

*Minireview***The *RB2/p130* Gene: The Latest Weapon in the War against Lung Cancer?<sup>1</sup>****Pier Paolo Claudio, Mario Caputi, and Antonio Giordano<sup>2</sup>**

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**Abstract**

**Lung cancer is the second cause of death after cardiovascular diseases and is the major cause of cancer deaths in the Western world. Large scale screening trials conducted 15–20 years ago using chest X-rays and sputum cytology were able to detect stage I cancers but failed to impact on survival. This is because of the early metastatic potential of small primary tumors. It is important then to detect lung cancer at an earlier stage, studying and identifying genetic lesions that could indicate a new target(s) for gene therapy. The retinoblastoma-related gene *pRb2/p130*, a new tumor suppressor gene cloned in 1993, is emerging as one of the candidate markers and targets for gene therapeutic approach. Effective genetic therapy requires both a genetic material to be used therapeutically and a means to deliver it. A scope for this review is to examine some of the gene delivery systems mostly used, discussing their weaknesses and strengths, and to discuss the role of *pRb2/p130* in lung cancer.**

**Introduction**

Lung cancer is one of the leading causes of cancer death in the world (1). The high mortality rate for lung cancer probably results from the absence of standard clinical procedures for diagnosis of early tumoral stages compared with breast, prostate, and colon cancers (2). Early studies indicated that several distinct chromosomal loci (3p, 9p, 13q, 17p, and others) are implicated, suggesting that maybe sequential genetic events occur during initiation and progression of lung carcinogenesis

(2–4). Recent studies indicated instead that allelic loss of several other chromosomal regions could be involved in the pathogenesis of lung cancer. These chromosomal regions include 1p, 1q, 2q, 5p, 6p, 8p, 8q, 10q, 14q, 17q, 18q, and 22q (5–12). The malignant transformation of pulmonary epithelial cells is the result of multistep accumulation of genetic and molecular alterations highly related to tobacco carcinogens, involving key regulatory elements of the cell cycle and mechanism of proliferation and apoptosis. Oncogene activation (*ras*, *myc*, and autocrine growth factors loops) or more importantly tumor suppressor gene inactivation (*p53*, *pRb* family, and cyclin-dependent kinase inhibitor *p16*) at a genetic, epigenetic, or posttranslational level removes crucial constraints on cell division at the G<sub>1</sub> checkpoint and apoptosis, accelerating cell division (Refs. 13 and 14; Fig. 1). *p53* inactivation is one of the most common alterations in lung cancer (75% of genetic alterations). In fact, mutations of *p53* have been reported with frequencies up to 50% in NSCLC<sup>3</sup> and 70–80% in SCLC (15, 16). On the other hand, some authors have reported mutations or deletions of the *RB* gene in NSCLCs in >90% of the cases (16). *p53* missense mutation is highly concordant with *p53* stabilization and immunoreactivity; other gene products, like *pRb* and *Ras*, are either rapidly degraded or not detectable at the immunohistochemical level if mutated. By immunohistochemistry the expression of *p53*, *pRb*, *Ras*, and *Bcl-2* have been investigated in a panel of 65 samples of preneoplastic lesions of the bronchial epithelium. The frequency of *p53*-positive and *pRb*-negative microscopic fields was directly related to the morphological grading of the lesions. One of the main patterns found to be correlated with the severity of histopathological features was characterized by combined *p53* hyperexpression and *pRb* hypoexpression (17).

Lung cancer arises from a series of morphological and molecular changes that take several years to progress from a normal epithelium to an invasive cancer. The molecular changes include activation of dominant oncogenes as well as loss of recessive growth regulatory genes or antioncogenes (18). Interestingly, some authors could correlate the prognostic significance of the loss of *Rb* protein either alone or combined with *Ras* or with *p53* in patients with NSCLC. The individual with theoretically the best pattern of protein expression in their tumors *versus* those with theoretically the worst pattern of gene expression, *i.e.*, *Rb+/Ras-* *versus* *Rb-/Ras+* and *Rb+/p53-* *versus* *Rb-/p53+*, showed a longer period of survival. The correlation between *Rb* and *Ras* appeared to be a better prognostic factor compared with the *Rb/p53* status in NSCLCs. In patients affected by squamous cell carcinoma, neither *Rb/Ras* nor *Rb/p53* status was a significant prognostic factor in this cohort instead (19).

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<sup>3</sup> The abbreviations used are: NSCLC, non-small cell lung cancer; AAV, adeno-associated virus; rAAV, recombinant AAV; wt, wild type; TAG, large T antigen; rSV40, recombinant SV40; *Rb*, retinoblastoma.

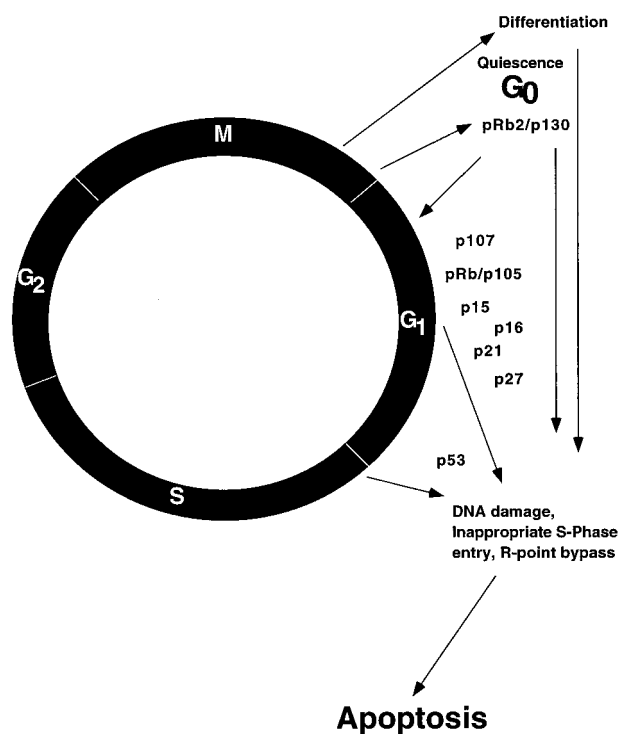


Fig. 1 Schematic representation of the involvement of tumor suppressor genes in the cell cycle.

Therapeutically, radiation and chemotherapy have been the two main treatment modalities for advanced NSCLC (20). New techniques in radiation as well as introduction of cisplatin into chemotherapeutic regimens for NSCLC has changed the life span of these patients, but each of these techniques has shown their limitations already. Combined treatments with radiation and chemotherapy have become a powerful alternative for patients with unresectable and locally advanced NSCLC (20). Until now, none of these treatments have changed the natural history of lung cancer patients.

Gene replacement therapy is potentially a very powerful tool, targeting specific molecular mediators of cancer development and progression. The field of gene therapy has been rapidly expanding since the first submission of gene therapy trials in the 1990s, which provided encouraging results (21). In the last decade, much effort has been dedicated to improving protocols in human gene therapy. Many significant goals have been achieved, although there are still several issues that investigators have to address to develop new efficient therapeutic approaches to treat cancer. The number of approved protocols in clinical trials for cancer is increased considerably in the last decade and have been extended to several types of cancer such as sarcoma, lung cancer, melanoma, and brain, ovarian, and breast tumors (22, 23). Phase I studies are in progress today using replication-defective adenoviral expression vectors encoding wt p53 in patients with incurable NSCLC (24, 25). The viruses have been delivered by bronchoscopic intratumoral injection or by computed tomography-guided percutaneous intraluminal injection of the vector solution. Until now, no toxic

effects have been registered using these viral vectors. Different genes are currently under investigation for their use in human gene therapy such as p53, p16, p21, Bax, pRb, and others (13, 24–30).

### Gene Therapy Models

There is a broad variety of gene delivery systems that have been designed to introduce either DNA or oligonucleotides in mammalian cells. They comprise viral-based systems such as: retroviral-based vector systems (comprising lentivirus; Refs. 31–38), adenovirus (21, 39–43), AAV (AAV vectors; Refs. 44–53), SV40 virus (54–56), herpes simplex viral vectors (57–59), human cytomegalovirus (60), EBV (61), poxvirus (62, 63), negative-strand RNA viruses (influenza virus; Ref. 64), alphaviruses (65), and herpesvirus saimiri virus (66). Other nonvirally based systems are composed of cationic liposomes and receptor-mediated poly-lysine-DNA complexes (67–71). Some of these systems are well characterized for gene therapy use such as retroviruses, adenoviruses, AAV, and SV40 viruses, whereas others are still not very well known today such as poxvirus, negative-strand RNA viruses (influenza virus), alphaviruses, and herpesvirus saimiri virus. Each of these systems has advantages and disadvantages (Table 1).

Scientists in all laboratories are working to produce safe and suitable gene transfer systems for human gene therapy. In fact, the most important concern in designing gene delivery systems is that they must be nonpathogenic and nontoxic in patients so that they can be used in the clinics. For these reasons, as a general rule, all of the viral vectors have been modified to be replication defective and do not contain genes that encode for factors that may be hazardous or toxic for humans. Gene therapy models have been already tried, first *in vitro* and then *ex vivo* and *in vivo*. As mentioned previously, the variety of gene delivery systems is too ample to be described in great details. However, some of these viral-based systems, which in our opinion are more likely to be used in human gene therapy and to implement the efficiency of gene transfer technology in the near future, are worth discussing in this review.

### Retroviral Vectors

Retroviruses are among the most efficient vector systems for transducing genes into mammalian cells, and they have been successfully used to deliver therapeutic genes into humans (31–34, 72–77). The best-characterized retroviral vector system is based on the amphotropic Moloney murine leukemia virus (78–80). This retroviral vector system meets all of the requirements to achieve an efficient transduction *in vitro*; the integration of the viral genome allows for long-term gene expression, and the viral, relatively high titer obtainable ( $10^6$ – $10^7$  cfu/ml) allows for optimal *in vitro* transduction.

Retroviruses do not elicit immune response in the host, but unfortunately there are some limitations to their use *in vivo*. In fact, the random integration of the viral genome may eventually result in mutagenesis, and the levels of viral titers are not yet sufficient for efficient *in vivo* gene transfer. In fact, the optimal levels of titer for gene transfer are in the range of  $10^{10}$  cfu/ml. A third issue that we should consider is that retroviruses are rapidly degraded by complement, which again limits their use in

Table 1 Gene delivery systems

Vectors	Advantages	Disadvantages
Retrovirus	<ul style="list-style-type: none"> <li>● Relatively high titers (<math>10^6</math>–<math>10^7</math> cfu/ml).</li> <li>● Broad cell tropism.</li> <li>● Stable gene expression.</li> <li>● No toxic effect on infected cells.</li> <li>● Insert capacity in the range of 10 kb.</li> <li>● Not immunogenic.</li> </ul>	<ul style="list-style-type: none"> <li>● Infect only dividing cells.</li> <li>● Random insertion of the foreign gene in the host genome.</li> <li>● Possible homologous recombination and formation of replication-competent viruses.</li> <li>● Inactivated by complement.</li> <li>● Labile.</li> </ul>
Adenovirus	<ul style="list-style-type: none"> <li>● Very high titers (<math>10^{10}</math> pfu/ml).</li> <li>● High levels of gene expression.</li> <li>● Infect dividing and nondividing cells.</li> <li>● Large insert capacity (7–8 kb).</li> </ul>	<ul style="list-style-type: none"> <li>● Elicits a strong host immune response.</li> <li>● Lack of viral genome integration in the host and short-term gene expression.</li> <li>● Complicated to produce.</li> </ul>
AAVs	<ul style="list-style-type: none"> <li>● Infect dividing and nondividing cells.</li> <li>● Viral integration specific for human chromosome 19.</li> <li>● Nonpathogenic, nontoxic.</li> <li>● Not immunogenic.</li> </ul>	<ul style="list-style-type: none"> <li>● High titers difficult to obtain.</li> <li>● Limited capacity for foreign genes.</li> </ul>
SV40 virus	<ul style="list-style-type: none"> <li>● Infect dividing and nondividing cells.</li> <li>● Stable gene expression.</li> <li>● Not immunogenic. Virus particles do not elicit neutralizing antibodies; multiple administrations can be given.</li> <li>● Easily produced at high titers (<math>10^{10}</math> pfu/ml).</li> <li>● No adverse effect observed to date.</li> </ul>	<ul style="list-style-type: none"> <li>● These vectors are still not well characterized.</li> <li>● Limited capacity for foreign genes (5 kb).</li> </ul>

gene therapy *in vivo* (81). Many strategies have been tried to improve the efficiency of retroviral gene transduction *in vivo*. In fact, scientists had concentrated on the problem of optimizing retroviral titers, which were achieved to some extent by transient expression systems (82–85).

Besides the obvious safety advantage of ruling out the possibility of helper virus formation by homologous recombination, because the packaging components were placed on different plasmids, the transient expression systems offered the possibility of improving the retroviral titers from 10- to 50-fold with respect to those obtained with conventional packaging cell lines (22). An attempt to concentrate the viral particles failed, because retroviruses are among the most fragile viruses, and current concentrating techniques are still aggressive for this type of virus.

The requirement for host cells to actively divide to allow viral genes to integrate into the host genome (34) may be theoretically advantageous for cancer gene therapy. This would limit exogenous gene delivery to rapidly proliferating cancer cells while sparing delivery to other nonproliferating cells within the affected organ. The aforementioned limitations still restrict the use of retroviruses in human gene therapy.

### Adenovirus

Adenoviruses are large, nonenveloped DNA viruses, with a double-stranded genome of 36 kb (39). Human adenovirus was isolated for the first time in 1953, and since then 49 serotypes have been identified and classified in six different groups according to their similarities in genome organization and hemagglutinin activities (86). Some adenovirus serotypes are capable of causing tumors in animals. The first recombinant adenoviral vectors were engineered in 1985 and were derived by serotypes 2 and 5, because they are not known to cause severe disease in humans and do not cause tumors in animals (40, 41). Since then, scientist focused on the possibility of introducing adenoviral vectors in human gene therapy protocols. The first adenoviral application in gene therapy was carried out in the 1990s for

diseases linked to genetic deficiencies (ornithine transcarbamylase and cystic fibrosis; Ref. 21). Many different approaches have been tried in cancer gene therapy using adenoviral vectors either *in vitro* or *in vivo* in animals. Infection of cancer cells by recombinant adenovirus is dependent on the virus:cell ratio (87). These vectors can be produced at high titers ( $10^{10}$  pfu/ml), which can ultimately be concentrated to higher titers because adenoviruses are more stable than retroviruses. The possibility of encapsulating large *trans*-genes, to infect nondividing cells, to obtain high levels of *trans*-gene expression, and the lack of integration of the viral genome into the host genetic information render this viral vector suitable for gene therapy trials. However, the foreign gene is expressed only transiently, because the viral genome is exclusively extrachromosomal. For this reason, adenoviral vectors are not suitable for long-term expression. Additionally, when adenoviral vectors are administered to animals as well as to humans, they are responsible of a strong immune reaction, which may cause inflammatory and toxic reaction and may vanish the therapeutic purposes (88–96). Of course, the immunogenic problem poses a severe limitation in the use of chronic diseases such as cancer, in which repeated exposure to the viral vector may be needed. Accordingly, some scientists thought to use the immunogenic capabilities of this class of viruses to target specific cells with the action of the host immune system. These studies are still in progress. Other strategies that are under investigation are linked to the capacity of native adenoviruses to produce some proteins, which are able to bind and inactivate cellular key cell cycle regulators, such as wt p53. Basically, these researchers genetically engineered adenoviruses in which these proteins (such as E1A) are modified in their transforming capacity but retain their ability to bind only the mutated forms of p53, leading the target cell to a lytic cycle and therefore selectively killing the cancerous cells. These studies are also currently in progress. Other strategies attempted are directed to reduce the virus load in combination with a short-term immune suppression (97, 98).

Another challenge for the researchers is to improve the

transduction efficiency and the infective capacity of these viral vectors. One field that the scientists are exploring is the possibility to enhance the viral transduction capacity of cancer cells by a pretreatment with cisplatin, a common chemotherapeutic drug. The cisplatin plus *p53* gene transfer strategy yielded significantly greater apoptosis and tumor growth suppression in an animal model in which a lung tumor cell line was grown than wt *p53* gene transfer alone (77, 87).

## AAV

AAV belongs to the family of the Parvoviridae, which is a nonenveloped virus of 20–25-nm diameter and contains a linear, single-stranded DNA genome of 4.6 kb. Despite the fact that this virus is widespread, it has not been linked to any human disease (44, 99). rAAVs have attracted considerable interest as vectors for gene therapy because they lack all viral genes and are able to infect a wide variety of cells, both dividing and quiescent (45, 47–53). Additionally, rAAV are not immunogenic and are able to raise long-lasting gene expression *in vivo*, even after a single virus injection (100). Several authors reported persistence of expression of foreign genes transduced with rAAV from 180 days up to 18 months (101–104). Another interesting hallmark of this virus is that in the absence of helper virus, wt AAVs have the ability to integrate their viral genome in the host, and that this integration is site specific (q arm of the chromosome 19), generating a latent infection (49, 51). However, genetically engineered rAAVs lose this desired safety feature of site-directed genome integration and accommodate only 4.9-kb *trans*-genes (105). One of the major disadvantages in rAAV generation has been the requirement for coinfection with an unrelated virus, such as adenovirus, to provide essential helper functions for the productive life cycle. This procedure of AAV production is efficient, but it results in a number of problems. The possibility of contaminating adenoviral particles is the major concern. Removal of the contaminating part by physical techniques, such as CsCl<sub>2</sub> gradients or column chromatography, and an adenoviral heat inactivation have been attempted. However, the potential for residual contamination and the presence of immunogenic adenoviral denatured proteins in the preparation is still a concern for human trials (106). To circumvent this issue, the generation of rAAV without wt adenovirus as a helper virus has been reported by Samulsky *et al.* (107) since 1989. Recently, the same group has described a more advanced and efficient method of rAAV generation that is completely free of adenovirus helper virus and with high yields of viral particles (108). The novelty of this method consists of the use of a plasmid construct that contains a mini-adenoviral genome capable of propagating rAAV in the presence of AAV *Rep* and *Cap* genes. Cotransfection of 293 cells with the new mini adeno-helper and AAV packaging plasmids results in titers of adenovirus-free AAV as high as  $1 \times 10^5$  viral particles/cell. This advance in AAV generation should impact the study of rAAV vectors as therapeutic vehicles, addressing the major safety concerns of cross-contamination and immunogenicity.

## SV40 Viruses

SV40 viruses are nonenveloped DNA papova viruses. Their circular, double-stranded DNA genome is 5.2 kb. It lacks

terminal repeat regions that characterize many other viral vectors with linear genomes. SV40 early (EP) and late promoters (LP) are on opposite strands. The SV40-EP drives expression of TAg and small t antigen (tAg), but even if their genes are encoded by the same DNA, their transcripts are spliced differently. The SV40-LP is responsible for transcription of the three structural, or capsid, genes *VP1*, *VP2*, and *VP3*. Similar to the early genes, the late genes are also produced by overlapping, differently spliced transcripts. They are able to infect a broad variety of cells, including dividing and nondividing cells (54–56, 109). The capacity to integrate their viral genome in that of the host, accommodating *trans*-genes up to 5 kb and the lack of immune response, are interesting features for their possible application in gene therapy (54, 55, 109). Engineered SV40 vectors can be easily produced at high titers ( $10^{10}$  pfu/ml and more), and they may transfer sustained expression of foreign genes to bone marrow as well as other organs for at least 3 months and are suitable for either *in vitro* or *in vivo* gene transfer (54). Stable, efficient gene transfer to cells is a prerequisite for gene therapy of a number of diseases, both hereditary and acquired, and requires an efficient, nonreplicating, transfer agent. In general, to be a useful gene transfer vector, any chosen system must be efficient in delivering the genetic material but at the same time also safe for both the patients and the operator. Our understanding of the biology of wt SV40 provides the background that is necessary to discern both the strengths and weaknesses of SV40 as a gene delivery vector. Immunogenicity is one of the major concerns of today's gene therapy protocols. SV40 virus after binding enters the cells by pinocytosis and is transported immediately to the nucleus, where it is uncoated. The viral proteins are, therefore, hidden from the host immune system. Wild-type SV40 virus elicits an immune response only after a large amount of TAg and the proteins of the viral capsid are produced and exposed on the host cellular membrane. Without TAg, this step will not occur; hence, rSV40s, by lacking TAg in their genome, will not produce any of the immunogenic particles.

The safety of any gene delivery vectors is another very important concern, and rSV40 vectors are no exception. Like retroviruses, SV40 integrates randomly into cellular genomes, and it may either activate or inactivate cellular genes. Clearly, any vector that integrates may disrupt a critical gene, causing the cell to become dysfunctional or die. On the other hand, insertional activation of a gene could result in cellular proliferation and oncogenesis. In fact, retrovirus, such as the Moloney murine leukemia virus, can cause tumors with this mechanism. Retroviruses contain in their genome long terminal repeat sequences with outward-directed promoter activity that are adjacent to integration sites, which makes the potential for positional activation of an undesired gene a statistically possible event.

SV40 sequences at each integration site do not possess outward-directed promoter activity. Cellular genes would be accidentally activated if virus sequences at an integration site were just downstream from the early promoter. Thus, the likelihood of activation of cellular genes is less for SV40 than for Moloney murine leukemia virus vectors.

An additional noteworthy potential concern is the capacity of TAg to bind and functionally inactivate important cell cycle proteins such as p53 and the Rb family of proteins (110, 111),

which would theoretically impair the usefulness of SV40 virus as a gene therapy vector at first thought. At second thought, however, recombinant SV40 viruses, by missing TAG in their genome, also lack this oncogenic potential.

The last important safety issue is the possibility of contamination of the recombinant virus with wt SV40. It is true that wt SV40 could theoretically be produced by recombination between the rSV40 and the wt SV40 genomes in the packaging cells and that this would constitute an unfortunate event. However, there are still many debates on the oncogenic capacity of the SV40 in humans. In fact, wt SV40 has been demonstrated oncogenic only in the hamster animal model until now (112). Despite a few reports of some human tumors carrying SV40 TAG-like sequences (110, 111, 113, 114), multiple epidemiological studies have found no evidence that wt SV40 causes tumors in humans (115–119). Additionally, recently Fang *et al.* (20) have reported a system for packaging SV40 vectors without viral coding sequences. In their work, they describe the ability of recombinant adenovirus expressing SV40 capsids to effectively package plasmids that contain SV40 replication origin (120). More recently, it has been described that SV40 capsid proteins synthesized in insect cells are capable of packaging plasmids into SV40 pseudovirions *in vitro*. Thus, SV40 replication-competent virus can be effectively eliminated (121).

In conclusion, granted its apparent safety to date, SV40 vectors constitute a promising category of viral shuttles for potential application in gene therapy.

### The *RB2/p130* Gene and Its Prospect in Lung Cancer Therapy

Still today, gene therapy is not an official tool available to conventional medicine. Different research models have been proposed and studied to reduce the gap existing between conventional and experimental therapies (22, 24, 26–33, 35–37, 42, 43, 53–55, 59, 61–64, 72, 73, 76, 77, 88, 90, 92, 93, 95, 96, 98, 122–132).

As a model to study carcinogenesis, mice are among the most suitable. In particular, on one hand, mice develop lung tumors similar in their histogenesis and molecular features to peripheral adenocarcinomas in humans, and on the other hand, mice are an easy model to grow *in vivo* tumors and to study different delivery systems suitable for human gene therapy. The advantage of this model system is that events early in the tumorigenesis can be studied in their molecular events. In fact, decreased expression of p15, p16, Rb, cyclin D1, Apc, Mcc, and Gjal occur in the murine and the human species as well (133).

Both p53 and the prototypic tumor suppressor gene *RB* are mutated in a number of human tumors, and numerous studies are now beginning to identify their roles in cancer development, as well as in normal cell physiology. In cell lines that lack a functional pRb or p53 protein, the restoration of the function of the proteins suppresses the neoplastic properties of the cells (25, 130, 134–140).

Attempts at human gene therapy in different types of cancers using retroviral or adenoviral vectors to deliver either a wt p53 or pRb/p105 (RB1), replacing the malfunctioning gene, have been used frequently (25, 77, 129, 135, 140–144).

New targets are under investigation today. *RB2/p130* is a

member of the Rb family, which elicits growth-suppressive properties that are not fully functionally redundant with the other two known Rb members (RB/p105 and p107; Refs. 145 and 146). In a very recent study, using a tetracycline-regulated gene expression system to control the expression of *RB2/p130* (a member of the Rb family) in a JC virus-induced hamster brain tumor cell line, it was demonstrated for the first time that *RB2/p130* could be used to reduce the growth of tumors when grown s.c. in nude mice. Induced expression of pRb2/p130 in this *in vivo* study brought about a 3.2-fold or 69% reduction in tumor mass in nude mice (125). Another study in *nude* mice showed that ectopic expression of pRb2/p130 suppresses the tumorigenicity of the erb-2-overexpressing SKOV3 ovarian tumor cell line (147).

From the same group, the effects of expressing pRb2/p130 *in vivo* in a lung adenocarcinoma cell line have been also analyzed using a retroviral delivery approach (148). Retroviruses are among the most efficient vector systems for transducing genes into mammalian cells, and they have been successfully used to deliver therapeutic genes into humans (34, 135). The requirement for host cells to actively divide to allow viral genes to integrate into the host genome (34) may be advantageous for cancer gene therapy and would limit exogenous gene delivery to rapidly proliferating cancer cells while sparing delivery to other nonproliferating cells within the affected organ. *In vivo* retroviral transduction of the *RB2/p130* gene in established tumors, derived from injection of the lung adenocarcinoma cell line H23 grown s.c. in nude mice, reduced the mass 12-fold with respect to the control viruses (148).

In support of an involvement of the *RB2/p130* gene in human cancer as a tumor suppressor gene, it maps to human chromosome 16q12.2, an area in which deletions have been found in several human neoplasias including breast, ovarian, hepatic, and prostatic cancers (149). Intriguingly, immunohistochemical data examining the expression of the Rb family proteins in 235 retrospective specimens of lung carcinoma and/or on lung fine needle aspiration biopsies suggested an independent role for the reduction or loss of pRb2/p130 expression in the formation and/or progression of lung carcinoma (150–152). Moreover, low expression levels of pRb2/p130 have been found to correlate with poor prognosis and high aggressiveness in other cancer types (153–155). Additionally, mutations in the *RB2/p130* gene in a human cell line of small cell carcinoma as well as in primary lung tumors and different primary tumors have been reported (148, 154, 156–159).

Taken together, these results support the hypothesis that *RB2/p130* is a tumor suppressor gene. These findings have significant clinical implications involving prognosis and implementation of lung cancer therapeutic strategies.

Another mechanism of tumor suppressor gene inactivation recently proposed is that the oncoprotein of SV40, the SV40 TAG, targets and inactivates proteins such as the Rb family and p53, leading to transformation of human cell lines *in vitro* and the production of tumors in rodents (110, 111, 160, 161). Although mesotheliomas are among the most aggressive human cancers, alterations of important cell cycle “controllers,” such as the Rb family genes, have never been reported in these tumors. The same authors described the presence of SV40-like sequences in archival specimens of mesothelioma, demonstrating

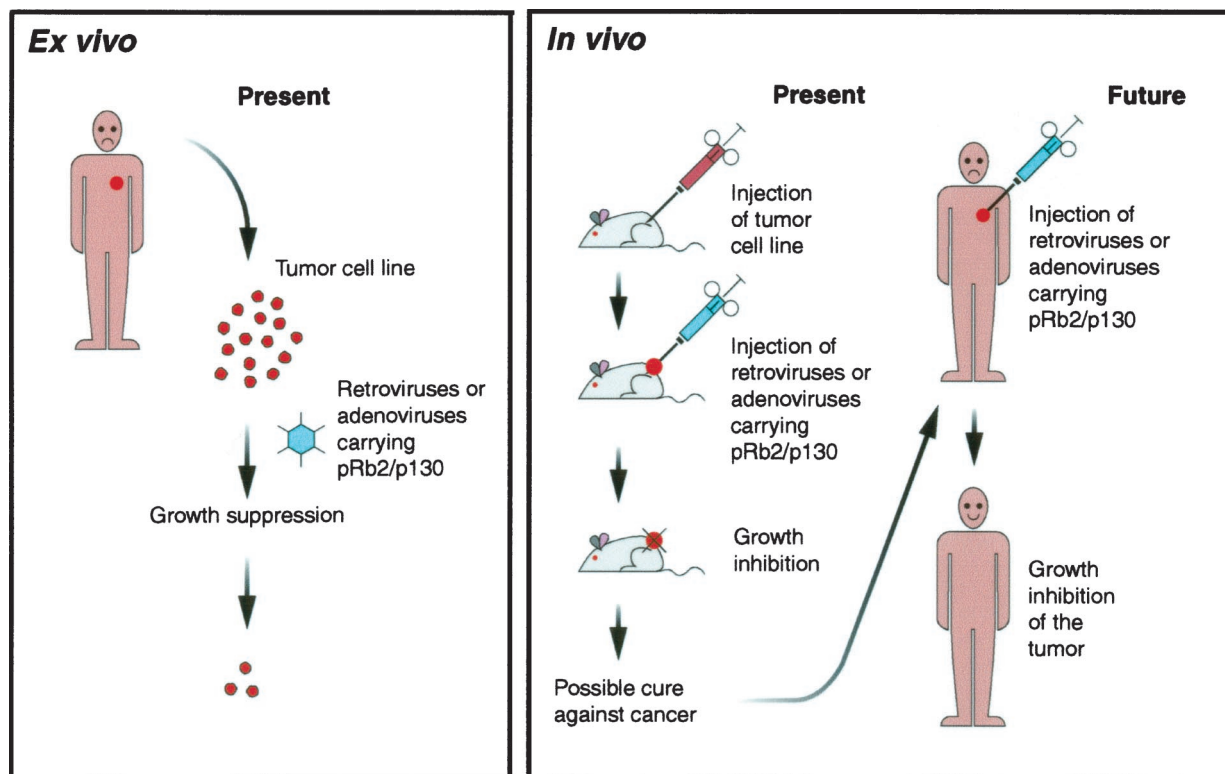


Fig. 2 Schematic representation of the therapeutic possibilities of *RB2/p130* in lung cancer. Present studies and future prospects are shown.

that SV40 TAg, isolated from frozen biopsies of human mesotheliomas, binds each of the Rb family proteins, pRb, p107, and pRb2/p130, and also p53 (110, 111). This obviously raises the question of whether the tumorigenic potential of the SV40 TAg in some human mesotheliomas may arise from the tumor's ability to interact with and thereby inactivate several tumor and/or growth-suppressive proteins.

The absence of mutations in Rb family proteins and the unusual high level of expression of pRb, pRb2/p130, and p107 in the mesothelioma specimens can be explained by the physical association of these proteins with SV40 TAg, which should lead to their inactivation. All members of the Rb gene family share the ability to interact physically with certain oncoproteins of DNA tumor viruses. These viral oncoproteins compete with the E2F family of transcription factors for binding to the Rb family proteins (162). Release of the E2F family members from the pocket structure leads to the induction of genes needed to proceed through the cell cycle. The finding that *p53*, a well-characterized tumor suppressor gene, is also a target of SV40 TAg (111, 163) further supports the model of viral transformation, via the binding of the viral oncoproteins, to a specific region of cell growth suppressor genes. The SV40 TAg, found in these and other mesothelioma samples, by targeting and inactivating p53 and the Rb family proteins (two critical, negative cell cycle-regulatory elements), could lead to carcinogenesis.

A paradigm is forming that the removal or inactivation of a functional pRb/p105, pRb2/p130, or p53 protein by way of

tumor viral oncoproteins, as is the case in SV40 TAg-associated mesothelioma (110, 111, 163), or by way of genetic alteration as is the case in lung cancer (2, 4, 15, 16, 18, 164), may be a critical event in the malignant transformation of a cell.

Considering the impact of lung cancer in terms of morbidity and mortality on the Western world (165), it is possible to suggest that RB1 (pRb/p105), RB2 (pRb2/p130), and p53, alone or in combination, could serve as valuable tools on establishing the molecular diagnosis and/or prognosis of lung cancer. Additionally, identification of mutations within these tumor suppressor loci in lung cancer could have possible implications on guiding and designing standard as well as novel therapeutic regimes, such as targeted gene transfers assisted by viruses. Because the use of *RB2/p130* viral-mediated gene transfer proved to be efficient in both an *in vitro* and in an *in vivo* animal model, it would be worthwhile to also test its effectiveness in humans. This gene therapy study could be performed also in combination with other genes, such as *p53*, that proved to be somehow efficient in lung cancer to study possible synergistic actions that could improve the outcomes of lung cancer patients (Fig. 2).

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