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Viruses – seeking and destroying the tumor program

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DNA viruses have enormous utility in cancer research, both as tools for tumor target discovery as well as agents for lytic cancer therapies. This is because there is a profound functional overlap between the DNA viral and tumor cell programs. DNA viruses encode proteins that elicit growth deregulation in infected cells similar to that engendered by mutations in tumor cells. Evolution has refined viral proteins to target the critical cellular hubs that regulate growth. Thus, viral proteins are discriminating biochemical probes that can be used to identify and characterize novel tumor targets. Moreover, the overlap between the DNA viral and tumor programs can also be exploited for the development of lytic cancer therapies. Discovering whether tumor cells selectively complement the replication of viral mutants can reveal novel oncolytic viral therapies, as well as unexpected tumor properties. For example, altered RNA export was recently uncovered as a novel tumor cell property that underlies ONYX-015 replication, a promising oncolytic adenoviral therapy. A perspective is provided on how adenovirus could be systematically exploited to map the requisite role, or indeed the redundancy, of cellular pathways that act in an integrated program to elicit pathological replication. This knowledge has important applications for the rational design of the next generation of oncolytic viruses, as well as the discovery of efficacious combination cancer therapies.
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Introduction

Cell fate is determined by networks of growth-regulatory pathways that exert integrated or additive effects upon each other, and critical downstream effectors. Tumor mutations, or virally encoded proteins, act as a program to disrupt such networks to elicit aberrant DNA replication that is uncoupled from powerful tumor suppressor mechanisms. Thus, DNA viral proteins and tumor cell mutations functionally converge in perturbing similar cellular pathways. Indeed, many of the critical tumor targets were first identified through the study of DNA viruses. p53 was first discovered as a cellular protein that interacted with SV40 Large T (LT) antigen (Lane and Crawford, 1979; Linzer and Levine, 1979), E2F as a cellular factor that bound to the adenoviral E2 promoter following E1A expression (Kovesdi *et al*., 1987) and PI3-kinase as an activity associated with polyoma Middle T (MT) antigen (Kaplan *et al*., 1987). Thus, viral proteins are novel tools with which to identify the critical cellular targets, or hubs, that act together to engender unscheduled replication.

Our knowledge of many of the critical cellular lesions that drive neoplastic transformation has prompted a major development in rational targeted molecular therapies (Sawyers, 2004). For example, Herceptin is a recombinant monoclonal antibody that targets the ERBb2 (Her-2) growth factor receptor, which is amplified in over 20–30% of breast cancers (Yu and Hung, 2000). However, over 65% of patients with ERBb2-overexpressing breast tumors fail to respond to Herceptin as a single agent (Vogel *et al*., 2002), a resistance that has been linked to additional tumor cell mutations (Nagata *et al*., 2004). Thus, just as cancer is not a disease of any single mutation, no single agent is likely to cure it. With the possible exception of the leukemias, therapies that target single oncogenes have been limited by pre-existing neoplastic mutations or drug-induced selection of resistant tumor cells (Sawyers, 2004). The development of combination therapies that target the tumor cell program at multiple hubs, and/or cytolytic agents that trigger the rapid and specific death of tumor cells, is therefore, critical to overcome such shortcomings.

To these ends, the study of DNA viruses has much to contribute (O'Shea, 2005). DNA viruses are a natural, and tractable, genetic 'system' in which to study the complex network dynamics that underlie aberrant cellular replication, perhaps one of the greatest challenges in contemporary cancer research. In addition, a precise knowledge of the cellular networks perturbed in the viral and tumor cell replication programs is crucial for the engineering of viral mutants that fail to replicate in normal cells but undergo selective lytic replication in tumor cells that complement the viral defect. Such selective oncolytic viral agents have many potential advantages, including the capability to be self-perpetuating, to kill tumors through regulated lytic death and to spread to distant micro-metastases (Chiocca, 2002; Dobbelstein, 2004). In this review, I will discuss the similarities between the DNA virus and tumor cell

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programs with respect to the cellular pathways they each perturb and, as such, how DNA viral proteins can be used to reveal novel tumor targets and therapeutic modalities. In addition, I will describe how the application of systems biology to the study of DNA viruses could impact the development of both oncolytic viral agents and our knowledge of the complex networks deregulated in tumor cells, an understanding of which is critical for cancer therapy.

The teleology and functional convergence of the viral and tumor cell programs

Both viruses and tumor cells encode a program that engenders a common end point: their limitless propagation. This is a manifestation of a number of pathologies that result from the coercion and disruption of integrated growth-regulatory networks. Some of the hallmarks of cancer shared by the majority of human tumors include: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, defective DNA damage/repair pathways and tissue invasion/metastasis. For a comprehensive review, see Hanahan and Weinberg (2000). Each of these traits is acquired through mutations, although the nature and chronological order in which such mutations arise may vary for different tumor types/stages.

DNA viruses, which include adenovirus, human papillomavirus (HPV), Herpes virus, polyoma and SV40 infect quiescent cells, but due to their limited genetic capacity rely on the cellular DNA replication/protein translation machinery for their propagation. Thus, one the of hallmarks of the small DNA viruses is that they express viral proteins that force infected quiescent cells into unscheduled S-phase entry, so that viral genomes are replicated concomitantly with host DNA. Many DNA viruses undergo lytic replication, ultimately resulting in the death of infected host cells. Therefore, at first it may seem counterintuitive that viruses encode early proteins which actually desensitize infected host cells to apoptosis. However, this most likely reflects the necessity of uncoupling evolutionarily linked tumor-suppressive mechanisms, such as apoptosis, which are triggered by cellular networks that are forced to drive unscheduled DNA replication (Lowe *et al*., 2004). As obligate intracellular parasites, viruses have also evolved to replicate in host cells irrespective of an inhospitable cellular microenvironment. Since function often limits mechanisms, DNA viral proteins and tumor cell mutations converge in perturbing many of the same molecular players to execute their program of growth deregulation, examples of which I discuss below.

Self-sufficiency in growth signals

Commitment to cell growth and proliferation is normally determined by the ability to sense and respond to extracellular growth factor and nutrient signals in the microenvironment. However, both viruses and tumor cells subvert these control mechanisms to replicate even in the absence of such signals (Figures 1 and 2). Deregulation of growth factor receptor signaling pathways is one of the most common oncogenic lesions in human cancer, liberating tumor cells from a dependency on extrinsic mitogenic signals and also normal tissue homeostatic mechanisms. Oncogenic lesions in the growth factor signaling pathway include activating mutations/overexpression of growth factor receptors or their downstream cellular transducers/effectors. In tumor cells, members of the EGFR family are often overexpressed (Yu and Hung, 2000), which can elicit ligand-independent signaling and hyperactivation. Mutations in the downstream transducers of growth factor signaling are also common. For example, deregulated Ras–Raf–ERK/PI3-kinase–PTEN–PKB–mTOR signaling can also supplant the requirement for extrinsic growth factor stimulation (reviewed in Vivanco and Sawyers, 2002; Downward, 2003). In addition to growth factors, nutrient signals are also necessary for normal cell growth and replication (Yen and Pardee, 1979). mTOR, a kinase that regulates the translation of important growth-regulatory mRNAs, is a critical cellular node that integrates both the nutrient and growth factor signaling pathways (Fingar and Blenis, 2004). Inherited mutations in TSC1/TSC2 or LKB-1 result in the cancer predisposition syndromes tuberous sclerosis and Peutz–Jeghers' disease, respectively, and are thought to result in constitutive mTOR activation even in the absence of nutrient/growth factor signals (Inoki *et al*., 2005).

Viruses also render infected primary cells autonomous for the presence of extracellular nutrient/growth factor signals. For example, bovine papillomavirus E5 activates the PDGF growth factor receptor in a novel way, by binding to its transmembrane domain (see Freeman-Cook and DiMaio, 2005). Viruses also deregulate downstream transducers/effectors of growth factor signaling. Polyoma virus MT, adenovirus E4-ORF1, HTLV tax and CMV IE1/2 all activate PI3-kinase (Kaplan *et al*., 1987; Liu *et al*., 2001; Yu and Alwine, 2002; Frese *et al*., 2003). Recently, viral proteins that mimic nutrient signaling to activate mTOR-mediated translation have also been identified. Adenovirus E4-ORF4 mimics glucose signaling to activate mTOR (O'Shea *et al*., 2005), while HPV 16 E6 degrades TSC2, constitutively activating mTOR even in the absence of nutrient/growth factor signals (Lu *et al*., 2004). The herpes viruses also activate growth factor and mTOR protein translation pathways. HSV-1 and CMV stimulate the phosphorylation of the critical downstream target of mTOR, 4E-BP1, together with the ERK/p38 target mnk-1, which play a pivotal role in enhancing translation and driving viral replication in infected quiescent primary cells (Walsh and Mohr, 2004; Walsh *et al*., 2005). This is discussed further by (Mohr, 2005). Thus, there are many similarities between virally infected cells and tumor cells in that they both functionally perturb pathways that allow their replication to occur autonomously of extrinsic growth signals.

The RB tumor suppressor pathway and insensitivity to anti-growth signals

One of the hallmarks of cancer and viral replication is deregulated cell-cycle entry. The RB family of tumor suppressor proteins (RB, p107, p130) are a hub for many of the growth and anti-growth signaling networks, and play a critical role in determining whether a cell will proliferate or not. RB is inactivated by a series of phosphorylation events, mediated by cyclin-dependent kinase (cdk) complexes that are activated and induced in response to mitogenic signals, such as growth factors (Trimarchi and Lees, 2002). In contrast, the presence of TGFβ is an anti-growth signal that can inhibit RB phosphorylation by inducing the expression of the cyclin : cdk inhibitor p15 (Hannon and Beach, 1994). The cyclin : cdk inhibitors, p16, p27 and p21, also play a key role in gating cell-cycle entry, and are induced in response to diverse signals, including oncogenic and genotoxic stress (Figure 1).

Disruption of the RB checkpoint, either directly or indirectly, is believed to be a requisite event for tumorigenisis. RB is a tumor suppressor that is absent or mutated in at least one-third of human tumors. Tumors with wild-type RB have almost invariably deregulated other components of the pathway (reviewed in Sherr and McCormick, 2002). For example, loss of p16 functions, through mutations, promoter hypermethylation or epigenetic silencing (Kamb *et al*., 1994; Nobori *et al*., 1994), or upregulation of cyclin/cdk expression, are frequent lesions.

Viruses also render infected cells resistant to anti-growth signals (Figure 2). Adenovirus E1A ablates the anti-growth functions of TGFβ1 by downregulating the expression of its cellular receptor (TGFβ1-RII) (Tarakanova and Wold, 2003). Herpes virus 8 encodes a novel viral D type cyclin that binds to cdk6, which inactivates RB. However, in contrast to cellular cyclin D : cdk complexes, the viral cyclin : cdk complex cannot be inactivated by the p16/p21/p27 cell-cycle inhibitors (Swanton *et al*., 1997). The RB checkpoint is a hub for targeted inactivation by disparate DNA viruses. Adenovirus E1A, SV40/polyoma LT, and HPV E7 proteins use a homologous LXCXE motif to bind to the pocket region of RB/p107/p130 and activate E2F, reviewed in (Helt and Galloway, 2003). The LXCXE motif was first discovered through studies with E1A (Whyte *et al*., 1988), but subsequently identified in cellular proteins, such as histone deacetylase (HDAC), which also binds to RB (Ferreira *et al*., 1998). Thus, HDAC and E1A/LT/E7 binding to RB is thought to be mutually exclusive. In addition to binding the RB family of tumor suppressors, E1A, LT and E7 also share another property, in that they all interact with histone acetyl transferases (HAT), such as p300 (Iyer *et al*., 2004). Indeed, E1A binding to either p300 or RB is sufficient to drive quiescent cells into S phase (Howe *et al*., 1990). Does this remarkable physical convergence of DNA viral proteins in binding to both RB and HATs reflect some distant shared ancestry, or is it because the functions of these proteins in perturbing cellular networks that regulate DNA replication limit the number of possibilities for their design, canalizing their evolution? The former explanation would seem unlikely as these viruses/viral proteins are otherwise highly divergent. However, the latter explanation suggests that tumor cells would also have to deregulate both the RB checkpoint and chromatin remodeling factors, an understanding of which could provide key insights for the development of therapies that prevent their replication.

'And death shall have no dominion' in the viral and tumor programs

To achieve unchecked proliferation implies simultaneously avoiding premature and abortive cell death. In the evolution of multicellular organisms, apoptosis has been linked as an innate tumor suppressor mechanism to aberrant replication elicited by malfunctioning cellular networks. Uncoupling the apoptotic machinery from oncogenic replication is therefore a hallmark of both the tumor and viral programs.

Apoptosis, triggered by extrinsic and intrinsic signals, activates the cleavage of a cascade of intracellular proteases – known as caspases, which act as a module to execute the destruction of the cellular membrane, cytoplasmic blebbing and nuclear degradation (reviewed in Danial and Korsmeyer, 2004). The extrinsic death pathway is activated through ligand binding to cell-surface death receptors such as Fas, Trail and TNF, which are apically bound to caspase 8 by their cytoplasmic tails (Peter and Krammer, 2003). The intrinsic death pathway is the primary death pathway and is engaged by a diverse array of cellular stresses. This pathway is gated by the balance of a family of BH3-containing proteins, such as the ratio of proapoptotic Bax/Bak and antiapoptotic B-cell lymphoma 2 (BCL2)/BCL_{XL}. Apoptosis occurs when the proapoptotic members dominate, which together form a channel that permeabilizes the mitochondrion, releasing cytochrome *c* and provoking caspase activation (Figure 1) (reviewed in Cory and Adams, 2002).

Tumor cells acquire resistance to apoptosis through a variety of mechanisms. For example, in a large percentage of tumor cell lines, a decoy receptor for Fas ligand is upregulated, titrating it away from the Fas death receptor (Pitti *et al*., 1998). BCL2 was first discovered as a chromosomal translocation to the immunoglobulin locus in follicular lymphoma (Tsujimoto *et al*., 1984), which activates its constitutive expression, resulting in the inhibition of apoptosis. There is little doubt that one of the major mechanisms whereby tumor cells evade apoptosis is through inactivation of the p53 tumor suppressor pathway. p53 induces apoptosis through the transcriptional induction of downstream targets such as the proapoptotic Bcl2 members, including Puma, Noxa, Bid and Bax (Fridman and Lowe, 2003), and may also act directly at the mitochondrion to release cytochrome *c* (Mihara *et al*., 2003). p53 is a hub for a web of cellular networks that

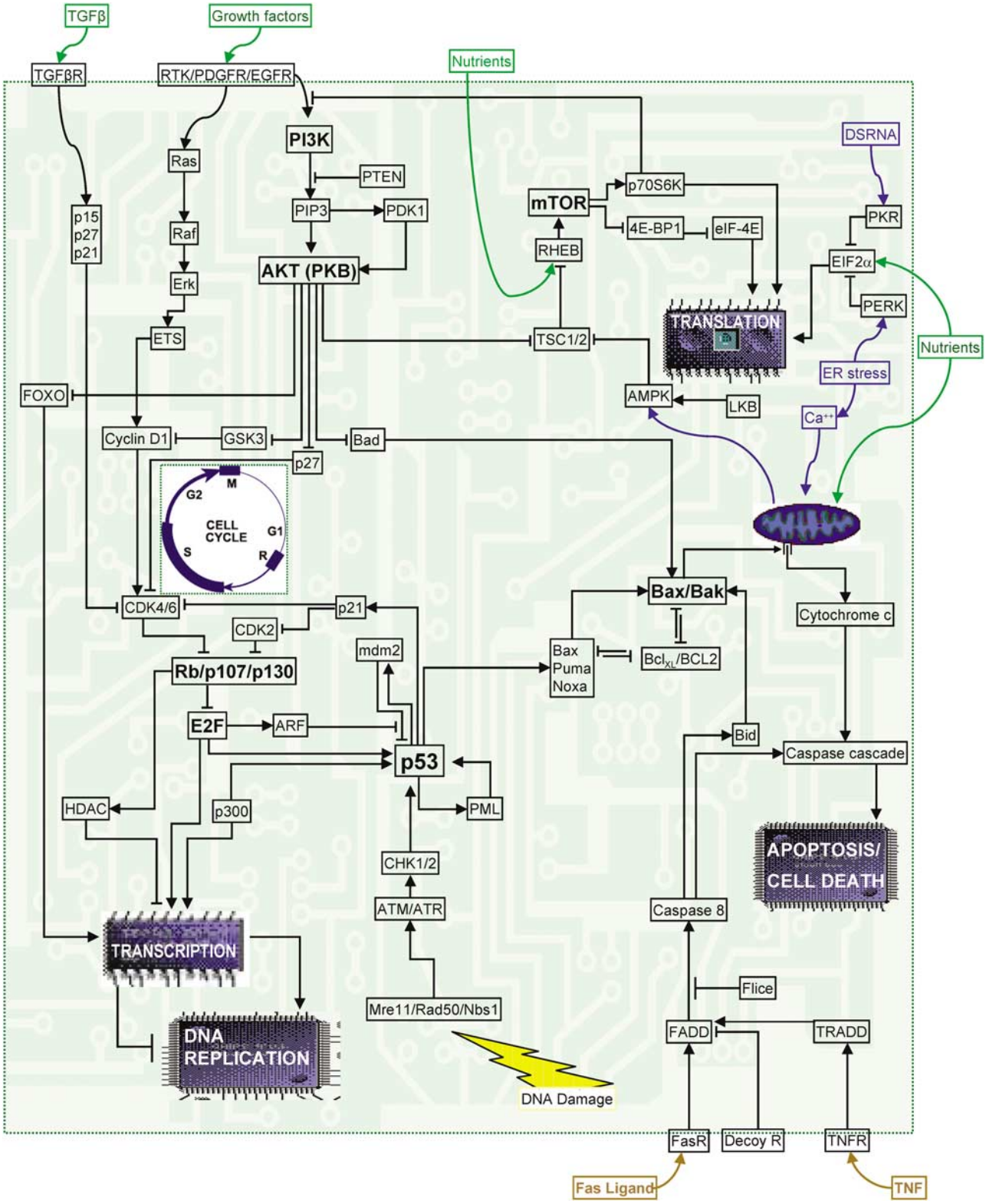

Figure 1 The integrated molecular circuitry of pivotal growth-regulatory pathways. Cell fate is determined by embedded networks of growth-regulatory pathways that exert integrated or additive effects upon each other, and critical downstream effectors. Some of the key pathways discussed in this review are represented, albeit in a redacted form. These pathways are highly integrated, but for clarity, growth factor signaling pathways are for the most part depicted in the upper left quadrant; the RB/p53 checkpoints in the lower left quadrant; apoptotic signaling pathways in the lower right quadrant and protein translation pathways to the upper right quadrant. Tumor cell mutations deregulate many of these pathways and cooperate to uncouple the cellular networks that elicit DNA replication from anti-growth signals, senescence and apoptosis

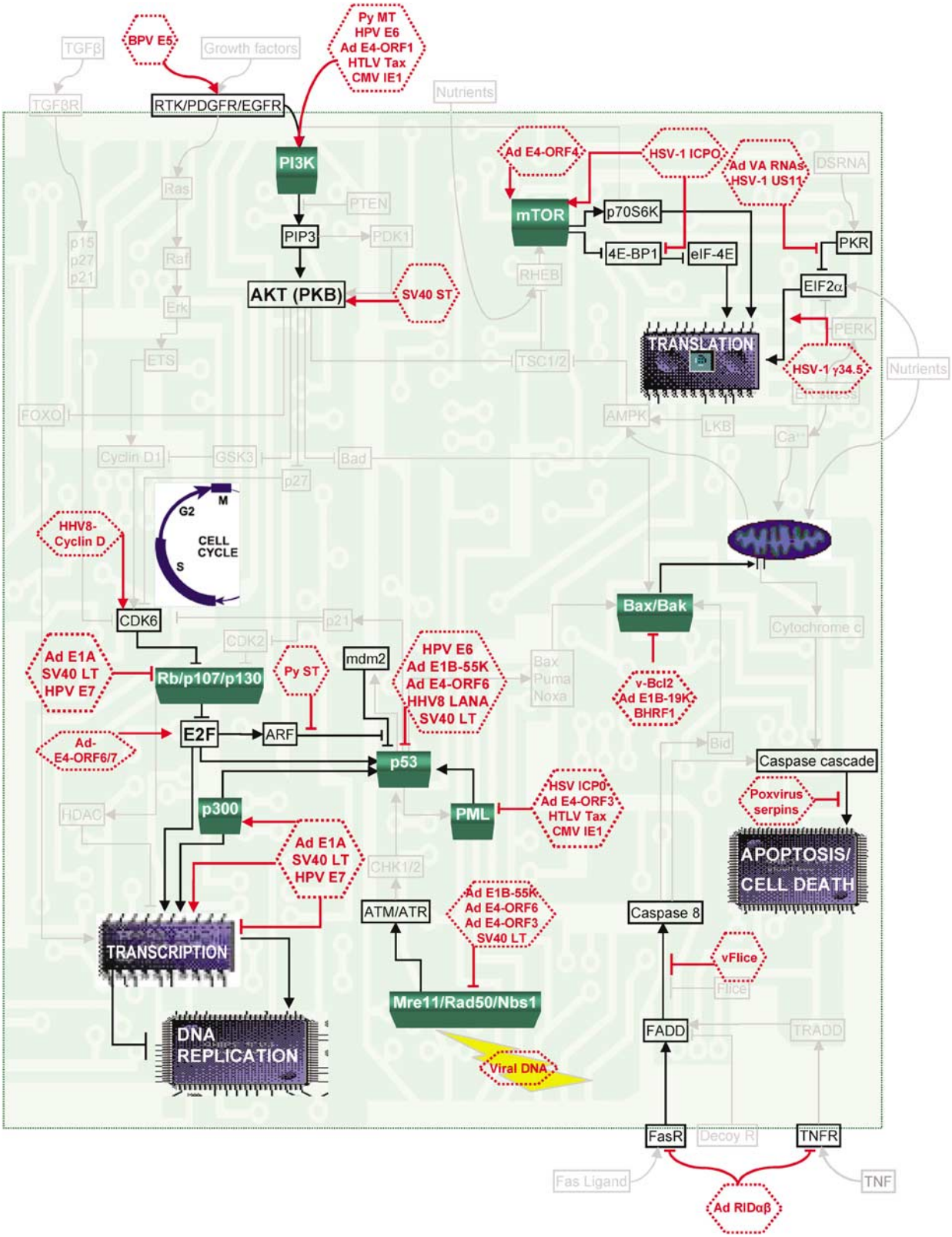

Figure 2 DNA viruses-discovering the hubs and program for pleiotropic growth deregulation. DNA viruses encode a program of proteins that act within complex cellular networks to execute an orchestrated program of growth deregulation that is similar to that engendered in tumor cells by mutations. Thus, viruses and viral proteins are a natural system that can be exploited to discover the critical cellular hubs and the necessity of perturbing them in the context of a pleiotropic growth deregulation program. This knowledge has important applications both in the rational design of oncolytic viruses and the discovery of efficacious combination cancer therapies

respond to stresses such as hypoxia, nutrient deprivation and DNA damage, incumbent signals in the incipient tumor. Oncogenes activate the p53 checkpoint through the induction of ARF, which prevents MDM2-mediated degradation of p53 (Lowe and Sherr, 2003). Intriguingly, ARF is not expressed in normal proliferating tissues, but is selectively induced in aberrant cellular replication, although how such signals are distinguished at the *ARF* promoter remains unclear (Zindy *et al*., 2003; Aslanian *et al*., 2004). Thus, the *p53* tumour suppressor gene is mutated in more than 50% of human cancers, and mutations in other genes that affect p53 function/activation, for example, *ARF*, occur in many, if not all, tumors that retain wild-type *p53* (Sherr and McCormick, 2002).

Viruses encode proteins that prevent premature apoptosis, aborting their productive replication in infected cells (Figure 2). Many viruses encode proteins that inactivate members of the TNF family of receptors (Benedict and Ware, 2001). For example, the adenovirus E3–RID complex mediates the internalization and lysosomal degradation of Fas and Trail receptors (Tollefson *et al*., 1998, 2001; Benedict *et al*., 2001). This may be necessary to avoid cell death triggered by immune surveillance, or sensitization to TNF-induced cell death by viral oncogenes such as E1A, which downregulates Flip, a negative regulator of TNF receptor signaling (Perez and White, 2003). Poxviruses encode caspase inhibitory serpins or viral FLICE inhibitors as well as soluble TNF receptors, and several DNA viruses encode functional homologs of antiapoptotic BCL-2 family members, for example, adenovirus E1B-19K, the herpes viruses Epstein–Barr BHRF1 and HHV8 v-BCL-2 (reviewed in Cuconati and White, 2002).

In addition, many DNA viruses encode proteins that bind and inactivate p53 through a variety of mechanisms (O'Shea and Fried, 2005). Indeed, p53 was first identified as a cellular protein that interacted with SV40 LT (Lane and Crawford, 1979; Linzer and Levine, 1979). Adenovirus encodes two viral proteins, E1B-55K and E4-ORF6, that form a complex and, together with cellular proteins involved in ubiquitination, bind and degrade p53 (Querido *et al*., 1997, 2001; Harada and Berk, 1999). The HPV E6 protein degrades p53 by binding to both p53 and the cellular E3 ubiquitin ligase, E6AP. While E6AP does not normally function to degrade p53, HPV E6 recruits and redirects E6–AP's ubiquitin ligase activity to target p53 (Scheffner *et al*., 1990, 1992; Huibregtse *et al*., 1991). Recently, E6 has also been shown to repress p53-activated transcription, independently of E6–AP-mediated p53 degradation, via inhibition of p300-mediated acetylation (Thomas and Chiang, 2005). In contrast to the other small DNA viruses, mouse polyoma virus inactivates p53 indirectly, by inhibiting ARF-mediated induction of p53. The PyST PP2A is required to prevent ARF-mediated activation of p53, perhaps indicating a previously unrecognized role for PP2A in regulating the ARF–p53 tumor suppressor pathway (Moule *et al*., 2004). In addition, the herpes virus 8 protein LANA has been linked to p53 transcriptional inactivation (Friborg *et al*., 1999). Thus, disparate DNA viruses have functionally converged to encode proteins that bind and inactivate p53, underscoring the importance of the p53 tumor suppressor pathway in monitoring normal cell growth and DNA replication.

Forever young – abrogating the senescence program

In addition to apoptosis, the induction of cellular senescence could also thwart aberrant replication. Cellular senescence is characterized by a permanent irreversible cell-cycle arrest (distinct from the reversible cell-cycle arrest in quiescent cells), and specific changes both in the morphology of the cell and epigenetic status of its chromatin. Senescence results from the natural erosion of telomeres in progressive cell divisions (replicative senescence), but can also occur independently of the latter in response to oncogenic or genotoxic stress. *In vitro*, the cell-cycle arrest associated with cellular senescence can be triggered via the induction of *p16/ARF* by activated oncogenes, such as Ras, and has, therefore, been proposed as a tumor suppressor mechanism that guards against malignant transformation (Campisi, 2005). Nevertheless, whether oncogene induced senescence is a *bona fide* tumor suppressor mechanism remains contentious as it has yet to be definitively observed *in vivo*. However, there is little doubt that human cells undergo replicative senescence, as in every cell cycle 50–100 base pairs are lost from the telomeric ends of chromosomes. Ultimately, as cells age, the progressive shortening of telomeres can lead to end-to-end chromosomal fusions, a crisis that prevents their further replication.

In contrast to primary cells, tumor cells evolve to become the insidious 'Dorian Greys' within the body, impervious to senescence and with seemingly limitless replicative potential. Since the induction of p16/ARF can elicit RB/p53-mediated cell-cycle arrest, it is perhaps unsurprising that their inactivation in tumor cells is thought to play a key role in disabling the normal senescence program (Lowe and Sherr, 2003). The PML tumor suppressor, first identified as the translocation partner of RARα in promyelocytic leukemia (de The *et al*., 1990), has also been implicated in the senescence program (Pearson *et al*., 2000; Bischof *et al*., 2002). Moreover, telomere shortening and replicative senescence is prevented in tumor cells by either the direct upregulation of telomerase (hTERT) (Kyo and Inoue, 2002) or through an alternative pathway that involves recombination-based interchromosomal exchanges (Bryan *et al*., 1995).

In that viruses encode proteins which inactivate the RB/p53 checkpoints, they interfere with the senescence program. For example, suppressing either HPV E6- or E7-mediated inactivation of the p53 and RB checkpoints, respectively, triggers the rapid senescence of HeLa cervical carcinoma cells (Horner *et al*., 2004; Psyrri *et al*., 2004). E1A overrides oncogene-induced senescence by inactivating RB, as well as by preventing the formation of senescence-associated repressive

heterochromatin structures, through its interaction with chromatin remodeling factors, such as p300/p400 (Narita *et al*., 2003). Similarly, HPV E7 also circumvents PML-induced senescence independently of its binding/inactivation of RB (Bischof *et al*., 2005). Indeed, many viruses encode proteins that disrupt components of the PML bodies, including adenovirus E4-ORF3, HSV-ICPO, CMV-IE1/IE2 and HTLV tax (reviewed in Everett, 2001). Moreover, the HPV E6/E6–AP complex, in addition to degrading p53, also induces the transcriptional upregulation of hTERT, which requires an intact myc/USF E-box in the hTERT promoter (McMurray and McCance, 2003; Veldman *et al*., 2003). Recent studies with E6 led to the identification of NFX1–91 as a novel hTERT transcriptional repressor that is targeted for degradation by the E6/E6–AP complex (Gewin *et al*., 2004). In addition, SV40 ST and herpes virus 8 LANA have also been reported to induce telomerase activity (Foddis *et al*., 2002; Yuan *et al*., 2002; Verma *et al*., 2004). Whether such viral proteins' functions act to facilitate viral replication irrespective of cellular/organismal age is a matter for conjecture. It is more likely that the ability of viral proteins to break senescence is an example of evolutionary exaptation, that is a property they have acquired but evolved for an entirely distinct function. Notwithstanding, this does not undermine their utility as tools with which to identify critical modulators of cellular senescence.

'O What a tangled Web We Weave (W.W.W.)…

As is apparent from even the redacted set of examples discussed above, a tumor-like state is the sum of a number of mutations/viral proteins affecting cellular pathways that are integrated within highly complex networks (Figure 1). The convergence of the viral and tumor cell programs suggests that the design of cellular networks may constrain the number of strategies by which it is possible to engender aberrant DNA replication, channeling their evolution. What is the nature of cellular networks then? And would understanding the interplay of cellular networks in the dynamics of viral replication provide us with a key tool to affect, or ablate, the pathological rewiring that is acquired in tumorigenesis?

Recent advances in the theory of networks biology provides a conceptual and mathematical framework with which to study the 'network of networks' that determine the behavior of the cell (reviewed in Barabasi and Oltvai, 2004). At an abstract level, the components of cellular pathways are reduced to a series of nodes that are connected by links, with each link representing the interaction between two components. The nodes and links together form a network. If biological networks were random, then on average each node would have the same average number of links. However, biological networks are nonrandom, in that some nodes are hubs with a tremendously large number of links to other nodes. For example, in the protein interaction network in yeast, the majority of proteins interact with only one or two others, but a few are hubs and interact with a large number (Yook *et al*., 2004). In this sense, the network appears to have no scale and is often termed 'scale free'. The architectural features and hubs apparent in cellular networks are shared by other complex networks such as the world wide web and computers.

The existence of cellular hubs is explained by a network's evolution (Barabasi and Albert, 1999). In protein interaction networks, evolutionarily older proteins generally have more links than their younger counterparts (Wagner, 2003). In addition, all nodes are not equal. Preferential attachment, or the principle of 'it's not what you know but who you know', also explains the existence of hubs; the evolution and natural selection of gene duplication events is more likely to occur at highly connected nodes (Eisenberg and Levanon, 2003). This inhomogenous wiring of biological networks has far-reaching implications for understanding cellular functions. Since the majority of nodes are not hubs, it means that the random failure/mutation of a large number of nodes can be tolerated without affecting the overall functioning or interconnectivity of the cellular network as a whole. However, such error tolerance comes at a high price and makes biological networks extremely vulnerable to concerted attacks that select and remove the hubs which maintain the networks connectivity (Albert *et al*., 2000). The latter are all characteristics of a virus and, I would suggest, the selection of mutations that occur in the evolution of a tumor.

Viruses – identifying the hubs in the tumor cell program or 'it takes one to know one'

However, neither cancer biologists nor clinicians need network theorists to predict what they have already proven to be only all too true in practice: that targeting any one malfunctioning node in cancer is unlikely to be effective. This is because cellular hubs are embedded in a web of overlapping, and hence often redundant, networks. For example, from the model depicted in Figure 1, drugs that inhibit PI3-kinase might be conjectured to have limited efficacy in tumor cells with additional mutations in RB, Ras, p53, etc. The hope then lies in the prediction that a combined attack on 2–3 well-chosen hubs (Albert *et al*., 2000) that destroy the diseased networks connectivity would crash the tumor cell program. But what combination of targets will be effective? Thus, while we may have identified many of the critical players in tumorigenesis, a major challenge is to understand how they interact in the pathological program. For this, we need a system in which to query the network dynamics of deregulated growth. Mouse models are one such system and have provided many valuable insights. However, it is not practical to look at spontaneous naturally occurring tumors in mice. Therefore, the conditions for tumorigenesis are artificially imposed to resemble lesions that can occur in human tumors, but, unfortunately, may not reflect the context in which they naturally arise. Often, there is an extended

latency period, in which additional mutations necessary to elicit the neoplastic phenotype are acquired. In addition, it is literally the nature of the beast that makes it difficult to determine *a priori* the molecular network dynamics of, for example, loss of p53 and activated Ras, other than to know that together they 'can' give rise to tumors.

Historically, some of our first insights into the links, or cooperation, between oncogene and tumor-suppressor pathways came from rodent cell transformation assays with combinations of cellular and DNA viral oncogenes (Rassoulzadegan *et al*., 1982; Land *et al*., 1983; Ruley, 1983). Indeed, human cellular transformation has not been achieved in the absence of viral proteins, which continue to provide fundamental insights (see Ahuja *et al*., 2005; Arroyo and Hahn, 2005). Obviously, such assays do not reflect the natural context in which the growth-deregulating properties of viral proteins arose, or indeed interact. However, understanding the requirements for early viral proteins, and the cellular pathways they co-opt/perturb, in infected quiescent primary cells is a natural biological system that could be exploited to map the network dynamics of aberrant replication.

The systems biology of DNA viruses could offer a number of insights for tumor biology/therapy. Many viruses replicate in diverse primary cell types, and therefore must encode a universal growth deregulation program that operates regardless of any tissue-specific wiring differences. Thus, the cellular network perturbations engendered by such viruses are likely to be applicable to tumors of diverse tissue origins. As noted above, viruses target hubs. Therefore, viral proteins are biochemical probes that can identify critical cellular players, as evidenced by the discovery of p53 through its interaction with SV40 LT (Lane and Crawford, 1979; Linzer and Levine, 1979). Relatively simple viral genetics can then be used to map the interdependence of viral proteins, and by inference the cellular pathways they co-opt, in driving aberrant replication in primary cells. For example, is there a combination of viral proteins/cellular functions that upon inhibition prevents DNA replication, or triggers apoptosis, in virally infected primary cells? This is akin to 'synthetic lethal' screens in yeast that have been proposed for identifying drug combinations that prevent DNA replication (Hartwell *et al*., 1997), but which could be used directly in primary human cells. Moreover, a detailed molecular understanding of the viral program is also of paramount importance for the development of effective oncolytic viral strategies. Therefore, for the remainder of this review, I will illustrate some of these potential applications with particular reference to the adenoviral program.

Adenovirus – a pleiotropic growth deregulation program

Human adenovirus subgroup C replicates in a broad array of human cell types, and with a genome of approximately 36 kb occupies a relative 'sweet spot' in terms of the number of viral genes versus the complexity of the proteins that such genes encode (33–36). Adenovirus encodes a number of early viral proteins (Table 1), ostensibly to drive infected quiescent primary cells into unscheduled replication, while simultaneously preventing premature apoptosis. The E1 proteins include E1A, E1B-55K and E1B-19K (discussed by Berk, 2005). E1A is the master regulator of the adenoviral program, and activates the transcription of the other early viral genes, as well as the critical E2F S-phase transcriptional program. As discussed previously, E1A uses an LXCXE motif to bind and inactivate the tumor suppressor proteins p107, p130 and RB (Helt and Galloway, 2003). In addition, E1A binds to chromatin remodeling factors such as the HATs p300/CBP (Whyte *et al*., 1989) and the p400/TRRAP complex (Fuchs *et al*., 2001).

Adenovirus E1B-55K and E4-ORF6, together with cellular proteins involved in ubiquitination, bind and degrade the tumor suppressor p53 (Querido *et al*., 1997, 2001; Harada and Berk, 1999). However, even in the absence of E4-ORF6, E1B-55K is a dominant transcriptional repressor of p53 (Martin and Berk, 1999). The E1B-55K/E4-ORF6 complex also degrades Mre11 (Stracker *et al*., 2002), which accumulates at the sites of double-strand DNA breaks triggering a DNA damage/repair response. Viral genomes are recognized by the DNA damage/repair machinery in the cell due to either their unusual DNA structures/intermediates or the replication of naked double-stranded DNA termini (Weitzman *et al*., 2004). Thus, E1B-55K/E4-ORF6-mediated degradation of Mre11 prevents the 'repair' and end-joining of viral genomes, together with the downstream activation of ATM (discussed by Weitzman and Ornelles, 2005). Adenovirus E1B-19K is a functional homolog of the antiapoptotic cellular BCL-2 family of proteins, but an even more potent inhibitor of cell death (reviewed in Cuconati and White, 2002).

Surely then, adenovirus E1 proteins that together inactivate p53, RB and Bax/Bak are all that is required to invoke unscheduled DNA replication in quiescent primary cells (Figure 2). Why then does adenovirus encode additional early viral proteins, such as the E4 gene products, without which its replication is highly defective (Halbert *et al*., 1985; Weinberg and Ketner, 1986; Yoder and Berget, 1986)? No doubt, one of the answers is that such proteins counter cellular antiviral responses (Weitzman and Ornelles, 2005). In addition, E4 proteins themselves promote aberrant replication. Understanding the requirements for these other early viral proteins, their cooperation in cellular networks disrupted for p53/RB/apoptosis functions by E1 proteins, is therefore, a fertile strategy to identify novel cellular pathways critical for cell growth that may also be disrupted in tumor cells. In a recent study (O'Shea *et al*., 2005), we applied such a principle to try to gain new insights into the regulation of protein translation and nutrient/growth factor signaling pathways to mTOR, an emerging and important tumor target (Inoki *et al*., 2005).

Table 1 Adenovirus early viral proteins cellular targets and functions

	Early viral protein	*Cellular targets*	*Functions*
1	E1A (13S, 12S, 11S, 9S)	p21, cdk 2, p400/TRRAP, p300, CBP, NFkB, STAT1, ATF2, YY1, MAZ, TAF (II)135, TBP, SUR-2/Med23, CtBP, DYRK	Activation of E2F and cell cycle entry Activates transcription of early viral genes Inhibits interferon-induced transcription Suppression of differentiation Inhibition of senescence
2	E1B-55K	p53, Mre11, HDAC	p53 degradation/inactivation Degradation of Mre11 Late viral RNA export
3	E1B-19K	Bax, Bak,	Inhibition of apoptosis
4	E4-ORF1	MAGI-1,MUPP1, DLG, PI-3 kinase	Activation of PI-3 kinase
	E4-ORF2	Unknown	Unknown
5	E4-ORF3	PML, CBP, Mre11	Perturbation of Mre11/DNA repair Mislocalization of PML Modulation of transcription
6	E4-ORF4	PP2A, c-jun, src, mTOR, SR proteins	Modulation of PP2A activity Activation of mTOR and src Modulation of SR regulated splicing
7	E4-ORF6	p53, p73, Mre11, Cullin-5, Elongin B/C	Degradation/inactivation of p53 Degradation of Mre11 Late viral RNA export
8	E4-ORF6/7	E2F transcription factors	Dimerization and activation of E2F(s)
9	E3-gp19K	MHC Class I	Retains MHC Class I in the endoplasmic reticulum
9	E3-RID$\alpha\beta$ (10.6K,14.5K)	Fas, TNF family of receptors, EGFR	Internalization and lysosomal degradation of Fas, TNF receptors and EGFR

(1) Reviewed by Berk (this issue) and Russell (2000); Frisch and Mymryk (2002); Helt and Galloway (2003); (2) Berk (this issue); (3) reviewed in Cuconati and White (2002); (4) Lee *et al*. (1997, 2000); Glaunsinger *et al*. (2001); Frese *et al*. (2003); O'Shea *et al*. (2005); (5) Weitzman and Ornelles (this issue); (6) Branton and Roopchand (2001); Estmer Nilsson *et al*. (2001); O'Shea *et al*. (2005); (7) Berk (this issue) and Dobner *et al*. (1996); Higashino *et al*. (1998); Querido *et al*. (2001); Harada *et al*. (2002); (8) Helin and Harlow (1994); O'Connor and Hearing (2000); (9) reviewed in Lichtenstein *et al*. (2004)

mTOR activation induces the phosphorylation of 4EBP1 and $p70^{S6K}$, which stimulates the translation of mRNAs with a high degree of secondary structure in their 5′ untranslated regions, including that of many important growth-regulatory messages. Examples of mTOR-regulated mRNAs include *cyclin D1* (Muise-Helmericks *et al*., 1998), *ornithine decarboxylase* (Seidel and Ragan, 1997) and *c-myc* (West *et al*., 1998). In this way, mTOR activation is thought to serve as an important checkpoint for normal cell growth (Avruch *et al*., 2005). We found that adenovirus bypasses a nutrient/growth factor checkpoint for protein translation in infected primary cells (O'Shea C *et al*., 2005a) and encodes two early viral proteins, E4-ORF1 and E4-ORF4, which stimulate mTOR activity in distinct but complementary ways (O'Shea *et al*., 2005). E4-ORF1 had previously been identified as a potent activator of PI3-kinase (Frese *et al*., 2003), and mimics growth factor signaling to mTOR. However, surprisingly, an E4-ORF1 mutant virus that fails to activate PI3-kinase still stimulates mTOR activity, in infected primary cells. This led us to find that E4-ORF4, which binds and modulates PP2A (Branton and Roopchand, 2001), substitutes for glucose-mediated signaling to mTOR (O'Shea *et al*., 2005). Recently, deregulated nutrient signaling pathways have been implicated in tumorigenesis. In the inherited cancer predisposition syndrome Peutz–Jeghers' disease, inactivating mutations in *LKB* result in constitutive mTOR activation, even upon nutrient withdrawal (Shaw *et al*., 2004). Interestingly, *PP2A A* subunit mutations and B56γ downregulation have been found in a subset of human tumors (Wang *et al*., 1998; Calin *et al*., 2000; Ito *et al*., 2003). Thus, E4-ORF4 is a unique tool with which to identify novel cellular targets and a potential role for PP2A in the nutrient signaling pathway to mTOR and cancer.

The *E4* region also encodes *E4-ORF2*, *E4-ORF3*, *E4-ORF6* and *E4-ORF6/7*. As of yet, there is no known function of E4-ORF2. E4-ORF6 works in a complex with E1B-55K to degrade p53 and Mre11, as discussed above. Recently, E4-ORF6 has also been shown to exert effects independently of E1B-55K in inhibiting the cellular DNA damage/repair pathway (Hart *et al*., 2005). As well as E4-ORF6/E1B-55K-mediated degradation of Mre11, adenovirus encodes another protein, E4-ORF3, which inactivates the Mre11 signaling pathway through a novel mechanism. In addition, E4-ORF3 disrupts the PML nuclear bodies (Doucas *et al*., 1996), an event also mitigated by the PML-RARα chromosomal translocation product, which is believed to play a causal role in acute promyelocytic leukemia. The molecular mechanisms underlying E4-ORF3's functions could provide novel insights into tumor cell lesions in PML and the DNA damage-signaling pathway. Finally, E4-ORF6/7 promotes the heterodimerization and activation of the E2F family of transcription factors (Neill and Nevins, 1991; Helin and Harlow, 1994), and can functionally compensate for E1A-mediated inactivation of RB in adenovirus infected cells (O'Connor and Hearing, 2000). This in turn begs the question as to whether there is a functional equivalent of E4-ORF6/7 in tumor cells.

The *E3* viral genes encode immunomodulatory proteins that are dispensable for adenovirus replication *in vitro*. E3-gp19k binds and retains MHC-class I in the endoplasmic reticulum to abrogate cytotoxic T-cell-mediated killing of adenoviral infected cells (Andersson *et al*., 1985). MHC-class I downregulation is also associated with the escape of tumor cells from immune surveillance mechanisms. In addition, as noted above, the adenovirus RID complex comprising the E3R1Dα and β proteins provokes the lysosomal degradation of the Fas and Trail death receptors to abrogate apoptosis (Tollefson *et al*., 1998, 2001; Benedict *et al*., 2001).

Putting oncolytic viral therapies in context

Taken together, the known functions of adenoviral early proteins include: inactivation of the tumor suppressors RB, p107, p130, p53; abrogation of DNA damage signaling; inhibition of apoptosis through both the intrinsic and extrinsic death pathways; activation of oncogenic nutrient/growth factor signaling pathways to PI3-kinase/mTOR; deregulated PP2A signaling, a critical target in human transformation assays (discussed by Arroyo and Hahn, 2005); modulation of transcription through chromatin remodeling factors and E2F and disruption of PML nuclear bodies. In the face of such a concerted attack on cellular hubs (Figure 2), it is little wonder that adenovirus overrides all the multiple checkpoints embedded in cellular networks to induce a 'tumor-like' state in quiescent primary epithelial cells within 24–36 h post-infection. Consequently, crippling any single early viral protein/cellular hub interaction might be predicted to have a limited impact on replication. Indeed, this has been the experience in the development of oncolytic viral therapies, two examples of which I discuss below.

ONYX-411-E2F selective oncolytic viral therapy

There are three main strategies that are being used to develop adenoviruses as agents that undergo selective lytic replication in tumor cells: the complementation of viral mutants by tumor cell mutations, transcriptional retargeting and transductional retargeting, each of which is reviewed in this issue of Oncogene (see Ko *et al*., 2005; Mathis *et al*., 2005; McCormick, 2005). The first strategy exploits the functional overlap between DNA viral proteins and tumor cell mutations. For example, adenoviral mutants that fail to disrupt critical cellular checkpoints such as, RB, would be predicted to be defective for their replication in normal cells, but undergo selective replication in tumor cells that have inactivated the RB pathway. Thus, an adenovirus mutant, *dl*922/47 (Whyte *et al*., 1989), which encodes an E1A mutant protein (ΔLXCXE) that fails to bind and inactivate RB, was expected to be an oncolytic viral therapy for RB mutant tumor cells. However, *dl*922/47 induces S-phase entry, viral DNA replication and late protein expression similar to that of wild-type virus in quiescent primary epithelial cells (Johnson *et al*., 2002). This may seem surprising given the prevailing RB-centric view of cell-cycle entry. However, it is entirely consistent with cell-cycle entry occurring as a result of the integration of multiple cellular pathways that crosstalk, or bypass each other, in the molecular circuitry of the cell. To create an RB mutant-tumor selective virus, ONYX-411, required the use of an E2F promoter to transcriptionally regulate *E1A* (ΔLXCXE RB mutant), as well as the *E4* genes (Johnson *et al*., 2002). This is because the expression of the *E4* genes, even in the absence of E1A, engendered viral replication in primary cells. ONYX-411 fails to replicate in primary cells, but undergoes selective lytic replication in tumor cells that have deregulated E2F, and its promise as a therapeutic agent is discussed further by (Ko *et al*., 2005; McCormick, 2005).

Nevertheless, there are some concerns that the presence of cellular DNA sequences in oncolytic viral vectors may promote potentially dangerous recombination events between viral and host DNA. In addition, promoter modifications to the E4 region result in more genetically unstable adenoviruses that make them less suitable from a manufacturing/clinical perspective (reviewed by Working *et al*., 2005). Thus, identifying, and ablating, the E4 gene functions that stimulate S-phase entry would be an alternative strategy by which to achieve the selectivity of ONYX-411 in the absence of promoter modifications to the E4 region. One candidate for such a role is E4-ORF6/7, which dimerizes and activates E2F transcription factors (Neill and Nevins, 1991; Helin and Harlow, 1994; O'Connor and Hearing, 2000). An alternative candidate is E4-ORF4. Recently we found that an E4-ORF4 mutant virus, which fails to activate mTOR and its downstream target $p70^{S6K}$, resulted in delayed S-phase entry in quiescent primary cells, despite the induction of E2F transcriptional targets (O'Shea *et al*., 2005). A requirement for $p70^{S6K}$ activation in S-phase entry has previously been demonstrated (Lane *et al*., 1993), and rapamycin is known to arrest cells in the G1 phase of the cell cycle (Huang *et al*., 2001). Our favored hypothesis is that E4-ORF4-mediated activation of mTOR regulates the translation, much like E2F regulates the transcription of a panel of mRNAs important for the onset of DNA synthesis (Bracken *et al*., 2004). Some of the chemotherapeutic agents commonly used in the clinic target E2F transcriptional targets, for example, 5-fluorouracil and thymidylate synthetase. Therefore, identifying whether there are critical mRNAs that are translationally regulated for cell-cycle entry could uncover novel cellular targets for cancer drug development. Infection of primary cells with a ΔE4-ORF4 mutant adenovirus, which readily distinguishes between E2F and mTOR activation in the onset of aberrant DNA replication, may be a novel strategy with which to elucidate such targets.

dl1520/ONYX-015 oncolytic therapy

The prototype for oncolytic adenoviral therapy is *dl*1520/ONYX-015, a mutant that lacks the E1B-55K

gene product and, therefore, fails to degrade p53 in infected cells (Barker and Berk, 1987; Bischoff *et al*., 1996). ONYX-015 was expected to be restricted by p53 in normal cells, but undergo selective lytic replication in p53-deficient tumor cells. Consistent with this, ONYX-015 replication is attenuated in primary cells, but replicates to wild-type virus levels in many tumor cell lines. On this basis, ONYX-015 entered phase I and II clinical trials, where it was shown to be safe with evidence of promising clinical activity from several indications (Khuri *et al*., 2000; Kirn, 2001; Reid *et al*., 2002; Ries and Korn, 2002; Rudin *et al*., 2003). Nevertheless, ONYX-015 has yet to be evaluated in phase III clinical trials and, therefore, remains an unproven therapy. Two major factors that stalled the further clinical evaluation of ONYX-015 were the controversy surrounding its p53 tumor selectivity together with its variable tumor cell replication (Goodrum and Ornelles, 1998; Hall *et al*., 1998; Rothmann *et al*., 1998; Harada and Berk, 1999; Turnell *et al*., 1999; Nemunaitis *et al*., 2000; Ries *et al*., 2000; Hann and Balmain, 2003; McCormick, 2003). Recently, the role of p53 in determining ONYX-015 tumor selectivity has been resolved. In ONYX-015 infected cells, p53 is induced, but not activated, and therefore plays a minimal role in limiting viral replication, at least *in vitro* (Hobom and Dobbelstein, 2004; O'Shea C *et al*., 2004). In addition to its role in p53 degradation, E1B-55K has late functions that include the preferential export and translation of late viral mRNAs, both necessary events for a productive viral infection (Babiss *et al*., 1985; Pilder *et al*., 1986; Leppard and Shenk, 1989). Recently, using a novel adenovirus mutant, ONYX-053, which distinguishes between E1B-55K-mediated p53 inactivation and late functions (Shen *et al*., 2001), it was demonstrated that differential late viral RNA export in tumor versus normal cells is the major determinant of ONYX-015 oncolytic selectivity (O'Shea C *et al*., 2004). Permissive tumor cells that support ONYX-015 replication export late viral RNAs in the absence of E1B-55K, a propensity not shared by primary cells (O'Shea C *et al*., 2004).

Thus, altered RNA export is an unexpected tumor cell property that is selectively targeted by ONYX-015 cancer therapy. Unfortunately, not all patients' tumors respond to ONYX-015 therapy. In contrast to permissive tumor cell lines, refractory tumor cells fail to provide the late functions of E1B-55K in the selective export and/or translation of capsid RNAs, such as *fiber* and *hexon* (Goodrum and Ornelles, 1998, 1999; Harada and Berk, 1999; O'Shea C *et al*., 2005b), as well as *100K* RNA (O'Shea C *et al*., 2005b). The 100K protein acts downstream of E1B-55K to inhibit cellular translation by displacing mnk from eIF-4G, while binding to the 5′UTR of late viral RNAs to drive their selective translation (Hayes *et al*., 1990; Cuesta *et al*., 2004). Late viral RNAs share a common 5′UTR that plays an important role in facilitating both their translation and export in adenovirus-infected cells (Yueh and Schneider, 1996; Huang and Flint, 1998). The 5′UTR of late adenoviral mRNAs shares significant structural homology with the 5′UTR of mammalian *Hsp70* mRNA, which underlies their selective translation via a common ribosome shunting mechanism in a cellular heat shock response, or viral infection, respectively (Yueh and Schneider, 2000). In many ways, the cellular response to heat shock resembles the late stages of adenovirus infection; the translation of cellular mRNAs is inhibited, while heat shock mRNAs are preferentially translated. *dl*1520/ONYX-015 is known to be a 'cold sensitive' virus, exhibiting defective late protein translation and replication in tumor cells incubated at 32°C (Ho *et al*., 1982; Leppard and Shenk, 1989; Harada and Berk, 1999). Recently, we examined if this might instead be a 'heat-dependency' phenotype, and if heat shock could rescue ONYX-015 late viral protein expression in resistant tumor cell lines. The induction of a heat shock response, by incubation at 39.4°C, rescues late viral RNA export/translation and renders refractory tumor cells permissive to ONYX-015 oncolytic replication (O'Shea C *et al*., 2005b). This suggests that heat shock and late viral RNAs may impinge on a common molecular mechanism, not only for their translation but also their nuclear export, which is deregulated in many tumor cells. Fortuitously, but for unknown reasons, heat shock inhibits both wild-type and ONYX-015 viral replication in normal cells (O'Shea C *et al*., 2005b). Indeed, febrile responses have recently been proposed as a factor that may have significantly affected the clinical outcome of ONYX-015 therapy (Thorne *et al*., 2005). Thus, agents that elicit a cellular heat shock response could have a major impact on the efficacy of ONYX-015 as a cancer therapy, which has important clinical applications.

Nevertheless, the potential for, or the promise of, a p53-selective oncolytic virus remains to be realized. Loss of E1B-55K induces the levels but not the activity of p53 in adenovirus-infected cells (Hobom and Dobbelstein, 2004; O'Shea C *et al*., 2004), suggesting that p53 is suppressed through an additional mechanism. One candidate for such a role is E4-ORF6, the binding partner of E1B-55K that has also been shown to interact with p53 (Dobner *et al*., 1996). However, viral mutants with compound mutations in *E1B-55K* and *E4-ORF6* also fail to induce p53 transcriptional targets (Hobom and Dobbelstein, 2004). An alternative candidate is E1A, which binds to p300 (Chiou and White, 1997; Thomas and White, 1998), a coactivator for p53-mediated transcription (Espinosa and Emerson, 2001). However, an *E1A* Δ*p300*/Δ*E1B-55K* adenovirus mutant fails to activate p53 in infected primary cells (O'Shea C *et al*., 2004). Even so, E1A modulates cellular transcription in multiple ways, as discussed by Berk in this issue. Alternatively, an additional signal, for example, DNA damage, may be required to activate p53 in ONYX-015-infected cells. UV/γ irradiation fails to induce p53 transcriptional targets in ONYX-015-infected cells, indicating that p53 activity is dominantly suppressed (unpublished data). However, E4-ORF3 may inhibit DNA damage/repair signaling (Stracker *et al*., 2002) to p53. Understanding the mechanism(s) whereby adenovirus inactivates p53 could provide new therapeutic

insights into how p53 activity may be modulated in tumor cells, and ultimately inform the design of novel p53-selective oncolytic viral strategies.

Taken together, although we have a reasonable understanding of the function(s) of individual viral proteins, we still have a lot to learn about their downstream functional and biological consequences within the context of the overall viral program. Historically, much of what we do know is derived from studies in tumor cell lines, such as HPV-transformed HeLa. However, the profound overlap between the tumor and viral programs implies that cancer cell lines may complement, and thereby inadvertently mask, critical viral functions. The tropism for subgroup C adenoviruses is thought to be nonciliated respiratory epithelial cells, the majority of which are in a quiescent G0 phase of the cell cycle within the human body. As evidenced by the above examples, understanding the requisite role of adenovirus proteins for viral replication in the natural context of primary cells, together with the potentially applied context of tumor cells, is of paramount importance for the rational design of the next generation of oncolytic viral therapies.

Systematically exploiting adenovirus to discover novel tumor targets and therapies

While we may not yet understand why adenovirus has evolved multiple mechanisms to disrupt specific cellular checkpoints, it is reasonable to assume that this is because all pivotal growth-regulating checkpoints are embedded in a web of redundant and integrated cellular processes, each of which must be perturbed. A major question then is whether tumor cells also reflect the inherent complexity of a 'simple' adenovirus. Certainly, tumor cells deregulate many of the same pathways, as discussed previously. However, it is a daunting prospect to consider how we would treat a tumor with the pleiotropic growth deregulation that is engendered in adenoviral infected cells. Thus, we can hope that the adenoviral program is 'overkill'. After all, adenovirus encodes multiple redundant proteins to activate E2F and mTOR, and inactivate apoptosis and DNA damage signaling. However, genetic redundancy is not usually a feature that is accommodated in the evolution of a minimal viral genome, but instead is likely to reflect the necessity of perturbing overlapping cellular networks with multifarious feed-back/-forward loops. It is the very same cellular network that is disrupted in both human tumors and adenoviral infection. Rather than 'hope', better perhaps to assume that the adenovirus program is at least a worse case scenario for many aspects of growth deregulation in tumor cells, for in this assumption lies a powerful prediction, which is that by finding the correct combination of viral proteins/cellular targets that selectively prevents the aberrant replication engendered by adenovirus infection, we might also discover efficacious combination tumor therapies. Using genetics we could approach such a task with humility, deferring to the virus to tell us the necessary combinations for novel and efficacious cancer therapies. While it is beyond the scope of this review to describe such a strategy, the principle is similar to 'synthetic lethal' screens in yeast for the identification cellular target/drug combinations that prevent DNA replication (Hartwell *et al*., 1997).

Furthermore, establishing whether tumor cells complement the replication of viral mutants is in itself a simple way to discover novel tumor targets. For example, studies with the E1B-55K deleted *dl*1520/ONYX-15 have revealed altered RNA export in tumor cells as a novel therapeutically exploitable target (O'Shea C *et al*., 2004). Clearly, the ability to export late adenoviral RNAs would be of no selective advantage to tumor cells *per se*. Therefore, this unexpected tumor property is likely to be an example of exaptation. This would infer that late viral RNAs may share a molecular resemblance with RNAs important for cell growth or tumorigenesis. As noted above, *Hsp70*, *18S rRNA* and late viral RNAs have structurally similar 5′UTRs (Yueh and Schneider, 2000), and heat shock rescues the translation and export of late viral RNAs in ONYX-015-infected resistant tumor cell lines (O'Shea C *et al*., 2005b). The upregulation of Hsps in tumor cells is well documented and is considered a promising tumor target (Jolly and Morimoto, 2000). Therefore, it is intriguing to speculate that permissive tumor cells may constitutively upregulate Hsps via post-transcriptional mechanisms, rendering them permissive to ONYX-015 oncolytic therapy. Such effects could be mediated via increased RNA export and/or translation. Indeed, in some tumor cell lines, translation rather than RNA export may be the major determinant (Harada and Berk, 1999), and the cell cycle may also exert important context-dependent effects (Goodrum and Ornelles, 1997, 1999). Notwithstanding, determining the molecular mechanisms underlying ONYX-015 oncolysis has important implications for our understanding of tumorigenesis as well as the development of novel cancer therapies. Indeed, this is becoming a recurring theme in the development of oncolytic viruses. The oncolytic selectivity of HSV-1 γ34.5 mutants, VSV and reovirus is for tumor cells that have deregulated interferon/PKR/Ras-mediated effects on viral RNA translation, again an unanticipated tumor property that is a novel therapeutic target (see Barber, 2005; Mohr, 2005; Shmulevitz *et al*., 2005).

It is also interesting to consider that the 'worse case scenario' adenovirus growth deregulation program may be the 'best case scenario' in which to discover broadly efficacious cancer therapies. For example, PI3-kinase is an appealing target for cancer therapy. However, to date, no PI3-kinase inhibitors have been approved, in part due to limiting toxicity, associated with the inhibition of PI3-kinase in many normal cellular functions, such as glucose metabolism. An alternative strategy is to develop drugs that inhibit the critical downstream targets of PI3-kinase in tumorigenesis. But what are the critical targets of PI3-kinase in a tumor cell program that disrupts multiple cellular networks? For example, as illustrated in Figure 1,

PI3-kinase inactivates the p27 cyclin-cdk inhibitor and proapoptotic Bad protein (via Akt), suggesting that the latter may be useful drug targets in PTEN mutant tumor cells. However, inhibiting PI3-kinase-mediated effects on p27 or Bad are likely to be of limited therapeutic value in tumor cells that have also deregulated RB or BCL-2. Could we use adenovirus then as a system in which to determine what it is that E4-ORF1-mediated activation of PI3-kinase 'does' that inactivation of RB and Bax/Bak by E1A and E1B-19K fail to do, in eliciting aberrant replication in infected primary cells? In essence, adenovirus infection may not only be a useful system in which to plot the dynamics of growth deregulation, but also to identify the Achilles hub(s) within the tumor program.

Late viral functions – lytic drug opportunities and combination therapies

The similarities between the tumor and viral programs have provided fundamental insights into tumor biology, and, as discussed above, is a paradigm that could be exploited for the development of novel cancer therapies. However, the analogy, and hence the utility, only stretches so far. Clearly, the study of DNA viruses is unlikely to increase our understanding of tumor cell angiogenesis or metastasis. However, it may not be necessary to target every diseased hub, just critical combinations that destroy the pathological program (Albert *et al*., 2000). Obviously, viruses also have distinct properties that do not extend to tumor cells, such as late viral functions (post-DNA replication) in viral DNA encapsidation and virion maturation.

However, understanding late viral functions is not necessarily without application or interest for tumor biology and therapy. Indeed, although adenovirus induces a rapid tumor-like state in infected primary cells, it nonetheless succeeds in lysing such cells to release mature virions. In essence, adenovirus solves the dilemma as to how to kill the tumor program. The solution to the viral dilemma may be the drug opportunity of which many oncologists dream. What are the late viral proteins/functions that elicit the lysis of the host cell? The E3 11.6K protein, or adenoviral death protein (ADP), is expressed late, and plays a key role in the lysis of infected cells (Tollefson *et al*., 1996). It is a nuclear membrane/Golgi glycoprotein that is thought to trigger lytic death via its cytoplasmic tail (Tollefson *et al*., 2003), although the precise molecular mechanisms remain to be elucidated. In addition to its early functions discussed previously, E4-ORF4 also plays a late role in the death of adenovirus-infected cells (Marcellus *et al*., 1998). How can E4-ORF4 have such diametrically opposed functions in promoting both DNA replication and cell death? The answer is likely to be that these functions of E4-ORF4 are not affected simultaneously, but are temporally regulated by either other early/late viral proteins or differences in cellular effectors/substrates at the early, G0/G1, versus late, G2/M, phase of the cell-cycle/viral infection. Interestingly, ectopic expression of E4-ORF4 has been shown to selectively kill tumor cells, suggesting its use as a novel cancer therapy (Shtrichman *et al*., 1999; Branton and Roopchand, 2001). Understanding the molecular mechanisms whereby adenoviral late proteins affect the death of infected cells is, therefore, a novel platform for the development of cytolytic drugs for the treatment of cancer.

The rational identification of drugs that phenocopy late viral functions is not without precedent. As noted above, elevated temperatures rescue E1B-55K late functions and selectively sensitize refractory tumor cells to ONYX-015 oncolytic viral therapy (O'Shea C *et al*., 2005b). This suggested that drugs which elicit a cellular heat shock response might be a powerful therapeutic combination with ONYX-015. Geldanamycin and 17-AAG, which inhibit Hsp90, trigger a cellular heat shock response (Hegde *et al*., 1995) and are currently undergoing clinical evaluation as cancer therapies. We found that these drugs, which can be administered systemically, rescue E1B-55K late functions and selectively sensitize refractory tumors cells to ONYX-015 oncolytic therapy (O'Shea C *et al*., 2005b). Such agents could greatly augment and broaden ONYX-015's clinical utility as a cancer therapy. Moreover, this underscores the potential for rationally combining oncolytic viruses with pharmacological agents to achieve novel efficacious therapeutic modalities.

Conclusions

Taken together, studies encompassing *in vitro* transformation assays, mouse models, human cancer genetics and DNA viruses suggest that there is a limited and recurring repertoire of cellular hubs that must be perturbed to drive aberrant replication. The challenge then is to join the dots, to envision how together they create the tumor cell picture so that we may irrevocably erase it. To this end, adenovirus is an ideal system in which to map the complex network dynamics of pleiotropic growth deregulation. This knowledge has important applications for the discovery of novel tumor targets, as well as the rational design of oncolytic viruses and combination therapies that effectively treat patients suffering from cancer.

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References

Ahuja D, Sáenz-Robles MT and Pipas JM. (2005). *Oncogene*, **24,** 7729–7745.

Albert R, Jeong H and Barabasi AL. (2000). *Nature*, **406,** 378–382.

Andersson M, Paabo S, Nilsson T and Peterson PA. (1985). *Cell*, **43,** 215–222.
Arroyo JD and Hahn WC. (2005). *Oncogene*, **24,** 7746–7755.
Aslanian A, Iaquinta PJ, Verona R and Lees JA. (2004). *Genes Dev.*, **18,** 1413–1422.
Avruch J, Lin Y, Long X, Murthy S and Ortiz-Vega S. (2005). *Curr. Opin. Clin. Nutr. Metab. Care*, **8,** 67–72.
Babiss LE, Ginsberg HS and Darnell Jr JE. (1985). *Mol. Cell. Biol.*, **5,** 2552–2558.
Barabasi AL and Albert R. (1999). *Science*, **286,** 509–512.
Barabasi AL and Oltvai ZN. (2004). *Nat. Rev. Genet.*, **5,** 101–113.
Barber GN. (2005). *Oncogene*, **24,** 7710–7719.
Barker DD and Berk AJ. (1987). *Virology*, **156,** 107–121.
Benedict CA, Norris PS, Prigozy TI, Bodmer JL, Mahr JA, Garnett CT, Martinon F, Tschopp J, Gooding LR and Ware CF. (2001). *J. Biol. Chem.*, **276,** 3270–3278.
Benedict CA and Ware CF. (2001). *Virology*, **289,** 1–5.
Berk AJ. (2005). *Oncogene*, **24,** 7673–7685.
Bischof O, Kirsh O, Pearson M, Itahana K, Pelicci PG and Dejean A. (2002). *EMBO J.*, **21,** 3358–3369.
Bischof O, Nacerddine K and Dejean A. (2005). *Mol. Cell. Biol.*, **25,** 1013–1024.
Bischoff JR, Kirn DH, Williams A, Heise C, Horn S, Muna M, Ng L, Nye JA, Sampson-Johannes A, Fattaey A and McCormick F. (1996). *Science*, **274,** 373–376.
Bracken AP, Ciro M, Cocito A and Helin K. (2004). *Trends Biochem. Sci.*, **29,** 409–417.
Branton PE and Roopchand DE. (2001). *Oncogene*, **20,** 7855–7865.
Bryan TM, Englezou A, Gupta J, Bacchetti S and Reddel RR. (1995). *EMBO J.*, **14,** 4240–4248.
Calin GA, di Iasio MG, Caprini E, Vorechovsky I, Natali PG, Sozzi G, Croce CM, Barbanti-Brodano G, Russo G and Negrini M. (2000). *Oncogene*, **19,** 1191–1195.
Campisi J. (2005). *Cell*, **120,** 513–522.
Chiocca EA. (2002). *Nat. Rev. Cancer*, **2,** 938–950.
Chiou SK and White E. (1997). *J. Virol.*, **71,** 3515–3525.
Cory S and Adams JM. (2002). *Nat. Rev. Cancer*, **2,** 647–656.
Cuconati A and White E. (2002). *Genes Dev.*, **16,** 2465–2478.
Cuesta R, Xi Q and Schneider RJ. (2004). *J. Virol.*, **78,** 7707–7716.
Danial NN and Korsmeyer SJ. (2004). *Cell*, **116,** 205–219.
de The H, Chomienne C, Lanotte M, Degos L and Dejean A. (1990). *Nature*, **347,** 558–561.
Dobbelstein M. (2004). *Curr. Top. Microbiol. Immunol.*, **273,** 291–334.
Dobner T, Horikoshi N, Rubenwolf S and Shenk T. (1996). *Science*, **272,** 1470–1473.
Doucas V, Ishov AM, Romo A, Juguilon H, Weitzman MD, Evans RM and Maul GG. (1996). *Genes Dev.*, **10,** 196–207.
Downward J. (2003). *Nat. Rev. Cancer*, **3,** 11–22.
Eisenberg E and Levanon EY. (2003). *Phys. Rev. Lett.*, **91,** 138701.
Espinosa JM and Emerson BM. (2001). *Mol. Cell*, **8,** 57–69.
Estmer Nilsson C, Petersen-Mahrt S, Durot C, Shtrichman R, Krainer AR, Kleinberger T and Akusjarvi G. (2001). *EMBO J.*, **20,** 864–871.
Everett RD. (2001). *Oncogene*, **20,** 7266–7273.
Ferreira R, Magnaghi-Jaulin L, Robin P, Harel-Bellan A and Trouche D. (1998). *Proc. Natl. Acad. Sci. USA*, **95,** 10493–10498.
Fingar DC and Blenis J. (2004). *Oncogene*, **23,** 3151–3171.
Foddis R, De Rienzo A, Broccoli D, Bocchetta M, Stekala E, Rizzo P, Tosolini A, Grobelny JV, Jhanwar SC, Pass HI, Testa JR and Carbone M. (2002). *Oncogene*, **21,** 1434–1442.
Freeman-Cook L and DiMaio D. (2005). *Oncogene*, **24,** 7756–7762.
Frese KK, Lee SS, Thomas DL, Latorre IJ, Weiss RS, Glaunsinger BA and Javier RT. (2003). *Oncogene*, **22,** 710–721.
Friborg Jr J, Kong W, Hottiger MO and Nabel GJ. (1999). *Nature*, **402,** 889–894.
Fridman JS and Lowe SW. (2003). *Oncogene*, **22,** 9030–9040.
Frisch SM and Mymryk JS. (2002). *Nat. Rev. Mol. Cell. Biol.*, **3,** 441–452.
Fuchs M, Gerber J, Drapkin R, Sif S, Ikura T, Ogryzko V, Lane WS, Nakatani Y and Livingston DM. (2001). *Cell*, **106,** 297–307.
Gewin L, Myers H, Kiyono T and Galloway DA. (2004). *Genes Dev.*, **18,** 2269–2282.
Glaunsinger BA, Weiss RS, Lee SS and Javier R. (2001). *EMBO J.*, **20,** 5578–5586.
Goodrum FD and Ornelles DA. (1997). *J. Virol.*, **71,** 548–561.
Goodrum FD and Ornelles DA. (1998). *J. Virol.*, **72,** 9479–9490.
Goodrum FD and Ornelles DA. (1999). *J. Virol.*, **73,** 7474–7488.
Halbert DN, Cutt JR and Shenk T. (1985). *J. Virol.*, **56,** 250–257.
Hall AR, Dix BR, O'Carroll SJ and Braithwaite AW. (1998). *Nat. Med.*, **4,** 1068–1072.
Hanahan D and Weinberg RA. (2000). *Cell*, **100,** 57–70.
Hann B and Balmain A. (2003). *J. Virol.*, **77,** 11588–11595.
Hannon GJ and Beach D. (1994). *Nature*, **371,** 257–261.
Harada JN and Berk AJ. (1999). *J. Virol.*, **73,** 5333–5344.
Harada JN, Shevchenko A, Pallas DC and Berk AJ. (2002). *J. Virol.*, **76,** 9194–9206.
Hart LS, Yannone SM, Naczki C, Orlando JS, Waters SB, Akman SA, Chen DJ, Ornelles D and Koumenis C. (2005). *J. Biol. Chem.*, **280,** 1474–1481.
Hartwell LH, Szankasi P, Roberts CJ, Murray AW and Friend SH. (1997). *Science*, **278,** 1064–1068.
Hayes BW, Telling GC, Myat MM, Williams JF and Flint SJ. (1990). *J. Virol.*, **64,** 2732–2742.
Hegde RS, Zuo J, Voellmy R and Welch WJ. (1995). *J. Cell Physiol.*, **165,** 186–200.
Helin K and Harlow E. (1994). *J. Virol.*, **68,** 5027–5035.
Helt AM and Galloway DA. (2003). *Carcinogenesis*, **24,** 159–169.
Higashino F, Pipas JM and Shenk T. (1998). *Proc. Natl. Acad. Sci. USA*, **95,** 15683–15687.
Ho YS, Galos R and Williams J. (1982). *Virology*, **122,** 109–124.
Hobom U and Dobbelstein M. (2004). *J. Virol.*, **78,** 7685–7697.
Horner SM, DeFilippis RA, Manuelidis L and DiMaio D. (2004). *J. Virol.*, **78,** 4063–4073.
Howe JA, Mymryk JS, Egan C, Branton PE and Bayley ST. (1990). *Proc. Natl. Acad. Sci. USA*, **87,** 5883–5887.
Huang S, Liu LN, Hosoi H, Dilling MB, Shikata T and Houghton PJ. (2001). *Cancer Res.*, **61,** 3373–3381.
Huang W and Flint SJ. (1998). *J. Virol.*, **72,** 225–235.
Huibregtse JM, Scheffner M and Howley PM. (1991). *EMBO J.*, **10,** 4129–4135.
Inoki K, Corradetti MN and Guan KL. (2005). *Nat. Genet.*, **37,** 19–24.
Ito A, Koma YI and Watabe K. (2003). *Histol. Histopathol.*, **18,** 1313–1319.
Iyer NG, Ozdag H and Caldas C. (2004). *Oncogene*, **23,** 4225–4231.
Johnson L, Shen A, Boyle L, Kunich J, Pandey K, Lemmon M, Hermiston T, Giedlin M, McCormick F and Fattaey A. (2002). *Cancer Cell*, **1,** 325–337.

Jolly C and Morimoto RI. (2000). *J. Natl. Cancer Inst.*, **92,** 1564–1572.
Kamb A, Gruis NA, Weaver-Feldhaus J, Liu Q, Harshman K, Tavtigian SV, Stockert E, Day III RS, Johnson BE and Skolnick MH. (1994). *Science*, **264,** 436–440.
Kaplan DR, Whitman M, Schaffhausen B, Pallas DC, White M, Cantley L and Roberts TM. (1987). *Cell*, **50,** 1021–1029.
Khuri FR, Nemunaitis J, Ganly I, Arseneau J, Tannock IF, Romel L, Gore M, Ironside J, MacDougall RH, Heise C, Randlev B, Gillenwater AM, Bruso P, Kaye SB, Hong WK and Kirn DH. (2000). *Nat. Med.*, **6,** 879–885.
Kirn D. (2001). *Expert Opin. Biol. Ther.*, **1,** 525–538.
Ko D, Hawkins L and Yu D-C. (2005). *Oncogene*, **24,** 7763–7774.
Kovesdi I, Reichel R and Nevins JR. (1987). *Proc. Natl. Acad. Sci. USA*, **84,** 2180–2184.
Kyo S and Inoue M. (2002). *Oncogene*, **21,** 688–697.
Land H, Parada LF and Weinberg RA. (1983). *Nature*, **304,** 596–602.
Lane DP and Crawford LV. (1979). *Nature*, **278,** 261–263.
Lane HA, Fernandez A, Lamb NJ and Thomas G. (1993). *Nature*, **363,** 170–172.
Lee SS, Glaunsinger B, Mantovani F, Banks L and Javier RT. (2000). *J. Virol.*, **74,** 9680–9693.
Lee SS, Weiss RS and Javier RT. (1997). *Proc. Natl. Acad. Sci. USA*, **94,** 6670–6675.
Leppard KN and Shenk T. (1989). *EMBO J.*, **8,** 2329–2336.
Lichtenstein DL, Toth K, Doronin K, Tollefson AE and Wold WS. (2004). *Int. Rev. Immunol.*, **23,** 75–111.
Linzer DI and Levine AJ. (1979). *Cell*, **17,** 43–52.
Liu Y, Wang Y, Yamakuchi M, Masuda S, Tokioka T, Yamaoka S, Maruyama I and Kitajima I. (2001). *Oncogene*, **20,** 2514–2526.
Lowe SW, Cepero E and Evan G. (2004). *Nature*, **432,** 307–315.
Lowe SW and Sherr CJ. (2003). *Curr. Opin. Genet. Dev.*, **13,** 77–83.
Lu Z, Hu X, Li Y, Zheng L, Zhou Y, Jiang H, Ning T, Basang Z, Zhang C and Ke Y. (2004). *J. Biol. Chem.*, **79,** 35664–35700.
Marcellus RC, Lavoie JN, Boivin D, Shore GC, Ketner G and Branton PE. (1998). *J. Virol.*, **72,** 7144–7153.
Martin ME and Berk AJ. (1999). *Mol. Cell. Biol.*, **19,** 3403–3414.
Mathis JM, Stoff-Khalili MA and Curiel DT. (2005). *Oncogene*, **24,** 7775–7791.
McCormick F. (2005). *Oncogene*, **24,** 7817–7819.
McCormick F. (2003). *Cancer Biol. Ther.*, **2,** S157–60.
McMurray HR and McCance DJ. (2003). *J. Virol.*, **77,** 9852–9861.
Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P and Moll UM. (2003). *Mol. Cell*, **11,** 577–590.
Mohr I. (2005). *Oncogene*, **24,** 7697–7709.
Moule MG, Collins CH, McCormick F and Fried M. (2004). *Proc. Natl. Acad. Sci. USA*, **101,** 14063–14066.
Muise-Helmericks RC, Grimes HL, Bellacosa A, Malstrom SE, Tsichlis PN and Rosen N. (1998). *J. Biol. Chem.*, **273,** 29864–29872.
Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, Hortobagyi GN, Hung MC and Yu D. (2004). *Cancer Cell*, **6,** 117–127.
Narita M, Nunez S, Heard E, Lin AW, Hearn SA, Spector DL, Hannon GJ and Lowe SW. (2003). *Cell*, **113,** 703–716.
Neill SD and Nevins JR. (1991). *J. Virol.*, **65,** 5364–5373.
Nemunaitis J, Ganly I, Khuri F, Arseneau J, Kuhn J, McCarty T, Landers S, Maples P, Romel L, Randlev B, Reid T, Kaye S and Kirn D. (2000). *Cancer Res.*, **60,** 6359–6366.
Nobori T, Miura K, Wu DJ, Lois A, Takabayashi K and Carson DA. (1994). *Nature*, **368,** 753–756.
O'Connor RJ and Hearing P. (2000). *J. Virol.*, **74,** 5819–5824.
O'Shea C, Klupsch K, Choi S, Bagus B, Soria C, Shen J, McCormick F and Stokoe D. (2005). *EMBO J.*, **24,** 1211–1221.
O'Shea CC. (2005). *Curr. Opin. Genet. Dev.*, **15,** 18–26.
O'Shea CC, Choi S, McCormick F and Stokoe D. (2005a). *Cell Cycle*, **4,** 883–888.
O'Shea CC and Fried M. (2005). *Cell Cycle*, **4,** 449–452.
O'Shea CC, Johnson L, Bagus B, Choi S, Nicholas C, Shen A, Boyle L, Pandey K, Soria C, Kunich J, Shen Y, Habets G, Ginzinger D and McCormick F. (2004). *Cancer Cell*, **6,** 611–623.
O'Shea CC, Soria C, Bagus B and McCormick F. (2005b). *Cancer Cell*, **8,** 61–74.
Pearson M, Carbone R, Sebastiani C, Cioce M, Fagioli M, Saito S, Higashimoto Y, Appella E, Minucci S, Pandolfi PP and Pelicci PG. (2000). *Nature*, **406,** 207–210.
Perez D and White E. (2003). *J. Virol.*, **77,** 2651–2662.
Peter ME and Krammer PH. (2003). *Cell Death Differ.*, **10,** 26–35.
Pilder S, Moore M, Logan J and Shenk T. (1986). *Mol. Cell. Biol.*, **6,** 470–476.
Pitti RM, Marsters SA, Lawrence DA, Roy M, Kischkel FC, Dowd P, Huang A, Donahue CJ, Sherwood SW, Baldwin DT, Godowski PJ, Wood WI, Gurney AL, Hillan KJ, Cohen RL, Goddard AD, Botstein D and Ashkenazi A. (1998). *Nature*, **396,** 699–703.
Psyrri A, DeFilippis RA, Edwards AP, Yates KE, Manuelidis L and DiMaio D. (2004). *Cancer Res.*, **64,** 3079–3086.
Querido E, Blanchette P, Yan Q, Kamura T, Morrison M, Boivin D, Kaelin WG, Conaway RC, Conaway JW and Branton PE. (2001). *Genes Dev.*, **15,** 3104–3117.
Querido E, Marcellus RC, Lai A, Charbonneau R, Teodoro JG, Ketner G and Branton PE. (1997). *J. Virol.*, **71,** 3788–3798.
Rassoulzadegan M, Cowie A, Carr A, Glaichenhaus N, Kamen R and Cuzin F. (1982). *Nature*, **300,** 713–718.
Reid T, Galanis E, Abbruzzese J, Sze D, Wein LM, Andrews J, Randlev B, Heise C, Uprichard M, Hatfield M, Rome L, Rubin J and Kirn D. (2002). *Cancer Res.*, **62,** 6070–6079.
Ries S and Korn WM. (2002). *Br. J. Cancer*, **86,** 5–11.
Ries SJ, Brandts CH, Chung AS, Biederer CH, Hann BC, Lipner EM, McCormick F and Korn WM. (2000). *Nat. Med.*, **6,** 1128–1133.
Rothmann T, Hengstermann A, Whitaker NJ, Scheffner M and zur Hausen H. (1998). *J. Virol.*, **72,** 9470–9478.
Rudin CM, Cohen EE, Papadimitrakopoulou VA, Silverman Jr S, Recant W, El-Naggar AK, Stenson K, Lippman SM, Hong WK and Vokes EE. (2003). *J. Clin. Oncol.*, **21,** 4546–4552.
Ruley HE. (1983). *Nature*, **304,** 602–606.
Russell WC. (2000). *J. Gen. Virol.*, **81,** 2573–2604.
Sawyers C. (2004). *Nature*, **432,** 294–297.
Scheffner M, Munger K, Huibregtse JM and Howley PM. (1992). *EMBO J.*, **11,** 2425–2431.
Scheffner M, Werness BA, Huibregtse JM, Levine AJ and Howley PM. (1990). *Cell*, **63,** 1129–1136.
Seidel ER and Ragan VL. (1997). *Br. J. Pharmacol.*, **120,** 571–574.
Shaw RJ, Bardeesy N, Manning BD, Lopez L, Kosmatka M, DePinho RA and Cantley LC. (2004). *Cancer Cell*, **6,** 91–99.

Shen Y, Kitzes G, Nye JA, Fattaey A and Hermiston T. (2001). *J. Virol.*, **75,** 4297–4307.
Sherr CJ and McCormick F. (2002). *Cancer Cell*, **2,** 103–112.
Shtrichman R, Sharf R, Barr H, Dobner T and Kleinberger T. (1999). *Proc Natl. Acad. Sci. USA*, **96,** 10080–10085.
Shmulevitz M, Marcato P and Lee PW. (2005). *Oncogene*, **24,** 7720–7728.
Stracker TH, Carson CT and Weitzman MD. (2002). *Nature*, **418,** 348–352.
Swanton C, Mann DJ, Fleckenstein B, Neipel F, Peters G and Jones N. (1997). *Nature*, **390,** 184–187.
Tarakanova VL and Wold WS. (2003). *J. Virol.*, **77,** 9324–9336.
Thomas A and White E. (1998). *Genes Dev.*, **12,** 1975–1985.
Thomas MC and Chiang CM. (2005). *Mol. Cell*, **17,** 251–264.
Thorne SH, Brooks G, Lee YL, Au T, Eng LF and Reid T. (2005). *J. Virol.*, **79,** 581–591.
Tollefson AE, Hermiston TW, Lichtenstein DL, Colle CF, Tripp RA, Dimitrov T, Toth K, Wells CE, Doherty PC and Wold WS. (1998). *Nature*, **392,** 726–730.
Tollefson AE, Scaria A, Hermiston TW, Ryerse JS, Wold LJ and Wold WS. (1996). *J. Virol.*, **70,** 2296–2306.
Tollefson AE, Scaria A, Ying B and Wold WS. (2003). *J. Virol.*, **77,** 7764–7778.
Tollefson AE, Toth K, Doronin K, Kuppuswamy M, Doronina OA, Lichtenstein DL, Hermiston TW, Smith CA and Wold WS. (2001). *J. Virol.*, **75,** 8875–8887.
Trimarchi JM and Lees JA. (2002). *Nat. Rev. Mol. Cell. Biol.*, **3,** 11–20.
Tsujimoto Y, Finger LR, Yunis J, Nowell PC and Croce CM. (1984). *Science*, **226,** 1097–1099.
Turnell AS, Grand RJ and Gallimore PH. (1999). *J. Virol.*, **73,** 2074–2083.
Veldman T, Liu X, Yuan H and Schlegel R. (2003). *Proc. Natl. Acad. Sci. USA*, **100,** 8211–8216.
Verma SC, Borah S and Robertson ES. (2004). *J. Virol.*, **78,** 10348–10359.
Vivanco I and Sawyers CL. (2002). *Nat. Rev. Cancer*, **2,** 489–501.
Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ and Press M. (2002). *J. Clin. Oncol.*, **20,** 719–726.
Wagner A. (2003). *Proc. Biol. Sci.*, **270,** 457–466.
Walsh D and Mohr I. (2004). *Genes Dev.*, **18,** 660–672.
Walsh D, Perez C, Notary J and Mohr I. (2005). *J. Virol.*, **79,** 8057–8064.
Wang SS, Esplin ED, Li JL, Huang L, Gazdar A, Minna J and Evans GA. (1998). *Science*, **282,** 284–287.
Weinberg DH and Ketner G. (1986). *J. Virol.*, **57,** 833–838.
Weitzman MD, Carson CT, Schwartz RA and Lilley CE. (2004). *DNA Repair (Amsterdam)*, **3,** 1165–1173.
Weitzman MD and Ornelles DA. (2005). *Oncogene*, **24,** 7686–7696.
West MJ, Stoneley M and Willis AE. (1998). *Oncogene*, **17,** 769–780.
Whyte P, Buchkovich KJ, Horowitz JM, Friend SH, Raybuck M, Weinberg RA and Harlow E. (1988). *Nature*, **334,** 124–129.
Whyte P, Williamson NM and Harlow E. (1989). *Cell*, **56,** 67–75.
Working PK, Lin A and Borellini F. (2005). *Oncogene*, **24,** 7792–7801.
Yen A and Pardee AB. (1979). *Science*, **204,** 1315–1317.
Yoder SS and Berget SM. (1986). *J. Virol.*, **60,** 779–781.
Yook SH, Oltvai ZN and Barabasi AL. (2004). *Proteomics*, **4,** 928–942.
Yu D and Hung MC. (2000). *Oncogene*, **19,** 6115–6121.
Yu Y and Alwine JC. (2002). *J. Virol.*, **76,** 3731–3738.
Yuan H, Veldman T, Rundell K and Schlegel R. (2002). *J. Virol.*, **76,** 10685–10691.
Yueh A and Schneider RJ. (1996). *Genes Dev.*, **10,** 1557–1567.
Yueh A and Schneider RJ. (2000). *Genes Dev.*, **14,** 414–421.
Zindy F, Williams RT, Baudino TA, Rehg JE, Skapek SX, Cleveland JL, Roussel MF and Sherr CJ. (2003). *Proc. Natl. Acad. Sci. USA*, **100,** 15930–15935.