Tumour supressor genes and retinoblastoma

Throughout our life time growth is constant. Barring a few cells, most cells continue to grow, divide and replace themselves. Such processes require a strict regulation and this is brought about by cell cycle processes. These processes are carried out by proteins which control the growth and development. Proteins are coded by the genes and when the genetic information is altered or is mutated, it leads to a protein which is dysfunctional and the cell might lose its restraint on growth processes. This in turn leads to an uncontrolled growth of the cells which then causes cancer.

Cancer is a disease of the genes. At a molecular level, there are two major reasons which have been attributed for cancer induction. The first one is the gain of function in genes and the other one is loss of function of genes. The genes which gain function, that is, get activated after certain specific events are known as proto-oncogenes. They are typically activated in cancer cells conferring new properties, such as hyperactive growth and division, protection against programmed cell death, loss of contact inhibition between cells, and the ability to become established in diverse and adverse environments. Those genes which lose their functions due to specific events are termed tumor suppressor genes (Eeles et al 2004). Tumor suppressor genes are inactivated in cancer cells, resulting in loss of normal functions, such as accurate DNA replication, cell cycle control, adhesion within tissues.

Tumor suppressor genes are named such because when they are expressed in a normal cell they maintain the cell in a differentiated state and do not allow unchecked proliferation of cells. Therefore, at least one functional copy is required to prevent tumor initiation. That there are tumor suppressor

genes was proposed when it was experimentally found by Harris et al. (1969) in cell fusion experiments wherein a fusion between a normal and a tumor cell rendered the cell non-cancerous and therefore, it was proposed there must be tumor suppressor genes which suppress cell growth in a dominant fashion (Skapek et al, 1997). As long as the normal copy is functional, the protein expressed maintains normal functioning of the cells. Many tumor suppressors have been identified and extensively characterized. TP53, WT1, NF1, BRCA, VHL, APC, MEN1 are a few examples of tumor suppressor genes.

Tumor suppressor genes are divided into three categories- the gatekeepers, the caretakers and the landscapers. Gatekeepers are genes that directly regulate the growth of tumors by inhibiting their growth or by promoting apoptosis. In contrast, inactivation of caretakers does not directly promote growth of tumors. Rather, inactivation of caretakers leads to genetic instability that only indirectly promotes growth by causing an increased mutation rate. Landscaper genes do not directly affect cancer cell growth but contribute to an abnormal stromal environment that contributes to neoplastic transformation of the overlying epithelium.

This project deals with one gatekeeper gene, known as retinoblastoma susceptibility gene (RB1). RB1 is the first tumor suppressor gene to be identified and cloned (Lee at al., Eeles et al, 2004; Andrade et al, 2006). It is the gene which when mutated predisposes a person to a common malignancy of the eye, retinoblastoma.

Retinoblastoma is the most common intraocular cancer in children. It generally manifests in children before the age of five years with a majority of the tumors occurring by the age of 2 years (Lee et al., Eeles et al, 2004).

Clinical features of retinoblastoma:

As the name implies, retinoblastoma is one of the rare embryonic neoplasms originating in the retina. It is the most common intraocular tumor in children (Valverde et al, 2005). It was first described as a specific entity by James Wardrop. In majority of cases, the first sign at presentation is the characteristic cat’s eye reflex, which is usually noted by direct visualization. This white, pink-white, or yellow-white pupillary reflex, termed leukocoria, results from replacement of vitreous by the tumor or by a tumor growing in the macula.

Another common symptom, strabismus or squint (exotropia or esotropia) can occur alone when small macular tumors interfere with the vision, or can be associated with leukocoria.

Uncommon presenting signs for retinoblastoma are red, painful eye with secondary glaucoma, orbital cellulitis (infection of the soft tissues of the eyelids), unilateral mydriasis (excessive dilation of the pupil due to disease or due to trauma), and heterochromia (the iris of the two eyes show different color).

In rare cases, presenting symptoms include hypopyon (pus in the anterior chamber of eye), hyphema (blood in the anterior eye chamber), keratitis, and vitreous hemorrhage (Vogelstein and Kinzler, 2002).

A complete evaluation for retinoblastoma includes ophthalmologic examination, radiographic evaluation (skull X-ray, CT scan, MRI), and more recently, genetic testing. Fundus examination of first degree relatives is also done to look for the presence of retinoma or a regressed tumor which may indicate a hereditary component of the disease.

Retinoblastoma can be exophytic or endophytic. It is exophytic when tumor occurs between the choroid and the retina and it is endophytic when it extends from retina towards the vitreous chamber.

Therapy depends on the stage at which it is discovered. Based on that, treatment is given which includes enucleation, external beam radiotherapy, cryotherapy, episcleral plaques, xenon and argon laser photocoagulation, and chemotherapy. The choice of treatment depends on the factors such as:

- Multifocal or unifocal disease,
- Site and dimensions of the tumor,
- Diffused or focal vitreous seeding,
- Age of the individual,
- Histopathological finding.

Therefore, staging and grouping of the disease is very important.

Retinoblastoma can manifest in one of the eyes, both eyes and / or pineal body of the brain. When it is observed in one eye, it is referred to as unilateral retinoblastoma. When it is present in both the eyes, it is referred to as bilateral retinoblastoma. It is called trilateral retinoblastoma when the pineal gland is also involved.

Why should retinoblastoma occur in one eye in some individuals and both the eyes in others? One more observation was that most of the individuals who came with retinoblastoma in both the eyes were diagnosed at an age earlier than those who presented with unilateral retinoblastoma. What is different in the genotypes of these two types of individuals that warrant a delayed onset of disease in the unilateral retinoblastoma cases? This was explained by Knudson in his study.

Genetics in retinoblastoma:

Retinoblastoma occurs with a frequency of 1 in 13, 500 (Mateu et al., 1997) to1 in 20, 000 (Di Commo et al., 2000). It is seen in both hereditary and nonhereditary forms (Knudson, Lee et al.) and shows no significant variation between races, countries, or level of industrialization (Mateu et al., 1997). However, contradictory views have been expressed by Mastrangelo as he questions the credibility of the data. Previous studies indicated that a gene predisposing to retinoblastoma manifests in young children was localized to chromosome 13. The gene was probably at band q14, since several retinoblastoma patients had shown to carry constitutional deletions of this region of chromosome 13.”

Retinoblastoma can be hereditary as well as sporadic. Those who have a mutation at one of the alleles of RB1 in germline cells are said to be predisposed to the disease. Such cases are categorized under hereditary retinoblastoma. Both hereditary as well as non-hereditary forms of retinoblastoma may show tumors in one or both the eyes. In the hereditary form of retinoblastoma, a germline mutation is transmitted as high

penetrance (90%) autosomal dominant trait (Martinez et al). Most reported cases are sporadic (Knudson, 1971; Mateu et al, 1997).

Retinoblastoma is caused by two mutational events at the retinoblastoma (RB1) locus (Knudson, 1971). That is, biallelic inactivation of RB1 due to mutations is a crucial event in the development of retinoblastoma (Andrade et al, 2006).

Knudson's hypothesis:

Alfred Knudson realized the implications of the fact that individuals with hereditary bilateral retinoblastoma were diagnosed at a younger age than those children with non-heritable disease, mostly unilateral retinoblastoma. The mean age of individuals on diagnosis was found to be 19 months in unilateral retinoblastoma cases whereas it was found to be 5 months in bilateral retinoblastoma cases. Also, in most cases of familial retinoblastoma, tumor develops in both the eyes. To explain these two important phenomena, age of onset and tumor development (unifocal or multifocal) in one or both eyes, Knudson proposed the " two hit hypothesis".

The hypothesis seeks to explain the occurrence of hereditary and nonhereditary retinoblastoma and its correlation with the age of onset of the disease. According to Knudson, the first mutational hit can be inherited through the germline or can be somatically acquired, whereas the second occurs somatically in both cases and leads to tumor in cells that is double defective at the RB1 locus (Knudson, 1971; Mateu et al., 1997).

Thus in case of hereditary retinoblastoma, the individual must have inherited the mutation through the germline and hence, during the course of development may show the second mutational hit leading to tumorigenesis. As this individual already has a mutation in germline, it shows the second hit early in the life time. Statistical analyses indicated that as few as two mutational ' hits' were rate limiting for the development of retinoblastoma tumors. The occurrence of the first mutation (M1) in the germline and all developing retinal cells gives retinoblastoma tumor a ' head start' in hereditary cases (only M2 must arise in a retinal cell), compared to non-hereditary tumors where both M1 and M2 must arise in a single retinal cell.

On the other hand, in unilateral retinoblastoma cases, the individual has not inherited a germline mutation at the RB1 locus. During the course of development, the cells of such patients gather both the mutations in somatic cells with some exceptions. Thus these tumors arise later than the hereditary forms of retinoblastoma.

Knudson performed a statistical analysis of some patients presenting with retinoblastoma. The number of tumors in each eye was calculated and he devised a distribution keeping number of tumors in one eye, m= 3. Each tumor which is seen originates from a single cell. Thus, a mutation rate can be roughly calculated. If the total number retinal cells are n, m/n is the probability of a cell undergoing mutation at one of the alleles.

Now, retinoblastoma is derived from the inner and outer neuroblastic layer. The order of magnitude of retinoblasts is reflected by the magnitude of the number of ganglion cells which are derived from the early differentiated

inner nuclear layer of the retina. The estimated number of ganglion cells has been put at 2 x 106 per retina. Thus, using this as an approximation for the total number of cells, the probability that a cell will inherit one mutation is 0. 75 x 10-6. Since a majority of hereditary cases occur in the first two years of life, the probability expressed per year at either member of the autosomal gene pair would be one fourth of this value, or approximately 2x 10-7 per year. This estimates the rate of second mutation in mutated cells (Knudson, 1971). It is seen that the rate at which the second mutation occurs is relatively lower than the first mutational hit.

Retinoblastoma in hereditary cases which present themselves as bilateral retinoblastoma show high penetrance. In such cases, the vast majority of high penetrance mutations are “ null” alleles where the mutations abrogates which destabilizes RB mRNA, presumably due to pre mature truncation of translation, so that no pRb is detected. However, some mutations show low penetrance. The low penetrance phenotype can result from several different types of RB alleles.

Germline deletion of the whole RB gene often results in unilateral retinoblastoma, presumably because an unknown adjacent critical gene is also deleted, without which the RB-/- cell cannot survive. Only cells in which M2 is a different intragenic RB mutation on an allele with the adjacent critical gene still intact can survive to form retinoblastoma.

Some mutations reduce expression of wild type pRB by targeting the promoter or splice sites.

In-frame mutations result in a stable pRB with some aberrant functions.

THE RB1 GENE:

The RB gene family includes at least three members- RB1, RBL1, and p130. The RBL1 and p130 also show similar protein binding characteristics as RB1 and therefore they come under one gene family (Mulligan and Jacks, 1998). All three genes code for pocket proteins because their main sequence similarity resides in the pocket domain, which mediates interactions with the cellular and viral proteins to exert biological functions of this family (Pogoriler et al., 2006). The RB1 gene has been localized on chromosome 13 of humans on the long arm with locus 14. 2 (Cavenee et al, 1971). The RB1 gene codes for a protein which is 928 amino acids long. There are 27 exons in RB1 and occupies nearly 200 kb of the genome. The gene transcribes a 4. 7 kb mRNA. The exon size varies across the gene. The largest is exon 27 which is 1892bp long while exon 24 has only 30bp in its exonic region (NCBI database: www. ncbi. nlm. nih. gov/).

Comings et al. suggested that RB1 is a tumor suppressor gene which is recessive at cellular level (Comings, 1971; Di Commo et al., 2000). The RB1 promoter present upstream of the exons does not show the typical TATA box binding domain. The promoter sequence of RB1 reveals that the sequence between -300 and +400 is GC rich (Hong et al, 1989). As the promoter lacks a TATA element, it might explain the presence of three transcriptional initiation sites. Deletion analysis of the promoter by Hong et al demonstrated that the sequence stretching from +13 to +83 suffices the promoter activity. Another characteristic of the promoter region is that the G+C rich region shows similarity with many “ housekeeping genes” (Hong et al, 1989). This

outlines the fact that the RB1 gene is expressed constitutively in almost all tissues of our body.

The Leiden Open source Variation Database (LOVD) maintains the reported mutations in the RB1 gene. Almost 940 mutations and polymorphisms have been reported in RB1 by many scientists (www. rb1-lsdb. d-lohmann. de/). Single base pair mutations are the most frequent M1 mutations and account for nearly 40% of the confirmed mutations, followed by short and large mutations. The second hit might be associated with loss of heterozygosity (LOH) mutations, promoter hypermethylation, or even a second independent base substitution (Andrade et al, 2006; Lohmann et al., 1996). The most common point mutation found in the studies is the change from C> T at the CGA sites which codes for amino acid arginine. This change leads to premature termination of the protein (Lohmann et al., 1996).

Expression of Rb protein:

Initially, RB gene was considered to be expressed ubiquitously in all tissues of the body considering its role in maintaining cell differentiation (Karantza et al, 1993). However, later studies revealed differential expression of Rb protein.

In situ Hybridization studies done with Rb expression during embryogenesis show, that the Rb family of proteins is differentially expressed in only certain specific cell lineages. According to studies, Rb1 mRNA transcripts were detected not only during in the ganglionic cell layer of retina but also during neurogenesis, hematopoiesis, myogenesis, lens development prior to and during differentiation. In the liver and the CNS, RB1 is co-expressed along

with p107 protein. Consequently, RB-/-, p107-/- cells undergo cell apoptosis. RB1 transcripts were also detected throughout myogenesis. pRB has also been found to be expressed during spermatogenesis (Yan et al, 2001).

Since RB1 mutations specifically arise in the human eye, analysis has been performed for the developing eye. The results suggested that RB1 transcripts were detected in the ganglion cell layer of the developing retina from embryonic day 14 through 18 (Jiang et al, 1997). Preliminary studies had indicated that Rb expression in developing retina initiates as the cells commit to differentiation, but pRb has been detected only in certain subsets of retinal cells (Di Commo et al, 2000).

In conclusion, pRb expression is important for terminal mitosis in peripheral nervous system, keratinocytes, and skeletal muscles (Di Commo et al, 2000).

THE RETINOBLASTOMA SUSCEPTIBILITY PROTEIN:

The RB1 gene transcribes a 4. 7 kb mRNA which encodes a phosphoprotein which is 928 amino acids long. It is an example of a pocket protein as there are sites or pockets which interact with other proteins. The protein is a negative regulator of the cell cycle. The pRb migrates in SDS PAGE as a multiple, closely spaced bands with molecular weights between 110 and 114 kDa (Skapek et al, 1997).

The pRb can be divided into three domains protease resistant, protease soluble and structural domains comprising of the N terminal, R motif, and A/B pocket (Di Commo et al, 2000).

The N Terminal:

The N terminal region extends from amino acid 1-379. Although the N terminal is well conserved among Rb orthologs and paralogs, it has been studied far less. However, a significant number of mutations in this region occur in retinoblastomas, strongly implicating it in tumor suppression. Another study by Goodrich et al, 2003, suggested that integrity of the domain is necessary for rescue from both developmental defects as well as tumor susceptibility. The crystal structure of the domain reveals a globular entity formed by two rigidly connected cyclin folds similar to the pocket domains which suggests that Rb evolved through domain duplication. A coherent conformation of the Rb holoprotein has been suggested in which the N terminal domain and the pocket domains interact directly (Hassler et al).

The A/B domain:

The pocket domain consists of two non consecutive stretches of amino acids, A (amino acids 380-577) and B (amino acids 645-785) (Xiao et al, 2003). It is coded by the exons 12 through 22 of the RB1 gene (Brichard et al, 2006). This region has been shown to interact with many proteins. This domain has been shown to be critical for many interactions of pRb including interaction with a variety of cellular proteins like E2F transcription factor (extensively characterized), tethering of pRb to nuclear structures (Skapek et al., 1997), phosphorylation during the G1 phase of cell cycle. Between the A and B domain is a small stretch of 75 amino acids, the spacer region. A small deletion within the spacer region or replacement of the spacer region with a random sequence has no effect on the function of pRb. However, deletion of entire spacer affects the physical interaction between the two domains.

There is another pocket referred to as the C pocket domain in the large A/B domain of the pRb protein. This C pocket lies within the minimal functional domain of RB (Rb amino acids – 395 to 876) (Whitaker et al, 1998).

The C terminal:

The C terminal stretches from amino acid 786 to 928. Of the sixteen sites for phosphorylation by cdk's identified, six lie in the C terminal. It has been demonstrated that phosphorylation of S788 and S795 destabilizes the E2F complex interaction directly while phosphorylation of T821 and T826 induces an intramolecular interaction with Rb pocket that destabilizes the remaining interactions indirectly (Rubin et al.). The C terminal also contains a Nuclear Localization Signal (NLS) as well as cyclin binding motif [R/K]XL that are important for Rb phosphorylation (Di Commo et al, 2000).

RB AND CELL CYCLE:

pRB is a negative regulator of the cell cycle. The cell cycle consists of DNA synthesis (S phase) and mitosis (M phase) separated by two gap intervals, G1 and G2. When they are not cycling, cells are in a quiescent phase, G0, and extra- and intracellular signals are required to re-enter the cell cycle. Serum-starved cells in G0 will enter G1upon growth factor stimulation, and will return to G0 if mitogens are removed prior to a point in late G1. Beyond this ' restriction point' (R) cells traverse through S, G2 and M, and will not stop even if serum is removed. There are two checkpoint controls which ensure proper cell cycle progression. The first one is referred to as the G1S checkpoint that ensures that there are proper environmental conditions for cell division. Importantly, many of the extracellular and intracellular signals

that regulate passage through R point converge on the pRB pathway (Di Commo et al.) The second one is called as the G2M checkpoint which ensures that all the requirements for cell division are fulfilled.

Fig: Schematic diagram showing RB phosphorylation pathway. (1) E2F activity repressed in G0 phase imparting cell cycle inhibition. (2) Mitogens trigger a signaling pathway leading to activation of cdk 4/6- cyclin D, cdk 2- cyclin E complexes which in turn phosphorylate RB sequentially rendering it inactive. (3) E2F mediated gene expression allows progression through S phase. (4) During transition to mitosis, the action of mitosis reactivates RB to hypophosphorylated form.

The regulation of pRB is by phosphorylation. Hypophosphorylated pRb is in its active form whereas on phosphorylation it loses its activity. The protein contains 16 CDK recognition motifs (S/TP) for phosphorylation, six of which are located in the C-terminus (Rubin et al, 2005). Hypophosphorylated pRB binds target proteins and arrests cells in G1. This block is relieved by a crescendo of CDK-mediated phosphorylation that begins as cells in G1 approach R, and is abruptly reversed at the end of M phase.

The major targets for hypophosphorylated pRB are E2F and co repressors such as Histone Deacetylases (HDAC’s). E2F belongs to a family of transcription factors who have evolutionarily conserved domains including the one for DNA binding and a dimerization domain. E2F is found as a heterodimer with another class of proteins- Differentiation Regulated Transcription Factors (DRTF-b). E2F-pRB co repressor complexes maintain the gene repression (Lees et al, 1993) that is required for progression

through R, such as cyclin E. To pass this checkpoint, these repressor complexes are disrupted in two stages by the sequential action of cyclin D and cyclin E activated CDKs.

As cells exit G0, cyclin D levels rise, causing activation of CDK4/6 and phosphorylation of multiple C-terminal sites on pRB (Gorges et al, 2008). The C-terminal region of pRB contains a series of (R/K)XL cyclin docking motifs. Ser 795 is the first site to be phosphorylated, and is critical for inactivating growth suppression by pRB. Following D-CDK4/6 phosphorylation, the increase in negative charge promotes an intramolecular interaction between the C-terminus and a series of positively charged lysine residues (the ‘ lysine patch’) that surround the LXCXE binding groove in the B domain of the pocket. Associated LXCXE proteins, such as HDACs, are also dislodged. However, E2F which does not use LXCXE motif to interact with the pRB does not dislodge. Removing HDAC’s is thought to relieve active repression of certain target genes such as cyclin E.

The genes which are required for DNA replication like gene for thymidine kinase, DNA polymerase and dihydrofolate synthase are also activated as a result of phosphorylation of RB1 and subsequent loss of interaction with E2F protein which now recruits other transcription factors. The cell thus enters the S phase. In the S phase of cell cycle, DNA gets replicated and synthesized. Histone synthesis also occurs during the S phase.

Once DNA replication is complete, the cell enters another gap, G2 where the cell continues to grow till the cell enters the M phase or mitosis phase. Significant protein synthesis takes place during the G2 phase. Proteins like

microtubules involved in mitosis stage are synthesized. At the end of the G2 is the G2/M checkpoint. Once it overcomes the second checkpoint, the cell enters actual cell division- mitosis.

RB1 PROTEIN INTERACTIONS IN CELL:

Cell cycle control at the G1 S checkpoint and growth suppression as well as differentiation into a particular subset of cell is considered the major function of RB1 protein. Transcription regulation is a control mechanism that is critical for fundamental processes such as cell growth and differentiation. Proteins involved in transcription regulation either bind to DNA sequences or act as co-activators or co-repressors. pRb is one such transcription regulator.

In accordance with its role as a tumor suppressor, introduction of Rb into Rb-/- cells diminishes their capacity for malignant transformation which underlines the fact that pRb plays a critical role in DNA replication, cellular senescence, differentiation, and apoptosis, placing pRb at the center of cellular proliferation and tumorigenesis through its interaction with various proteins.

At least three distinct protein binding activities have been identified and extensively studied: the large A/B pocket binds E2F (Benevolenskaya et al, 2005), the A/B pocket binds the LXCXE peptide motif, and the C pocket binds the nuclear c-Abl tyrosine kinase (Whitaker et al, 1998). Also, the protein also stabilizes the heterochromatin to maintain the overall chromatin structure. The C pocket is distinct from the A/B pocket domain as pRb can simultaneously bind to c-Abl as well as E2F (Whitaker et al, 1998).

The A/B domains are sufficient for E2F subunit binding to Rb. However, the E2F-DP1 heterodimer requires the presence of C terminal. The retinoblastoma protein inhibits E2F mediated transcription via two distinct mechanisms- 1.) pRb binds to E2F transactivation membrane and inhibits E2F's ability to promote transcriptional activation of E2F dependent genes. 2.) pRb actively represses expression of certain genes by recruiting HDAC's and other chromatin remodeling factors (Gorges et al, 2008). The C terminal has also been shown to bind to MDM2. MDM2 interaction with the extreme C terminal region has therefore been shown to contribute towards regulation of apoptosis (Janicke et al., 1996; Sdek et al, 2004). MDM 2 has been known to stimulate E2F transactivation activity and promote S phase entry of cells, independent of p53 (Sdek et al., 2004).

The retinoblastoma tumor suppressor protein has been known to bind directly and inhibit a transcriptionally important amino terminal kinase domain of TATA- binding protein associated factor TAFII250 (Siegert et al., 2000). TAF II250 is the largest of approximately 10 TAF subunits of Pol II specific human TFIID. It directly binds to TBP and is believed to be a central scaffold for assembly of TAF's and TBP into a stable TFIID complex (Siegert et al., 2000).

The retinoblastoma protein interacts even with transcription factors ATF2. ATF2 enhances the activity of TGF Î² promoter. The retinoblastoma protein also enhances the stimulatory effects of ATF 2. The site on Rb required for its effect alone and in combination with ATF's has been mapped mainly to A/B products and the C pocket (Li and Wicks, 2001).

It has been also reported that the amino terminal of BRCA 1 can efficiently bind to the ABC region (from amino acids 379-928) of the Rb protein. It has been shown that growth suppressor activity of BRCA1 takes place only in presence of a functional Rb protein (Aprelikova et al., 1999).

pRb has been known to interact with proto-oncogene c-jun. One of the transcriptional modulator target sites of pRB is the AP-1 binding site within the c-jun and collagenase promoters. c-Jun also physical interacts with pRb where the C terminal site of leucine zipper interacts with pRb (Nishitani et al., 1998). pRb also represses the expression of c-Fos gene the gene product of which , Fos, is one component of the heterodimeric transcription factor, AP-1. Thus, pRb can be functionally linked to c-jun for transcriptional regulation.

pRB when recruited to DNA via E2F is a potent transcriptional repressor due to its ability to recruit HDAC's (Kennedy et al., 2001) and histone methylases. Again, one pRB binding protein, EID1 is a potent inhibitor of histone acetylases, p300 and CBP and blocks differentiation (Benevolenskaya et al.). HDAC's interact directly with pRB by means of a LXCXE motif (Kennedy et al., 2001; Gorges et al., 2008).

p53 controls phosphorylation of pRB in a cell indirectly. On DNA damage induces the transcription of its target gene p21WAF1/CIP1. The p21 protein thus formed binds to two different proteins. First, it inhibits the activity of CDK's in cell which in turn keeps pRB in active state. Thus, p53 helps in negative regulationof cell cycle. Second, the p21 protein interacts with the PCNA which then leads to inhibition of cell cycle (Hsieh et al, 1999).

Androgen receptors show a unique interaction with pRb at the N terminal. Over expression of pRb leads to increased transcription activity of androgen receptor, AR. pRb also potentiates the activity of glucocorticoid recptors, GR. However, loss of pRb activity inhibits AR but not GR activity (Lu and Danielsen, 1998).

MUTATIONS IN RB GENE:

After RB1 gene was first cloned by Friend et al in 1986, mutations have been identified in the gene ranging from single base pair deletions, to small length insertions and deletions (Lohmann et al. 1996, Andrade et al. 2006). The mutations do not show any hotspots and they are widely distributed over the 27 exons of RB1 as well as the promoter region (Dalamon et al. 2003).

The most common mutation found to result in predisposition to retinoblastoma is the point mutation from C> T. At times the mutation at this point changes the codon from CGA to TGA, from Arginine to stop codon. This premature truncation of protein leads to abrogated expression of pRb in cells.

Hypermethylation of RB1 promoter has been also observed as a common epigenetic event in certain tumors (Gonzalez- Gomez et al., 2003).

Retinoblastoma Gene Mutation database (RBGMdb) maintains a comprehensive list of all mutations in the RB1 gene. Till now, 932 mutations have been reported in the RBGMdb. Most of the mutations are nonsense mutations (42%).

However, if recurrent nonsense mutations are not considered, the percentage comes down to 18%. RB1 mutations are scattered all along the genomic sequence. However, there are a few hotspots which show high recurrence. Most of the recurrences correspond to C> T changes in the sequence. Out of 351 nonsense mutations 271 (79%) are C> T transitions in eleven CGA (arginine) codons, in exon 8, 10, 11, 14, 17, 18, and 23. No mutations have been found in the three other CGA codons in exon 1 and 27.

In four of the mutated CGA codons, R251and R255 in exon 8, R451 and R455 of exon 14, a high frequency of constitutive hypermethylation has been demonstrated. In addition to hot spots, frameshift and point mutations leading to translational changes or splice site mutations are scattered along the retinoblastoma coding region and non-coding adjacent splicing sites. With the exception of exons 5, 14, 15, 24, 25 and the non-mutated exons 26 and 27, frameshift mutations are randomly distributed through the RB1 coding sequence.

Splicing mutations are also evenly distributed, but show preference for intronic sequences adjacent to exons 6, 12, 16, 17, 19 and 24. It is worth to mention that most missense substitutions (60 %) are located in cyclin box B, underlined by exons 19 to 21.