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Tumor Suppressor Genes

Donia Gamudi and Renald Blundell
Department of Physiology and Biochemistry,
University of Malta, Msida, Malta

Abstract: Tumour suppressor genes are a class of genes that have a crucial role in the genesis of neoplasia. These genes when transcribed and translated, result in the production of proteins that regulate the cell cycle, repair any DNA mutations and control apoptosis. When tumour suppressor genes are mutated, cells lose control and this leads to neoplastic growth. A good analogy is comparing a tumour suppressor gene with the brake pedal of a car. The tumour suppressor gene prevents the cell from dividing too quickly just as a brake keeps the car from going too fast.

Key words: Tumour suppressor genes, neoplasia, cell cycle, proteins, DNA mutations, neoplastic growth

INTRODUCTION

Identification of the genes that are mutated in the genesis of neoplasia is mandatory. Its only when one understands the aetiology of a disease that prevention and the best treatment can be provided. The two main classes of genes that have been implicated in the pathogenesis of neoplasia are the tumour suppressor genes and the oncogenes. The oncogenes were initially identified as genes in the viral DNA that cause transformation of their target cells. However, a major class of viral oncogenes have cellular counterparts that are involved in normal cell function. These cellular genes are called proto-oncogenes and when mutated they are known as oncogenes. The conversion of proto oncogenes into oncogenes portrays an example of gain of function mutation (Mueller and Young, 2002). The products of these proto-oncogenes are crucial for the ordered progression through the cell cycle. Their vital role is reflected in the fact that they have been highly conserved throughout evolution. The mechanisms that are involved in the activation of the proto-oncogenes into oncogenes are various. These include either point mutations in the proto-onocgene or else a duplication that results in multiple copies of the same gene or else a translocation. Contrary to the tumour suppressor genes, mutation in only one of the alleles is enough to induce neoplastic growth (Lodish, 2000). The second class of genes that are involved in neoplasia formation are the tumour suppressor genes. The role of the tumour suppressor genes in tumour formation was originally proposed in the 1960's when it was suggested that some forms of hereditary cancer might be initiated when a cell in a person heterozygous for a germ line mutation undergoes a second somatic mutation. This renders the cell homozygous for loss of function mutations in a tumour suppressor gene and this gives rise to a tumour. This is known as Knudson's two hit model of tumorigenesis. This is now widely accepted as the basis for both the hereditary and sporadic cancers (Gelehrter *et al*., 2000).

Although, both the tumour suppressor genes as well as the proto-oncogenes are crucial in determining the onset of cancer, genes do not act in vacuum but the action of a particular type of gene is highly dependent on the whole set of the other surrounding genes that constitute the genetic background. Also one has to keep in mind that neoplasia is a complex process. Genetic, environmental, dietary and microbial factors are all closely intertwined with one another and all have a role to play in neoplasia.

TUMOUR SUPPRESSOR GENES AND THEIR ROLE IN NEOPLASIA

The tumour suppressor genes as their name implies are a class of genes that regulate cell proliferation. Various types of tumour suppressor genes exit which can e widely classified into, genes that control cell division, genes that repair DNA and cell suicide genes (Lodish, 2000).

GENES THAT CONTROL CELL DIVISION

Some tumour suppressor genes help control cell growth and division. The Retinoblastoma gene (RB1) gene is an example of such a gene. Abnormalities of the RB1 gene can lead to retinoblastoma which is a type of eye malignancy in infants, as well as to other cancers. Because all the chromosomes are paired, there are always two copies of each gene. The inherited RB1 mutation only

Corresponding Author: Renald Blundell, Department of Physiology and Biochemistry, University of Malta, Msida, Malta

effects one of the genes. Since the person has one good gene and a mutated one, its said that the person is heterozygous for the trait (Tomlinson *et al*., 2001). In this situation, the person does not develop the disease. Then during the infant's development, a random mutation can occur in the normal copy of the RB1 gene. This process is known as loss of heterozygosity. When both genes are mutated, uncontrolled cell proliferation occurs as the products of some tumour suppressor genes like retinoblastoma and p53 restrict cells at the G1-S phase of the cell cycle preventing their entry into the proliferative phase of the cell cycle (Lodish, 2000).

GENES THAT REPAIR DNA

A second group of tumour suppressor genes is responsible for repairing DNA damage. Every time a cell prepares to divide, it must first duplicate its DNA. This process is not perfect and copying errors sometimes occur. Fortunately, cells have DNA repair genes which code for proteins that proofread DNA. But if the genes responsible for the repair are mutated, then the DNA can develop abnormalities that go unnoticed and thus unrepaired (Mueller and Young, 2002). The mutations responsible for HNPCC (Hereditary non polyposis colon cancer) are an example of DNA repair gene defects. Although, DNA repair enzymes do not directly function to inhibit cell proliferation, cells that have lost the ability to repair errors, gaps or broken ends in DNA accumulate mutations in many genes, including those that are critical in controlling cell growth and proliferation. Thus loss of function mutations in the genes encoding DNA repair enzymes promote inactivation of other tumour suppressor genes as well as activation of oncogenes (Lodish, 2000).

CELL SUICIDE GENES

If there is too much damage to a cell's DNA that can't be fixed by the DNA repair genes, the p53 tumour suppressor gene is responsible for destroying the cell by triggering apoptosis. If the p53 gene is mutated, cells with DNA damage that have not been repaired continue to grow and proliferate and can eventually become cancerous (Lewin, 2000). Abnormalities of the p53 gene are sometimes inherited such as in the Li Fraumeni Syndrome (LFS). People with LFS have a higher risk of developing a number of malignancies including soft tissue and bone sarcomas, brain tumours, breast cancer, adrenal gland cancer and leukemia. Many sporadic cancers such as lung, colon and breast also develop following a mutation in the p53 gene (Collins *et al*., 1997).

Tumour suppressor genes like p53 and p21 are also called anti oncogenes. They were first given this name because they reverse at least in cell culture the action of known oncogenes. The main important difference between oncogenes and tumour suppressor genes is that the onocgnes result from the activation of proto oncogenes but tumour suppressor genes lead to neoplasia when they are inactivated. Another major difference between the two is that while the overwhelming majority of oncogenes develop from sporadic mutations of the proto oncogenes during the life of the individual (acquired mutations), abnormalities of the tumour suppressor genes can be inherited as well as acquired (Gelehrter *et al*., 2000). In the following discussion, I will be considering mainly the role of tumour suppressor genes in neoplasia. However, one need to bear in mind that both the tumour suppressor genes as well as the protooncogenes are crucial in determining the onset of cancer. Genes do not act in a vacuum but the action of a particular type of gene highly depends on the whole set of the other surrounding genes that constitute the genetic background. The importance of tumour suppressor gene mutations and their link to cancer was originally proposed in the 1960s. It was suggested that some forms of hereditary cancer might be initiated when a cell in a person heterozygous for a germ line mutation undergoes a second somatic mutation thus rendering the cell homozygous for loss of function mutations in a tumour suppressor genes. This leads to uncontrolled proliferation and tumour formation (Tomlinson *et al*., 2001).

Loss of both alleles of a tumour suppressor gene also plays an important part in the pathogenesis of many common sporadic cancers. In this instance however, both alleles are inactivated by two somatic events occurring in the same cell. This two hit hypothesis was first applied for cancers that can occur both in the hereditary and sporadic forms such as retinoblastoma. But now the two hit hypothesis has been widely accepted as an important model in familial cancers including familial polyposis coli, familial breast cancer, neurofibromatosis type 1 (NF 1), colon carcinoma and the rare form of familial cancer known as lei fraumeni syndrome (Lodish, 2000). Although, in each of these disorders autosomal dominant inheritance of one mutated gene is generally the rule, loss of function of both copies of the responsible tumour suppressor gene is required for tumour development. The explanation of this seeming paradox is that cells heterozygous for a mutation still have a single functional copy of tumour suppressor gene which is enough to prevent abnormal cellular phenotype. However, a cell which already has one copy of the tumour suppressor gene which is altered or lost by inheritance of germline mutation will lose its ability to suppress tumour development if by chance it loses function of the other remaining allele. This second hit which is needed for the development of the tumour most often occurs as a result of a mutation but loss of function without a mutation such as occurs with transcriptional silencing has also been

observed in some cancer cells (Nordling, 1953). Since the development of the sporadic tumors involves two separate somatic events, with the second hit having to occur in the same cell lineage that has experienced the first or predisposing hit, sporadic tumors are relatively rare and occur later on in life. They also have the additional features of being unifocal and unilateral. This contrasts with the hereditary tumours which occur at a younger age and tend to be multifocal (Tomlinson *et al*., 2001). The two hit model is now widely accepted as the basis for both the hereditary and sporadic cancers. As explained previously the theory had to be expanded recently when it was discovered that a second hit in the normal allele is not always necessarily a mutation as it was initially thought. Silencing due to excessive DNA methylation associated with a loss of accessibility of the DNA to the transcription factors has been found to be an important alternative molecular mechanism for loss of function of a tumour suppressor gene (Nordling, 1953).

MECHANISMS INVOLVED IN LOSS OF HETEROZYGOSITY

Most studies that were done that confirmed the concept of loss of heterozygosity involved analysis of the DNA sequences in the region of the chromosome 13 in the peripheral blood as well as analysis of the retinoblastoma tumour material of children who had inherited the gene for retinoblastoma. These studies showed that children who developed retinoblastoma have a loss of an allele at the retinoblastoma locus in the tumour material. This is known as the Loss Of the Constitutional Heterozygosity (LOCH) (Mueller and Young, 2002). So in a somatic cell that contains one mutant and one normal allele of a tumour suppressor gene, how is the normal allele lost or inactivated? Point mutations are an unlikely cause because mutations of this type are usually repaired except in cells that have defective DNA repair mechanisms (Harvey *et al*., 1993). Loss of heterozygosity is better explained by missegregation of the chromosomes bearing the heterozygous tumour suppressor gene during mitosis. In this process which is also referred to as non disjunction, one daughter cell inherits only one normal chromosome (and probably dies) while the other inherits three chromosomes (the other normal chromosome as well as two bearing the mutant allele). Such missegregation is caused by failure of a mitotic checkpoint which would normally prevent a metaphase cell with an abnormal mitotic spindle from completing mitosis. Subsequent loss of one chromosome often occurs, restoring the 2n complement. If the normal chromosome is lost, the resultant cell will contain two copies of the mutant chromosome (Mueller and Young, 2002). Loss of heterozygosity can be caused by still other mechanisms which include a deletion of the chromosome carrying the corresponding allele or a cross over between the two homologous genes leading to homozygosity for the mutant allele.

TYPES OF TUMOUR SUPPRESSOR GENES

After having considered in detail what are tumour suppressor genes and their function its important to deal with the different types of tumour suppressor genes that have been discovered. These include DCC, NF1, NF2, VHL, MLH1 MSH2, MTS1, MTS2 and p53 tumour suppressor genes (Harvey *et al*., 1993). The DCC gene in the 18q region contains a putative tumour suppressor gene, DCC (deleted in colon carcinoma, 18q21). Allelic loss of chromosome 18, together with 17q, occurs in 70% of colorectal carcinomas with high frequency in ovarian adenocarcinomas (Turley *et al*., 1995). The DCC protein is homologous to neural crest adhesion molecules (N-CAM). Its allelic loss or reduced expression is detected in colorectal, gastric, esophageal, pancreatic, breast, haematological and glial malignancies. Loss of DCC gene is associated with alterations in cellular attachment. The DCC gene encodes for a membrane bound protein belonging to the immunoglobulin superfamily. Abnormalities of the DCC tumour suppressor gene and abolished DCC function may be critically involved in the development of gastric cancer. Recent studies found that the DCC gene product induces apoptosis by activating caspase-3. So, DCC may function as a tumour suppressor gene which controls programmed cell death (Turley *et al*., 1995).

The MLH1 MSH2 (Hereditary Non Polyposis Colon Cancer (HNPCC)) gene is defective in colon cancer. Its carried in approximately 1/200 individuals and its responsible for about 15% of all colon cancers. Two HNPCC genes loci have been detected, MSH2 and MLH1. The MSH2 gene is located on chromosome 2p22-21. It encodes a member of the MutS mismatch repair protein superfamily that binds to mismatched nucleotides in DNA. So they are able to prevent mutations during DNA replication. HNPCC patients show germline mutation in MSH2 at intronic splice acceptor site which is presumed to cause aberrant mRNA processing (Bronner *et al*., 1994). The VHL gene is the gene involve in von hippel landau disease which is a dominantly inherited familial cancer syndrome. VHL is located on chromosome 3p25-26 and it encodes a polypeptide of 28 kDa. The cellular location of the product is the plasma membrane. The protein function is not certain but the repeated acidic domain may contribute to VHL involvement in signal transduction or cell cell contacts (Pullman and Bodmer, 1992). One of the most important

tumour suppressor genes is p53. The p53 has been mapped to chromosome 17. In the cell p53 protein binds DNA. It recognises a 10 bp motif. Upon binding it stimulates another gene to produce a protein called p21 that interacts with a cell division-stimulating protein (cdk2). When p21 is complexed with cdk2 the cell cannot pass through to the next stage of cell division (Greenblat *et al*., 1994). Mutant p53 can no longer bind DNA in an effective way and as a consequence the p21 protein is not made available to act as the stop signal for cell division.

So the cells divide uncontrollably and form tumours. Although, as was previously described, tumour suppressor genes are generally viewed as being recessive at the cellular level so that mutation or loss of both tumour suppressor genes is a prerequisite for tumour formation, only one hit is needed for p53 to become tumorigenic. Since inactivation of one of the two suppressor loci can produce what appears to be a dominant effect, its said that p53 shows a dominant negative effect (Nigro, 1989). Two promoters have been demonstrated at the 5 prime end of the human p53 gene. The first is located upstream of the first exon and is responsible for the transcription of the major p53 mRNA species. The second promoter is stronger than the first and is located approximately 1,000 base pairs downstream of exon 4 (Clarke *et al*., 1993). These two promoters are the regulatory sites of the p53 gene.

If the regulatory protein p53 is activated by protein kinases, ATM and ATR (these kinases detect damaged DNA), it activates a CDK inhibitor, p21 (Collins *et al*., 1997). This CDK inhibitor, p21 can bind to and inhibit the cyclin E-CDK2, preventing phosphorylation of the retinoblastoma protein. Unphosphorylated retinoblastoma protein binds to and inactivates E2F blocking passage from G1-S (Clarke *et al*., 1993).

p53 AND ITS ROLE IN APOPTOSIS

The wild type p53 can interact with the TATA binding protein directly and interfere with the initiation of transcription. p53 exerts its tumour suppressor activity by down regulating genes governing proliferation and up regulating genes involved in preventing growth. p53 functions to delay transit from the G1 to S phase of the cell cycle. By delaying the transit time from the G1 to the S phase of the cell cycle and hence the onset of mitosis, p53 reduces the likelihood of incorporating mutated DNA in the next generation. If however DNA is so damaged that repair cannot take place, the cells undergo a p53 assisted process of cell death known as apoptosis. The latter is a specialised form of programmed cell death which leads to the elimination of the cells without releasing harmful substances in the surrounding area. Its characterised by chromosomal DNA fragmentation and cellular dissolution.

p53 can mediate apoptosis by various mechanisms, one of which is mediated by a subfamily of cysteine proteases known as caspases (Greenblat *et al*., 1994). In mammalian cells, a major caspase activation pathway is the cytochrome c initiated pathway. In this pathway, a variety of apoptotic stimuli cause cytochrome c release from the mitochondria which in turn induces a series of biochemical reactions that result in caspase activation and subsequent cell death. Anti apoptotic genes like Bcl-2 and proapoptotic genes like Bax regulate release of cytochrome c. Bax also contains the p53 binding sites in its promoter site and is upregulated in response to DNA damage and increased p53 (Clarke *et al*., 1993).

Another mechanism by which p53 can mediate apoptosis is through the generation of Reactive Oxygen Species (ROS). The tumour suppressor protein p53 is a redox-active transcription factor that organises and directs cellular responses in the face of a variety of stresses that lead to genomic instability. Reactive Oxygen Species (ROS), generated by cells as products or by products, can function either as signalling molecule or as cellular toxicants. Cellular generation of ROS is central to redox signalling. Studies have revealed that each cellular concentration and distribution of p53 has a distinct cellular function and that ROS act as both an upstream signal that triggers p53 activation and a downstream factor that mediates apoptosis (Greenblat *et al*., 1994). The final outcome of p53 activation depends on many factors. A cascade of events is triggered by the activation of p53 which all depend on the downstream effector genes that are activated by p53 (Greenblat *et al*., 1994).

After having considered the main tumour suppressor genes, the function of these proteins can summarise as follows:

- They cause repression of genes that are essential for the continuation of the cell cycle. If these genes are not expressed, the cell cycle will not continue, effectively inhibiting cell division
- Coupling the cell cycle to DNA damage. As long as there is damaged DNA in the cell it should not divide. If the damage can be repaired, the cell cycle can continue. Loss of function mutations in the genes encoding DNA repair enzymes promote inactivation of the of other tumour suppressor genes as well as activation of the oncogenes. If the damage cannot be repaired, the cell should initiate apoptosis or programmed cell death to remove the threat it poses to the individual

- Some of these proteins are involved in cell adhesion, preventing tumour cells from dispersing and so they play a very important role in inhibiting metastasis
- Other tumour suppressor genes have been found to trigger senescence, others induce terminal differentiation and maintain genomic stability (Harris, 1992)

CONCLUSION

Research on cancer is being conducted at a very fast pace. Understanding the pathogenesis of neoplasia at a molecular, biochemical and a genetic level will be the key to opening doors that lead to effective treatment of this dreaded disease.

REFERENCES

Bronner, C.F., S.M. Baker, P.T. Morrision, G. Warren and L.G. Smith *et al*., 1994. Mutation in the DNA mismatch repair gene is associated with hereditary non polyposis colon cancer. Nature, 368: 258-261.

Clarke, A.R., C.A. Purdie, D.J. Harrison, R.G. Morris, C.C. Bird, M.L. Hooper and A.H. Wyllie, 1993. *Thymocyte apoptosis* induced by p53-dependent and independent pathways. Nature, 362: 849-852.

Collins, K., T. Jacks and N.P. Pavletich, 1997. The cell cycle and cancer. Proc. Natl. Acad. Sci. USA., 94: 2776-2778.

Gelehrter, T.D., F.S. Collins and D. Ginsburg, 2000. Principles of Medical Genetics. 2nd Edn., Williams and Wilkins, Pennsylvania, USA pp: 257-267.

Greenblat, M.S., W. Bennett, M. Hollstein and C.C. Harris, 1994. Mutations in the p53 tumour suppressor gene: Clues to cancer aetiology and molecular pathogenesis. Cancer Res., 54: 4855-4878.

Harris, C.C., 1992. Mechanism of Carcinogenesis in Risk Identification. International Agency for Research on Cancer, Lyon, Cedex, France, pp: 67-79.

Harvey, M., M.J. Mc-Arthur, C.A. Montgomery, J.S. Butel A. Bradley and L.A. Donehower, 1993. Spontaneous and carcinogen induced tumorigenesis in p53 deficient mice. Nature Genet., 5: 225-229.

Lewin, B., 2000. Genes VII. 1st Edn., Oxford University Press Inc., New York.

Lodish, H., 2000. Molecular Cell Biology. W.H. Freeman and Company, New York, USA.

Mueller, R.F. and I.D. Young, 2002. Emery's Elements of Medical Genetics. 11th Edn., Churchill Livingstone, London.

Nigro, J.M., S.J. Baker, A.C. Preisinger, J.M. Jessup and R. Hosteller *et al*., 1989. Mutations in the p53 gene occur in diverse human tumour types. Nature, 342: 705-708.

Nordling, C.O., 1953. A new theory on cancer-inducing mechanism. Br. J. Cancer, 7: 68-72.

Pullman, W.E. and W.F. Bodmer, 1992. Cloning and characterisation of a gene that regulates cell adhesion. Nature, 356: 529-532.

Tomlinson,P.M., R. Roylance and R.S. Houlston, 2001. Two hits revised again. J. Med. Genet., 38: 81-85.

Turley, H., F. Pezzella, S. Kocilkowski, M. Comley and L. Kaklamanis *et al*., 1995. The distribution of the deleted in colon cancer (DCC) protein in human tissue. Cancer Res., 55: 5628-5633.