



ELSEVIER

DNA tumor viruses — the spies who lyse us

Clodagh C O'Shea

Identifying the molecular lesions that are 'mission critical' for tumorigenesis and maintenance is one of the burning questions in contemporary cancer biology. In addition, therapeutic strategies that trigger the lytic and selective death of tumor cells are the unfulfilled promise of cancer research. Fortunately, viruses can provide not only the necessary 'intelligence' to identify the critical players in the cancer cell program but also have great potential as lytic agents for tumor therapy. Recent studies with DNA viruses have contributed to our understanding of critical tumor targets (such as EGFR, PP2A, Rb and p53) and have an impact on the development of novel therapies, including oncolytic viral agents, for the treatment of cancer.

Addresses

UCSF Comprehensive Cancer Center, 2340 Sutter Street, Box 0128, San Francisco, CA 94115, USA

Corresponding author: O'Shea, Clodagh (coshea@cc.ucsf.edu)

Current Opinion in Genetics & Development 2005, 15:18–26

This review comes from a themed issue on
Oncogenes and cell proliferation
Edited by Michelle D Garrett and Sibylle Mitnacht

Available online 23rd December 2004

0959-437X/\$ – see front matter

© 2005 Elsevier Ltd. All rights reserved.

DOI 10.1016/j.gde.2004.12.010

Introduction

If medicine has been the great tutor of biology (William Harvey) then viruses have been the grand dons of oncology. More than 40 years ago it was known that DNA viruses could induce tumors in animals and transform non-permissive host-cells. Encoding relatively few genes, viruses provided a simple genetic system to study the transformation (see Glossary) of animal cells. DNA viruses are obligate intracellular parasites whose lytic cycle is totally dependent on their ability to commandeer the replicative machinery of their host. Thus, like tumor cells, DNA tumor viruses have had to evolve mechanisms that uncouple cellular replication from the many intracellular and extracellular factors that normally control it so tightly. Consequently, DNA viruses and tumor cells subvert many of the same cellular checkpoints (see Glossary). For example, in tumor cells, the Rb/p53 checkpoints are inactivated through mutations; DNA viruses encode specific viral proteins to achieve the same end (Figure 1). p53 was first identified as a cellular protein that

interacts with SV40 large T (LT) antigen, and E2F as a cellular factor that bound to the adenoviral *E2* promoter following *E1A* expression. Our understanding of oncogene cooperation and the Rb/p53 tumor suppressor pathways was greatly facilitated by the study of transforming viral proteins such as polyoma T antigens, adenovirus E1A/E1B-55K, human papillomavirus (HPV) E7/E6 and SV40 LT. In addition, the identification of src as a cellular target, not only of transforming retroviruses but also of polyoma middle T (MT), underscored its importance in cellular transformation and as a target for tumor therapy. Thus, the study of DNA viruses led to the identification of many of the critical targets of cancer therapy.

In a post-genomic era, equipped with the human genome sequence and a battery of futuristic technologies with which to determine the differences between tumor and normal cells, the age of viruses in cancer research may appear to have passed. However, technology does not always buy clarity, as evidenced by genomic analyses that have revealed thousands of differences between tumor and normal cells. Do we need to target all of these differences to scourge ourselves of cancer? Or does the instability inherent to tumor genomes generate a vast number of confounding mutations, many of which are largely irrelevant for generation or maintenance of the neoplastic phenotype? DNA viruses could help us find some of the answers. Owing to their relatively small genomes, DNA viruses are forced to abrogate complex and integrated cellular checkpoints with only a limited repertoire of viral proteins. Thus, viruses often encode more than one viral protein — or a single viral protein with multiple functions — to co-ordinate all the critical cellular activities necessary to drive quiescent cells into S-phase while simultaneously preventing apoptosis. By continuing to uncover the cellular targets of viral proteins we discover critical therapeutic targets for cancer therapy. Moreover, given that the oncogenic properties of DNA tumor viruses have evolved outside of the obligatorily integrated framework of host cellular processes, they can acquire novel strategies to perturb cellular checkpoints. This will reveal new insights as to how these checkpoints could be regulated and modulated for tumor therapy.

Viruses also have enormous promise as agents that undergo selective lytic replication in tumor cells. A profound functional overlap exists between DNA viruses and tumor cells with respect to the cellular checkpoints they must perturb to ensure their replication. This common ground forms the basis for several oncolytic viral therapies. DNA virus mutants that fail to inactivate normal checkpoints are being developed as lytic agents that

Glossary

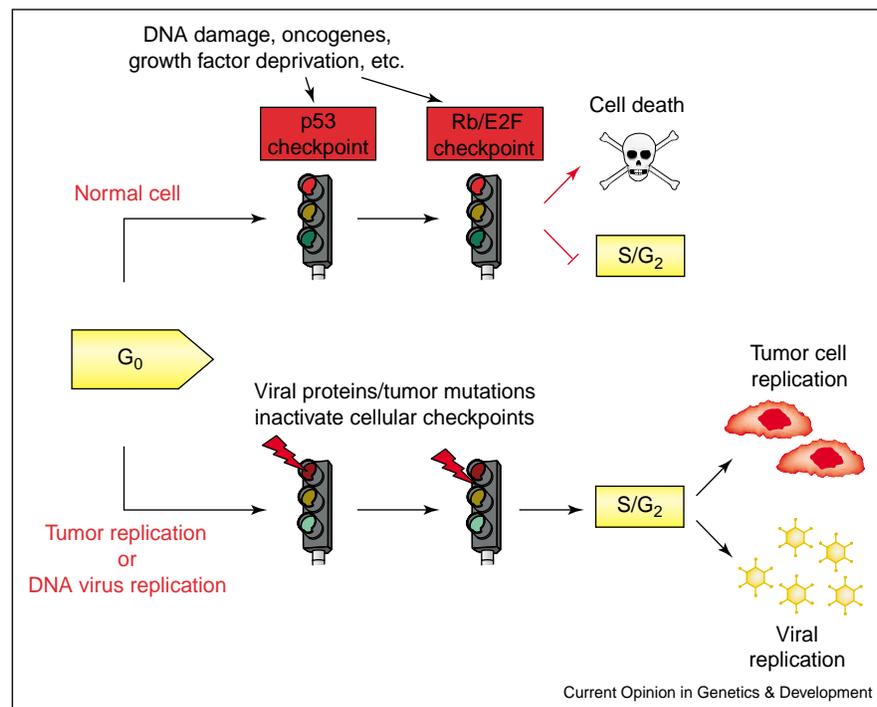
Arf – A critical tumor suppressor that prevents mdm2-mediated degradation of p53 (see also RH Costa *et al.*, this issue).
Cellular checkpoints – Proteins that play a key role in cell-cycle stress responses.
HAT – Histone acetyl transferase (for example, p300 or CBP); the enzyme responsible for acetylation of histone tails.
Mdm2 – an E3 ubiquitin ligase that represses and degrades p53 (see also X Lu, this issue).
mTOR – the mammalian target of rapamycin and a kinase that regulates the translation of mRNAs important for cell growth.
Oncolytic virus – a virus that selectively lyses tumor cells.
Rb family of tumor suppressor proteins – often called pocket proteins, these include Rb, p107 and p130, and share the property of binding and regulating E2F. In normal cellular proliferation, their ability to bind E2F is tightly regulated by cell-cycle dependent phosphorylation events. See reference [25] for an excellent review.
Transformation – the process whereby a primary cell is transformed into a tumor cell, as evidenced by several criteria including reduced growth factor dependence and anchorage-independent cell growth.

selectively replicate in tumor cells in which these checkpoints are already inactivated (Figure 2). Such agents would offer a novel and specific approach to cancer therapy, with the potential to be self-perpetuating, to kill tumors through regulated lytic death, and to spread to distant micro-metastases.

The utility of viruses, both as tools with which to identify and modulate critical tumor targets, or as lytic cancer therapies, is intimately linked. In this review, I discuss how recent studies with viruses and viral proteins have provided elemental insights into our understanding of diverse cellular processes perturbed in tumorigenesis, and how this impacts the development of novel cancer therapies including oncolytic viral strategies.

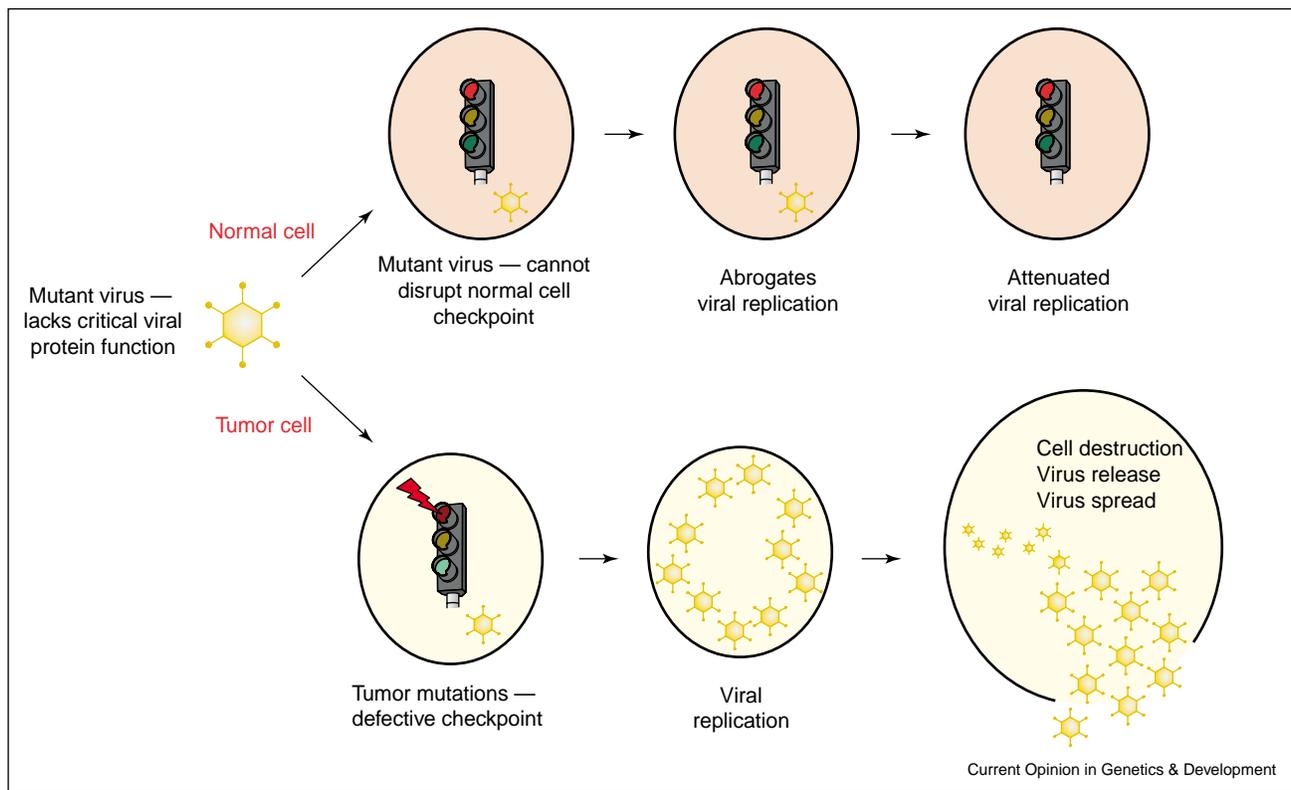
Unlocking the portals to tumor cell perdition

Regulation of growth factor receptor signaling is one of the most common oncogenic lesions in human cancer and, therefore, an attractive therapeutic target. Growth factor binding to members of the epidermal growth factor receptor (EGFR) tyrosine kinase family (ERBs 1–4) induces receptor oligomerization and activation. However, in tumor cells, high levels of EGFR lead to its hyperactivation. For example, ERB2 is upregulated in more than 20–30% of breast cancers, where it is associated with a highly aggressive form of the disease and a poor prognosis [1]. Small molecule inhibitors of EGFR kinase activity are undergoing clinical evaluation [2]. Trastuzumab (Herceptin®) is a recombinant monoclonal antibody that binds to ERB2, leading to its internalization and downregulation, and has been approved for metastatic breast cancer therapy. However, over 65% of patients with ERB2-overexpressing metastatic breast cancer fail to

Figure 1

DNA viruses and tumor cells inactivate similar cellular checkpoints to facilitate their replication. Normal cellular replication is regulated by a series of checkpoints, which are disrupted in both tumor cell and viral replication. For example, in tumor cells the Rb/p53 checkpoints are inactivated through mutations, while DNA viruses encode specific viral proteins to achieve the same end.

Figure 2



Oncolytic virus strategy based on the inactivation of cellular tumor suppressors. DNA viruses and tumor cells need to inactivate many of the same cellular checkpoints for their replication. This is the basis of several oncolytic viral therapies that target the loss of tumor suppressors: DNA viral mutants that fail to inactivate normal checkpoints selectively replicate in tumor cells in which these checkpoints are already disrupted.

respond to trastuzumab as a single agent [3]. The mechanisms underlying tumor resistance might include factors such as PTEN (phosphatase and tensin homolog) mutations [4] or upregulation of other members of the EGFR family. Therefore, the development of therapeutic modalities that target EGFR signaling through multiple or alternative mechanisms is critical for broad clinical efficacy.

Viral proteins bind to growth factor receptors to mediate not only their uptake into the cell but also to activate signaling pathways necessary for viral replication. Recently, EGFR was revealed as a key cellular receptor for the herpesvirus cytomegalovirus (CMV) [5••]. The CMV glycoprotein gB displaces EGF and induces EGFR downregulation. gB lacks an EGF-like domain but displays an epitope that shares some homology to a distinct region of EGF. Modeling the binding of viral proteins such as gB to the EGFR could have great utility in the development of therapeutic agents that target growth factor receptors through novel mechanisms. A precedent for such an approach comes from a recent study with the bovine papillomavirus E5 protein [6••], which binds to the transmembrane domain of the platelet-derived

growth factor (PDGF) receptor inducing its dimerization, activation and, ultimately, cellular transformation [7,8]. Using the E5 protein sequence as a molecular scaffold, a library of small proteins was constructed. This allowed the identification of novel transmembrane proteins that bind to the PDGF receptor, and the creation of a set of molecular rules for engagement of the PDGF transmembrane domain [6••]. Therefore, viral proteins present a novel molecular scaffold that could perhaps be engineered to either inhibit growth factor receptors (viral proteins are generally thought to activate receptors) or be tethered with prodrugs that are selectively internalized by tumor cells. In addition, identifying the protein domains and sequences that viral proteins use to interact with their cellular targets might reveal cellular proteins with homologous domains that play a cognate role in regulating normal or tumor cell growth.

The corollary is also true; pharmacological agents that inhibit growth factor receptor signaling could be used to treat virally associated cancers and pathologies. For example, cervical cancer, hepatocellular carcinoma and nasopharyngeal carcinoma are associated with HPV 16/18, Hepatitis B virus and Epstein Barr virus, respectively.

HPV E5 affects EGFR signaling, while both hepatitis HBx and Epstein Barr LMP-1 (latent membrane protein-1) result in EGFR upregulation [9]. The mechanisms whereby HBx and LMP-1 upregulate EGFR might yield new insights into EGFR deregulation in tumor cells, and EGFR inhibitors could have important therapeutic applications in treating virally associated carcinomas.

Finally, the selective targeting of replication-competent viruses to the cellular receptors upregulated on tumor cells is an attractive therapeutic strategy. For example, the addition of ligand binding domains to viral capsid proteins is being explored. Such a strategy will allow viruses to be selectively re-targeted to the tumor cells that have upregulated the receptors that interact with these ligands [10]. However, this can often interfere with viral packaging or internalization. Therefore, it is intriguing to speculate that the natural tropism of viruses such as CMV for EGFR, or HTLV (human T-cell lymphotropic virus) for GLUT-1 [11^{*}], could be exploited to target the uptake and replication of modified forms of these viruses to tumor cells in which these receptors are upregulated.

Interrogating the signals for cellular transformation

The successful *in vitro* transformation of human cells was finally achieved by the combined expression of the catalytic subunit of telomerase, Ras and the SV40 early region (which encodes two viral proteins, LT and small t [ST] [12]). Initially, the critical transforming protein of the SV40 early region was thought to be LT, which binds and inactivates both Rb and p53. However, subsequently, it emerged that the ability of ST to interact with the cellular serine threonine protein phosphatase 2A (PP2A) is also required [13–15].

PP2A is a heterotrimeric complex comprising A, B and C subunits, of which there are at least 60 possible combinations (see also V Janssens *et al.*, this issue). The large number of B subunits is thought to allow for different substrate specificities and is displaced by ST, leading to the inhibition of PP2A. A role for PP2A in tumorigenesis had been previously implied from studies with okadaic acid, a potent tumor promoter that inhibits PP1 and PP2A phosphatases. In addition, PP2A A subunit mutations occur in a subset of human tumors [16,17]. However, the complex regulation of PP2A had confounded any clear understanding of the role of PP2A in cancer. The specific displacement of the B56 γ from PP2A might well be the critical cellular target of ST in transformation [18^{**}] (see also JS Boehm and WC Hahn, this issue) and, indeed, this subunit is downregulated in several lung tumor cell-lines and truncated in malignant melanoma [19]. Identifying the substrates of PP2A affected by either ST expression or B56 γ suppression might reveal important targets for cancer therapy. For example, mem-

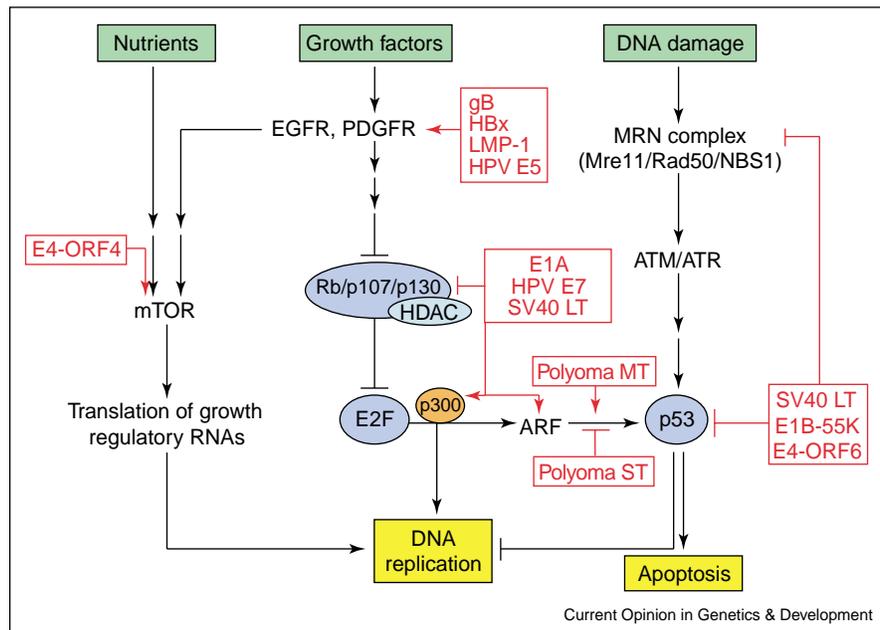
bers of the B56 family interact with cyclin G to regulate mdm2 [20] (see Glossary) and the APC tumor suppressor protein [21]. In addition, PP2A might play a critical role in myc activation/stabilization. A myc mutant, T58A, which cannot be dephosphorylated by PP2A, can substitute for ST in transformation [22^{**}]. Thr58 mutations are found in *v-myc* genes recovered from transforming retroviruses, and *c-myc* gene translocations in Burkitt's lymphoma [23,24]. It will be interesting to determine whether Burkitt's lymphoma cells that retain wild type *c-myc* genes have deregulated or mutated PP2A.

Similar to Rb and p53, PP2A is a common target of DNA viral proteins, highlighting its importance in cellular growth control. Understanding how different viral proteins modulate PP2A could yield important insights into its regulation and its role during normal and tumor cell growth. For example, adenovirus encodes the viral protein E4-ORF4, which interacts with — but unlike ST does not displace — the B subunit of PP2A. E4-ORF4 mimics the signals normally supplied by nutrients to bypass an mTOR checkpoint (see Glossary) for protein translation in viral replication (CC O'Shea *et al.*, unpublished). mTOR is an important downstream effector of growth factor signaling and a promising therapeutic target (Figure 3). Nutrient signaling also activates mTOR but the mechanism of this activation is poorly understood. Therefore, a viral protein that mimics the presence of nutrients is a unique tool with which to discover critical cellular targets in the nutrient signaling pathway, and to tell us whether they are also deregulated in cancer. This also suggests that an *E4-ORF4* mutant virus might be a novel and potentially efficacious oncolytic virus (see Glossary) for the treatment of tumors that have deregulated the growth factor/PP2A/mTOR pathway.

Replicating and reprogramming the DNA operating system

One of the hallmarks of viral replication and cancer is deregulated cell cycle entry. In normal cells, the Rb family of tumor suppressor proteins (see Glossary), Rb/p107/p130, bind to E2F, thereby regulating its transcriptional activity to prevent unscheduled S-phase entry [25]. Components of the Rb tumor suppressor pathway [26] are almost invariably inactivated through mutations in tumor cells, whereas viral proteins such as adenovirus E1A, SV40 LT, HPV and E7 bind and inactivate Rb directly. These viral proteins use a homologous LXCXE motif to bind the pocket region of Rb/p107/p130, and subsequently activate E2F through various mechanisms [27] (Figure 3). Therefore, it was proposed that an adenovirus that encoded an E1A Δ LXCXE mutant would be unable to replicate in primary cells, but undergo selective lytic replication in tumor cells. However, surprisingly, an *E1A* Δ LXCXE viral mutant is competent for both E2F activation and viral replication in primary cells [28]. Another adenoviral protein encoded by the *E4* region,

Figure 3



DNA viruses: an integrated circuit of growth deregulating functions. Viral proteins (red) perturb critical cellular pathways at multiple levels to induce DNA replication and prevent apoptosis. This superficial redundancy might instead reflect a complexity inherent to the disruption of integrated cellular pathways that is mirrored also in tumor cells.

E4-ORF6/7, dimerizes E2F. Therefore, a novel adenovirus, ONYX-411, in which the *E2F* promoter regulates the expression of both *E1A ΔLXCXE* and *E4* viral genes was developed. ONYX-411 selectively replicates in tumor cells and such a strategy has great potential as a systemic oncolytic viral therapy [28]. Recently, oncolytic viruses that use other tumor selective promoters to regulate *E1A* expression have also been described [29–31].

The transcription of genes necessary for S-phase entry is likely to be regulated not only by the disruption of Rb/E2F binding but also by the remodeling of surrounding chromatin. Although the regulation of chromatin remodeling is poorly understood, histone methylation is thought to result in tightly packed chromatin, which is less accessible to transcription factors. Histone acetylation has the opposite effect and is negatively regulated by histone deacetylases (HDACs), and positively regulated by histone acetyltransferases (HATs; see Glossary). Although the LXCXE motif was first discovered through studies with E1A, cellular proteins such as HDAC also use an LXCXE-like motif to bind Rb [32]. Thus, viral proteins compete with HDAC for Rb binding, perhaps preventing the deacetylation of core histones at the promoters of genes that play a critical role in DNA replication. The avian adenovirus CELO (chicken embryo lethal orphan virus) encodes Gam1, a protein that actually binds and disables HDAC directly. Gam1

is normally critical for viral replication but a *Gam1*-defective virus can be rescued by HDAC inhibitors [33]. Therefore, the modulation of HDAC activation by viral proteins might promote DNA replication. Understanding how viral proteins modulate chromatin remodeling factors may reveal key insights into tumorigenesis and the use of HDAC inhibitors for cancer therapy.

Histone acetylation is positively regulated by HATs such as p300, which was first discovered as a cellular protein that binds to E1A. SV40 LT and HPV E7 also bind to HATs. This is necessary for their ability to transform cells and induce S-phase entry [34]. Whether it is the inhibition, activation or selective recruitment of HATs that is critical for E1A-, E7- and LT-mediated effects could be attributable to context-dependent effects. However, support for the recruitment/activation model comes from a recent study, which demonstrates that E1A/p130 binding in quiescent cells is accompanied by Histone 3 acetylation and the recruitment of activating E2Fs [35••]. The ability of E1A to modulate both Rb and chromatin remodeling factors is required to prevent the formation of repressive heterochromatin structures and to bypass oncogene-induced senescence [36••]. Disparate DNA viruses have converged functionally to encode viral proteins that simultaneously affect Rb/E2F and chromatin remodeling factors. Understanding why this convergence occurred has important implications for the development of both

oncolytic viruses and effective combination therapies that target Rb dysfunction at multiple levels.

Inactivating the guardians of the genome

p53 is activated by oncogenes or DNA damage (see also X Lu, this issue), resulting in the cell cycle arrest or apoptosis of cells that may have acquired potentially tumorigenic lesions [37,38]. Therefore, the p53 tumor suppressor pathway is inactivated in nearly all human tumors [39]. Oncogenes such as Ras, myc and E1A induce Arf (see Glossary), which prevents the degradation of p53 by mdm2, an E3 ubiquitin ligase (Figure 3). Although Arf is an E2F target, it is not induced in normal cellular proliferation, but specifically responds to aberrant proliferative signals [40**,41**]. E1A also stabilizes p53 by sequestering p300, leading to the recent discovery that p300 acts as an E4 ubiquitin ligase for p53. This suggests that inhibiting the p300 E4 function might be of therapeutic value in treating tumors in which p53 is rapidly turned over due to mdm2 amplification [42**].

Tumor cell inactivation of the p53/DNA damage pathway results in genomic instability. For example, mutations in ATM (ataxia telangiectasia-mutated kinase) or NBS1 result in chromosomal instability and the cancer predisposition syndromes ataxia telangiectasia and Nijmegen breakage syndromes, respectively. Viruses also target the DNA damage/repair machinery of the cell, which is activated by the presence of viral genomes. Mre11 is part of the MRN complex, comprising Mre11, Rad50 and Nbs1, which accumulates at the sites of double-strand DNA breaks. In adenovirus infection, E1B-55k and E4-ORF6 act together as a complex to bind and degrade Mre11. [43], thereby preventing the end-joining of viral genomes and the activation of ATM [44] (Figure 3). This has revealed a key role for the MRN complex not only in sensing DNA damage but also in the activation of ATM [45**]. SV40 also targets the MRN complex. SV40 uses LT to escape the strict cellular control of one round of DNA replication per cell cycle and massively amplify the viral genome — and often cellular DNA in infected cells. The ability of LT to induce DNA 're-reduplication' was recently shown to be through LT binding and repression of Nbs1 [46**]. The mechanisms whereby viral proteins disrupt the DNA damage/repair response could help us understand and target the genomic instability that is a hallmark of tumor cells.

The activation of p53 by both viral oncogenes and genomes could have a devastating impact on viral replication. Therefore, viruses encode proteins such as LT (SV40), E6 (HPV) or E1B-55K and E4-ORF6 (adenovirus) that bind and/or degrade p53. Mouse polyoma virus is closely related to SV40 but is unusual among the small DNA tumor virus family in that none of its three transforming T antigens directly bind and inactivate p53. However, a possible resolution to this apparent disparity comes from a

study demonstrating that although MT induces high levels of Arf the expression of ST prevents any consequent induction of p53 [47**]. The ability of polyoma ST to disrupt the Arf/p53 tumor suppressor pathway appears to be mediated through the interaction of ST with PP2A, and may indicate a novel role for PP2A in the p53 tumor suppressor pathway.

There is a great need to identify lytic cancer therapies, and p53 is a major tumor target. ONYX-015 (*d/1520*) is an adenovirus mutant that lacks the *E1B-55K* gene and, therefore, fails to degrade p53 during adenoviral replication [48,49]. On this basis, ONYX-015 was conceived as an oncolytic virus that would selectively replicate in p53-defective tumor cells. In clinical trials, ONYX-015 has proven safe, with evidence of promising activity from several indications [50–53]. Nevertheless, the role of p53 in governing ONYX-015 tumor selectivity has proved highly controversial. A recent study demonstrates that loss of E1B-55K leads to the induction, but not activation, of p53 in ONYX-015-infected primary cells [54**]. This suggests that adenovirus has evolved an additional strategy to inactivate p53, which has important implications for the design of p53-selective oncolytic viruses. The tumor selective replication of ONYX-015 is instead determined by loss of an E1B-55K late function in the export and expression of late viral RNAs required for virion production [54**]. Tumor cells that support ONYX-015 replication are able to efficiently export late viral RNAs in the absence of E1B-55K, a property not shared by primary cells. This reveals altered RNA export in tumor cells as a new and therapeutically exploitable target. Therefore, novel tumor properties can be revealed by discovering whether tumor cells, as opposed to normal cells, selectively complement the functions of viral mutants.

Conclusions

DNA viruses are a simple genetic tool with which to dissect the complexities of tumorigenesis. Viruses encode a minimum number of proteins and yet target the critical cellular players necessary for S-phase entry while preventing premature apoptosis. These same cellular players are also likely to be targeted in cancer cells. Therefore, by discovering and understanding the functional interaction of viral proteins with cellular proteins, we identify new tumor targets and therapeutic modalities. In addition, viruses provide us with a natural systems biology approach to study the integration of the often overlapping pivotal growth-regulating pathways that are perturbed in both viral and tumor replication. This knowledge is also critical for the development of viruses that undergo selective lytic replication in tumor cells, and as such, has the potential to fulfill many of the aspirations of cancer research. However, if oncolytic viruses are to enjoy widespread use in the clinic, then significant advances will have to be made in both the technologies that allow their large-scale production, and strategies that pre-

vent their neutralization by the host humoral response. Nevertheless, working together, cancer researchers, virologists, the biotechnology sector and immunologists would comprise an irresistible force capable of meeting such challenges in the war of attrition on cancer. Therefore, in many ways, DNA viruses may prove to be the ultimate 'double-agents', that will reveal the tumor cells' secrets and ultimately become the spies that will lyse them.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Yu D, Hung MC: **Overexpression of ErbB2 in cancer and ErbB2-targeting strategies.** *Oncogene* 2000, **19**:6115-6121.
2. Noble ME, Endicott JA, Johnson LN: **Protein kinase inhibitors: insights into drug design from structure.** *Science* 2004, **303**:1800-1805.
3. Vogel CL, Cobleigh MA, Tripathy D, Guthel JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M *et al.*: **Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer.** *J Clin Oncol* 2002, **20**:719-726.
4. Nagata Y, Lan KH, Zhou X, Tan M, Esteve FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT *et al.*: **PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients.** *Cancer Cell* 2004, **6**:117-127.
5. Wang X, Huang SM, Chiu ML, Raab-Traub N, Huang ES: **Epidermal growth factor receptor is a cellular receptor for human cytomegalovirus.** *Nature* 2003, **424**:456-461.
This study demonstrates that CMV binds to the EGFR, resulting in viral internalization and EGFR downregulation from the cell surface. The authors identify gB as the viral protein that binds to the EGFR (to monomeric erb1 and hetero-oligomers of erb1-3) and can displace EGF. EGFR inhibitors prevent viral entry and replication and could have important therapeutic applications in preventing fatal CMV-associated pathologies in immunocompromised individuals.
6. Freeman-Cook LL, Dixon AM, Frank JB, Xia Y, Ely L, Gerstein M, Engelman DM, DiMaio D: **Selection and characterization of small random transmembrane proteins that bind and activate the platelet-derived growth factor β receptor.** *J Mol Biol* 2004, **338**:907-920.
The authors use the 44 amino acid BPV E5 protein as a molecular scaffold to screen a library of polypeptides in which hydrophobic residues have been randomly substituted for the natural E5 sequences. They identify novel transmembrane proteins that bind and activate the PDGF receptor — these proteins are diverse in sequence but most likely similar in structure to the E5 protein. This approach could be modified to help identify small proteins that modulate the activity of cellular receptors in different ways.
7. Petti L, Nilson LA, DiMaio D: **Activation of the platelet-derived growth factor receptor by the bovine papillomavirus E5 transforming protein.** *EMBO J* 1991, **10**:845-855.
8. DiMaio D, Lai CC, Mattoon D: **The platelet-derived growth factor β receptor as a target of the bovine papillomavirus E5 protein.** *Cytokine Growth Factor Rev* 2000, **11**:283-293.
9. Miller WE, Raab-Traub N: **The EGFR as a target for viral oncoproteins.** *Trends Microbiol* 1999, **7**:453-458.
10. Everts M, Curiel DT: **Transductional targeting of adenoviral cancer gene therapy.** *Curr Gene Ther* 2004, **4**:337-346.
11. Manel N, Kim FJ, Kinet S, Taylor N, Sitbon M, Battini JL: **The ubiquitous glucose transporter GLUT-1 is a receptor for HTLV.** *Cell* 2003, **115**:449-459.
GLUT-1 is often upregulated on tumor cells. HTLV is associated with leukemia and HTLV envelope proteins inhibit glucose transport and perturb glucose metabolism. The authors demonstrate that GLUT-1 is

a cellular receptor for HTLV and indicate that defects in glucose transport may underlie some of the pathologies associated with HTLV.

12. Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA: **Creation of human tumour cells with defined genetic elements.** *Nature* 1999, **400**:464-468.
13. Yu J, Boyapati A, Rundell K: **Critical role for SV40 small-t antigen in human cell transformation.** *Virology* 2001, **290**:192-198.
14. Hahn WC, Dessain SK, Brooks MW, King JE, Elenbaas B, Sabatini DM, DeCaprio JA, Weinberg RA: **Enumeration of the simian virus 40 early region elements necessary for human cell transformation.** *Mol Cell Biol* 2002, **22**:2111-2123.
15. Hahn WC, Weinberg RA: **Modelling the molecular circuitry of cancer.** *Nat Rev Cancer* 2002, **2**:331-341.
16. Wang SS, Esplin ED, Li JL, Huang L, Gazdar A, Minna J, Evans GA: **Alterations of the PPP2R1B gene in human lung and colon cancer.** *Science* 1998, **282**:284-287.
17. Calin GA, di lasio MG, Caprini E, Vorechovsky I, Natali PG, Sozzi G, Croce CM, Barbanti-Brodano G, Russo G, Negrini M: **Low frequency of alterations of the α (PPP2R1A) and β (PPP2R1B) isoforms of the subunit A of the serine-threonine phosphatase 2A in human neoplasms.** *Oncogene* 2000, **19**:1191-1195.
18. Chen W, Possemato R, Campbell KT, Plattner CA, Pallas DC, Hahn WC: **Identification of specific PP2A complexes involved in human cell transformation.** *Cancer Cell* 2004, **5**:127-136.
The authors demonstrate that short hairpin RNA-mediated knockdown of B56 γ expression has similar effects to ST in inhibiting PP2A activity, promoting anchorage-independent growth and inducing cellular transformation. By contrast, B56 γ overexpression attenuates the tumorigenicity of ST-expressing cells. This suggests that the B56 γ subunit may be a key cellular target of ST in mediating cellular transformation.
19. Ito A, Koma YI, Watabe K: **A mutation in protein phosphatase type 2A as a cause of melanoma progression.** *Histol Histopathol* 2003, **18**:1313-1319.
20. Okamoto K, Li H, Jensen MR, Zhang T, Taya Y, Thorgerisson SS, Prives C: **Cyclin G recruits PP2A to dephosphorylate Mdm2.** *Mol Cell* 2002, **9**:761-771.
21. Seeling JM, Miller JR, Gil R, Moon RT, White R, Virshup DM: **Regulation of β -catenin signaling by the B56 subunit of protein phosphatase 2A.** *Science* 1999, **283**:2089-2091.
22. Yeh E, Cunningham M, Arnold H, Chasse D, Monteith T, Ivaldi G, Hahn WC, Stukenberg PT, Shenolikar S, Uchida T *et al.*: **A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells.** *Nat Cell Biol* 2004, **6**:308-318.
This is an interesting paper, which demonstrates that MYC stability appears to be negatively regulated by PP2A-mediated dephosphorylation at Ser62 (an ERK target). The ability of PP2A to mediate dephosphorylation of Ser62 is regulated by the Pin1 prolyl isomerase, which binds to MYC in a phospho-Thr58 dependent manner (a GSK-3 target). The absence of PIN1 or inhibition of PP2A leads to c-MYC stabilization. In addition, a Thr58 MYC mutant can substitute for ST in transformation assays. This has important implications for understanding the cooperation of RAS and MYC in both cellular transformation and tumorigenesis.
23. Papas TS, Lautenberger JA: **Sequence curiosity in v-myc oncogene.** *Nature* 1985, **318**:237.
24. Bhatia K, Huppi K, Spangler G, Siwarski D, Iyer R, Magrath I: **Point mutations in the c-Myc transactivation domain are common in Burkitt's lymphoma and mouse plasmacytomas.** *Nat Genet* 1993, **5**:56-61.
25. Trimarchi JM, Lees JA: **Sibling rivalry in the E2F family.** *Nat Rev Mol Cell Biol* 2002, **3**:11-20.
26. Lowe SW, Sherr CJ: **Tumor suppression by Ink4a-Arf: progress and puzzles.** *Curr Opin Genet Dev* 2003, **13**:77-83.
27. Helt AM, Galloway DA: **Mechanisms by which DNA tumor virus oncoproteins target the Rb family of pocket proteins.** *Carcinogenesis* 2003, **24**:159-169.
28. Johnson L, Shen A, Boyle L, Kunich J, Pandey K, Lemmon M, Hermiston T, Giedlin M, McCormick F, Fattaey A: **Selectively**

- replicating adenoviruses targeting deregulated E2F activity are potent, systemic antitumor agents.** *Cancer Cell* 2002, **1**:325-337.
29. Lanson NA Jr, Friedlander PL, Schwarzenberger P, Kolls JK, Wang G: **Replication of an adenoviral vector controlled by the human telomerase reverse transcriptase promoter causes tumor-selective tumor lysis.** *Cancer Res* 2003, **63**:7936-7941.
30. Post DE, Van Meir EG: **A novel hypoxia-inducible factor (HIF) activated oncolytic adenovirus for cancer therapy.** *Oncogene* 2003, **22**:2065-2072.
31. Fuerer C, Iggo R: **Adenoviruses with Tcf binding sites in multiple early promoters show enhanced selectivity for tumour cells with constitutive activation of the wnt signalling pathway.** *Gene Ther* 2002, **9**:270-281.
32. Ferreira R, Magnaghi-Jaulin L, Robin P, Harel-Bellan A, Trouche D: **The three members of the pocket proteins family share the ability to repress E2F activity through recruitment of a histone deacetylase.** *Proc Natl Acad Sci USA* 1998, **95**:10493-10498.
33. Chiocca S, Kurtev V, Colombo R, Boggio R, Sciripi MT, Brosch G, Seiser C, Draetta GF, Cotten M: **Histone deacetylase 1 inactivation by an adenovirus early gene product.** *Curr Biol* 2002, **12**:594-598.
34. Iyer NG, Ozdag H, Caldas C: **p300/CBP and cancer.** *Oncogene* 2004, **23**:4225-4231.
35. Ghosh MK, Harter ML: **A viral mechanism for remodeling chromatin structure in G₀ cells.** *Mol Cell* 2003, **12**:255-260.
- The recruitment of HDAC and a histone methyltransferase (e.g. SUV39H) has been proposed as an important mechanism whereby Rb represses the cyclin E and A promoters. The authors demonstrate that E1A remodels chromatin in G₀ cells creating an S-phase environment. E1A binds to E2F promoters where it eliminates p130/E2F4 and also results in the exchange of H-3 Lys-9 methylation for H-3 Lys-9 acetylation. This suggests that, in addition to inactivating the pocket proteins, specific chromatin modifications may be necessary to activate transcription from E2F promoters.
36. Narita M, Nunez S, Heard E, Lin AW, Hearn SA, Spector DL, Hannon GJ, Lowe SW: **Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence.** *Cell* 2003, **113**:703-716.
- Oncogene-induced senescence might reflect a tumor suppressive mechanism. This is an interesting paper, which describes a new marker of senescence, distinctive senescence-associated heterochromatin foci (SAHF), which are associated with the stable repression of E2F transcriptional targets. SAHF formation is dependent on an intact Rb pathway and is prevented by the expression of E1A. Interestingly, the authors demonstrate that while a ΔN E1A mutant did not prevent Ras-induced cell cycle arrest or senescence-associated β -gal accumulation, it retained the ability to prevent SAHF formation. This suggests that Rb and the cellular proteins that bind to the E1A N-terminus (perhaps p300 or p400 binding) have distinct functions that underlie the ability of E1A to prevent senescence, and which it would be of considerable interest to understand.
37. Oren M: **Decision making by p53: life, death and cancer.** *Cell Death Differ* 2003, **10**:431-442.
38. Levine AJ: **p53, the cellular gatekeeper for growth and division.** *Cell* 1997, **88**:323-331.
39. Sherr CJ, McCormick F: **The RB and p53 pathways in cancer.** *Cancer Cell* 2002, **2**:103-112.
40. Zindy F, Williams RT, Baudino TA, Rehg JE, Skapek SX, Cleveland JL, Rousset MF, Sherr CJ: **Arf tumor suppressor promoter monitors latent oncogenic signals in vivo.** *Proc Natl Acad Sci USA* 2003, **100**:15930-15935.
- This is an important study, which uses a mouse strain that expresses GFP in place of p19^{ARF} to demonstrate that Arf is specifically activated by abnormal proliferative signals in incipient tumors but is low or undetectable in most normal cell and tissue types.
41. Aslanian A, Iaquinta PJ, Verona R, Lees JA: **Repression of the Arf tumor suppressor by E2F3 is required for normal cell cycle kinetics.** *Genes Dev* 2004, **18**:1413-1422.
- Using E2F3-deficient mouse embryonic fibroblasts, the authors demonstrate that E2F3 is a key transcriptional repressor of Arf under normal proliferative conditions. Oncogenic activation of Arf by E1A results in the recruitment of endogenous activating E2Fs such as E2F1 to the Arf promoter and overrides E2F3-mediated repression. This paper establishes a direct role for distinct E2F complexes in the oncogenic activation of Arf.
42. Grossman SR, Deato ME, Brignone C, Chan HM, Kung AL, Tagami H, Nakatani Y, Livingston DM: **Polyubiquitination of p53 by a ubiquitin ligase activity of p300.** *Science* 2003, **300**:342-344.
- The authors investigate E1A-induced p53 stabilization and find that E1A expression promotes the mono- but not poly-ubiquitination of p53. They show that the ability of E1A to prevent p53 polyubiquitination is dependent on E1A/p300 binding. The authors also demonstrate that p300 can act as an E4 ubiquitin ligase for p53. They suggest that, although p300 might be a p53 co-activator following DNA damage, under normal conditions p300 might promote p53 degradation.
43. Weitzman MD, Carson CT, Schwartz RA, Lilley CE: **Interactions of viruses with the cellular DNA repair machinery.** *DNA Repair (Amst)* 2004, **3**:1165-1173.
44. Stracker TH, Carson CT, Weitzman MD: **Adenovirus oncoproteins inactivate the Mre11-Rad50-NBS1 DNA repair complex.** *Nature* 2002, **418**:348-352.
45. Carson CT, Schwartz RA, Stracker TH, Lilley CE, Lee DV, Weitzman MD: **The Mre11 complex is required for ATM activation and the G₂/M checkpoint.** *EMBO J* 2003, **22**:6610-6620.
- In [44], the adenoviral proteins E1B-55K and E4-ORF6 were shown to degrade Mre11 in adenovirus infection and prevent viral genome concatemerization. In [45**], these adenoviral proteins are used as a tool to demonstrate a role for the Mre11 complex in the upstream activation of ATM and G₂/M arrest in response to double strand breaks.
46. Wu X, Avni D, Chiba T, Yan F, Zhao Q, Lin Y, Heng H, Livingston D: **SV40 T antigen interacts with Nbs1 to disrupt DNA replication control.** *Genes Dev* 2004, **18**:1305-1316.
- SV40-infected cells escape the strict cellular control of one round of DNA replication per cell cycle, which allows massive amplification of the viral genome. In addition, it can often result in endoreduplication of cellular DNA and hyperploidy. The authors demonstrate that LT interacts with Nbs1 preventing Nbs1-mediated suppression of DNA re-replication. They show that SV40 origin containing DNA hyper-replicates in Nbs1-deficient cells. Interestingly, they also find that an N-terminal fragment of Nbs1 induces tetraploidy in Nbs1 deficient, but not proficient, cells — perhaps indicating that wild type Nbs1 opposes its interaction with certain cellular proteins.
47. Moule MG, Collins CH, McCormick F, Fried M: **Role for PP2A in ARF signaling to p53.** *Proc Natl Acad Sci USA* 2004, **101**:14063-14066.
- This paper demonstrates that polyoma uses a unique strategy to abrogate activation of p53. Polyoma ST inhibits Arf-mediated induction of p53 through its PP2A binding domain, suggesting that PP2A might play a role in modulating the Arf/p53 tumor suppressor pathway.
48. Barker DD, Berk AJ: **Adenovirus proteins from both E1B reading frames are required for transformation of rodent cells by viral infection and DNA transfection.** *Virology* 1987, **156**:107-121.
49. Bischoff JR, Kirn DH, Williams A, Heise C, Horn S, Muna M, Ng L, Nye JA, Sampson-Johannes A, Fattaey A et al.: **An adenovirus mutant that replicates selectively in p53-deficient human tumor cells.** *Science* 1996, **274**:373-376.
50. Khuri FR, Nemunaitis J, Ganly I, Arseneau J, Tannock IF, Romel L, Gore M, Ironside J, MacDougall RH, Heise C et al.: **A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer.** *Nat Med* 2000, **6**:879-885.
51. Reid T, Galanis E, Abbruzzese J, Sze D, Wein LM, Andrews J, Randlev B, Heise C, Uprichard M, Hatfield M et al.: **Hepatic arterial infusion of a replication-selective oncolytic adenovirus (d1520): phase II viral, immunologic, and clinical endpoints.** *Cancer Res* 2002, **62**:6070-6079.
52. Ries S, Korn WM: **ONYX-015: mechanisms of action and clinical potential of a replication-selective adenovirus.** *Br J Cancer* 2002, **86**:5-11.

53. Rudin CM, Cohen EE, Papadimitrakopoulou VA, Silverman S Jr, Recant W, El-Naggar AK, Stenson K, Lippman SM, Hong WK, Vokes EE: **An attenuated adenovirus, ONYX-015, as mouthwash therapy for premalignant oral dysplasia.** *J Clin Oncol* 2003, **21**:4546-4552.
54. O'Shea CCJ, Johnson LJ, Bagus B, Choi S, Nicholas C, Shen A, Habets G, Shen J, Soria C, Kunich J, Ginzinger D, McCormick F: **Late viral RNA export rather than p53 activation determines ONYX-015 tumour selectivity.** *Cancer Cell* 2004.

ONYX-015 was the first replication-competent oncolytic adenovirus to enter the clinic, where it was found to be safe, with evidence of promising tumoricidal activity. However, the role of p53 in governing ONYX-015 tumor selectivity has proved highly controversial. This paper demonstrates that loss of E1B-55K leads to the induction, without the activation, of p53 and consequently does not restrict ONYX-015 replication in primary cells. The authors demonstrate that differential export of late viral RNAs between normal and tumor cells is the major determinant of ONYX-015 oncolytic selectivity.