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Viruses: tools for tumor target discovery, and agents for oncolytic therapies – an introduction

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Viruses, traditionally synonymous with disease, are some of the oncologist's greatest allies in providing the necessary intelligence and weaponry to wage the war on cancer. Over half a century ago, it was known that both RNA and DNA viruses could induce tumors in animals. Encoding relatively few genes, viruses are a simple genetic system with which to study the transformation of animal cells. RNA retroviruses were found to transform animal cells by 'capturing' and activating cellular proto-oncogenes, leading to the discovery of the first oncogene, v-src. The nucleic acid cousins of the retroviruses, DNA viruses, have also provided fundamental insights into tumorigenesis. With exquisite symmetry to its discovery in transforming retroviruses, src was also identified as a critical target of the transforming polyomavirus Middle T antigen. DNA viruses encode proteins that induce a rapid 'tumor-like' state in infected cells to facilitate their pathological replication. As such, DNA viral proteins and tumor cell mutations converge in subverting many of the same cellular pathways and checkpoints. Thus, many of the critical tumor targets were first identified through their interaction with DNA viral proteins, including p53 and PI3-kinase. Furthermore, our first insights into the cellular pathways that cooperate to elicit transformation were with combinations of DNA viral and cellular oncogenes. Thus, studies with viruses elucidated many of the critical tumor targets, and were our first steps down the path to rational-targeted therapies such as the bcr-abl (src kinase) inhibitor, Gleevec.

Nevertheless, in this brave new world of high throughput genomics, the utility of viruses as tools in cancer research may to some seem comparatively blunt instruments. After all, microarrays can be used to directly discover the differences between neoplastic and primary cells. However, 'more' is not always better. Such analyses, as often as not, leave us with a conundrum: of the myriad of differences observed, which are the key changes that maintain or drive the neoplastic phenotype? Unfortunately, the instability inherent to cancer genomes, together with their relatively short evolutionary time-span, can make it difficult to discern *a priori* the 'joy-riders' from the molecular culprits that actually drive tumorigenesis. Distinguishing between that which is coincidental and causal is paramount for the development of effective tumor therapies, which is why retroviral insertional mutagenesis screens and DNA viral proteins are still such invaluable tools. Nevertheless, enumerating critical tumor targets is of little value to a cancer patient in the absence of effective therapies that treat them. Factors that have limited the clinical successes of cancer therapies include multiple tumor cell mutations as well as the lack of cytolytic drugs. Thus, there is a great need to develop novel therapeutic modalities, rational drug combinations, and lytic agents for the treatment of cancer.

The stage is set then for the next Act in cancer research in which viruses play a starring role. This issue of Oncogene features reviews on viruses and viral proteins and the many roles they are playing both as tools for the discovery/characterization of critical cellular targets, as well as novel therapeutic agents that undergo selective lytic replication in tumor cells (Figure 1). The first article describes how viruses encode a cast 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 (O'Shea, 2005). This overlap can be exploited to map the pathological networking of growth regulatory pathways as well as identifying the critical cellular hubs that may also be targeted in tumor cells. Adenovirus infected primary cells can be used as a 'system' to understand the redundancy intrinsic to integrated cellular networks that elicit aberrant growth. This is of paramount importance for the rational design of the next generation of oncolytic viral therapies as well as efficacious combination cancer therapies.

Historically, retroviruses led to the discovery of the first oncogenes. However, as Uren *et al.* discuss the future is brighter than ever for their utility as cutting edge tools in cancer research. Retroviral insertional mutagenesis is a powerful strategy that can reveal, without prejudice, critical cellular targets that drive, or are required for, tumorigenesis in mouse models. The development of somatic insertional mutagens, which can

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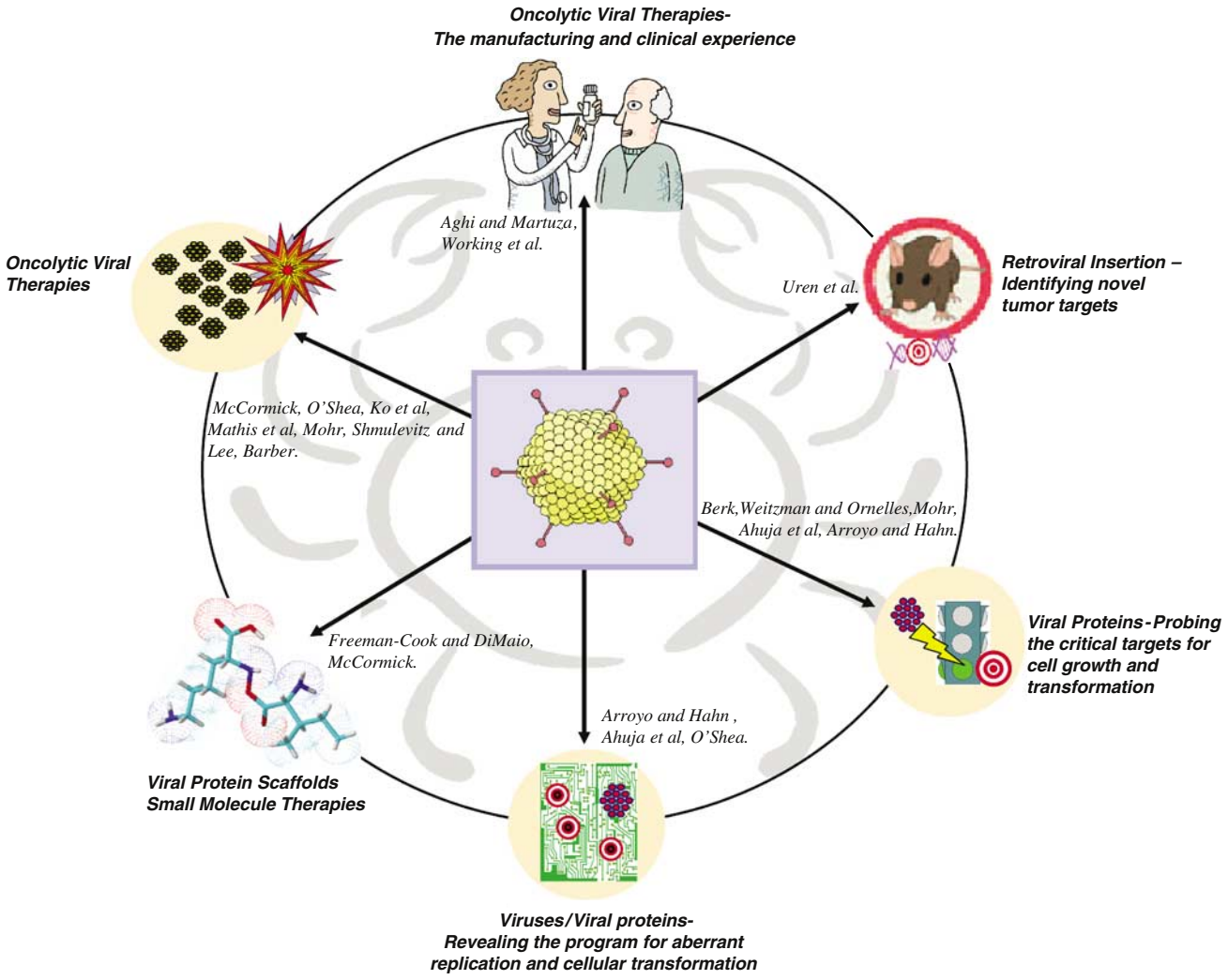

Figure 1 Viruses-waging the war on cancer on multiple fronts. This issue of Oncogene reviews the enormous utility of viruses and viral proteins as tools with which to discover novel tumor targets, pivotal growth regulatory pathways and the program for cellular transformation, together with their applications as novel oncolytic cancer therapies. The use of viruses in these nominally different areas of cancer research is intimately linked and highly complementary

be used to study tumours of diverse tissue-origin, together with improved methodologies to identify insertion sites, has advanced the state of the art to a new level, and is revealing a plethora of exciting tumor targets (Uren *et al*., 2005).

Retroviruses are not only the only viruses that can identify the key cellular players in aberrant replication. Evolution over many millennia, has streamlined the DNA viral program to encode the minimal number of proteins necessary to engender pathological cellular replication. Thus, viral proteins are discerning biochemical probes with which to reveal and characterize the requisite events in cell growth and transformation. Viral proteins perturb cell growth through diverse cellular processes and pathways. Arnie Berk describes how the transforming adenoviral E1 proteins are providing fundamental new insights into the regulation of cellular transcription and replication. Lessons learnt here have important applications for understanding similar phenotypes in the aberrant growth of tumor cells (Berk, 2005). Weitzman and Ornelles discuss how the DNA damage pathway is not only a powerful antigrowth response triggered by cellular DNA damage, but also by the replication of viral genomes. Recent studies indicate that the DNA damage pathway may be deregulated in the majority of tumor cells. Thus, viral proteins that inactivate the DNA damage pathway and/or sensitize cells to radio-/chemo-therapy are of considerable interest (Weitzman and Ornelles, 2005).

Like DNA replication, protein synthesis is tightly controlled, and plays a critical role in regulating cell growth. Ian Mohr describes how viruses subvert the cellular protein translation machinery at multiple levels. Several herpesvirus proteins disable cellular antiviral responses that would otherwise act to prevent protein translation and viral multiplication in infected primary

cells (Mohr, 2005). However, the replication of herpesvirus mutants that fail to inactivate PKR-mediated inhibition of protein translation is selectively complemented in tumor cells. One such mutant, HSV-1 γ34.5, is currently being tested in the clinic as novel oncolytic viral therapy (Aghi and Martuza, 2005). Indeed, HSV-1 γ34.5 oncolytic herpesviruses are not alone in their selectivity for tumor cells in which protein translation is deregulated, but are joined by the RNA oncolytic viruses, vesicular stomatitis virus (VSV) and reovirus. Glen Barber discusses how VSV is a potent antitumor agent that appears to replicate in tumor cells with defects in the interferon and PKR signaling pathways (Barber, 2005). Shmulevitz and Lee describe how tumor cells with mutant Ras selectively translate reovirus RNAs, facilitating their oncolytic replication. Small molecule therapies that inhibit oncogenic Ras have to date proved elusive. Therefore, a reovirus oncolytic therapy for tumor cells with activated Ras is an exciting prospect (Shmulevitz and Lee, 2005). Collectively, these reviews also suggest that interferon/PKR/Ras-mediated effects on translation may be a novel tumor target. Understanding the molecular basis of γ34.5 HSV-1/VSV/reovirus oncolysis could provide key insights into the latter and has important implications for cancer therapy.

DNA viral proteins have provided fundamental insights into the cooperation and integration of pivotal growth regulatory pathways. Recently, the *in vitro* transformation of human cells was finally achieved by the expression of telomerase, Ras and the SV40 early region, which encodes Large T (LT) and Small T (ST). Therefore, the molecular mechanisms whereby SV40 LT and ST mediate cellular transformation are of paramount importance. Certainly, inactivation of p53 as well as the Rb family of tumor suppressors is critical for LT-mediated transformation. However, as discussed by Ahuja *et al*., this is probably the 'tip of the iceberg' in terms of LT functions that act together to orchestrate pleiotropic growth deregulation. Understanding why LT-mediated transformation is cell-type specific and context dependent, as well as the role of LT's chaperone functions in remodeling p107/E2F complexes are likely to provide key insights (Ahuja *et al*., 2005). However, in addition to LT, ST's ability to modulate PP2A signaling is also required for the transformation of human cells (Arroyo and Hahn, 2005). This has revealed PP2A as one of the missing links in cellular transformation, and its role in tumorigenesis is discussed by Arroyo and Hahn.

Thus, viral proteins have enormous utility in identifying and probing the function(s) of critical cellular players in cell growth and transformation. Could we use this in another way then, exploiting the often dominant and unusual interactions of viral proteins with critical tumor targets as templates for the development of novel drugs/peptides? Freeman-Cook and DiMaio discuss how the papillomavirus E5 protein, which binds and dimerizes the PDGF transmembrane domain, has been used as a molecular scaffold to design an entirely new class of small, modular transmembrane proteins with novel biological activities (Freeman-Cook and DiMaio, 2005). This is an exciting precedent for exploiting the novel binding properties of viral proteins as a model for the development of innovative therapies in the future.

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. The first strategy exploits the functional overlap between DNA viral proteins and tumor cell mutations. Thus, adenoviral mutants that fail to disrupt critical cellular checkpoints for their replication in normal cells, for example p53 and RB, may selectively replicate in tumor cells in which these checkpoints are defective (O'Shea, 2005; McCormick, 2005). Ko *et al*. discuss the promise of transcriptional targeting strategies, in which critical viral genes are regulated by tumor selective promoters, such as E2F, PSA, telomerase, restricting their lytic replication to tumor cells in which these promoters are constitutively activated (Ko *et al*., 2005). Mathis *et al*. describe an alternative strategy, based on the selective retargeting of oncolytic adenoviruses to tumor cell receptors/cell surface markers. This also has important applications in rendering tumor cells that lack the adenovirus receptor, CAR, permissive to infection by oncolytic adenoviral agents, may minimize any potential toxicity and help in evading pre-existing neutralizing antibodies to the natural adenoviral capsid proteins (Mathis *et al*., 2005). It is important to note that these strategies are not necessarily mutually exclusive with each other. Thus, absorbing the lessons, taking advantage of the advances, and perhaps combining elements from each of these strategies could significantly improve the next generation of oncolytic adenoviral therapies.

Nevertheless, what are the realities of oncolytic viruses ever becoming part of the standard portfolio of agents used to treat cancer patients? Are they more dream therapies than real therapies? Indeed, the recent clinical successes of Gleevec suggest that kinase inhibitors may be the therapeutic modality of choice. However, as discussed by Frank McCormick, small molecule therapies also have their limitations, and oncolytic viruses have some distinct advantages (McCormick, 2005). Oncolytic viral therapies are relatively recent and an entirely new kind of 'drug', which required enabling technologies for their manufacture, and experience in their clinical application. Working *et al*. describe how over the last few years the biotechnology industry has met the challenges, straddling many of the hurdles they initially faced in the large-scale manufacture of clinical grade oncolytic viruses (Working *et al*., 2005). The last 9 years have also seen oncolytic viruses from five different viral families being tested in phases I and II clinical trials. Aghi and Martuza review the clinical safety, efficacy and experience with such agents in the treatment of patients suffering from cancer (Aghi and Martuza, 2005). These reviews provide important perspectives on the remaining challenges, the practicalities, but also the enormous

potential, for the use of oncolytic viral agents as cancer therapies.

This issue juxtaposes reviews on viruses as both tools for tumor target discovery as well as agents for lytic cancer therapies. This is because the utility of viruses in these nominally two areas of cancer research are intimately linked and highly complementary; each with much to gain from the other (Figure 1). By discovering whether tumor cells selectively complement the functions of viral proteins, or the replication of viral mutants, we reveal not only novel tumor properties but potentially efficacious oncolytic viral therapies that target them. Together, the reviews in this issue offer an exciting insight on the many roles viruses are likely to play in impacting our understanding and treatment of cancer in the future. Rather than cause disease, viruses may yet help us to cure one.

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