We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

9

Viral Oncogenes and the Retinoblastoma Family

M. Geletu and L. Raptis*
Departments of Microbiology and Immunology and Pathology,
Queen's University, Kingston, Ontario
Canada

1. Introduction

The discovery of viruses is tightly linked to the most significant advances in Molecular cell biology, including cancer research. Cell division is a fundamental biological phenomenon, therefore it is not surprising that human cancer is an evolutionarily ancient disease, that attracted attention in early civilizations. The remains of a 4 million-old fossilized hominid show evidence of bone tumors (Diamandopoulos, 1996), while some of the oldest written accounts of cancer are recorded in the code of Hamourabi (1750 BCE), Egyptian papyri (1600 BCE) and others. Unfortunately, in ancient Egypt knowledge was the realm of priests, so the writings of the time attributed the etiology of disease to the "will of Gods". In ancient Greece, medicine was freed from the bonds of religion; Hippocrates (460-370 BCE) tried to use logical thinking to propose the humoral theory of cancer and his ideas influenced philosophers and scientists for the next ~1,800 years. During this time, knowledge of Mathematics and Physics was remarkably advanced; the acoustics of amphitheaters, built in the 5th century BC are as good as the best of today's structures. Still, these amazing minds believed in spontaneous generation of life, a theory that lasted till Louis Pasteur (1822-1895) who demonstrated that living things cannot be generated automatically (Javier and Butel, 2008).

In 1892 Ivanofsky and Beijerinck became the fathers of the new field of Virology by showing that an infectious pathogen of tobacco plants, the Tobacco Mosaic agent, retained infectivity after passage through filters of unglazed porcelaine, known to retain bacteria (Levine, 2001). These landmark discoveries were quickly followed by the discovery of the first cancer viruses, the chicken leukemia virus by Ellerman and Bang in 1908, and the Rous Sarcoma virus in 1911. Since that time, virus research and cancer research have been closely integrated, since complicated biological problems that constitute the basic mechanisms of life are highly conserved, since they cannot change radically once solved by evolution. As viruses need to use the molecular machinery of the host to replicate, they have provided us with valuable tools to study the host, including mechanisms of cellular replication, ie cancer. Historically, two classes of viruses, the retroviruses and DNA tumor viruses have been involved in landmark discoveries in cancer, and have played a fundamental role as models in molecular biology, as well as experimental cancer research.

* Corresponding Author

Early work by Sarah Stewart and Bernice Eddy demonstrated that the mouse polyoma virus produced in cell culture could cause tumors upon injection in newborn hamsters (Eddy et al., 1958). Soon thereafter, Vogt and Dulbecco developed tissue culture methods and it was shown that it could transform normal, cultured cells to acquire properties of cells derived from polyoma virus-induced tumors, such as growth to many layers, growth in the absence of anchorage to a solid support and tumorigenicity in syngeneic animals (Vogt and Dulbecco, 1960). At the same time, Simian Virus 40 (SV40) gained notoriety because it was found by Eddy to be a contaminant of poliomyelitis and adenovirus vaccines, which had been administered to millions of healthy individuals worldwide. The public health implications of this revelation provided the initial impetus for an in depth study of SV40 biology. Later work showed that SV40 DNA sequences as well as infectious virus are in fact found in human tumors and may have contributed to oncogenesis. The fact that SV40 uses mostly cellular machinery to carry out important steps in viral infection, made it into a powerful probe to examine many fundamental questions in eukaryotic molecular biology.

In addition to their importance in cell biology, due to their potent transforming ability, DNA tumor viruses have been studied extensively. In fact, work on the mechanism of neoplasia caused by these viruses has yielded a plethora of information on cell growth controls and led to the discovery of two families of antioncogenes, p53 and the retinoblastoma susceptibility (Rb) gene products.

2. Replication of DNA tumor viruses

The DNA tumor viruses belong in the papova (the name is derived from **pa**pilloma, **po**lyoma, Simian **va**cuolating viruses) or adenovirus families. They all have non-enveloped particles and both groups are highly tumorigenic in experimental animals.

Polyoma viruses cause disease in a variety of species, with a very limited host range. The prototype of this group is the mouse polyoma virus, but three polyomaviruses have also been described in humans: JCV, the etiologic agent of progressive, multifocal leucoencephalopathy, a fatal demyelinating disease, BKV, causing nephropathy in immunocompromised individuals, (Hirsch, 2005) and SV40, the contaminant of polio vaccines, whose prevalence in humans is not clear (Garcea and Imperiale, 2003).

The response of cultured cells to DNA tumor virus infection depends upon the species being infected. In the case of SV40, monkey cells support the production of infectious virus, which leads to their death (lytic cycle), whereas rodent cells produce only the early proteins, ie the proteins expressed before viral DNA replication has commenced in a lytic infection, and acquire a neoplastically transformed phenotype. Similarly, polyoma virus grows lytically in mouse cells but transforms rat or hamster cells in culture.

Viruses of the polyoma family have circular dsDNA genomes of approximately 5,000bp, contained in icosahedral capsids. Their genome contains two coding regions, the early genes, expressed before viral DNA replication in a lytic infection, and the late genes which are expressed after viral DNA replication is underway. Both transcription units are regulated by a common non-coding control region that contains the transcription start sites, binding sites for the transcription factors and the origin of DNA replication. The early region encodes the alternatively spliced transforming proteins large T (or Tumor)-antigen and small t-antigen, while the mouse polyoma virus also expresses a 56kDa middle tumor

antigen, which is its main transforming protein. The late genes encode mostly structural coat proteins (VP1, VP2, VP3, Figure 1).

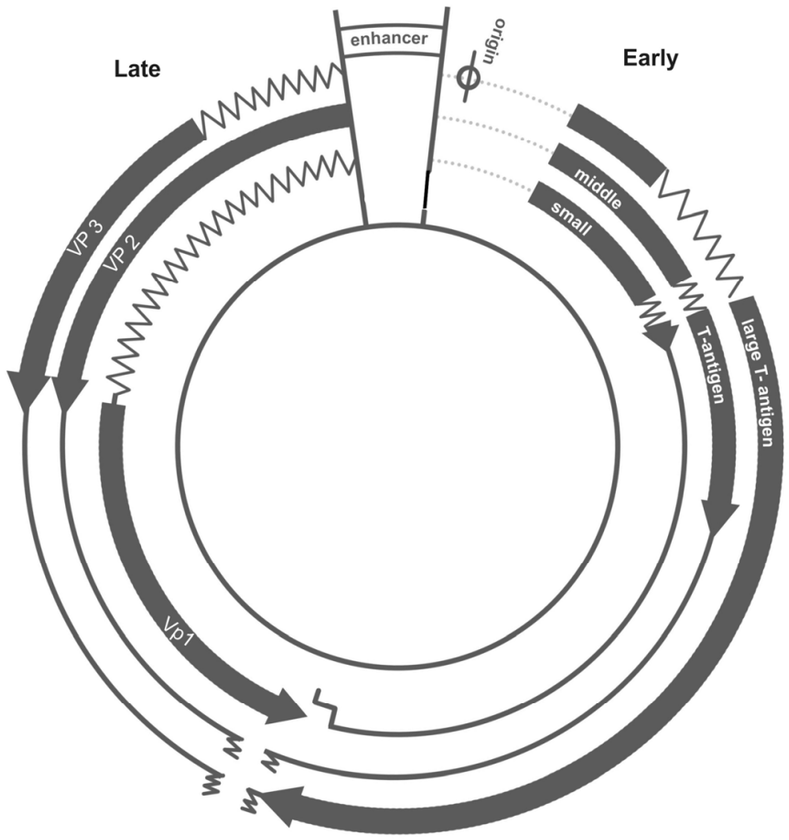


Fig. 1. Genomic organisation of the 5297 bp mouse polyoma virus. The early region is at the right and the late region at the left. The transcriptional enhancer and origin of DNA replication are also shown. Soon after infection, the differentially spliced, early genes (T antigens) are expressed (right side), followed by replication of viral DNA. After DNA replication, the late genes are expressed (left), coding mostly for the structural proteins, VP1, VP2, VP3. Squiggly lines indicate the introns (Cole, 1995).

In the host species, polyomaviruses spread by lytic infection of permissive cells. Lytic infection requires the large T-antigen, a ~100 kDa nuclear phosphoprotein which binds the origin and is essential for viral DNA replication [reviewed in (Cole, 1995)]. Polyomaviruses rely on cellular enzymes for the replication of their DNA, since their genome does not code for replication proteins. These proteins are confined to the S phase of the cell cycle, and the large T-antigens modulate cellular signaling pathways by interacting with a plethora of cellular proteins that promote cell cycle progression into S phase. Due to this property, the large T-antigens are also important players in the transformation of virus-infected cells. The most well-known interaction is the ability of the T-antigens to associate with, and interfere with the functions of the two tumor suppressor proteins, pRb and p53.

In non-permissive, cultured cells infection is abortive and neoplastic transformation of the cell may occur. Transformation requires expression of the early region, in particular the

large T-antigen in the case of the human polyoma viruses (Fig. 1). The mouse polyoma middle-tumor antigen associates with and activates the cellular Src protein, but its large T antigen is also important in viral DNA replication, as well as transformation in certain systems. The tumor antigens are also key players in the highly efficient oncogenesis *in vivo* by these viruses, ie when virus is inocculated into animals or when the early region is introduced into transgenic mice.

Adenoviruses have linear dsDNA's of approximately 35,000bp enclosed in icosahedral particles with spikes on the vertices.The human adenoviruses infect and can grow lytically in human cells but can cause tumors in rodents and transform a variety of rodent cells in culture. Their genome is linear, double-stranded DNA of ~35,000 bp with a number of transcription units. Early after infection at least four promotors are activated (E1, E2, E3, E4), while at late times there is activation of the major late promotor, coding mostly for a number of coat proteins (Fig. 2). The adenovirus early region E1A and E1B genes are important in transformation of cultured cells and tumorigenicity, but E1A is also required in a lytic infection, for the transactivation of all other Adenoviral genes. E1A proteins bind the Rb family, while E1B associates with and inactivates p53. The adenoviruses display extensive RNA splicing, therefore it is not surprising that splicing was discovered for the first time in adenoviruses [(Broker, 1984), reviewed in (Shenk, 1995)].

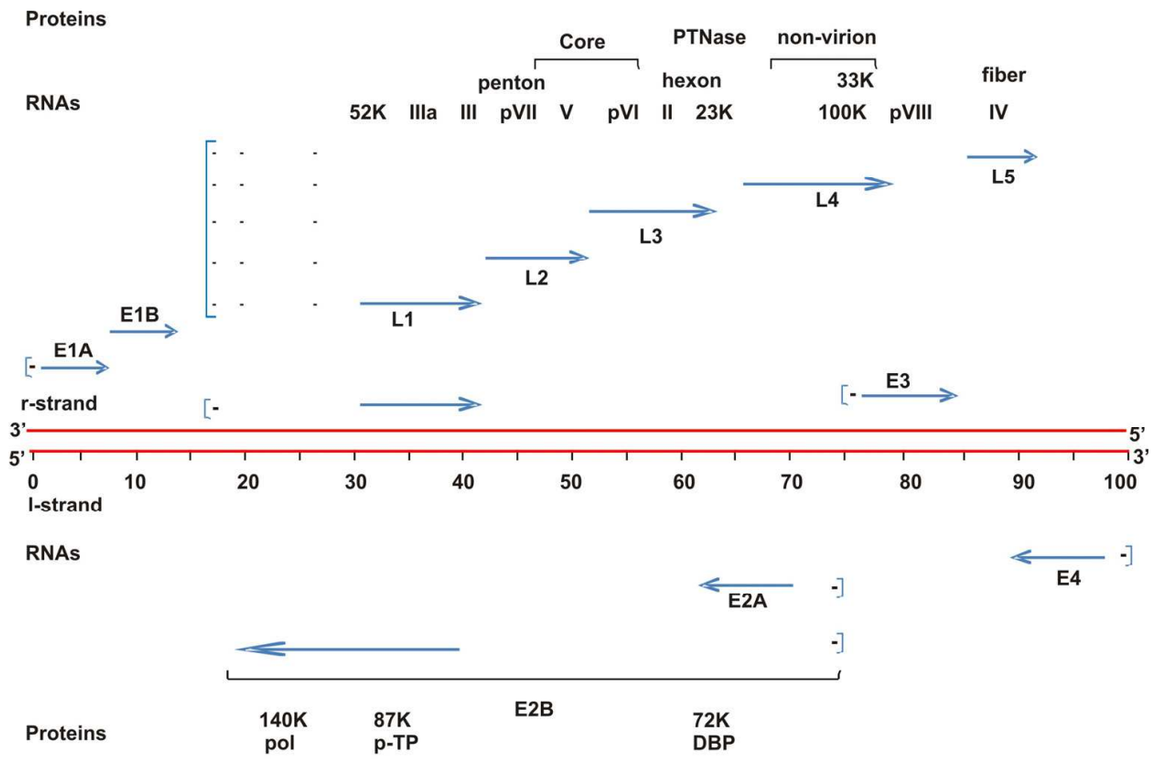


Fig. 2. Transcription and translation map of adenovirus type 2. The early mRNAs are designated E, late mRNAs are designated L. The main genes involved in transformation are the E1A and E1B at the left of the genome, giving rise to several proteins with differential splicing (not shown). (From Broker, 1984).

r-strand: rightwards transcribed, l-strand, leftwards transcribed.

The papillomaviruses comprise a group of nonenveloped DNA viruses that induce mostly benign lesions of the skin (warts) and mucous membranes (condylomas) in humans and animals. However, some members such as human papilloma viruses 16 and 18 have been implicated in the development of epithelial malignancies, especially cancer of the uterine cervix and other tumors of the urogenital tract. The papillomaviruses are small, nonenveloped, icosahedral DNA viruses that replicate in the nucleus of squamous epithelial cells. The virion has a single molecule of double-stranded, circular DNA of approximately 8,000 base pairs. The E6 and E7 are the main oncogenes of the high-risk HPVs. E7 binds with and inactivates Rb, while E6 binds p53 and leads to its degradation (Howley and Lowy, 2007)

3. Interaction of the DNA tumor virus oncogenes with the retinoblastoma family

The demonstration that DNA tumor viruses can cause tumors in animals led to an intensive investigation into the mechanism of tumor induction. The type of tumor that developed in an animal following viral inocculation often depended upon the site of injection; early findings demonstrated that injection of adenovirus-12 directly into the vitreous body of newborn rats, mice or baboons induced retinoblastoma-like tumors that expressed adenovirus gene products (Kobayashi and Mukai, 1973; Mukai et al., 1977; Mukai et al., 1980; Kobayashi et al., 1982). However, no adenovirus or JC polyoma virus was ever found in human retinoblastomas. Still, adenovirus research and the development of monoclonal antibodies against the tumor antigens of these viruses greatly facilitated the identification of cellular proteins specifically binding to the viral oncogenes. One of them was the *Rb* gene product, whose inactivation was independently demonstrated to lead to retinoblastoma formation in humans.

Seminal studies on retinoblastoma by Knudson *et al* (Knudson, Jr., 1971) laid the foundation for the tumor suppressor hypothesis. Statistical analysis of age and family history made him conclude that two independent mutation events are required for retinoblastoma development. It was later proposed that the two mutations occurred in the two alleles of the same gene, *Rb1* that is, retinoblastoma is a recessive cancer where one abnormal chromosome was inherited, while the corresponding, wild-type chromosomal segment was lost in the tumor cells (Godbout et al., 1983; Benedict et al., 1983). Genetic linkage studies demonstrated anomalies on chromosome 13q14, close to the esterase D locus (Sparkes et al., 1983). Cloning of the retinoblastoma cDNA followed and it was shown that it encodes a 110 kDa nuclear phosphoprotein (Lee et al., 1987). Additional studies showed that the *Rb1* gene from retinoblastoma tumors had deletions and mutations, consistent with a model where gene inactivation ie loss of function leads to tumor formation [reviewed in (Burkhart and Sage, 2008)].

Several groups tried to identify cellular proteins that bind to the E1A gene products. Branton *et al* developed a series of anti-peptide antisera and identified several co-immunoprecipitated proteins, including a doublet of approximately 105 kDa (Yee and Branton, 1985). Most importantly, these proteins could be affinity-purified from uninfected cells using E1A expressed in bacteria. This observation indicated that the 105 kDa protein(s) was of cellular, rather than viral, origin. Moreover, their expression did not depend upon adenovirus infection, or expression of any viral proteins (Egan et al., 1988). It was further

shown that residues 111-127 and 30-60 of E1A were required for binding to the 105 kDa protein (Egan et al., 1988). A breakthrough finding followed: A monoclonal antibody was raised using as an immunogen E1A that had been immunoprecipitated from E1A-expressing, 293 cells, therefore potentially containing cellular proteins bound to E1A. As it turned out, this antibody recognised the 105kDa, Rb protein. These data demonstrated that the Rb was, in fact, the 105 kDa, cellular protein associated with E1A. This observation offered the first demonstration of a physical association between an oncogene and an antioncogene (Whyte et al., 1988; Lee et al., 1987). Similar findings emerged on the SV40 system (DeCaprio et al., 1988). Most importantly, it was soon demonstrated that TAg mutants that were unable to transform, were unable to bind Rb (e.g. E107K). These mutants disrupted the sequence LxCxE, the site of Rb binding which is present in the large TAg's of both SV40 and polyoma, adenovirus E1A, the human papillomavirus E7 proteins, as well as the TAg's of several other human polyoma viruses (Munger et al., 1989; Dyson et al., 1989). Taken together, these findings demonstrated the cardinal importance of Rb binding in transformation by these oncogenes [reviewed in (DeCaprio, 2009)].

Examination of Rb's function demonstrated that Rb is unphosphorylated in quiescent (G0) cells, but its phosphorylation increases as cells progress in the cell cycle (Buchkovich et al., 1989). It was later found that it is the cyclin D/Cdk4, cyclin E/Cdk2, cyclin A/Cdk2 and cyclin B/cdc2 kinases, shown to be required for entry into the cell cycle, that phosphorylate, and thereby inactivate Rb (DeCaprio et al., 1992). It was also demonstrated that the SV40-TAg binds the under- or unphosphorylated form of Rb exclusively (Ludlow et al., 1990), which suggested that the G0 form of Rb served a growth-suppressive function, which was overcome by TAg. In addition, overexpression of Rb inhibits cell cycle progression from G1 to S (Goodrich et al., 1991). Finally, transgenic expression of SV40-TAg under control of luteinizing hormone-β induces retinoblastomas and TAg co-precipitated Rb in lysates from tumor cells, supporting the hypothesis that retinoblastoma can develop by the inactivation of Rb function by TAg (Windle et al., 1990).

Thus, the loss of the Rb growth suppressive function can be achieved by: 1. mutation (retinoblastomas), 2. phosphorylation (cell cycle), or 3. binding to viral oncogenes. It was later shown that two Rb-related proteins that also exhibit features of cell-cycle regulators, p107 and p130, can also bind the LxCxE sequence of E1A and TAg (Ewen et al., 1992; Dumont and Branton, 1992).

Several laboratories have mapped the Rb sequences required for binding the LxCxE sequence. In fact, the two thirds, C-terminal region of Rb can bind E1A and TAg, and it is referred to as the large-pocket, while the central part of Rb (379-792) constitutes the small pocket (Kaelin, Jr. et al., 1990; Hu et al., 1990) (Fig. 3). The central part of Rb was the site of mutations in retinoblastomas, and these forms also failed to bind E1A and TAg. This observation offers a strong correlation between loss of binding to the oncogene to loss of function (Kaelin, Jr. et al., 1990; Pietenpol et al., 1990). That is, the viral oncogenes were found to disrupt a normal function of Rb, that was necessary for tumor suppression.

Further studies on the mechanism of tumor suppression by Rb and transformation by these oncogenes led to the search for cellular proteins that could compete with TAg and E1A for Rb binding. One of these protein families is the E2F transcription factors. In fact, the hypophosphorylated form of Rb binds E2F and using Rb affinity columns it became clear

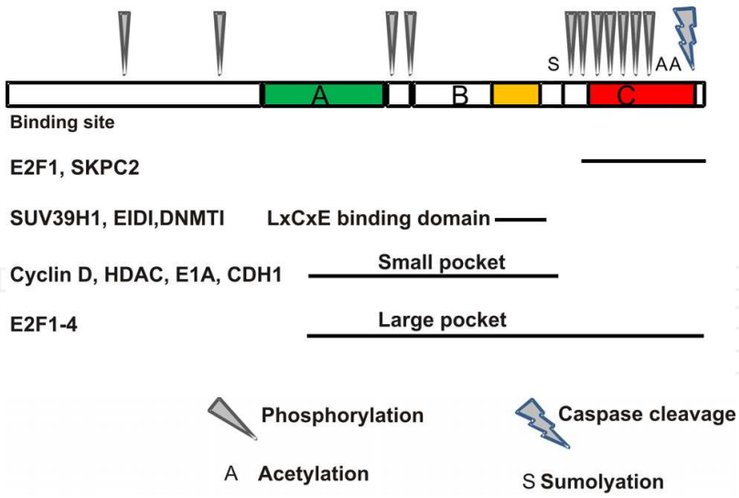


Fig. 3. A) The human pRb consists of 928 aminoacids. Deletion mutagenesis, as well as structural studies have uncovered regions that mediate its binding to individual partners. Most of them bind the pocket region. The Rb C-terminus binds specifically to E2F1 and this inhibits apoptosis. Rb can also be phosphorylated by CDK kinases as well as CHk2 (checkpoint homologue 2) and Raf 1 and this inhibits binding of most partners (Burkhart and Sage, 2008).

SKP2: S-phase kinase-associated protein-2; SUV39H1: methyltransferase, methylates lysine 9 of the amino terminus of histone H3; DNMT1: DNA methyltransferase-1; HDAC: histone deacetylase; CDH1: cadherin-1

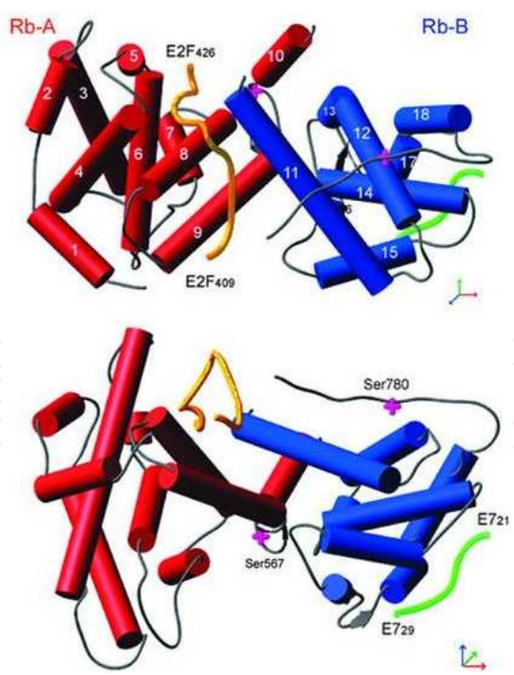


Fig. 3. B) Three-dimensional structure of the Rb/E2F complex. The helices of the A domain are shown in red and the B domain in blue. The main-chain trace of E2F is shown as a yellow worm (upper panel), while the main-chain trace of the papillomavirus E7 is shown as a green worm (lower panel) (Xiao et al., 2003).

that the viral oncogenes (E1A, TAg, E7) dissociate Rb from E2F (Chellappan et al., 1992; Chellappan et al., 1991). Rb binding correlated with repression of E2F transcriptional activity (Hiebert et al., 1992), and overexpression of E2F1 could promote entry into S phase in a manner similar to adenovirus E1A (Johnson et al., 1993). Therefore, the prevailing model is that E1A could serve to dissociate Rb from E2F, while the conserved LxCxE motif plays an important role in the high affinity binding of the viral oncoproteins to Rb. In fact, a minimal peptide of 9 residues corresponding to the LxCxE motif of HPV16-E7 could compete with Rb binding to E2F and to DNA (Jones et al., 1992). There is also evidence that TAg and E1A recruit the CBP/p300 histone acetyltransferase to remodel chromatin and actively start transcription (reviewed in (DeCaprio, 2009), Fig. 4).

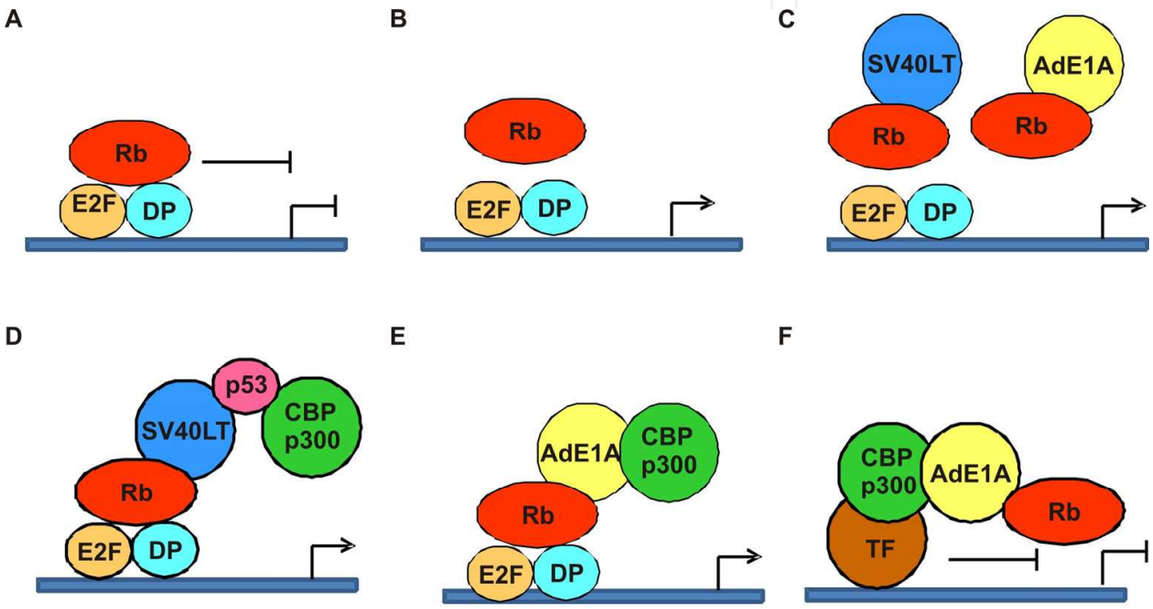


Fig. 4. Effect of TAg or adenovirus E1A upon Rb.

A. Active Rb binds the E2F/DP complex to repress transcription. **B**. Rb phosphorylation reduces binding to E2F/DP, permitting E2F activation. **C**. TAg or E1A binding removes Rb and permits E2F action. **D-E**: TAg or E1A can also bind the CBP/p300 histone acetyltransferase to increase gene expression. **F**. E1A can also bring Rb to CBP/p300 bound to transcription factors to repress promotors.

The crystal structure of Rb demonstrated that the A and B domains of the small pocket are bound to each other and are linked with a large area of highly conserved residues located in the fold between them. The LxCxE motif of HPV-E7 is bound to an exposed cleft within the B domain. The side chains of L,C and E make direct contact to Rb, which explains their high degree of conservation (Fig. 3B) (Xiao et al., 2003). Still, E1A and large TAg employ a different activity to displace E2F from Rb. The 100, N-terminal residues of LT form a J-domain, found in the family of DnaJ-Hsp40 molecular chaperones (Stubdal et al., 1997). The J domain recruits Hsc70 and activates its ATPase activity to promote chaperone activity. The J domain of large TAg cooperates with the LxCxE motif to dissociate Rb family members from E2F4 (Kim et al., 2001).

It was also found that E2F acts in a complex with another protein, the differentiation-regulated transcription factor-polypeptide 1 (DP1), which forms a heterotrimeric complex

with E2F1 and cooperates with E2F for promotor binding (Simonson and Herman, 1993). Since the original cloning, a large number of E2F and DP molecules have been identified that play a variety of roles [reviewed in (van den Heuvel and Dyson, 2008)].

4. Other viral gene products interacting with the retinoblastoma family

In addition to the DNA tumor viruses, viruses from other families, both DNA and RNA are known to interact with Rb directly. Following are some examples:

The Hepatitis C virus is a positive-strand RNA virus, which causes persistent infections that can lead to hepatocellular carcinoma (HCC). The viral RNA-dependent, RNA polymerase NS5B forms a complex with Rb, targeting it for degradation and this increases E2F activity in E2F activity. NS5B contains a LxC/AxE motif which overlaps with the active site of the polymerase, and its interaction with and inactivation of Rb may be part of the mechanism whereby HCV infection leads to carcinoma (Munakata et al., 2005).

The Hepatitis B virus (HBV) also causes hepatitis and chronic infections that can lead to HCC. It codes for the non-structural, HBx protein which transcriptionally represses p21 and p27, and binds directly to cyclin E and cyclin A, leading to cell cycle progression (Dayaram and Marriott, 2008).

The Rubella virus (RV) causes developmental abnormalities and birth defects. RV is a positive-strand RNA virus encoding NSP90, a non-structural protein with replicase activity, which binds to Rb through an Rb binding motif (LPCAE). This association plays a positive role in the replication of the virus and it has been postulated that this contributes to RV's teratogenicity (Forng and Atreya, 1999).

The human cytomegalovirus (HCMV) belongs in the Herpes family and it can cause developmental abnormalities. HCMV codes for UL97, a protein kinase which can phosphorylate and inactivate Rb in a manner similar to the cyclin-dependent kinases. Moreover, UL97 is not inhibited by the CDK inhibitor p21 and lacks amino-acid residues conserved in cdk's that permit the attenuation of kinase activity. That is, UL97 is a functional ortholog of the cyclin-dependent kinases that is immune from the normal cdk control mechanisms (Hume et al., 2008).

The Human T-cell leukemia virus (HTLV) is the only human retrovirus shown to be the cause of a human cancer, adult T-cell leukemia. The Tax protein of HTLV (40kDa) is sufficient to transform cultured cells, and it achieves this at least in part through inhibition of a number of cyclin-dependent kinase inhibitors. Tax binds directly and inhibits p15 and p16, and it represses transcription of p18 and p19. In addition, Tax interacts with cdk4 and facilitates its binding to cyclin D2, leading to enhanced kinase activity, enhanced phosphorylation and proteasomal degradation of Rb, hence E2F activation. Tax also interacts with hypophosphorylated Rb directly, and this results in premature proteasomal degradation (Dayaram and Marriott, 2008).

A number of plant viruses were also shown to bind to and require Rb function for replication. Geminiviruses are small, single-stranded DNA viruses infecting a wide range of plants. The viral genome is encapsidated into two joined icosahedral capsids. The beet curly top virus (BSCTV) codes for the C4 protein which, upon transgenic expression in *Arabidopsis* plants can increase the levels of most cyclins and CDK's, CAK's and the proliferating cell

nuclear antigen-1 (PCNA1). In addition, the Rb-related protein rbr1 and the CDK inhibitor ick1 are suppressed. Similarly, a protein of the Tomato golden mosaic virus, Rep, and the RepA protein of the Wheat dwarf virus are both able to bind the maize Rb-like proteins (Xie et al., 1995; Collin et al., 1996). Although the role of Rb in plants has not been firmly established, the fact that BSCTVcan induce cell division, points to the possibility that the effect of the C4 and Rep proteins upon Rb may be part of the mechanism of pathogenicity by these viruses (Park et al., 2010).

5. Consequences of E2F activation

Besides DNA tumor virus oncogenes, a large number of tyrosine kinases such as vSrc activate the E2F transcription factor indirectly, through activation of the CDK kinases. As a result, E2F is found to be hyperactive in many cancers. Transcriptional activation of E2F targets is achieved either through active transactivation or derepression of genes having E2F-binding sites on their promoters. A detailed Microarray analysis for E2F-activated genes yielded many targets, among which is a number of membrane receptor tyrosine kinases, such as PDGFRα, IGF1R, VEGF, and others (Young et al., 2003). In fact, it has long been demonstrated that transformed cells secrete autocrine factors, able to induce anchorage-independent growth to normal cells (Raptis, 1991; Ciardiello et al., 1990). These growth factors activate the membrane signalling apparatus, including the ras and phosphatidyl-inositol-3 kinase (PI3k) cascades, as well as the signal-transducer and activator of transcription-3 (Stat3) pathway. Following ligand binding, Stat3 binds to receptors of growth factors or cytokines and is phosphorylated on tyrosine-705 by the receptor itself or by the associated Jak or Src kinases. Two Stat3 molecules subsequently dimerize through reciprocal phosphotyrosine-SH2 domain interactions, the dimer migrates to the nucleus and initiates transcription of a number of genes (Yu et al., 2009; Raptis et al., 2009). Both the PI3 kinase and Stat3 constitute potent survival signals.

As a result of E2F activation, the SV40-TAg and adenovirus E1A have both been shown to activate and require ras (Raptis et al., 1997) and Stat3 (Vultur et al., 2005) for neoplastic transformation, as well as for the block of adipocytic differentiation (Cao et al., 2007b; Cao et al., 2007a). However, it is particularly remarkable that at the same time E2F is a potent *apoptosis inducer*, hence the high demand of transformed cells for survival signals, normally offered by Stat3, activated by the growth factor receptors. Therefore, direct Stat3 inhibition induces apoptosis of E1A or Tag-transformed cells preferentially, due to their higher E2F activity levels which promotes programmed cell death through p53-dependent or independent mechanisms. These findings underscore the importance of Stat3 the survival of tumor cells, and could have significant therapeutic implications (Fig. 5).

6. The Rb pathway in breast cancer

Although inherited, germline mutations in the Rb gene were identified mostly in retinoblastomas, Rb somatic mutations, mimicking Rb inactivation by the DNA tumor viruses have also been noted in a number of cancers, including breast cancer, so that, despite the fact that no DNA tumor viruses have ever been found in breast cancer, E2F is frequently activated.

Unlike the majority of cancers, the prognosis and treatment of breast cancer is significantly informed by a number of biomarkers, and the Rb pathway plays a prominent role [reviewed

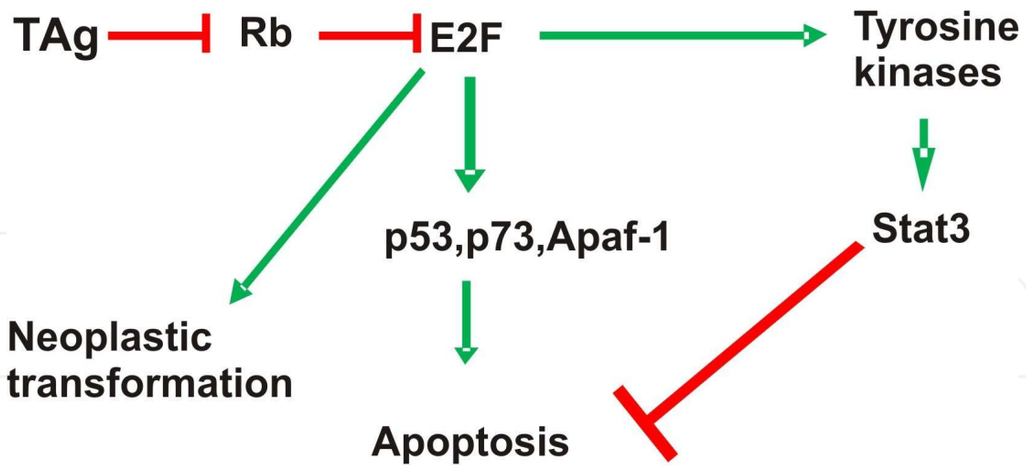


Fig. 5. E2F, activated through Rb inactivation by viral oncogenes, is known to be a potent activator of genes leading to cell division and neoplasia. Paradoxically however, E2F also induces apoptosis (through both p53-dependent and -independent mechanisms), but apoptosis inhibition would allow cell division to occur. In fact, E2F also activates a number of kinases such as IGF1-R and Src, which activate Stat3, a potent apoptosis inhibitor. As a result, Stat3 inhibition in cells with high E2F levels results in apoptosis (Sears and Nevins, 2002).

in (Musgrove and Sutherland, 2009)]. In particular, the status of the Estrogen receptor-α (ER) is an important determinant in treatment: ER-positive breast cancer has a more favorable prognosis and can be treated with selective ER antagonists (e.g. Tamoxifen) or aromatase inhibitors (e.g. Anastrozole), while ER-negative breast cancer is generally more aggressive, and with fewer treatment options. Still, a significant number of ER-positive cancers fail hormonal therapy and a great deal of effort has been expended in identifying pathways leading to Tamoxifen or aromatase inhibitor resistance.

In ER-positive breast cancer treatment, ER antagonists are effective at stopping cell division, indicating that such tumors are dependent upon estrogen for proliferation and survival (Musgrove and Sutherland, 2009). It was further shown that estrogen inhibition results in cell cycle arrest in the G0/ G1 phase of the cell cycle through attenuation of CDK/cyclin complexes at multiple levels (Foster et al., 2001). In particular, cyclin D1 is a direct transcriptional target of ER signalling (Eeckhoute et al., 2006). On the other hand, functional analyses have suggested that a multitude of cascades can contribute to acquired resistance to endocrine therapy, such as aberant ErbB2, Grb10 or Akt signalling (Miller et al., 2009), while p27kip1 and Rb inactivation can compromise the efficacy of ER inhibition (Cariou et al., 2000; Bosco et al., 2007). Specifically, a gene expression signature of Rb-dysfunction is associated with luminal B breast cancer, which exhibits a relatively poor response to endocrine therapy. Most importantly, recent reports demonstrated that a selective CDK4/6 inhibitor, PD-0332991 suppressed cell proliferation of a number of tamoxifen-resistant, cultured breast cancer cell lines. While ER antagonists in sensitive lines induce cell cycle arrest, CDK4/6 inhibition in tamoxifen-resistant lines induced a state having certain molecular characteristics of senescence in hormone therapy resistant cell populations. Therefore, PD-0332991 is an effective cell cycle inhibitor that could be especially valuable in ER+ breast cancers that are resistant to endocrine therapy, and is now being tested in phase II clinical trials (Thangavel et al., 2011).

7. Conclusions

Results on the mechanism of transformation by DNA tumor viruses have given valuable insights on the role of the Rb family in cell division. Evidence is now emerging that these conclusions are applicable to human cancers such as cancer of the breast, hence the Rb pathway may offer important targets for chemotherapy. It is reasonable to assume that the study of the viral oncogenes will offer additional insights into the role of Rb in cancer.

8. Acknowledgments

The financial support from the Canadian Institutes of Health Research (CIHR), the Canadian Breast Cancer Foundation (Ontario Chapter), the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canadian Breast Cancer Research Alliance, the Ontario Centers of Excellence, the Breast Cancer Action Kingston and the Clare Nelson bequest fund through grants to LR is gratefully acknowledged. MG was supported by a postdoctoral fellowship from the US Army Breast Cancer Program (#BC087586), the Ministry of Research and Innovation of the Province of Ontario and the Advisory Research Committee of Queen's University.

9. References

Benedict, W.F., Murphree, A.L., Banerjee, A., Spina, C.A., Sparkes, M.C., and Sparkes, R.S. (1983). Patient with 13 chromosome deletion: evidence that the retinoblastoma gene is a recessive cancer gene. Science *219*, 973-975.

Bosco, E.E., Wang, Y., Xu, H., Zilfou, J.T., Knudsen, K.E., Aronow, B.J., Lowe, S.W., and Knudsen, E.S. (2007). The retinoblastoma tumor suppressor modifies the therapeutic response of breast cancer. J. Clin. Invest *117*, 218-228.

Broker, T.R. (1984). Processing of RNA. In Animal virus RNA processing, D.Apirion, ed. CRC Press), pp. 181-212.

Buchkovich, K., Duffy, L.A., and Harlow, E. (1989). The retinoblastoma protein is phosphorylated during specific phases of the cell cycle. Cell *58*, 1097-1105.

Burkhart, D.L. and Sage, J. (2008). Cellular mechanisms of tumour suppression by the retinoblastoma gene. Nat. Rev. Cancer *8*, 671-682.

Cao, J., Arulanandam, R., Vultur, A., Anagnostopoulou, A., and Raptis, L. (2007a). Adenovirus E1A requires c-Ras for full neoplastic transformation or suppression of differentiation of murine preadipocytes. Molecular Carcinogenesis *46*, 284-302.

Cao, J., Arulanandam, R., Vultur, A., Anagnostopoulou, A., and Raptis, L. (2007b). Differential effects of c-Ras upon transformation, adipocytic differentiation and apoptosis mediated by the Simian Virus 40 Large Tumor Antigen. Biochemistry and Cell Biology *85*, 32-48.

Cariou, S., Donovan, J.C., Flanagan, W.M., Milic, A., Bhattacharya, N., and Slingerland, J.M. (2000). Down-regulation of p21WAF1/CIP1 or p27Kip1 abrogates antiestrogen-mediated cell cycle arrest in human breast cancer cells. Proc. Natl. Acad. Sci. U. S. A *97*, 9042-9046.

Chellappan, S., Kraus, V.B., Kroger, B., Munger, K., Howley, P.M., Phelps, W.C., and Nevins, J.R. (1992). Adenovirus E1A, simian virus 40 tumor antigen, and human papillomavirus E7 protein share the capacity to disrupt the interaction between

transcription factor E2F and the retinoblastoma gene product. Proc. Nat. Acad. Sci. USA *89*, 4549-4553.

Chellappan, S.P., Hiebert, S., Mudryj, M., Horowitz, J.M., and Nevins, J.R. (1991). The E2F transcription factor is a cellular target for the RB protein. Cell *65*, 1053-1061.

Ciardiello, F., Valverius, E.M., Colucci-D'Amato, G.L., Kim, N., Bassin, R.H., and Salomon, D.S. (1990). Differential growth factor expression in transformed mouse NIH-3T3 cells. J. Cell Biochem. *42*, 45-57.

Cole, C.N. (1995). Polyomavirinae: The viruses and their replication. In Virology, B.N.Fields, D.M.Knipe, and Hawley P.M., eds., pp. 917-946.

Collin, S., Fernandez-Lobato, M., Gooding, P.S., Mullineaux, P.M., and Fenoll, C. (1996). The two nonstructural proteins from wheat dwarf virus involved in viral gene expression and replication are retinoblastoma-binding proteins. Virology *219*, 324-329.

Dayaram, T. and Marriott, S.J. (2008). Effect of transforming viruses on molecular mechanisms associated with cancer. J. Cell Physiol *216*, 309-314.

DeCaprio, J.A. (2009). How the Rb tumor suppressor structure and function was revealed by the study of Adenovirus and SV40. Virology *384*, 274-284.

DeCaprio, J.A., Furukawa, Y., Ajchenbaum, F., Griffin, J.D., and Livingston, D.M. (1992). The retinoblastoma-susceptibility gene product becomes phosphorylated in multiple stages during cell cycle entry and progression. Proc. Natl. Acad. Sci. U. S. A *89*, 1795-1798.

DeCaprio, J.A., Ludlow, J.W., Figge, J., Shew, J.Y., Huang, C.M., Lee, W.H., Marsilio, E., Paucha, E., and Livingston, D.M. (1988). SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. Cell *54*, 275-283.

Diamandopoulos, G.T. (1996). Cancer: an historical perspective. Anticancer Res. *16*, 1595-1602.

Dumont, D.J. and Branton, P.E. (1992). Phosphorylation of adenovirus E1A proteins by the p34cdc2 protein kinase. Virology *189*, 111-120.

Dyson, N., Duffy, L.A., and Harlow, E. (1989). In vitro assays to detect the interaction of DNA tumor virus transforming proteins with the retinoblastoma protein. Cancer cells *7*, 235-240.

Eddy, B.E., Stewart, S.E., Young, R., and Mider, G.B. (1958). Neoplasms in hamsters induced by mouse tumor agent passed in tissue culture. J. Natl. Cancer Inst. *20*, 747-761.

Eeckhoute, J., Carroll, J.S., Geistlinger, T.R., Torres-Arzayus, M.I., and Brown, M. (2006). A cell-type-specific transcriptional network required for estrogen regulation of cyclin D1 and cell cycle progression in breast cancer. Genes Dev. *20*, 2513-2526.

Egan, C., Jelsma, T.N., Howe, J.A., Bayley, S.T., Ferguson, B., and Branton, P.E. (1988). Mapping of cellular protein binding sites on the products of early region E1A of human Adenovirus type 5. Mol. Cell. Biol. *8*, 3955-3959.

Ewen, M.E., Faha, B., Harlow, E., and Livingston, D.M. (1992). Interaction of p107 with cyclin A independent of complex formation with viral oncoproteins. Science *255*, 85-87.

Forng, R.Y. and Atreya, C.D. (1999). Mutations in the retinoblastoma protein-binding LXCXE motif of rubella virus putative replicase affect virus replication. J. Gen. Virol. *80 (Pt 2)*, 327-332.

Foster, J.S., Henley, D.C., Bukovsky, A., Seth, P., and Wimalasena, J. (2001). Multifaceted regulation of cell cycle progression by estrogen: regulation of Cdk inhibitors and Cdc25A independent of cyclin D1-Cdk4 function. Mol. Cell Biol. *21*, 794-810.

Garcea, R.L. and Imperiale, M.J. (2003). Simian virus 40 infection of humans. J. Virol. *77*, 5039-5045.

Godbout, R., Dryja, T.P., Squire, J., Gallie, B.l., and Phillips, R.A. (1983). Somatic inactivation of genes on chromosome 13 is a common event in retinoblastoma. Nature *304*, 451-453.

Goodrich, D.W., Wang, N.P., Qian, Y.W., Lee, E.Y., and Lee, W.H. (1991). The retinoblastoma gene product regulates progression through the G1 phase of the cell cycle. Cell *67*, 293-302.

Hiebert, S.W., Chellappan, S.P., Horowitz, J.M., and Nevins, J.R. (1992). The interaction of RB with E2F coincides with an inhibition of the transcriptional activity of E2F. Genes Dev. *6*, 177-185.

Hirsch, H.H. (2005). BK virus: opportunity makes a pathogen. Clin. Infect. Dis. *41*, 354-360.

Howley, P.M. and Lowy, D.R. (2007). Papillomaviruses. In Virology, D.M.Knipe and P.M.Howley, eds.

Hu, Q.J., Dyson, N., and Harlow, E. (1990). The regions of the retinoblastoma protein needed for binding to adenovirus E1A or SV40 large T antigen are common sites for mutations. EMBO J. *9*, 1147-1155.

Hume, A.J., Finkel, J.S., Kamil, J.P., Coen, D.M., Culbertson, M.R., and Kalejta, R.F. (2008). Phosphorylation of retinoblastoma protein by viral protein with cyclin-dependent kinase function. Science *320*, 797-799.

Javier, R.T. and Butel, J.S. (2008). The history of tumor virology. Cancer Res. *68*, 7693-7706.

Johnson, D.G., Schwarz, J.K., Cress, W.D., and Nevins, J.R. (1993). Expression of transcription factor E2F1 induces quiescent cells to enter S phase. Nature *365*, 349-352.

Jones, R.E., Heimbrook, D.C., Huber, H.E., Wegrzyn, R.J., Rotberg, N.S., Stauffer, K.J., Lumma, P.K., Garsky, V.M., and Oliff, A. (1992). Specific N-methylations of HPV-16 E7 peptides alter binding to the retinoblastoma suppressor protein. J. Biol. Chem. *267*, 908-912.

Kaelin, W.G., Jr., Ewen, M.E., and Livingston, D.M. (1990). Definition of the minimal simian virus 40 large T antigen- and adenovirus E1A-binding domain in the retinoblastoma gene product. Mol. Cell. Biol. *10*, 3761-3769.

Kim, H.Y., Ahn, B.Y., and Cho, Y. (2001). Structural basis for the inactivation of retinoblastoma tumor suppressor by SV40 large T antigen. EMBO J. *20*, 295-304.

Knudson, A.G., Jr. (1971). Mutation and cancer: statistical study of retinoblastoma. Proc. Natl. Acad. Sci. U. S. A *68*, 820-823.

Kobayashi, M., Mukai, N., Solish, S.P., and Pomeroy, M.E. (1982). A highly predictable animal model of retinoblastoma. Acta Neuropathol. *57*, 203-208.

Kobayashi, S. and Mukai, N. (1973). Retinoblastoma-like tumors induced in rats by human adenovirus. Invest Ophthalmol. *12*, 853-856.

Lee, W.H., Shew, J.Y., Hong, F.D., Sery, T.W., Donoso, L.A., Young, L.J., Bookstein, R., and Lee, E.Y. (1987). The retinoblastoma susceptibility gene encodes a nuclear phosphoprotein associated with DNA binding activity. Nature *329*, 642-645.

Levine, A. J. The origins of Virology. Fields Virology 1, 3-18. 2001.

Ludlow, J.W., Shon, J., Pipas, J.M., Livingston, D.M., and DeCaprio, J.A. (1990). The retinoblastoma susceptibility gene product undergoes cell cycle-dependent dephosphorylation and binding to and release from SV40 large T. Cell *60*, 387-396.

Miller, T.W., Perez-Torres, M., Narasanna, A., Guix, M., Stal, O., Perez-Tenorio, G., Gonzalez-Angulo, A.M., Hennessy, B.T., Mills, G.B., Kennedy, J.P., Lindsley, C.W., and Arteaga, C.L. (2009). Loss of Phosphatase and Tensin homologue deleted on chromosome 10 engages ErbB3 and insulin-like growth factor-I receptor signaling to promote antiestrogen resistance in breast cancer. Cancer Res. *69*, 4192-4201.

Mukai, N., Kalter, S.S., Cummins, L.B., Matthews, V.A., Nishida, T., and Nakajima, T. (1980). Retinal tumor induced in the baboon by human adenovirus 12. Science *210*, 1023-1025.

Mukai, N., Nakajima, T., Freddo, T., Jacobson, M., and Dunn, M. (1977). Retinoblastoma-like neoplasm induced in C3H/BifB/Ki strain mice by human adenovirus serotype 12. Acta Neuropathol. *39*, 147-155.

Munakata, T., Nakamura, M., Liang, Y., Li, K., and Lemon, S.M. (2005). Down-regulation of the retinoblastoma tumor suppressor by the hepatitis C virus NS5B RNA-dependent RNA polymerase. Proc. Natl. Acad. Sci. U. S. A *102*, 18159-18164.

Munger, K., Werness, B.A., Dyson, N., Phelps, W.C., Harlow, E., and Howley, P.M. (1989). Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. EMBO J. *8*, 4099-4105.

Musgrove, E.A. and Sutherland, R.L. (2009). Biological determinants of endocrine resistance in breast cancer. Nat. Rev. Cancer *9*, 631-643.

Park, J., Hwang, H.S., Buckley, K.J., Park, J.B., Auh, C.K., Kim, D.G., Lee, S., and Davis, K.R. (2010). C4 protein of Beet severe curly top virus is a pathomorphogenetic factor in Arabidopsis. Plant Cell Rep. *29*, 1377-1389.

Pietenpol, J.A., Stein, R.W., Moran, E., Yaciuk, P., Schlegel, R., Lyons, R.M., Pittelkow, M.R., Munger, K., Howley, P.M., and Moses, H.L. (1990). TGF-Beta1 Inhibition of c-myc Transcription and Growth in Keratinocytes is Abrogated by Viral Transforming Proteins with pRB Binding Domains. Cell *61*, 777-785.

Raptis, L. (1991). Polyomavirus middle tumor antigen increases responsiveness to growth factors. J. Virol. *65*, 2691-2694.

Raptis, L., Arulanandam, R., Vultur, A., Geletu, M., Chevalier, S., and Feracci, H. (2009). Beyond structure, to survival: Stat3 activation by cadherin engagement. Biochemistry and Cell Biology *87*, 835-843.

Raptis, L., Brownell, H.L., Wood, K., Corbley, M., Wang, D., and Haliotis, T. (1997). Cellular ras gene activity is required for full neoplastic transformation by Simian Virus 40. Cell Growth Differ. *8*, 891-901.

Sears, R.C. and Nevins, J.R. (2002). Signaling networks that link cell proliferation and cell fate. J. Biol. Chem. *277*, 11617-11620.

Shenk, T. (1995). Adenoviridae: The Viruses and Their Replication. In Fundamental Virology, 3rd Edition, B.N.Fields, D.M.Knipe, and P.M.Howley, eds. (Philadelphia: Lippincott-Raven).

Simonson, M.S. and Herman, W.H. (1993). Protein kinase C and protein tyrosine kinase activity contribute to mitogenic signaling by endothelin-1. Cross-talk between G protein-coupled receptors and pp60c-src. J. Biol. Chem. *268*, 9347-9357.

Sparkes, R.S., Murphree, A.L., Lingua, R.W., Sparkes, M.C., Field, L.L., Funderburk, S.J., and Benedict, W.F. (1983). Gene for hereditary retinoblastoma assigned to human chromosome 13 by linkage to esterase D. Science *219*, 971-973.

Stubdal, H., Zalvide, J., Campbell, K.S., Schweitzer, R.C., Roberts, T.M., and DeCaprio, J.A. (1997). Inactivation of RB related proteins P130 and P107 mediated by the J domain of Simian Virus 40 Large Tumor antigen. Mol. Cell. Biol. *17*, 4979-4990.

Thangavel, C., Dean, J.L., Ertel, A., Knudsen, K.E., Aldaz, C.M., Witkiewicz, A.K., Clarke, R., and Knudsen, E.S. (2011). Therapeutically activating RB: reestablishing cell cycle control in endocrine therapy-resistant breast cancer. Endocr. Relat Cancer *18*, 333-345.

van den Heuvel, S. and Dyson, N.J. (2008). Conserved functions of the pRB and E2F families. Nat. Rev. Mol. Cell Biol. *9*, 713-724.

Vogt, M. and Dulbecco, R. (1960). Virus-cell interaction with a tumor-producing virus. Proc. Natl. Acad. Sci. U. S. A *46*, 365-370.

Vultur, A., Arulanandam, R., Turkson, J., Niu, G., Jove, R., and Raptis, L. (2005). Stat3 is required for full neoplastic transformation by the Simian Virus 40 Large Tumor antigen. Molecular Biology of the Cell *16*, 3832-3846.

Whyte, P., Buchkovich, K.J., Horowitz, J.M., Friend, S.H., Raybuck, M., Weinberg, R.A., and Harlow, E. (1988). Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. Nature *334*, 124-129.

Windle, J.J., Albert, D.M., O'Brien, J.M., Marcus, D.M., Disteche, C.M., Bernards, R., and Mellon, P.L. (1990). Retinoblastoma in transgenic mice. Nature *343*, 665-669.

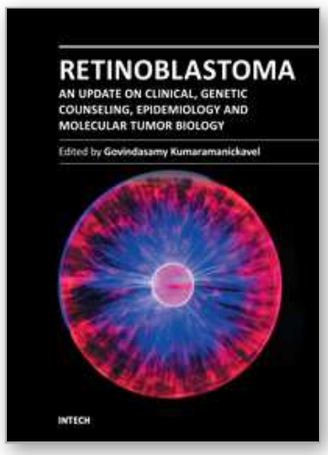
Xiao, B., Spencer, J., Clements, A., Ali-Khan, N., Mittnacht, S., Broceno, C., Burghammer, M., Perrakis, A., Marmorstein, R., and Gamblin, S.J. (2003). Crystal structure of the retinoblastoma tumor suppressor protein bound to E2F and the molecular basis of its regulation. Proc. Natl. Acad. Sci. U. S. A *100*, 2363-2368.

Xie, Q., Suarez-Lopez, P., and Gutierrez, C. (1995). Identification and analysis of a retinoblastoma binding motif in the replication protein of a plant DNA virus: requirement for efficient viral DNA replication. EMBO J. *14*, 4073-4082.

Yee, S.P. and Branton, P.E. (1985). Detection of cellular proteins associated with human adenovirus type 5 early region 1A polypeptides. Virology *147*, 142-153.

Young, A.P., Nagarajan, R., and Longmore, G.D. (2003). Mechanisms of transcriptional regulation by Rb-E2F segregate by biological pathway. Oncogene *22*, 7209-7217.

Yu, H., Pardoll, D., and Jove, R. (2009). STATs in cancer inflammation and immunity: a leading role for STAT3. Nat. Rev. Cancer *9*, 798-809.



Retinoblastoma: An Update on Clinical, Genetic Counseling, Epidemiology and Molecular Tumor Biology

Edited by Prof. Govindasamy Kumaramanickavel

ISBN 978-953-51-0435-3
Hard cover, 170 pages
Publisher InTech
Published online 28, March, 2012
Published in print edition March, 2012

Retinoblastoma is the first tumor suppressor gene discovered ever. The discovery opened a new avenue in the field of oncology leading to the identification of 35 tumor suppressor genes, till date in our genome. This book is an excellent compilation of both clinical and basic science information that meets the needs of a young clinician and a researcher at the same time. It also has abundant information on recent advances and cutting-edge knowledge in intracellular molecular cross-talking of retinoblastoma protein with various cellular viral-like proteins.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

M. Geletu and L. Raptis (2012). Viral Oncogenes and the Retinoblastoma Family, Retinoblastoma: An Update on Clinical, Genetic Counseling, Epidemiology and Molecular Tumor Biology, Prof. Govindasamy Kumaramanickavel (Ed.), ISBN: 978-953-51-0435-3, InTech, Available from: http://www.intechopen.com/books/retinoblastoma-an-update-on-clinical-genetic-counseling-epidemiology-and-molecular-tumor-biology/rb-and-viral-oncogenes

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen