experiment using murine p53, in an attempt to understand the significance of the phosphorylation of serine residue 389 by casein kinase II. Whilst carrying out this experiment they found mutations at this site may lead to loss of growth suppressor functions of p53 (Milne *et al*, 1992). In contrast, Rolley and Milner (1994), again using murine p53, conducted a similar experiment but replaced serine residue 389 with either aspartic acid (shown to prevent loss of p53 suppressor function) or with alanine (which cannot be phosphorylated and therefore eradicates p53 suppressor function). They found that the mutated forms of p53 (p53ala389 and p53asp389) were undistinguishable from the normal p53. However, they found that both mutant forms did not function normally; p53ala389 had lost its suppressor function but was still able to bind DNA normally. From this they concluded that phosphorylation by casein kinase II is important in order to induce sequence-specific DNA binding by p53, which in turn activates its growth suppressor function (Rolley & Milner, 1994).

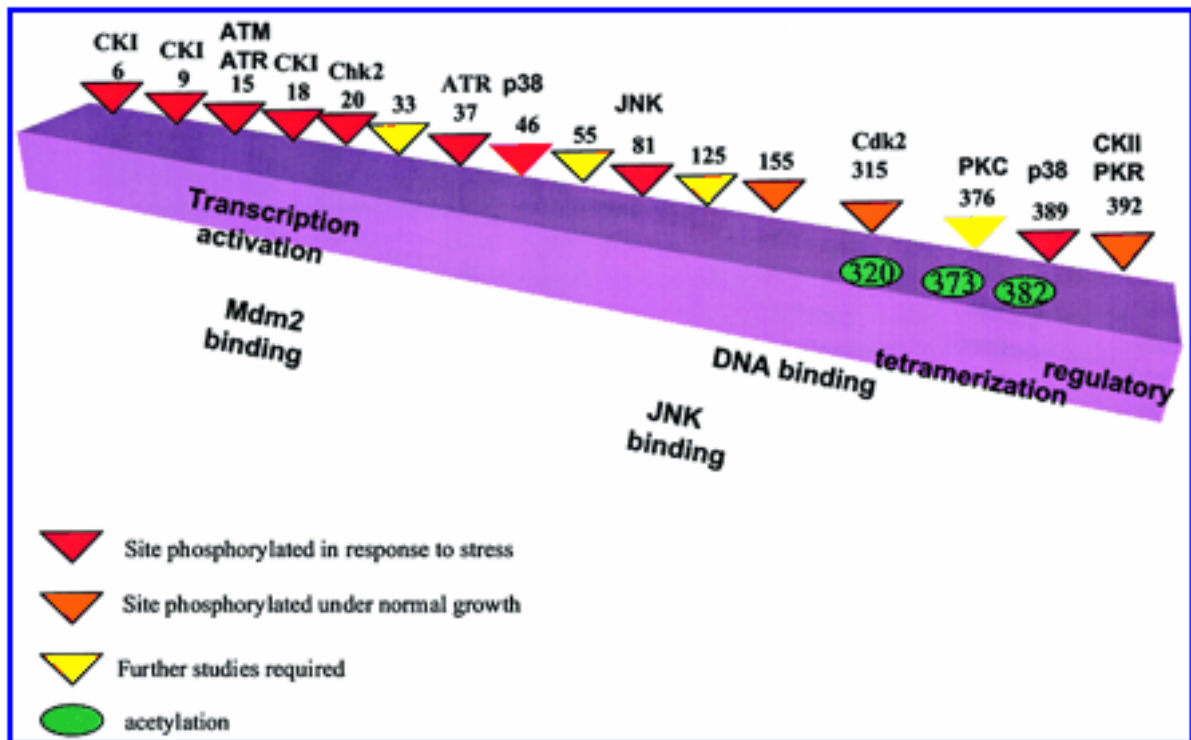


Figure 14: Illustrates the different phosphorylation sites on the p53

Adapted from Alarcon-Vargas & Ronai, (2002)

<http://carcin.oupjournals.org/cgi/content/full/23/4/541/F2>

7.4.3: p53 activation by acetylation.

Acetylation of p53 also occurs at the C-terminal domain, suggesting that it too induces sequence specific DNA binding activity of p53 (Lakin & Jackson, 1999).

Histone acetyl-transferase (HAT) enzymes such as CBP/p300 and PCAF are responsible for the acetylation of lysine residues 320, 373 and 382 (Lakin & Jackson, 1999, Pospisilova *et al*, 2004). Acetylation of p53 causes a conformational change in the protein, inducing sequence specific DNA binding activity (Brooks & Gu, 2003).

Acetylation of p53 is induced after genotoxic stress which activates and stabilizes p53. MDM2 can no longer bind to p53 once it has been activated by posttranslational modifications, resulting in an accumulation of p53 in the cell (Jin *et al*, 2002).

However, it has been found that acetylation of the tumour suppressor gene is inhibited

by MDM2. MDM2 was shown to combine with p300 resulting in inhibition of p53 acetylation, therefore aiding MDM2 in the degradation process of p53 (Kobet *et al*, 2000, Jin *et al*, 2002). Wang *et al* in 2004 found that MDM2 can also be acetylated by p300 and CBP which deactivates MDM2. Therefore p53 is no longer degraded, retaining its transcriptional activity (Wang *et al*, 2004).

Phosphorylation and acetylation play a crucial role in the activation and de-activation of p53 (Lakin & Jackson, 1999, Kobet *et al*, 2000, Wang *et al*, 2004).

7.5: p53 in human cancers

The tumour suppressor gene p53 is found mutated in 50% of human cancers (Yarnold, Stratton & McMillan, 1996, pg 93). Most mutations (more than 75%) that occur in p53 are missense mutations (i.e. base substitution which changes the whole polypeptide), occurring at different stages in different tumours. For example, in colon cancer p53 mutation can occur any time between adenoma to a carcinoma (Yarnold, Stratton & McMillan, 1996, pg 93). In colon cancers, up to 75-80% of the time both p53 alleles are lost, usually the first by deletion and the second by point mutation (Levine *et al*, 1991).

Somatic mutations are most common in human cancers, with up to 50% having somatic mutation in p53. These somatic mutations are known as base substitution (Sigal & Rotter, 2000, Kato *et al*, 2003). Missense mutations cause a change in the DNA binding sequence specific domain of p53, therefore it can no longer bind DNA (Sigal & Rotter, 2000). Mutated forms of p53 have oncogenic effects on normal p53, inhibiting regular functions such as growth arrest, apoptosis and genomic stability (Sigal & Rotter, 2000), therefore increasing the risk of tumour development.

Occasionally, p53 has been found to function adequately yet cancers still appear. This is found in many breast, colon and neuroblastoma cancers, where p53 is not

mutated but does not function properly (Macdonald & Ford, 1997, pg 121). Moll *et al* (1992) have explained that breast cancer appearing in the presence of normal p53 is due to the fact that p53 was found mostly in the cytoplasm in these cases, where it is unable to function as a transcriptional activator (Moll *et al*, 1992). p53 has a nuclear localisation sequence, to which a nuclear transport factor (importin alpha) binds, allowing p53 to be imported to the nucleus, where it can function normally (Kim *et al*, 2000). Kim *et al* (2000) have demonstrated that when importin alpha is mutated or missing p53 does not get imported to the nucleus (Kim *et al*, 2000). Li-Fraumeni syndrome (LFS) is a genetic disease often occurs due to a germline mutation in the p53 protein, followed by deletion or somatic mutation in the normal p53 allele. Individuals suffering from this disease develop cancer at very young age (Schneider & Li, 2004). Donehower *et al* (1992) carried experiment on mice, in which p53 null mice developed normally but were prone to development tumours.

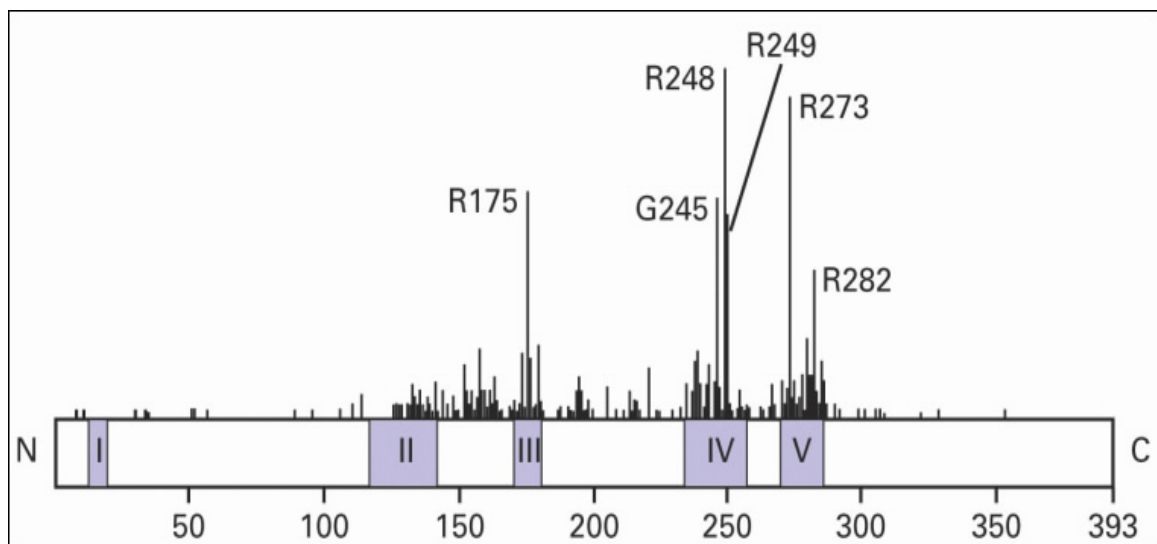


Figure 15: Illustrates the distribution of mutations on the p53 phosphoprotein

Taken and modified from <http://bcs.whfreeman.com/lodish5e/default.asp>

Most mutations are located between 130 and 290 amino acid residues of the p53 phosphoprotein (Levine *et al*, 1991).

Mutations occur at three particular amino acid residues more frequently than at other residues of the protein; these three residues are referred to as “hot spots”. In Figure 15; arrows indicate the three residues; the vertical bars represent the number of mutations which have occurred at each residue; the horizontal bar represents all 393 amino acid residues of p53 protein (Yarnold *et al*, 1996 pg 93, Levine *et al*, 1991).

8.0: Conclusion

p53 is regarded as the “Guardian of the Genome”, suggesting the importance of this gene. However, in many cases this guardian is non-functional, explaining why cells within the body lose their ability to respond normally to cellular processes, causing mutations and ultimately malignancy. In many cancers this gene cannot function properly for various reasons, some of which have been discussed above. Scientists and cancer researchers are now using gene therapy to treat cancer, by replacing the p53 gene in affected individuals.

Section C

9.0: Introduction

The aim of the practical component is to design a scientific journal that focuses on tumour suppressor genes (TSG). The design includes a discussion of scientific and intellectual direction of the journal, instructions to authors wishing to submit papers for publication, and criteria for submission of scientific articles.

To begin with the design of the journal, my primary aim was to gather as much information as possible from existing journals. These journals had instructions to authors, submission criteria and they had different covers which guided me when designing the covers of the journal. Advice and recommendations from publishers and authors gave me a greater insight into the procedures of design. Letters were written to both the authors and publishers as part of my market research (discussed later).

Scientific journals provide scientists all over the world with an opportunity to publish and communicate to the scientific community and the general public their intellectual and professional research ideas. The title of my journal will be 'Journal of Tumour Suppressor Genes', (T.S.G) the logo being 'T.S.G'. This journal will provide comprehensive information about tumour suppressor genes and their functions. The aim was to design a journal with a broad scope but also retaining focus on tumour suppressor genes. It is important that scientists publish their work as this helps promote recognition within their specific sector of research by other scientists (36).

Journals are extensively used by scientists, as they provide the means to find other literature that is related to, or similar to their field of study. Using journals saves a lot

of time for scientists as they can refer back to work that has already been carried out which can assist with their study. Many journals publish work which is original and has not been published in any other journals. My aim is to publish papers that are original and of a high standard. This includes papers that have been continued from previous research but have not met the appropriate criteria for publication. This gives an opportunity for potential authors as well as established authors to publish their work in T.S.G.

Many cancer journals have already been established, and many of these contain published and recognised research in the field of TSGs. Introducing a journal that predominantly focuses on tumour suppressor genes may attract authors wanting to submit papers for publication in this area as well as other relating factors involved in cancer. Many cancer research journals publish research on TSGs as well as other aspects of cancer related topics, therefore the subject of TSGs may remain hidden and undervalued. Due to this, the importance of tumour suppressor genes and their function in normal cellular growth control may be underestimated. When introducing a new journal for publication I expect the final format to be fresh, innovative, informative, sleek, understandable and approachable, with an appeal to all. I believe that T.S.G will fill a niche within the market as it is not only specific to tumour suppressor genes but also relates to cancer.

My intentions are to design a journal that publishes a wide range of the latest research carried out on TSGs. I anticipate that it will be well recognised within the community of scientists as well as on a global level. I hope to design an academic journal which will assist in teaching students and enable research scientists who may wish to share their findings with others to do so. The journal may be used by individuals from all

backgrounds, and does not necessarily cater for scientific professionals therefore increasing the prospective audience.

When a new journal is published it is normal for it not to have an impact factor due to the fact that it does not have an existing reputation. An impact factor of a journal shows how many times an article in that journal has been cited by authors in other journals. It shows the importance and the impact that the journal has in the research field around the world. Impact factors aid authors in decision making, for example, if more than one journal publishes relevant research of interest to them the impact factor is taken into consideration when publishing. I.F's (impact factors) are determined from the analysis of many different variables including how many people buy, copy, cite, access (internet), and distribute it. Obtaining these statistics show how successful the journal is and one can predict how successful it will be in the future.

Many journals have an international editorial board composed of scientists from all over the world. This is important as the editorial board comprises the individuals who decide what to publish and what not to publish in each journal. I believe that having an international editorial board will increase the audience and assist in promoting collaborative international research.

Many papers that are published in prestigious journals are peer reviewed. The peer review involves scientists in a similar field accrediting and justifying the research before publication. A submitted paper is checked for false information, plagiarism and also for very simple mistakes such as grammar and punctuation. The papers to be published in T.S.G will also be subjected to peer review ensuring that the submissions are of the highest standards and of excellent quality. There are certain stipulations a

submission is required to meet with regards to the final edited format. These conditions ensure that work submitted is compatible with the overall image of T.S.G. The editors are responsible for accepting or rejecting the paper once it has been proof read.

Due to inexperience I found myself looking towards established journals to provide an insight as to how to produce T.S.G. All journals have instructions to authors; in order to publish in the journal the authors must follow these instructions. Most instructions also give ideas about the types of papers that can be published in a journal. I decided to design a journal that publishes a variety of different types of papers such as reviews, editorials, letter to editors, short communications and full papers.

T.S.G will be available online as this would make it more accessible for authors. It is time efficient, reliable and it speeds up the reviewing process involved before publication. Authors can send papers at any time and from anywhere in the world as it can be accessed via the internet. Also authors don't have to wait a long time before they get a response from the journal, as this will be done via email and it can be checked at anytime. International authors would have an opportunity to submit papers online for publication, and the journal will be available to everyone who wishes to learn about TSGs and their functions. This increases the audience and the impact of the journal as it will be read by scientists as well as the general population. I have produced a website which can help envision how the overall layout of the final webpage will look.

One major factor that was considered when designing this journal was the publisher. The publisher must be established and well renowned so that in turn T.S.G can be

identified by association. It is important that the publishing company is considered as each publishing company prefers to have certain layouts for each of their journals. They have instructions for editors who wish to propose new journals which are similar to the instructions to authors wishing to submit papers for publication in a journal. As part of my market research I have contacted several publishers around the UK asking for their advice on how to publish a journal.

9.1: Methodology

In order to design the journal I carried out market research, which involved writing emails to authors and letters to publishers. In order to find out information on what criteria need to be met for a new journal launch, letters were written to sixteen publishers (addresses in appendix 1). There were several questions asked to provide me with sufficient information needed for the publication (letter found in appendix 1). Due to lack of replies I was unable to receive direct information on how to go about introducing this journal. However I received letters from publishers telling me to visit their websites, and some just refused to help (replies found in appendix 3). It was very disappointing that many of these publishers were not willing to share any form of information which could help me with this project. Most of the websites however had instructions to authors, which helped me put together information on the different types of papers and an idea of how to design my own instructions to authors. I was also able to decide whether the journal was going to be available on paper, online or both. By introducing my journal online and on paper, individuals are made more aware of the existence of such a journal. Having a journal available online also increases its recognition. I received a reply from Macmillan Publishers Ltd, however they were not able to help me at all. This was very disappointing as they are a large firm yet they were unable to give me any directions on how to introduce the journal of TSG. At the same time it is understandable that they would not have time to answer my request as they themselves are a notoriously busy company. However I was able to get information from other journal websites on the types of paper each journal publishes, the aim and scope of the journals, instruction to authors and the submission criteria. All of these have assisted me in designing the journal.

Also as part of my market research emails were also sent out to authors, to find out their recommendations about the journal (email letter found in appendix 1). Emails were sent out to 50 different authors, asking them whether they would be interested in publishing in the journal, and the factors they look for in a journal before they decide which journal to publish in. Many of the replies that I received had some similar points, such as the impact factors, the print qualities of images, the prospective readers of the journal and whether the paper covers relevant research suitable for the journal to publish.

9.2: Results and Analysis

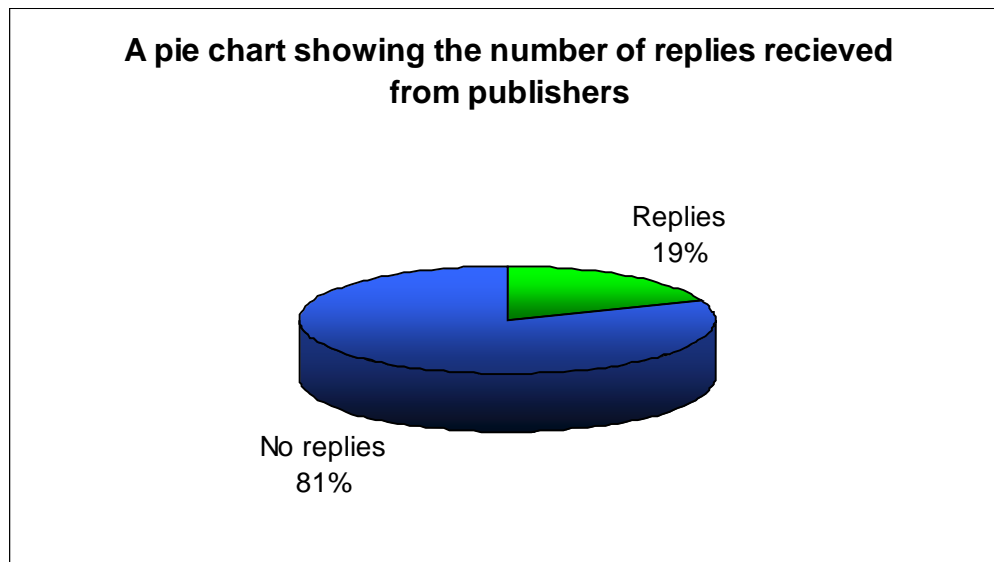
All replies that were received were analysed and the results were plotted in the form of a graph, the data is shown below.

9.2.1: Analysis of data received from publishers

Table 5: Represents replies received from publishers

| Number of Responses | |
|---------------------|------------|
| Replies | No Replies |
| 3 | 13 |

Pie chart 1: Illustrates the percentage of responses received from publishers



Out of sixteen publishers, three replies were received. As a result of this it became very difficult to interpret the results. Replies that were received were of very little help, as they did not have the time to sufficiently answer all my questions. Two of the three responses told me to visit their websites and I was informed that I would find answers to my question on there. However when the websites were visited I was able to collect little information with regards to what is required in a new journal proposal from a publisher. The main website that all this information was collected came from

the Taylor and Francis group (<http://www.tandf.co.uk/journals/publish.asp#proposal>).

A letter from Blackwell publishers (appendix 3) also gave me an insight into what they expect of a journal proposal.

9.2.2: Analysis of data received from Authors

Table 6: Represents replies received from authors

| Number of Responses | |
|---------------------|------------|
| Replies | No Replies |
| 9 | 42 |

Pie chart 2: Illustrates the percentage of responses received from authors

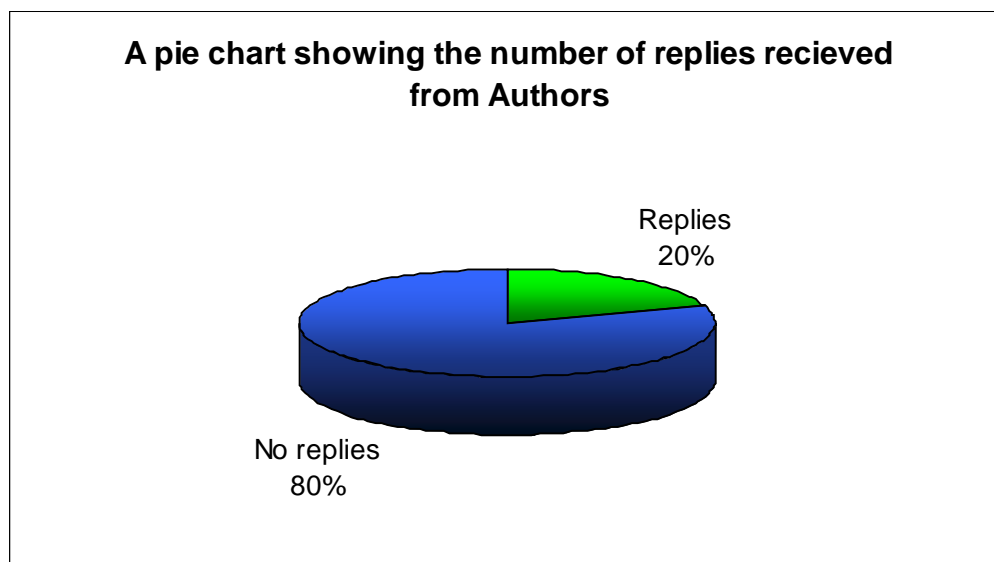
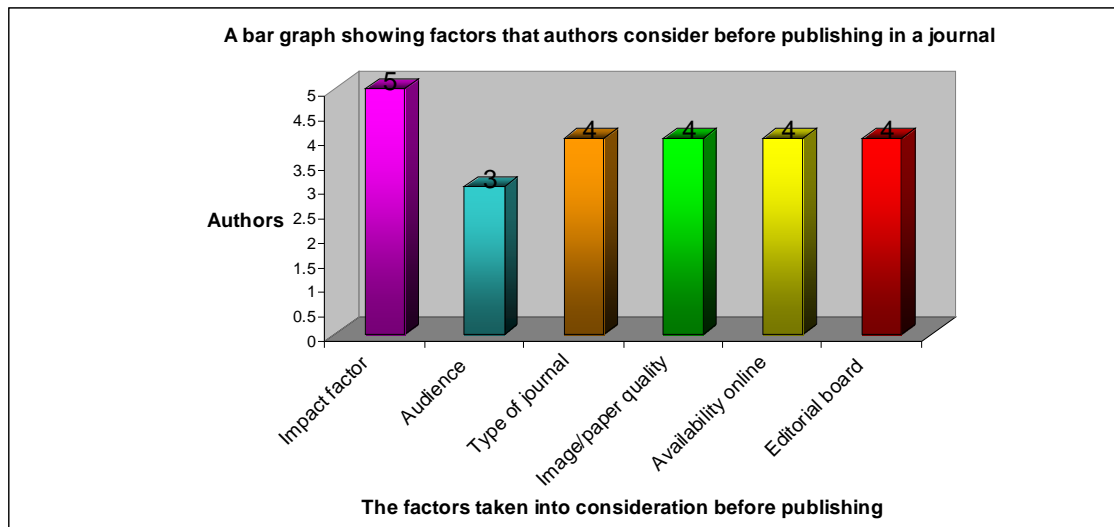


Table 7: Represents data for graph 1

| Impact factor | Audience | Type of journal | Image/paper quality | Availability online | Editorial board |
|---------------|----------|-----------------|---------------------|---------------------|-----------------|
| 5 | 3 | 4 | 4 | 4 | 4 |

Graph 1: Illustrates the points that are considered when selecting a journal for publication

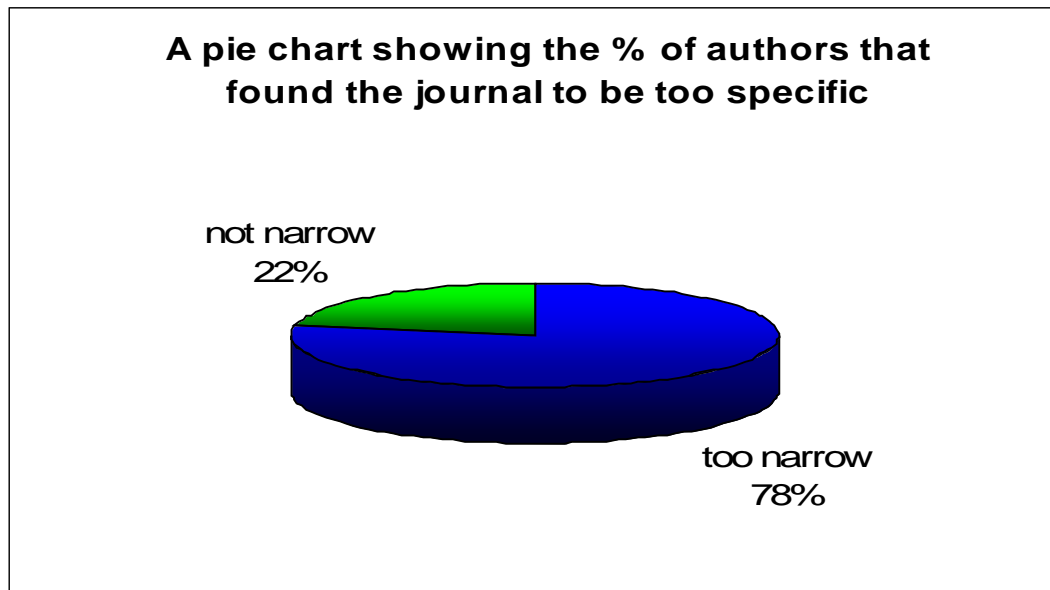


For the second part of the market research emails were sent out to authors. Out of fifty-one emails that were sent I received nine replies, this was a little disappointing but the replies achieved were very helpful. Pie chart 2 shows that 20% of the authors replied and 80% did not. With such a small number of replies it was very hard to interpret the results. However I was able to draw up a graph (graph 1) which represents points that authors consider before publishing in a journal. Many authors consider the impact factor of a journal before publishing within it. Many authors also considered the editorial board important for a new journal. If the editorial board consists of advanced scientific researchers with good reputations, authors are confident in publishing in the journal.

Table 8: Represents the data for pie chart 3

| Journal specificity | |
|---------------------|------------|
| Too Narrow | Not Narrow |
| 7 | 2 |

Pie chart 3: Illustrates the percentage of authors who recommend that this journal may be too specific



From the results obtained it was found that the authors thought that this journal was narrow and too specific to TSG. One of the authors who replied argued that the impact factor of the journal will not increase because of its specificity to the subject of tumour suppressors.

Over all the results that were obtained were not sufficient enough as I received a very small number of replies. Therefore due to this the journal design was done using advice from a sample of authors rather than the whole community of authors. A way in which this could have been improved is by having a larger sample for both authors and publishers.

9.3: Method of designing

In order to design the journal, I had to visit some websites of existing and well established journals, as I did not get the results I hoped for from my market research. Most of the replies received from the authors, all had similar answers. I was able to take in to account most of the points that authors consider before they publish in a journal. The journal is called 'Journal of Tumour Suppressor Genes', from the title it can be gathered that this journal is aimed to focus primarily on TSGs. In order to maintain a broad scope whilst retaining the focus on TSGs, I designed a journal that covers most recent research in TSGs. However as most research related to TSGs is interlinked with cancer, it would be difficult not to publish articles related to cancer. The radial diagram represents (figure 16) the topics that will be covered in this journal and how the other topics are related to tumour suppressors.

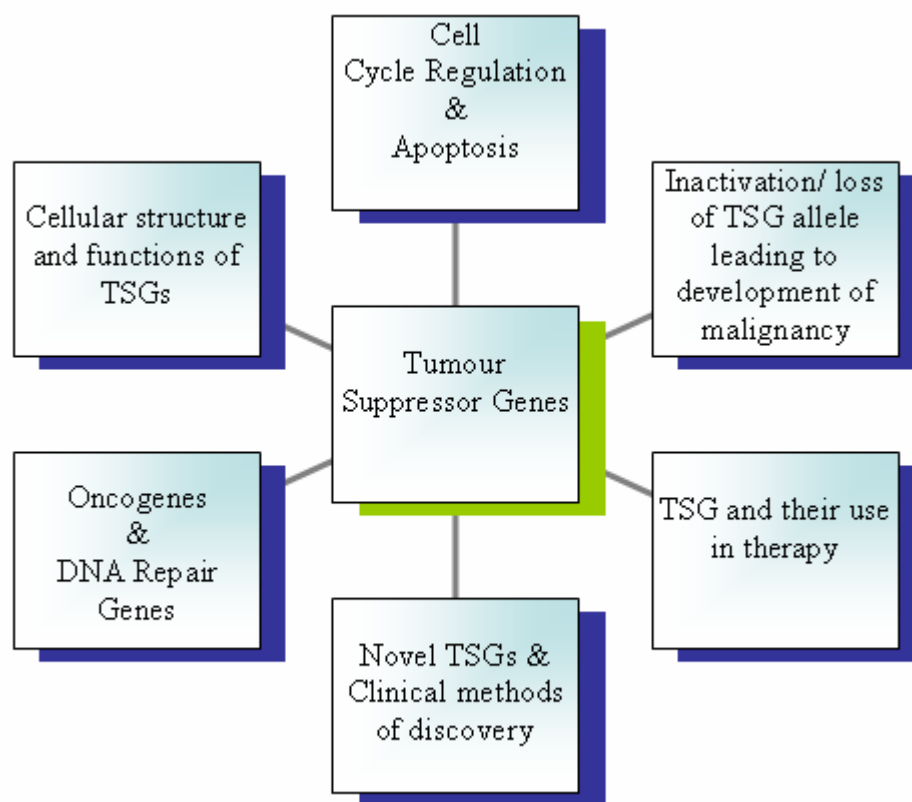
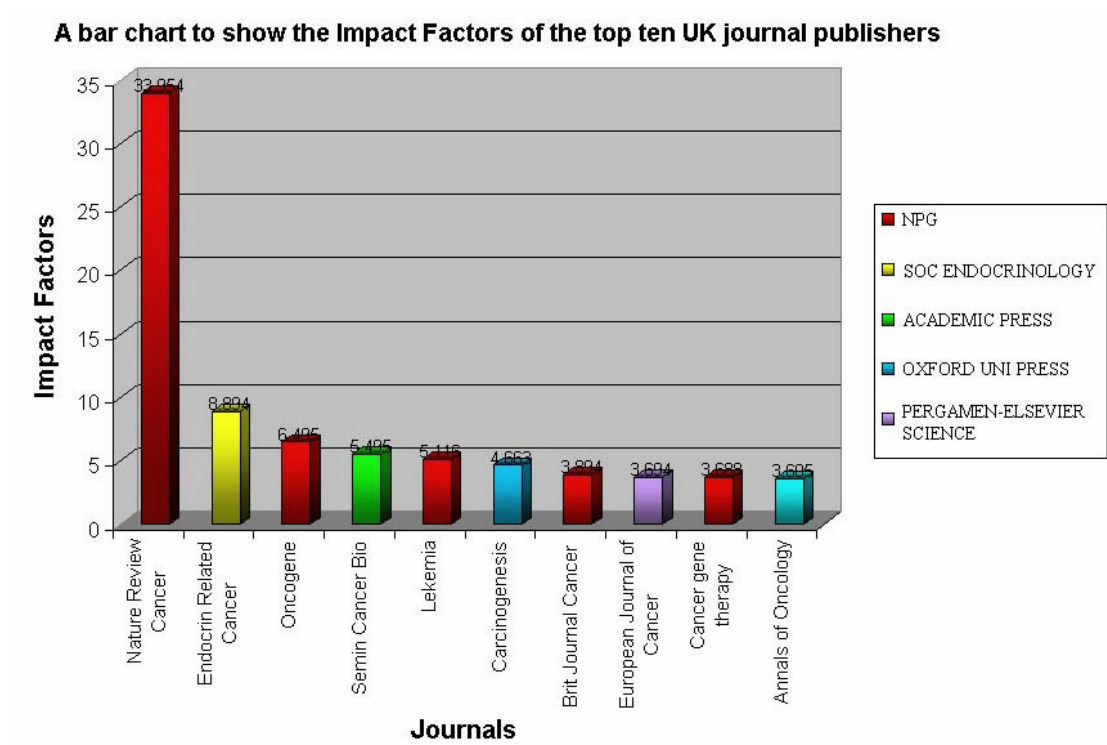


Figure 16: Illustrates a radial diagram of the aspects that are related to tumour suppressor genes.

As mentioned in the literature review TSGs play a pivotal role in cancer when both alleles of TSG are inactivated. TSGs are important in the control of cell cycle regulation and when these genes no longer function properly the cell continues to proliferate abnormally. This abnormal growth eventually produces tumours. Tumour suppressor genes are involved in regulating other genes which are responsible for apoptosis and DNA repair. This goes to show that TSGs are involved in controlling many biological functions within the body. Therefore it is possible to have a journal that focuses on TSG but does not limit us from investigating new advances and avenues of other research in relation to TSG. Although the journal relates to a very specific field of research, the scope of study is very large. Undergoing research constantly brings forth new information which enables us to have a better understanding of TSGs, further substantiating the need for my journal.

The highest impact factor of a journal in the subject field of oncology was achieved by Nature Review Cancer (NPG) of 33.954 (Web of Knowledge). Nature Review Cancer is published by Nature Publishing Group and it is not the only prestigious journal that is published by this company. The graph 2 shows the top ten oncology journals with the highest impact factors in the UK. As you can see by the bars displayed in red, out of the top ten impact factors for journals produced in the UK, NPG has 50% of the market share. NPG has the highest impact factor for its journal titled Nature Review Cancer, this also has the highest impact factor in the oncology field worldwide. This fact made me decide to publish my journal with NPG.

Graph 2: Illustrates the IF of the top ten Publishers



The impact factor of this journal will be non-existent as it is new and it would take time before research scientists and the general public become aware of it. Many journals don't have an impact factor to begin with, but, it may increase depending on the impact of the journal. As a result of these findings I am confident that publishing my journal with this publisher would give my journal a good head start into becoming a popular resource amongst scientists in the research field of oncology. Many established journals exist in the USA such as CA. A cancer journal for clinicians (IF = 33.056), Cancer Cell (IF = 18.913), Journal of National Cancer Institute (IF = 13.844) and there are several more. This goes to show that many prestigious journals exist in the U.S.A. private funding may be a contributing factor as to why this is so.

Many of these existing cancer journals are very well established and in order to publish within them the papers must be novel, spectacular and of great interest. Articles are rejected more frequently in prestigious journals. It has come to my

attention that the 'Oncogene' journal implies that the subject matter is very limited. This however is not the case as developmental research in this field is still ongoing (IF=6.495). From this I can realistically determine the need and relevance, drawing data to compare to my proposed journal. As oncogenes and tumour suppressor genes are linked the choice for comparison becomes valid.

As mentioned before there is currently little communication between researchers in this very specific field and the general public. The aim of my journal is to dedicate resources to help establish this field which inevitably creates more developmental research projects.

A common trait from all the responses I received advised me to invest in a governing editorial board. For many authors the editorial board decided whether they approached a journal. It seemed that boards with esteemed scientists and researchers instilled confidence. To design an editorial board of esteemed scientists, I referred to established journals and randomly selected existing editors, this is due to me not having enough knowledge of scientists who research in the field of oncology at present.

The publisher I chose for this journal is Nature Publishing Group as this publisher is at the forefront of many major scientific researches in the modern world. Macmillan Publishers Ltd is responsible for controlling the Nature Publishing Group.

Due to its reputable name and with it being recognised world wide, having a journal published by this company will be a great way of introducing it. As Nature Publishing Group is such a prestigious company and Macmillan Publishers Ltd publishes journals in more than 70 different countries making it easier for others internationally to know of this journal. This should help increase the number of scientists wishing to publish in this journal.

Again a great emphasis will be placed on online availability. This cannot be stressed enough as it proves to be the most effective and efficient method of communication to and from the editors of the journal. Particular attention will be paid to the ergonomics of the interface design, allowing people with all levels of IT skills to successfully navigate the browser. My results clearly show that authors prefer having an online journal, as this will be more time efficient and a great way of speeding up the reviewing process.

Preliminary designs were influenced by current established journals. However, much effort has been put into user specifications, since the whole format has evolved into a creative, contemporary, more user friendly concept.

The front covers that have been designed show the various ways that TSGs are involved in biological functions. Each was designed using Microsoft Publisher, Microsoft Power Point and Microsoft Paint. Two of the cover designs were drawn using various graphics software to show an overall idea on how p53 functions from my literature review and the other two designs were put forward by using various internet websites as guides.

In order to design my instructions to authors I have used many well known journals as a guide. I have chosen to publish many different types of papers i.e. rapid communications, full papers and reviews, as this will make my journal more approachable by both new and upcoming authors as well as experienced ones.

My market research showed me that online journal publishing was a highly successful and efficient way of getting an article in a journal published, therefore I decided that I would make my submission criteria and instructions to authors available online. To do this I learnt how to use Microsoft FrontPage, in which I produced the general template for my webpage. On my webpage you can see that I have my buttons

located on the left hand side. I felt that this would be the easiest way for people to navigate around my site. I have included a link to NPG to give the reader an idea of what the publishers do. There is also a facility to upload an article for the editors to look at and peer review. Although this functionality does not work I have provided a general concept of what it may look like. The website also shows my aims and the scope and my editorial board. I have enclosed my website on my disc along with an electronic copy of my dissertation.

9.4: Discussion

To conclude I feel that there is a great need for a specific journal based on TSGs. By producing journal T.S.G I will provide concise, relevant information in this subject field. One persistent obstacle I faced was the lack of response from both publisher and authors. This limitation meant that much of the information used to design the T.S.G journal was obtained from already established journals. I was able to obtain information on instructions to authors and submission criteria; these are required from all editors of different journals as this information is readily available online.

Many journals that were used to inspire me are published by Nature Publishing Group. I believe that if this journal was to be proposed, Nature Publishing Group would be interested, providing that the journal publishes papers of high interest and of satisfying levels. However I am unsure if this will be true as this journal has not actually been proposed. If I had more time, I would have liked to send this design to NPG and ask them for their opinions about the journal. Following this I may have been able to use their ideas to improve this journal design. Therefore, it is quite difficult to say how successful this journal would become in the future.

It has come to my attention that the journal *Oncogene* published by NPG, is a very specific journal which focuses on specific research about oncogenes and other related aspects of oncogenes. Journal T.S.G is also very specific as it focuses on research regarding TSGs and topics related to them (Figure16). By drawing comparisons from *Oncogene*, I can predict that my journal will have a similar impact upon the scientific community. With the correct publisher and through intellectual marketing, I envision that T.S.G would be successful. Although TSGs are investigated in many oncology journals, my journal focuses solely on TSGs. This means that even though other journals may see T.S.G in direct competition with themselves, this would not actually

be the case as TSGs make up a small percentage of their primary research focus. There are no journals devoted to just TSG research but this does not imply that there is no interest in the field TSGs. TSGs are notoriously difficult to identify and this may be a factor as to why oncogenes are much more widely researched. By introducing this journal I hope to increase awareness of how TSGs work and their importance in cancer research.

Overall from this I have learned that designing a journal takes a lot of time and effort. In order to produce a prestigious journal it requires many intermediate steps, all of which involve different individuals playing a crucial role. Chief Editors are involved in assigning these steps to the editorial board. To create my editorial board I randomly selected scientists from existing well esteemed journals. There was insufficient information as to how to create an editorial board which is why my selection was completely random.

In order to design the covers for the journal a lot of time was spent trying to learn how to use various types of software applications such as Adobe Photoshop and Microsoft Publisher. With more experience I feel I could have submitted more cover options which could have provided more insight. Much work was focused to try and create a genuine prototype of my design. Another problem I encountered was learning how to use Microsoft FrontPage in order to design my webpage, for the journal.

When designing the scope of the journal my knowledge of the p53 TSG and cancer was of a great help, however I still had to refer back to other journals. I personally believe that the topic of TSGs is not narrow as there are several aspects that can be covered involving these genes.

Research related to TSG will always be carried out in the future as these genes have several cellular functions within the body. TSG functions are crucial aspects for life, as they inhibit the cell growth, proliferation, and transcribe genes required to repair DNA damage. However in a majority of cancers these genes are non-functional, this is a major factor for substantial critical research.

To conclude, by introducing a journal that is truly devoted to TSGs we provide a great advantage to authors who solely research into TSG. Having their work published in T.S.G will increase the recognition and importance of their research. The journal will also increase awareness of TSGs within the community of professionals, students and the general public.

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