Cancer: Surviving on the edge

William C. Hahn[1,2,*]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Departments of Medicine, Brigham and Women's Hospital and Harvard Medical School, 44 Binney Street, Boston, Massachusetts 02115
[2]Broad Institute of MIT and Harvard, 320 Charles Street, Cambridge, Massachusetts 02141
*Correspondence: william_hahn@dfci.harvard.edu

Tumors arise from normal cells through the acquisition of multiple genetic alterations that endow cancer cells with the phenotypes associated with neoplasia. Although we still lack a complete understanding of the specific complement of mutations that together program the behavior of any particular cancer, several lines of evidence indicate that many of these alterations perturb regulatory networks critical for cell proliferation, growth, and survival. As such, cancer cells maintain a precarious balance among unfettered proliferation, genomic instability, cell cycle arrest, and apoptosis. This year's Beatson International Cancer Conference focused on recent advances in our understanding of the pathways that regulate senescence, apoptosis, and cancer.

The homeostasis of organs and tissues requires a complex orchestration of many cell-cell interactions. In addition to providing specific physiological functions, most tissues exhibit the capacity for the identification, deletion, and replacement of damaged cells. As such, within any specific tissue, equilibrium exists among renewal, differentiation, and cell death. Although the regulatory mechanisms that govern these states usually operate highly effectively, disruption of these control points plays an important role in the genesis of cancer, and may also contribute to the pathogenesis of aging.

Work from many laboratories has begun to identify and characterize the molecular interactions that regulate cell survival, renewal, and differentiation. In some cases, we now possess significant insight into the connections between specific molecules and cell behavior. In particular, recent advances in several fields have enhanced our understanding of the regulation of cell cycle progression, apoptosis, and the many factors that control proliferative lifespan. However, one theme that also emerges from these studies is that such regulatory pathways involve highly interdependent networks of signaling interactions. As a consequence, key checkpoints often control several possible functional outcomes. Thus, to understand the operation of these regulatory steps, we must not only identify the components of these regulatory and effector pathways, but also further characterize those interactions that modify the functional outcome of specific signaling pathways.

The study of cancer has provided, and will continue to provide, important insights into these regulatory pathways. Cancer cells harbor alterations in key steps that regulate cell proliferation, differentiation, and cell-cell communication. These mutations endow cancer cells with the phenotypes associated with the malignant state (Hanahan and Weinberg, 2000). By understanding how these genetic alterations affect the function of signaling and regulatory networks, we will begin to understand the function of these pathways in both normal and malignant cells. In addition, this knowledge promises to facilitate the rational design and implementation of molecularly targeted anticancer therapies.

Recent advances in our understanding of the pathways that control cell cycle progression, senescence, and apoptosis were the focus of the Beatson International Cancer Conference Institute, held June 20–23 in Glasgow, Scotland. The meeting was again sponsored by Cancer Research UK and the Association for International Cancer Research and brilliantly organized by the Beatson Institute for Cancer Research.

Multiple roles for telomeres and telomerase in regulating chromosomal stability and proliferative lifespan

Telomeres are nucleoprotein structures that cap chromosome ends. Abundant genetic evidence indicates that these structures play essential roles in maintaining genomic stability and that telomere dysfunction leads to genomic instability (Chan and Blackburn, 2002). Indeed, recent evidence suggests that dysfunctional telomeres induce some of the same mechanisms that repair double-strand DNA breaks, suggesting that these structures normally shield chromosome ends from recognition as a broken DNA fragment (de Lange, 2002).

In addition to this protective function, the status of telomere maintenance contributes to the control of replicative lifespan (Harley et al., 1994). Most normal human cells exhibit telomere attrition with prolonged passage in culture (Harley et al., 1990; Hastie et al., 1990) and only transiently express telomerase, the ribonucleoprotein reverse transcriptase that maintains telomere structure, at levels insufficient to elongate telomeres (Broccoli et al., 1995; Masutomi et al., 2003). In contrast, the majority of human cancers and immortal cell lines express constitutive telomerase activity and maintain stable telomere lengths with passage in culture (Kim et al., 1994). Overexpression of the rate-limiting catalytic subunit of telomerase, hTERT, in mortal human cells leads to stabilized telomere lengths (Bodnar et al., 1998; Vaziri and Benchimol, 1998), facilitates immortalization, and cooperates with other genetic alterations to transform human cells (Hahn et al., 1999), suggesting that the acquisition of constitutive telomerase activity contributes directly to the immortal phenotype and cancer.

However, the role of telomeres and telomere maintenance in cancer development is complex. One prediction from the observations described above is that telomere attrition, as is observed with repeated cell divisions, should serve to suppress tumor formation. Consistent with these predictions, normal human cells lacking constitutive telomerase expression never spontaneously immortalize (Shay and Wright, 1989), and instead enter an irreversible growth arrest termed replicative senescence. In addition, mice lacking the RNA subunit of telo-

merase (*mTERC*) show a diminished susceptibility to cancer development induced by chemical carcinogens in a skin papilloma model (Gonzalez-Suarez et al., 2000) or by functional loss of the *INK4A-ARF* locus (Greenberg et al., 1999).

However, telomere attrition in *mTERC* null mice also lacking the p53 tumor suppressor protein leads to rampant genomic instability (Chin et al., 1999a) and an increase in tumorigenicity with characteristic alterations observed in human epithelial cancers (Artandi et al., 2000). Moreover, although normal human cells enter replicative senescence and arrest with passage, cells harboring inactivating alterations of the p53 and retinoblastoma (RB) tumor suppressor pathways divide until they reach a second proliferative barrier, termed crisis, characterized by widespread cell death by apoptosis and karyotypic instability (Shay et al., 1991). The rare cells that survive crisis exhibit stabilized telomere lengths, most often through constitutive activation of telomerase (Counter et al., 1992). Thus, while these observations provide a broad framework for understanding the roles of telomeres and telomerase in cancer development, we still lack a detailed understanding of how telomere biology contributes to cancer.

In particular, dysfunctional telomeres may trigger several different effector pathways, including replicative senescence, apoptosis (crisis), genomic instability, and/or DNA damage repair, depending upon the context under which telomere uncapping occurs. One clue to understanding how telomeres accomplish this signal integration and processing involves specific protein complexes associated with the telomeric DNA. Titia de Lange (Rockefeller University, New York) described recent work from her laboratory concerning the roles of the complex anchored at the telomere by the telomere binding protein, TRF2. The TRF2 complex contains several proteins, including the Mre 11 complex (Zhu et al., 2000), the Werner's Syndrome helicase WRN (Opresko et al., 2002), hRap1 (Li et al., 2000), and the ERCC1/XPF nucleotide excision repair endonuclease (Zhu et al., 2003). The expression of dominantly interfering mutants of TRF2 rapidly induces telomere dysfunction and activates a DNA damage response at telomeres (Takai et al., 2003), which leads to either senescence or apoptosis in a cell type-specific manner. Thus, this experimental system permits one to study the consequences of telomere dysfunction under carefully controlled conditions. Using this system, Zhu et al. have shown that the TRF2 complex protects telomeres from nonhomologous end joining in part by sequestering 3′ telomeric overhangs from the ERCC1/XPF endonuclease (Zhu et al., 2003). These observations suggest that this complex plays an important role in determining the specific consequences of telomere dysfunction.

While TRF1 and TRF2 bind to double-stranded telomeric DNA, a third mammalian telomere binding protein, protection of telomeres 1 (POT1), binds single-stranded telomeric DNA and also regulates telomere integrity (Baumann and Cech, 2001). Consistent with studies in fission yeast, Chris Counter (Duke University, Durham) showed that suppresion of hPOT1 induces genomic instability and the formation of DNA bridges between cells after cytokinesis. Joachim Lingner (Swiss Institute for Experimental Cancer Research, Lausanne) showed that POT1 negatively regulates telomerase activity, and that this function requires the DNA binding domain of POT1, observations in agreement with work from the de Lange laboratory (Loayza and De Lange, 2003). Consistent with these findings, overexpression of a mutant POT1 lacking the DNA binding domain leads to telomere elongation. However, Roger Reddel (Children's Medical Research Institute, Sydney) and Chris Counter reported that under some conditions, overexpression of full-length POT1 (Colgin et al., 2003) or a POT1-hTERT fusion protein (Armbruster et al., 2004) leads to telomere elongation. Since POT1 is part of a large protein complex that also includes TRF1 (Liu et al., 2004; Ye et al., 2004), these findings suggest that POT1, through interactions with other proteins, serves to integrate signals regarding telomere status to control telomere length by regulating telomerase activity at the telomere.

Moreover, these observations reinforce the notion that telomere maintenance is regulated at several levels. Joachim Lingner described an assay that permits the measurement of telomere elongation of individual telomeres in *S. cerevisiae* (Teixeira et al., 2004). Using this assay, he showed that the number of nucleotides added by telomerase in any single cell cycle is independent of telomere length and that telomerase preferentially elongates shorter telomeres, observations consistent with genetic experiments in mice (Hemann et al., 2001). Kathy Collins (University of California, Berkeley) showed that the assembly of the telomerase holoenzyme requires several RNA processing steps (Fu and Collins, 2003). Significantly, defects in RNA processing lead to deficient telomerase assembly and activity (Mitchell et al., 1999) that contribute directly to the pathogenesis of dyskeratosis congenita, an inherited disorder characterized by aplastic anemia, skin and nail changes, and cancer susceptibility (Wong and Collins, 2003).

In addition, Maria Blasco (Spanish National Cancer Center, Madrid) showed that the state of telomeric chromatin affects telomere length regulation. Mice lacking the Suv39 histone methyltransferases harbor telomeres that are longer than in control littermates. This change in length correlates with telomeric chromatin that is less heterochromatic (Garcia-Cao et al., 2004). Woodring Wright (University of Texas, Southwestern, Dallas) also showed that areas of subtelomeric DNA exist that are resistant to nuclease digestion (Steinert et al., 2004). These X regions correlate with telomere length. Since recent ultrastructural studies demonstrate that telomeres form T loops that contain higher order chromatin structures (Nikitina and Woodcock, 2004), these studies suggest that further progress in understanding how telomere maintenance is achieved will require a deeper understanding of the proteins and interactions that control telomeric chromatin.

Taken together, these observations confirm the importance of telomere maintenance and telomerase activation for cancer development and underscore the potential utility of telomerase inhibition therapy for cancer. Calvin Harley (Geron Corporation, Menlo Park) presented an update of work on modified thiophosphoramidate oligonucleotide inhibitors of telomerase (Asai et al., 2003), which appear to have little nonspecific toxicity. In particular, he showed that GRN163L, a lipid-modified analog of their lead modified oligonucleotide GRN163, is 3- to 10-fold more effective than GRN163 in inhibiting telomerase activity in cancer cell lines and tumor xenographs. Based on these preclinical and additional pharmacokinetic studies, GRN163L is moving toward clinical trials.

Although most human cancers use telomerase to maintain telomeres, a significant minority use an alternative mechanism (ALT) that involves recombination to stabilize telomere length (Reddel, 2003). Roger Reddel (Children's Medical Research Institute, Sydney) presented compelling evidence that the specialized nuclear structure found in ALT cells that consists of

telomeric chromatin within PML nuclear domains, called the ALT-associated PML body (Yeager et al., 1999), correlates with ALT activity. He showed that these ALT-associated PML bodies are easily detectable in paraffin-embedded tissues and can be used to detect ALT cells in glioblastoma multiforme, where the presence of such bodies predicts a better prognosis (Hakin-Smith et al., 2003), and in many soft tissue sarcomas and osteosarcomas, where tumors that exhibit the ALT phenotype have outcomes similar to those of telomerase-expressing tumors. Thus, although further work will undoubtedly identify new targets amenable for use as cancer diagnostics or therapeutics, these efforts are important first steps.

The roles of ARF and p53 in senescence, apoptosis, and transformation

The p53 tumor suppressor protein plays an important role in limiting cancer development (Levine, 1997). Up to half of all human cancers show evidence of inactivating p53 mutations, and many of the remainder harbor mutations or alterations of proteins that regulate p53 or that are effectors of p53 function (Hofseth et al., 2004). Moreover, several DNA tumor viruses encode proteins (the simian virus 40 large T antigen, human papillomavirus E6, and adenovirus E1B proteins) that inactivate p53 and are required for cell transformation in permissive hosts (Levine, 1990). Experimentally, mice lacking p53 show remarkable tumor susceptibility, while mice heterozygous for p53 also readily form tumors in which the remaining allele shows loss of heterozygosity (Donehower et al., 1992; Jacks et al., 1994). In sum, these observations strongly implicate inactivation of the p53 pathway as an essential step in malignant transformation.

The p53 pathway responds to many different stimuli, including DNA damage, telomere dysfunction, oncogene activation, and hypoxia, by inducing either cell cycle arrest or apoptosis (Slee et al., 2004). As such, p53 acts as an important sensor to protect cells from genotoxic damage. Normal cells express low levels of relatively unstable p53; however, after exposure to genotoxic stimuli, p53 undergoes several posttranslational modifications (Slee et al., 2004). As a consequence, p53 is activated and stabilized and can serve as a transcription factor that regulates the expression of genes critical for apoptosis or cell cycle arrest (Levine, 1997). This framework begins to explain the importance of p53 in restricting malignant transformation, while recent work has begun to elucidate the molecular details of how this pathway is regulated.

MDM2 serves as an important regulator of p53 function. MDM2 binds p53 and targets it for degradation. MDM2 is itself regulated by ARF, one of two tumor suppressor gene proteins encoded by the *INK4A-ARF* locus (Sherr, 2001). By antagonizing MDM2, ARF promotes p53 stabilization and induces cell cycle arrest or apoptosis. Mice lacking the *ARF* gene but retaining the other *INK4A* tumor suppressor gene, $p16^{INK4A}$, spontaneously develop tumors and are highly susceptible to challenge by chemical carcinogens and oncogenes (Kamijo et al., 1997). Moreover, ARF induction acts as a key step in the stabilization of p53 by oncogenes such as RAS and Myc (Zindy et al., 1998). Charles Sherr (Howard Hughes Medical Institute at St. Jude Children's Research Hospital, Memphis) described recent observations from his laboratory that have begun to elucidate how ARF itself is regulated. Using mice in which the gene encoding the green fluorescent protein (GFP) was substituted for *ARF* genomic coding sequences, his group has found that the *ARF* promoter is tightly repressed in most tissues. Only ectopic retrolental masses and tumors that form in *ARF* null mice show evidence of GFP expression, implicating *ARF* activation as an rate-limiting inhibitory step in the development of such lesions (Zindy et al., 2003). In addition, several other ARF binding partners have recently been described. One of these proteins, nucleophosmin/B23, appears to play a critical role in ARF stability and its function as a tumor suppressor protein (Bertwistle et al., 2004). ARF protein turnover is also regulated through its N-terminal ubiquitination (Kuo et al., 2004). This provides a rare example of a naturally occurring lysine-less protein whose degradation is regulated by the ubiquitin-proteasome pathway.

In addition to interactions with ARF and MDM2, recent work suggests that several other interactions and modifications also play important roles in regulating p53 function and provide insight into the mechanisms by which particular p53 effector mechanisms are activated. David Dornan (Genentech, South San Francisco) showed that the human homolog of the *Arabidopsis* ubiquitin ligase, COP-1, binds p53 (Dornan et al., 2004). Suppression of COP-1 in U2OS osteosarcoma cells by RNA interference increases p53 levels and enhances apoptosis induced by ionizing radiation. Moreover, Melissa Jack and Patrick Lee (University of Calgary Health Sciences Center, Calgary) showed that DNA-PK and Chk2 independently and sequentially phosphorylate a latent fraction of p53 (Jack et al., 2004). Xin Lu (Ludwig Institute for Cancer Research, London) described new insights into the apoptotic-stimulating proteins of p53 (ASPP) family. The ASPP family members bind the DNA binding domain of p53 and specifically upregulate the expression of p53-dependent apoptotic genes, such as Bax and PIG3, in part by increasing the occupancy of p53 at certain promoters (Samuels-Lev et al., 2001). While ASPP1 and ASPP2 both activate transcription of p53-dependent genes involved in apoptosis, a third ASPP family member, iASPP, antagonizes this activation (Bergamaschi et al., 2003). Consistent with this function, expression of iASPP, similar to dominantly interfering mutants of p53, cooperates with several oncogenes to transform primary rat fibroblasts, and overexpression of iASPP has been reported in some human cancers (Bergamaschi et al., 2003).

The activation and stabilization of p53 leads to both apoptosis and cell cycle arrest. Thus, like telomere shortening, p53 controls different cell fates under different conditions. Over the past several years, many downstream regulators of p53 have been identified, and recent work has begun to identify the parameters and mechanisms that control these effector mechanisms. In particular, many proapoptotic and antiproliferative genes are transactivated by p53. Moreover, the p53 family members p63 and p73 also appear to play roles in p53-dependent apoptosis (Flores et al., 2002). However, evidence also exists that suggests that p53 induces apoptosis in a transcription-independent manner. Kevin Ryan (Beatson Institute for Cancer Research) described recent work investigating these transcription-independent functions of p53. Using a series of p53 truncation mutants, his laboratory showed that a 37 amino acid portion of p53 unable to transactivate several p53 transcriptional targets suffices to induce apoptosis when expressed in human cancer cell lines, at least in part, by altering the interaction of ASPP family members with the p53 family members p63 and p73. Although these studies, together with a large body of literature, confirm the large number and complexity of interactions that regulate p53, such approaches nonetheless offer

important clues to understanding p53 function in specific circumstances.

Moreover, these studies provide the foundation for potential strategies targeting the p53 pathway for therapeutic intervention. One assumption inherent in this approach is that restoring p53 function will provide primarily a tumor protective benefit. However, recent work has also implicated altered p53 function as a contributor to aging, suggesting that modulating the p53 pathway may affect more than just tumor resistance. To address these issues, Manuel Serrano's laboratory (Spanish National Cancer Center) has created transgenic mice expressing multiple copies of a large region of chromosomal region surrounding the p53 locus in order to assess the effect of superphysiological p53 (Garcia-Cao et al., 2002). Such mice express superphysiological levels of p53 upon exposure to ionizing radiation and show an enhanced ability to respond to radiation as measured by radiation-induced thymocyte apoptosis. In addition, such mice exhibit increased resistance to various stimuli that induce tumor formation, yet show normal longevity and phenotypes associated with aging. Although these studies appear to dissociate a potential role for senescence in aging, further detailed studies with these mice, as well as mice expressing estrogen receptor-p53 fusion genes to permit temporal regulation of p53 expression (Gerard Evan, University of California, San Francisco), will undoubtedly provide important insights into the relationship of tumor suppression and aging.

Roles of physiological responses to environmental stresses to cancer development

Throughout life, the cells and tissues that comprise our bodies are inundated with environmental agents that have the potential to damage cells and promote cancer development. In particular, DNA damage induced by carcinogens, ionizing radiation, or other genotoxic agents presents a potent force in cancer initiation. Indeed, the use of cytotoxic agents to treat patients with cancer leads to a dramatically higher lifelong risk of second cancers. Cells have evolved various protective mechanisms to sense and to protect the organism from these genotoxic insults, and although the p53 and telomere maintenance pathways participate in some of these responses, other pathways also play critical roles in sensing DNA damage and changes in the environment. Recent work from many laboratories has provided important new information about the mechanisms by which cells sense and respond to DNA damage.

Michael Kastan (St. Jude Children's Research Hospital, Memphis) offered recent insights into the early events that lead to the activation of the ATM kinase after induction of DNA damage by ionizing radiation. The ATM kinase plays a central role in the immediate response to DNA double-strand breaks and initiates the DNA damage response pathway in part through its ability to phosphorylate several effector mechanisms (Bakkenist and Kastan, 2004). Almost immediately after ionizing radiation, ATM itself becomes autophosphorylated at serine 1981, and this autophosphorylation is essential for ATM function (Bakkenist and Kastan, 2003). Interestingly, the Kastan laboratory has found that alterations in chromatin structure induced by treatments such as exposure to hypotonic saline, trichostatin, A or chloroquinine stimulates ATM autophosphorylation in the absence of DNA double-stranded breaks (Bakkenist and Kastan, 2003). These findings agree with recent work in mice lacking one or both copies of the histone H2AX, which show enhanced genomic instability and susceptibility to tumor formation (Bassing et al., 2003; Celeste et al., 2003), and together suggest that specific chromatin alterations are part of the sensor mechanisms for DNA damage.

Once activated, ATM initiates several effector pathways, including both S and G2 cell cycle checkpoints, the DNA damage repair machinery, and the p53 pathway. Recent work from several laboratories is now beginning to provide some understanding of how each of these specific effector pathways operates. Nick La Thangue (University of Glasgow, Glasgow) described the role of the ATM kinase target Strap (Demonacos et al., 2001). Phosphorylation of Strap by ATM alters its subcellular localization and facilitates the repair of DNA damage in part through its effects on the acetylation of p53 (Demonacos et al., 2004). Consistent with these observations, Strap remains localized in the cytoplasm in cells derived from patients with ataxia telangiectasia. ATM activation also leads to activation of the Chk1 and Chk2 kinases, which play important roles in both DNA damage responses and replication control. Using the DT40 chicken B cell lymphoma model, David Gillespie (Beatson Institute for Cancer Research, Glasgow) showed that unlike fission yeast, Chk1 plays a dominant role in stabilizing stalled replication forks and maintaining a mitotic checkpoint in vertebrates (Zachos et al., 2003). Michael Kastan also showed that phosphorylation of the SMC-1 kinase by ATM plays a critical role in mediating radiosensitivity and chromosomal instability (Kitagawa et al., 2004). Taken together, these studies begin to provide important clues to understanding how cells respond to DNA damage and other stresses.

Although many tumor suppressor pathways regulate cell proliferation and apoptosis in response to stresses induced by genotoxic agents and oncogene activation, emerging evidence exists that suggests that mutations that disrupt homeostatic mechanisms responsible for regulating nutrient metabolism and growth also contribute to cancer development. In particular, the mechanisms that control cell growth are distinct from, although interconnected with, those that regulate cell proliferation. Iswar Hariharan (University of California, Berkeley) described a large-scale genetic screen in Drosophila to look for genes that control cell growth. By looking for mutants that disrupt eye size regulation, his laboratory has found several genes that play important roles in growth regulation. For example, the Drosophila homologs of the tuberosclerosis genes Tsc1 and Tsc2 control cell size likely through their effects on the TOR pathway (Tapon et al., 2001). Since alterations in Tsc and PTEN associate with several types of human cancer (Kwiatkowski, 2003), these findings implicate the mechanisms that control cell growth control as important contributors to the cancer phenotype. In addition, *archipelago*, a F box/WD family member, affects cyclin E and Myc levels, and the human ortholog hCDC4 is found mutated in endometrial and colorectal cancers (Moberg et al., 2001, 2004). Taken together, these observations suggest that further characterization of the genes responsible for growth regulation will yield critical insights into pathways that participate in cancer development.

New tools and models

Much of our understanding of cancer derives from the study of tumor specimens isolated from cancer patients. Over the past decade, dramatic improvements in technologies for sequencing and identifying alterations in the cancer genome have permitted detailed study of the cancer genome and suggest that a complete catalog of cancer alterations may soon be possible. In par-

allel to these advances, increasingly sophisticated and relevant human and murine models are now available for the identification and characterization of the critical mutations that program specific cancer phenotypes.

Robert Weinberg (Whitehead Institute for Biomedical Research, Cambridge) delivered the conference keynote lecture and described recent progress in using cell-based models to understand carcinoma development. In particular, he showed how understanding the role of telomerase expression in cell immortalization has facilitated the development of human cancer models, particularly epithelial cancer models (Hahn and Weinberg, 2002). Recent work suggests that such experimental models, when cultured under appropriate conditions and placed orthotopically into murine hosts, permit the development of tumors with differentiation patterns reminiscent of human epithelial cancers (Kuperwasser et al., 2004). For example, Gordon Peters (Cancer Research UK London Research Institute, London) showed that this type of experimental scheme permits one to study pathways implicated in cancer pathogenesis. Specifically, using primary human cells derived from patients with inherited predisposition to melanoma due to mutations in the *INK4A-ARF* locus, he showed that loss of $p16^{INK4A}$ but not ARF function cooperates with expression of Myc, Ras, and telomerase to transform some types of human cells, and that tumorigenicity is enhanced with the concomitant loss of p53 function (Drayton et al., 2003). These studies, together with those from other laboratories using various combinations of introduced genes and cell types, promise to provide further insights into which specific combinations of genetic alterations cooperate to permit tumor formation.

While such studies permit the investigation and characterization of cancer-associated alterations that permit tumor formation, in most cases, these transformed human cells fail to recapitulate the capacity of cancers to invade surrounding tissues and metastasize to distant sites. Addressing this important issue, Robert Weinberg described the identification of the Twist transcription factor as one key factor that regulates invasion and metastasis (Yang et al., 2004). Using a series of murine cancer cell lines derived from a primary mammary tumor that exhibit differential abilities to invade and metastasize, his group used transcriptional profiling to identify genes whose expression correlated with metastatic behavior. One of these genes, Twist, when overexpressed in nonmetastatic cell lines, induced metastasis, while suppression of Twist expression in metastatic cell lines inhibited metastatic growths. Correlating with these findings, Twist is overexpressed in a subset of metastatic breast cancers. Although other factors certainly contribute to the complex phenotypes associated with invasion and metastasis, these studies begin to provide insight into the molecules important for these cancer phenotypes.

Until recently, cancer researchers have used overexpression of oncogenes or dominantly interfering mutants to create cell-based models of cancer. The demonstration that RNA interference operates efficiently in mammalian cells now provides an important tool to the study of specific molecules in transformation (Elbashir et al., 2001). Indeed, Agami and his colleagues have used retroviral delivery of short hairpin RNA (shRNA) molecules to confirm that loss of RB and p53 function cooperates to transform human cells (Voorhoeve and Agami, 2003). In addition to using shRNA technology to study specific molecules and their interactions, large collections of RNA interference reagents promise to provide genetic tools to identify molecules important for cancer development and to facilitate genotype-to-phenotype connections. Rene Bernards (Netherlands Cancer Institute, Amsterdam) described a series of experiments using an evolving library of shRNA reagents. Using a pooled library harboring siRNAs for each member of the family of deubiquitinating (DUB) enzymes, he showed that suppression of a single DUB, the familial cylindromatosis tumor suppressor (CYLD), enhances the transcriptional activity of NF-κB (Brummelkamp et al., 2003). Followup molecular and cellular studies confirmed that CYLD binds the IKK-γ subunit of IKB kinase complex and regulates the ubiquitination of TRAF2, thereby inhibiting apoptosis. Since aspirin derivatives inhibit NF-κB activity, these studies help not only elucidate the molecular mechanism underlying this rare disorder, but also provide a rational therapeutic treatment strategy. Using a larger library of shRNAs targeting nearly 8000 genes, Bernards also showed how such libraries will be useful for large-scale screening of phenotypes such as modulators of p53 function (Berns et al., 2004).

While such cell-based approaches certainly provide useful experimental systems that permit the rapid genetic and biochemical dissection of cancer pathways, the next generation of murine models of cancer promises to allow the detailed study of cancer physiology. Indeed, while traditional gene targeting of tumor suppressor genes in mice has confirmed roles of oncogene activation and tumor suppressor loss in cancer development, more sophisticated models that permit temporal, tissue- and cell-specific gene targeting in adult animals will facilitate the creation of experimental models that increasingly recapitulate the changes that occur in human cancer development. Tyler Jacks (Massachusetts Institute of Technology, Cambridge) described several new murine cancer models derived by introducing latent alleles of oncogenic K-RAS into the murine genome. One version of this type of allele spontaneously activates in a subset of cells and induces non-small cell lung cancer with high penetrance (Johnson et al., 2001). A variation of this strategy that permits the activation of this allele through adenoviral delivery of the Cre recombinase allowed his group to control the multiplicity and timing of lung cancer development and identify a specific cell type, one that has characteristics of both the Clara cells and type II pneumocytes as the target for transformation in the lung (Jackson et al., 2001). Other groups have used a similar strategy to produce experimental models of pancreatic cancer that recapitulate many of the phenotypes of the human disease (Aguirre et al., 2003; Hingorani et al., 2003).

Moreover, several laboratories have developed compound transgenic mice lacking combinations of specific tumor suppressor genes in specific tissues. These mice provide important clues to the tissue type specificity of gene mutation. in particular cancers. Anton Berns (Netherlands Cancer Institute, Amsterdam) showed that conditional loss of both p53 and RB in the murine lung leads to neuroendocrine tumors of the lung (Meuwissen et al., 2003). Such tumors resemble human small cell lung cancers immunohistochemically and, like human small cell lung cancers, demonstrate a high capacity to form metastatic extrapulmonary tumors. Taken together with the K-RAS studies, these observations suggest that particular cell types within the same tissue possess different responses to gene mutation. Moreover, in prior work, the Berns laboratory showed that a specific combination of gene mutation in the *INK4A-ARF* locus, namely complete loss of $p16^{INK4A}$ and heterozygous loss of ARF, conferred susceptibility to melanoma and other tumors, suggesting that haploinsufficiency at some tumor suppressor loci

may also play a role in the development of specific human cancers (Krimpenfort et al., 2001).

While these experimental models focus on the events necessary to initiate tumor development, an equally important question is what events are necessary to permit tumor maintenance. Gerard Evan (University of California, San Francisco) described work from his laboratory using transgenic mice harboring an estrogen receptor-c-Myc fusion gene in pancreatic β cells or the epidermis (Pelengaris et al., 2002; Flores et al., 2004). Activation of Myc by the addition of tamoxifen leads to massive β cell apoptosis that is abrogated by simultaneous expression of Bcl-XL (Pelengaris et al., 2002). In the presence of Bcl-XL, activation of Myc induces angiogenic, invasive tumors that are dependent upon Myc expression. Inactivation of Myc by tamoxifen withdrawal leads to tumor regression accompanied by vascular degeneration and apoptosis. These findings confirm that continued Myc expression is necessary for tumor maintenance, and that tumor cells survive on the cusp of unregulated proliferation and apoptosis. Moreover, together with studies from other laboratories (Chin et al., 1999b; Jain et al., 2002), these experimental models provide important systems to study the role of the oncogenes in programming particular cancer phenotypes.

Thus, although focused on the roles of senescence, cell cycle regulation, and apoptosis in cancer, this conference showcased a broad cross-section of cancer research and provided an opportunity for the attendees to integrate and discuss observations drawn from cell- and animal-based models, genetic and biochemical approaches, and the study of experimental models and human tumor specimens. This conference reinforced the notion that it is now increasingly possible to integrate these disparate approaches to elucidate a clearer picture of the molecular basis of cancer. While much further work remains to fully understand cancer initiation, maintenance, and progression, this conference certainly provided an excellent venue to exchange ideas. Moreover, although not focused on cancer therapeutics, many of the presentations provided new hypotheses and frameworks that may lead to new targeted, antineoplastic therapies.

References

Aguirre, A.J., Bardeesy, N., Sinha, M., Lopez, L., Tuveson, D.A., Horner, J., Redston, M.S., and DePinho, R.A. (2003). Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Dev. *17*, 3112–3126.

Armbruster, B.N., Linardic, C.M., Veldman, T., Bansal, N.P., Downie, D.L., and Counter, C.M. (2004). Rescue of an hTERT mutant defective in telomere elongation by fusion with hPot1. Mol. Cell. Biol. *24*, 3552–3561.

Artandi, S.E., Chang, S., Lee, S.-L., Alson, S., Gottlieb, G.J., Chin, L., and DePinho, R.A. (2000). Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. Nature *406*, 461–465.

Asai, A., Oshima, Y., Yamamoto, Y., Uochi, T.A., Kusaka, H., Akinaga, S., Yamashita, Y., Pongracz, K., Pruzan, R., Wunder, E., et al. (2003). A novel telomerase template antagonist (GRN163) as a potential anticancer agent. Cancer Res. *63*, 3931–3939.

Bakkenist, C.J., and Kastan, M.B. (2003). DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature *421*, 499–506.

Bakkenist, C.J., and Kastan, M.B. (2004). Initiating cellular stress responses. Cell *118*, 9–17.

Bassing, C.H., Suh, H., Ferguson, D.O., Chua, K.F., Manis, J., Eckersdorff, M., Gleason, M., Bronson, R., Lee, C., and Alt, F.W. (2003). Histone H2AX: A dosage-dependent suppressor of oncogenic translocations and tumors. Cell *114*, 359–370.

Baumann, P., and Cech, T.R. (2001). Pot1, the putative telomere end-binding protein in fission yeast and humans. Science *292*, 1171–1175.

Bergamaschi, D., Samuels, Y., O'Neil, N.J., Trigiante, G., Crook, T., Hsieh, J.K., O'Connor, D.J., Zhong, S., Campargue, I., Tomlinson, M.L., et al. (2003). iASPP oncoprotein is a key inhibitor of p53 conserved from worm to human. Nat. Genet. *33*, 162–167.

Berns, K., Hijmans, E.M., Mullenders, J., Brummelkamp, T.R., Velds, A., Heimerikx, M., Kerkhoven, R.M., Madiredjo, M., Nijkamp, W., Weigelt, B., et al. (2004). A large-scale RNAi screen in human cells identifies new components of the p53 pathway. Nature *428*, 431–437.

Bertwistle, D., Sugimoto, M., and Sherr, C.J. (2004). Physical and functional interactions of the Arf tumor suppressor protein with nucleophosmin/B23. Mol. Cell. Biol. *24*, 985–996.

Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S., and Wright, W.E. (1998). Extension of life-span by introduction of telomerase into normal human cells. Science *279*, 349–352.

Broccoli, D., Young, J.W., and de Lange, T. (1995). Telomerase activity in normal and malignant hematopoietic cells. Proc. Natl. Acad. Sci. USA *92*, 9082–9086.

Brummelkamp, T.R., Nijman, S.M., Dirac, A.M., and Bernards, R. (2003). Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-κB. Nature *424*, 797–801.

Celeste, A., Difilippantonio, S., Difilippantonio, M.J., Fernandez-Capetillo, O., Pilch, D.R., Sedelnikova, O.A., Eckhaus, M., Ried, T., Bonner, W.M., and Nussenzweig, A. (2003). H2AX haploinsufficiency modifies genomic stability and tumor susceptibility. Cell *114*, 371–383.

Chan, S.W., and Blackburn, E.H. (2002). New ways not to make ends meet: Telomerase, DNA damage proteins and heterochromatin. Oncogene *21*, 553–563.

Chin, L., Artandi, S.E., Shen, Q., Tam, A., Lee, S.L., Gottlieb, G.J., Greider, C.W., and DePinho, R.A. (1999a). p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. Cell *97*, 527–538.

Chin, L., Tam, A., Pomerantz, J., Wong, M., Holash, J., Bardeesy, N., Shen, Q., O'Hagan, R., Pantginis, J., Zhou, H., et al. (1999b). Essential role for oncogenic Ras in tumour maintenance. Nature *400*, 468–472.

Colgin, L.M., Baran, K., Baumann, P., Cech, T.R., and Reddel, R.R. (2003). Human POT1 facilitates telomere elongation by telomerase. Curr. Biol. *13*, 942–946.

Counter, C.M., Avilion, A.A., Le Feuvre, C.E., Stewart, N.G., Greider, C.W., Harley, C.B., and Bacchetti, S. (1992). Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J. *11*, 1921–1929.

de Lange, T. (2002). Protection of mammalian telomeres. Oncogene *21*, 532–540.

Demonacos, C., Krstic-Demonacos, M., and La Thangue, N.B. (2001). A TPR motif cofactor contributes to p300 activity in the p53 response. Mol. Cell *8*, 71–84.

Demonacos, C., Krstic-Demonacos, M., Smith, L., Xu, D., O'Connor, D.P., Jansson, M., and La Thangue, N.B. (2004). A new effector pathway links ATM kinase with the DNA damage response. Nat. Cell Biol., in press.

Donehower, L.A., Harvey, M., Slagle, B.L., McArthur, M.J., Montgomery, C.A., Jr., Butel, J.S., and Bradley, A. (1992). Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature *356*, 215–221.

Dornan, D., Wertz, I., Shimizu, H., Arnott, D., Frantz, G.D., Dowd, P., O'Rourke, K., Koeppen, H., and Dixit, V.M. (2004). The ubiquitin ligase COP1 is a critical negative regulator of p53. Nature *429*, 86–92.

Drayton, S., Rowe, J., Jones, R., Vatcheva, R., Cuthbert-Heavens, D., Marshall, J., Fried, M., and Peters, G. (2003). Tumor suppressor p16INK4a

determines sensitivity of human cells to transformation by cooperating cellular oncogenes. Cancer Cell *4*, 301–310.

Elbashir, S.M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., and Tuschl, T. (2001). Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature *411*, 494–498.

Flores, E.R., Tsai, K.Y., Crowley, D., Sengupta, S., Yang, A., McKeon, F., and Jacks, T. (2002). p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. Nature *416*, 560–564.

Flores, I., Murphy, D.J., Swigart, L.B., Knies, U., and Evan, G.I. (2004). Defining the temporal requirements for Myc in the progression and maintenance of skin neoplasia. Oncogene *23*, 5923–5930.

Fu, D., and Collins, K. (2003). Distinct biogenesis pathways for human telomerase RNA and H/ACA small nucleolar RNAs. Mol. Cell *11*, 1361–1372.

Garcia-Cao, I., Garcia-Cao, M., Martin-Caballero, J., Criado, L.M., Klatt, P., Flores, J.M., Weill, J.C., Blasco, M.A., and Serrano, M. (2002). Super p53" mice exhibit enhanced DNA damage response, are tumor resistant and age normally. EMBO J. *21*, 6225–6235.

Garcia-Cao, M., O'Sullivan, R., Peters, A.H., Jenuwein, T., and Blasco, M.A. (2004). Epigenetic regulation of telomere length in mammalian cells by the Suv39h1 and Suv39h2 histone methyltransferases. Nat. Genet. *36*, 94–99.

Gonzalez-Suarez, E., Samper, E., Flores, J.M., and Blasco, M.A. (2000). Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis. Nat. Genet. *26*, 114–117.

Greenberg, R.A., Chin, L., Femino, A., Lee, K.H., Gottlieb, G.J., Singer, R.H., Greider, C.W., and DePinho, R.A. (1999). Short dysfunctional telomeres impair tumorigenesis in the INK4a(delta2/3) cancer-prone mouse. Cell *97*, 515–525.

Hahn, W.C., and Weinberg, R.A. (2002). Modelling the molecular circuitry of cancer. Nat. Rev. Cancer *2*, 331–341.

Hahn, W.C., Counter, C.M., Lundberg, A.S., Beijersbergen, R.L., Brooks, M.W., and Weinberg, R.A. (1999). Creation of human tumor cells with defined genetic elements. Nature *400*, 464–468.

Hakin-Smith, V., Jellinek, D.A., Levy, D., Carroll, T., Teo, M., Timperley, W.R., McKay, M.J., Reddel, R.R., and Royds, J.A. (2003). Alternative lengthening of telomeres and survival in patients with glioblastoma multiforme. Lancet *361*, 836–838.

Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell *100*, 57–70.

Harley, C.B., Futcher, A.B., and Greider, C.W. (1990). Telomeres shorten during ageing of human fibroblasts. Nature *345*, 458–460.

Harley, C.B., Kim, N.W., Prowse, K.R., Weinrich, S.L., Hirsch, K.S., West, M.D., Bacchetti, S., Hirte, H.W., Counter, C.M., Greider, C.W., et al. (1994). Telomerase, cell immortality, and cancer. Cold Spring Harb. Symp. Quant. Biol. *59*, 307–315.

Hastie, N.D., Dempster, M., Dunlop, M.G., Thompson, A.M., Green, D.K., and Allshire, R.C. (1990). Telomere reduction in human colorectal carcinoma and with ageing. Nature *346*, 866–868.

Hemann, M.T., Strong, M.A., Hao, L.Y., and Greider, C.W. (2001). The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. Cell *107*, 67–77.

Hingorani, S.R., Petricoin, E.F., Maitra, A., Rajapakse, V., King, C., Jacobetz, M.A., Ross, S., Conrads, T.P., Veenstra, T.D., Hitt, B.A., et al. (2003). Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell *4*, 437–450.

Hofseth, L.J., Hussain, S.P., and Harris, C.C. (2004). p53: 25 years after its discovery. Trends Pharmacol. Sci. *25*, 177–181.

Jack, M.T., Woo, R.A., Motoyama, N., Takai, H., and Lee, P.W. (2004). DNA-dependent protein kinase and checkpoint kinase 2 synergistically activate a latent population of p53 upon DNA damage. J. Biol. Chem. *279*, 15269–15273.

Jacks, T., Remington, L., Williams, B.O., Schmitt, E.M., Halachmi, S., Bronson, R.T., and Weinberg, R.A. (1994). Tumor spectrum analysis in p53-mutant mice. Curr. Biol. *4*, 1–7.

Jackson, E.L., Willis, N., Mercer, K., Bronson, R.T., Crowley, D., Montoya, R., Jacks, T., and Tuveson, D.A. (2001). Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. Genes Dev. *15*, 3243–3248.

Jain, M., Arvanitis, C., Chu, K., Dewey, W., Leonhardt, E., Trinh, M., Sundberg, C.D., Bishop, J.M., and Felsher, D.W. (2002). Sustained loss of a neoplastic phenotype by brief inactivation of MYC. Science *297*, 102–104.

Johnson, L., Mercer, K., Greenbaum, D., Bronson, R.T., Crowley, D., Tuveson, D.A., and Jacks, T. (2001). Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. Nature *410*, 1111–1116.

Kamijo, T., Zindy, F., Roussel, M.F., Quelle, D.E., Downing, J.R., Ashmun, R.A., Grosveld, G., and Sherr, C.J. (1997). Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. Cell *91*, 649–659.

Kim, N.W., Piatyszek, M.A., Prowse, K.R., Harley, C.B., West, M.D., Ho, P.L., Coviello, G.M., Wright, W.E., Weinrich, S.L., and Shay, J.W. (1994). Specific association of human telomerase activity with immortal cells and cancer. Science *266*, 2011–2015.

Kitagawa, R., Bakkenist, C.J., McKinnon, P.J., and Kastan, M.B. (2004). Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. Genes Dev. *18*, 1423–1438.

Krimpenfort, P., Quon, K.C., Mooi, W.J., Loonstra, A., and Berns, A. (2001). Loss of p16Ink4a confers susceptibility to metastatic melanoma in mice. Nature *413*, 83–86.

Kuo, M.-L., den Besten, W., Bertwistle, D., Roussel, M.F., and Sherr, C.J. (2004). N-terminal polyubiquitination and degradation of the Arf tumor suppressor. Genes Dev. *18*, 1862–1875.

Kuperwasser, C., Chavarria, T., Wu, M., Magrane, G., Gray, J.W., Carey, L., Richardson, A., and Weinberg, R.A. (2004). Reconstruction of functionally normal and malignant human breast tissues in mice. Proc. Natl. Acad. Sci. USA *101*, 4966–4971.

Kwiatkowski, D.J. (2003). Rhebbing up mTOR: New insights on TSC1 and TSC2, and the pathogenesis of tuberous sclerosis. Cancer Biol. Ther. *2*, 471–476.

Levine, A.J. (1990). The p53 protein and its interactions with the oncogene products of the small DNA tumor viruses. Virology *177*, 419–426.

Levine, A.J. (1997). p53, the cellular gatekeeper for growth and division. Cell *88*, 323–331.

Li, B., Oestreich, S., and de Lange, T. (2000). Identification of human Rap1: Implications for telomere evolution. Cell *101*, 471–483.

Liu, D., Safari, A., O'Connor, M.S., Chan, D.W., Laegeler, A., Qin, J., and Songyang, Z. (2004). PTOP interacts with POT1 and regulates its localization to telomeres. Nat. Cell Biol. *6*, 673–680.

Loayza, D., and De Lange, T. (2003). POT1 as a terminal transducer of TRF1 telomere length control. Nature *423*, 1013–1018.

Masutomi, K., Yu, E.Y., Khurts, S., Ben-Porath, I., Currier, J.L., Metz, G.B., Brooks, M.W., Kaneko, S., Murakami, S., DeCaprio, J.A., et al. (2003). Telomerase maintains telomere structure in normal human cells. Cell *114*, 241–253.

Meuwissen, R., Linn, S.C., Linnoila, R.I., Zevenhoven, J., Mooi, W.J., and Berns, A. (2003). Induction of small cell lung cancer by somatic inactivation of both Trp53 and Rb1 in a conditional mouse model. Cancer Cell *4*, 181–189.

Mitchell, J.R., Wood, E., and Collins, K. (1999). A telomerase component is defective in the human disease dyskeratosis congenita. Nature *402*, 551–555.

Moberg, K.H., Bell, D.W., Wahrer, D.C., Haber, D.A., and Hariharan, I.K. (2001). Archipelago regulates Cyclin E levels in Drosophila and is mutated in human cancer cell lines. Nature *413*, 311–316.

Moberg, K.H., Mukherjee, A., Veraksa, A., Artavanis-Tsakonas, S., and Hariharan, I.K. (2004). The Drosophila F box protein archipelago regulates dMyc protein levels in vivo. Curr. Biol. *14*, 965–974.

Nikitina, T., and Woodcock, C.L. (2004). Closed chromatin loops at the ends of chromosomes. J. Cell Biol. *166*, 161–165.

Opresko, P.L., von Kobbe, C., Laine, J.P., Harrigan, J., Hickson, I.D., and Bohr, V.A. (2002). Telomere-binding protein TRF2 binds to and stimulates the Werner and Bloom syndrome helicases. J. Biol. Chem. *277*, 41110–41119.

Pelengaris, S., Khan, M., and Evan, G.I. (2002). Suppression of Myc-induced apoptosis in beta cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. Cell *109*, 321–334.

Reddel, R.R. (2003). Alternative lengthening of telomeres, telomerase, and cancer. Cancer Lett. *194*, 155–162.

Samuels-Lev, Y., O'Connor, D.J., Bergamaschi, D., Trigiante, G., Hsieh, J.K., Zhong, S., Campargue, I., Naumovski, L., Crook, T., and Lu, X. (2001). ASPP proteins specifically stimulate the apoptotic function of p53. Mol. Cell *8*, 781–794.

Shay, J.W., and Wright, W.E. (1989). Quantitation of the frequency of immortalization of normal human diploid fibroblasts by SV40 large T-antigen. Exp. Cell Res. *184*, 109–118.

Shay, J.W., Pereira-Smith, O.M., and Wright, W.E. (1991). A role for both RB and p53 in the regulation of human cellular senescence. Exp. Cell Res. *196*, 33–39.

Sherr, C.J. (2001). The ink4a/arf network in tumour suppression. Nat. Rev. Mol. Cell Biol. *2*, 731–737.

Slee, E.A., O'Connor, D.J., and Lu, X. (2004). To die or not to die: How does p53 decide? Oncogene *23*, 2809–2818.

Steinert, S., Shay, J.W., and Wright, W.E. (2004). Modification of subtelomeric DNA. Mol. Cell. Biol. *24*, 4571–4580.

Takai, H., Smogorzewska, A., and de Lange, T. (2003). DNA damage foci at dysfunctional telomeres. Curr. Biol. *13*, 1549–1556.

Tapon, N., Ito, N., Dickson, B.J., Treisman, J.E., and Hariharan, I.K. (2001). The Drosophila tuberous sclerosis complex gene homologs restrict cell growth and cell proliferation. Cell *105*, 345–355.

Teixeira, M.T., Arneric, M., Sperisen, P., and Lingner, J. (2004). Telomere length homeostasis is achieved via a switch between telomerase- extendible and -nonextendible states. Cell *117*, 323–335.

Vaziri, H., and Benchimol, S. (1998). Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. Curr. Biol. *8*, 279–282.

Voorhoeve, P.M., and Agami, R. (2003). The tumor-suppressive functions of the human INK4A locus. Cancer Cell *4*, 311–319.

Wong, J.M., and Collins, K. (2003). Telomere maintenance and disease. Lancet *362*, 983–988.

Yang, J., Mani, S.A., Donaher, J.L., Ramaswamy, S., Itzykson, R.A., Come, C., Savagner, P., Gitelman, I., Richardson, A., and Weinberg, R.A. (2004). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell *117*, 927–939.

Ye, J.Z., Hockemeyer, D., Krutchinsky, A.N., Loayza, D., Hooper, S.M., Chait, B.T., and de Lange, T. (2004). POT1-interacting protein PIP1: A telomere length regulator that recruits POT1 to the TIN2/TRF1 complex. Genes Dev. *18*, 1649–1654.

Yeager, T.R., Neumann, A.A., Englezou, A., Huschtscha, L.I., Noble, J.R., and Reddel, R.R. (1999). Telomerase-negative immortalized human cells contain a novel type of promyelocytic leukemia (PML) body. Cancer Res. *59*, 4175–4179.

Zachos, G., Rainey, M.D., and Gillespie, D.A. (2003). Chk1-deficient tumour cells are viable but exhibit multiple checkpoint and survival defects. EMBO J. *22*, 713–723.

Zhu, X.D., Kuster, B., Mann, M., Petrini, J.H., and Lange, T. (2000). Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 and human telomeres. Nat. Genet. *25*, 347–352.

Zhu, X.D., Niedernhofer, L., Kuster, B., Mann, M., Hoeijmakers, J.H., and de Lange, T. (2003). ERCC1/XPF removes the 3′ overhang from uncapped telomeres and represses formation of telomeric DNA-containing double minute chromosomes. Mol. Cell *12*, 1489–1498.

Zindy, F., Eischen, C.M., Randle, D.H., Kamijo, T., Cleveland, J.L., Sherr, C.J., and Roussel, M.F. (1998). Myc signaling via the ARF tumor suppressor regulates p53-dependent apoptosis and immortalization. Genes Dev. *12*, 2424–2433.

Zindy, F., Williams, R.T., Baudino, T.A., Rehg, J.E., Skapek, S.X., Cleveland, J.L., Roussel, M.F., and Sherr, C.J. (2003). Arf tumor suppressor promoter monitors latent oncogenic signals in vivo. Proc. Natl. Acad. Sci. USA *100*, 15930–15935.