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Contents

Immunology 1
The Perils of Histone Loss

Molecular Biology 2
Tissue Inhibitor of Metalloproteinase 2 (TIMP-2): The Janus of Angiogenesis Inhibitors

Administrative Links 3

Translational Research 4
Improved Wound Healing in Irradiated Skin

From the Director's Office . .5
A Comparative Molecular Pathology Research Training Program is Successfully Launched

Cancer and Cell Biology 6
Telomerase Repression as a Tumor Suppressor Function

On the Tenure Track 8
Jeff Gildersleeve, PhD

NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTES OF HEALTH
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■ IMMUNOLOGY

The Perils of Histone Loss

Celeste A, Difilippantonio S, Difilippantonio MJ, Fernandez-Capetillo O, Pilch DR, Sedelnikova OA, Eckhaus M, Ried T, Bonner WM, and Nussenzweig A. H2AX haploinsufficiency modifies genomic stability and tumor susceptibility. *Cell* 114: 371-83, 2003.

Just as the nucleotide is the fundamental repeating unit of DNA, the nucleosome forms the basic building block of chromatin. Within each nucleosome, 147 nucleotide pairs of double-stranded DNA are wrapped 1.7 times around a central core of eight histone protein molecules (an octamer consisting of two copies each of H2A, H2B, H3, and H4 histones). Each core histone contains a globular domain, which is necessary for histone–histone and histone–DNA contacts, as well as a tail motif in the carboxyl and amino terminal regions, which contains sites for posttranslational modification. Although histones are highly conserved proteins, cells contain alternative versions of the canonical core histones with distinct amino acid sequences. Recent studies have indicated that these histone variants have specialized biological functions beyond their structural role in packaging DNA in the nucleus.

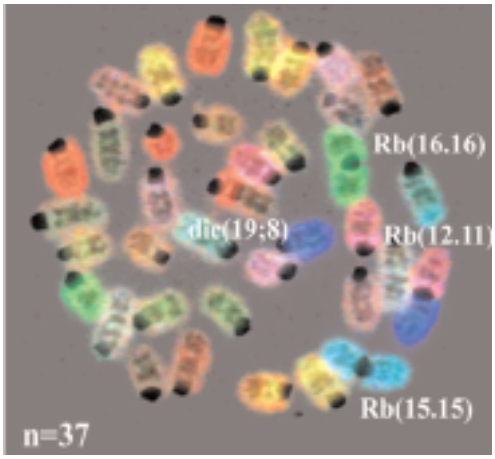

Figure 1. Spectral karyotype analysis of an H2AX$^{-/-}$p53$^{-/-}$ mouse thymic lymphoma. Each chromosome is painted with a distinct fluorescent color. Structural aberrations, resulting in chromosome–chromosome fusions, include dicentric (dic) chromosomes and Robertsonian (Rb) fusions. Thirty-seven mouse chromosomes are present in this metaphase spread, instead of the normal chromosome content of forty. The dark areas are the centromeres.

Almost 6 years ago, a seminal paper by William Bonner's group at NCI revealed an unexpected role for the histone H2AX variant in response to damaged DNA (Rogakou EP. *J Biol Chem* 273: 5858-68, 1998). Two-dimensional gel analysis of histones extracted from mammalian cells demonstrated that a novel mass component (γ-H2AX, or phosphorylated H2AX) rapidly formed in response to treatments that clipped both DNA strands, thereby generating DNA double-strand breaks. The unique phosphorylation site in H2AX was mapped to an evolutionarily conserved serine residue located four amino acids from the carboxyl tail. Subsequent studies demonstrated that γ-H2AX is induced in response to DNA breaks originating from diverse origins, including external damage, stalled DNA replication forks, apoptosis, and dysfunctional telomeres. In addition, γ-H2AX is activated at sites of physiological recombination in germ cells and lymphocytes. Because DNA breaks are potent substrates for chromosomal rearrangements that can push a cell toward malignancy, the question arose as to whether phosphorylation of histone H2AX plays some role in maintaining genomic integrity.

In 2002 our lab, and independently Fred Alt's group at Harvard Medical School, generated mice null for H2AX (Celeste A et al. *Science*

296: 922-7, 2002; Bassing CH et al. *Proc Natl Acad Sci U S A* 99: 8173-8, 2002). Somewhat unexpectedly, these mice were viable, although the chromosomes in cells from these mice contained many breaks and translocations. Despite this massive genomic instability, loss of H2AX did not result in tumor development, perhaps because other cellular systems detected the damage and rapidly eliminated these potentially dangerous cells via cell cycle arrest or apoptosis. The p53 tumor suppressor normally carries out these protective functions, so we crossed the H2AX-deficient mice with p53-knockout mice. Our lab, and independently the Alt lab, found that mice deficient in both H2AX and p53 developed tumors earlier and at a higher rate than did mice deficient in p53 alone. Moreover, these tumors were characterized by frequent chromosomal rearrangements and oncogene amplifications (Figure 1) (Celeste A et al. *Cell* 114: 371-83, 2003; Bassing CH et al. *Cell* 114: 359-70, 2003). Interestingly, loss of a single *H2AX* allele in p53-deficient mice was sufficient to increase tumorigenesis, and the tumors in these mice showed a broader spectrum than did tumors arising in p53-deficient animals missing both *H2AX* genes. Together, the data indicated that H2AX does not behave like a "classical" tumor suppressor in that the functioning of both gene alleles is essential for optimal protection against tumorigenesis.

The finding that decreased *H2AX* gene dosage triggers genomic instability may have implications for human cancer. The human *H2AX* gene is located in a region of chromosome 11 commonly deleted or translocated in several hematological malignancies and solid tumors. Other potential cancer-causing genes are in this region, but the evidence from the mouse model indicates that H2AX may be a major player in cancer-causing genomic instability. One important avenue for basic research will be to determine precisely how chromatin structure is modified by the phosphorylation marks on H2AX and how this modification facilitates DNA repair.

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■ MOLECULAR BIOLOGY

Tissue Inhibitor of Metalloproteinase 2 (TIMP-2): The Janus of Angiogenesis Inhibitors

Seo DW, Li H, Guedez L, Wingfield PT, Diaz T, Salloum R, Wei B, and Stetler-Stevenson WG. TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism. *Cell* 114: 171-80, 2003.

Janus: The Roman god of gates, having two faces looking in opposite directions.

Tumor growth and inflammatory responses cannot be sustained without blood vessels to supply oxygen and other nutrients. Thus, the disruption of angiogenesis (the formation of new blood vessels) has been proposed as a mechanism to treat cancer and inflammatory disease (Hanahan D and Folkman J. *Cell* 86: 353-364, 1996).

The angiogenic response shares functional similarities with tumor cell invasion in that both require remodeling of the extracellular matrix (ECM) mediated by matrix metalloproteinases (MMPs). MMP activities are kept in check by the endogenous tissue inhibitors of MMPs (TIMPs). TIMPs have suppressed both tumor growth and angiogenesis in animal models, which suggests that MMP inhibitors might prove useful in treating cancer. However, results from clinical trials of synthetic MMP inhibitors have been disappointing (Coussens LM et al. *Science* 295: 2387-92, 2002).

One reason for these disappointing results is that MMP functions are not limited to ECM remodeling and removal of matrix barriers to cell migration: MMPs also target latent growth factors, cell surface receptors, cell adhesion receptors, and clotting factors. In addition, MMPs can both stimulate and inhibit angiogenic responses.

A decade ago, we demonstrated that TIMP-2 inhibits the mitogenic response of human endothelial cells following stimulation with basic fibroblast growth factor 2 (FGF-2) (Murphy AN et al. *J Cell Physiol* 157: 351-8, 1993). This effect was not observed using TIMP-1 or a synthetic MMP inhibitor, which suggested a novel and unique biological activity of TIMP-2. TIMP-2 also inhibits angiogenic responses *in vivo* (Valente P et al. *Int J Cancer* 75: 246-53, 1998).

The disruption of angiogenesis (the formation of new blood vessels) has been proposed as a mechanism to treat cancer and inflammatory disease.

We investigated the role of MMP inhibition in mediating the anti-angiogenic effects of TIMP-2. In collaboration with Paul Wingfield, PhD, and colleagues at the National Institute of Arthritis and Musculoskeletal and Skin Diseases, we produced a mutant TIMP-2 lacking MMP inhibitor activity, designated Ala+TIMP-2 (Wingfield PT et al. *J Biol Chem* 274:

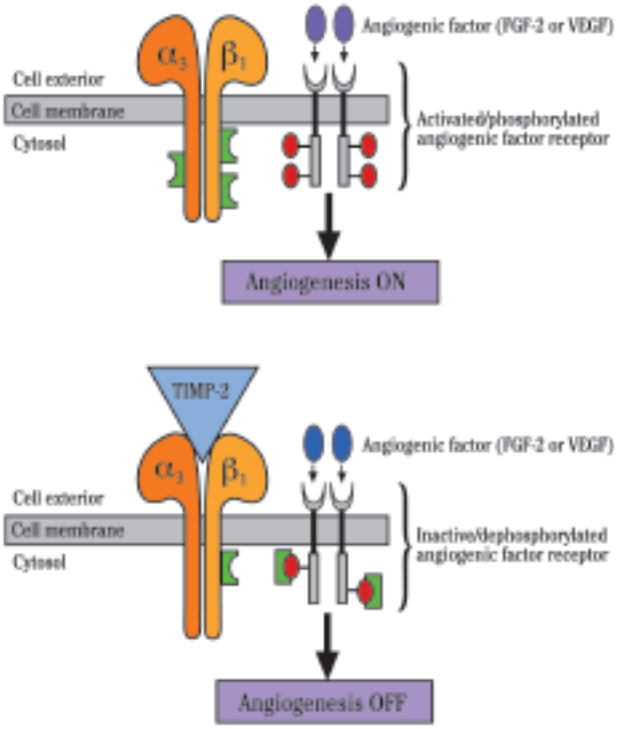

Figure 1. Model for tissue inhibitor of metalloproteinase 2 (TIMP-2) inhibition of angiogenesis. TIMP-2 binding to human microvascular endothelial cell is mediated by $\alpha_3\beta_1$ integrin on the cell surface. TIMP-2 binding to $\alpha_3\beta_1$ integrin results in activation and translocation of protein tyrosine phosphatase activity, which blocks the phosphorylation of angiogenic factor receptors and thereby inhibits angiogenesis. Red ovals represent phosphate groups. Green blocks represent SHP-1, the SH2 domain containing protein tyrosine phosphatase. FGF-2, basic fibroblast growth factor 2; VEGF, vascular endothelial growth factor.

21362-8, 1999). Both TIMP-2 and Ala+TIMP-2 inhibited endothelial cell proliferation in response to FGF-2 or vascular endothelial growth factor A (VEGF-A), and both TIMPs bound to human microvascular endothelial cells in a saturable and reversible fashion. In binding competition experiments TIMP-2 did not bind to the FGF or VEGF receptor. Clearly, TIMP-2 inhibition of endothelial cell growth and angiogenesis was independent of MMP-inhibitory activity and required cell surface binding to an unidentified receptor.

To identify this receptor, we screened a series of anti-integrin subunit antibodies for competition with TIMP-2 binding to human microvascular endothelial cells. Our results helped identify $\alpha_3\beta_1$ integrin as a TIMP-2 binding partner. We then investigated how this integrin mediates the growth-inhibitory effects of TIMP-2. We found that TIMP-2 inhibited phosphorylation of either the FGF or VEGF receptors, indicating suppression of receptor activation. The effect was reversed by inclusion of sodium orthovanadate, an inhibitor of protein tyrosine phosphatase (PTP) activity.

To investigate the role of PTP activity, we examined levels of PTP activity directly associated with the $\alpha_3\beta_1$ integrin as well as the FGF and VEGF receptors, with and without TIMP-2 treatment. TIMP-2 and Ala+TIMP-2 both reduced PTP activity associated with this integrin and increased activity associated with the FGF and VEGF receptors. Further, the SH2 domain containing PTP, SHP-1, was associated with $\alpha_3\beta_1$ integrin before TIMP-2 treatment. After exposure to TIMP-2, the SHP-1 association with $\alpha_3\beta_1$ integrin decreased, whereas its association with the FGF or VEGF receptors increased. These results suggest a model for TIMP-2 suppression of growth factor-stimulated endothelial cell growth (Figure 1). Both TIMP-2 and Ala+TIMP-2 inhibited angiogenic responses to FGF-2 or VEGF-A *in vivo*. Furthermore, this inhibition is suppressed by orthovanadate, again indicating that MMP inhibitory activity is not a major component of TIMP-2 anti-angiogenic activity.

What are the medical implications of these findings? The development of synthetic inhibitors of MMP for cancer treatment was based in part on the observation that TIMPs inhibit tumor growth in animal models. These inhibitors were selected for their TIMP-like ability to block MMP proteolytic activity. Our recent work, however, suggests that the anti-angiogenic—and probably the anti-tumor—activity of TIMP-2 is independent of the MMP-inhibitory activity. This independence would explain why using MMP inhibitors in the clinic has failed. However, our findings suggest new strategies for developing effective anti-tumor therapies based on these new biological activities of TIMP-2. For example, we are currently investigating the *in vivo* anti-angiogenic and anti-tumor activity of TIMP-2 peptide fragments or analogues through their ability to induce PTP activity. Our study demonstrates that TIMP-2, like the Roman god Janus, has two faces: one inhibiting MMP activity, the other inhibiting cell growth and angiogenesis.

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■ ADMINISTRATIVE LINKS

CCR Intranet: Useful Information and Links for Scientific Staff
Don't forget the CCR intranet home page (http://ccr.cancer.gov/default.asp) offers principal investigators and other scientific staff useful links and information, including information and templates for upcoming site visits, listservs for faculties and working groups, a link to the Institutional Review Board website, information on patents and inventions, and media guidelines and tips. The home page also includes the CCR news center, which offers links to research briefs of recent, important papers, archived issues of *CCR Frontiers in Science*, press releases, and the NCI news center.

Improved Wound Healing in Irradiated Skin

Flanders KC, Major CD, Arabshahi A, Aburime EE, Okada MH, Fujii M, Blalock TD, Schultz GS, Sowers A, Anzano MA, Mitchell JB, Russo A, and Roberts AB. Interference with transforming growth factor-beta/Smad3 signaling results in accelerated healing of wounds in previously irradiated skin. *Am J Pathol* 163: 2247-57, 2003.

Radiation therapy and surgery are frequently combined in treating malignancies, but delayed healing of wounds in previously irradiated tissue may present clinical complications. Agents that induce more rapid wound closure while reducing inflammation and scarring would improve surgical outcomes and enhance patients' recovery.

Transforming growth factor β1 (TGF-β1) is a multifunctional cytokine that orchestrates the wound healing response (Figure 1). At a wound site, platelet degranulation releases TGF-β1, which recruits inflammatory cells (monocytes and neutrophils) and fibroblasts into the wound bed. Inflammatory cells cleanse the wound and secrete other cytokines involved in wound repair, while fibroblasts produce extracellular matrix (ECM) that contracts the wound to aid closure. Because TGF-β1 induces its own production in inflammatory cells and fibroblasts, elevated TGF-β1 concentration is maintained in the wound bed. Additionally, TGF-β1 inhibits keratinocyte growth, ensuring that the wound will not re-epithelialize until it has been cleansed by the inflammatory cells, thus reducing the likelihood of infection.

We previously showed that incisional wounds heal more rapidly in Smad3-null mice than in wild-type littermates (Ashcroft GS et al. *Nat Cell Biol* 1: 260-6, 1999). Smad3 is an important intracellular mediator of signals for the TGF-β receptor. Cells null for Smad3 lose their ability to autoinduce TGF-β1, respond to its chemotactic effects, and be growth inhibited by TGF-β1. Therefore, wounds in Smad3-null mice re-epithelialize more rapidly, with reduced inflammation and ECM accumulation (Figure 1).

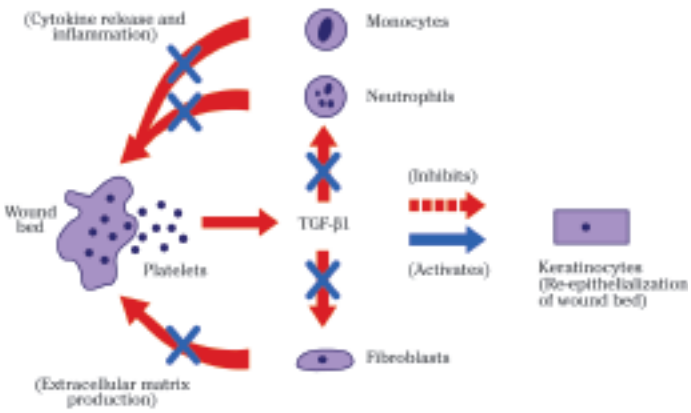

Figure 1. Biological actions in wild-type mice of transforming growth factor β1 (TGF-β1) in wound healing, which include recruitment of inflammatory cells and release of cytokines (e.g., platelet-derived growth factor, interleukin 1, tumor necrosis factor, and basic fibroblast growth factor), recruitment of fibroblasts and extracellular matrix production, and inhibition of re-epithelialization (*red arrows*). In Smad3-null mice, however, inflammation and extracellular matrix production are inhibited (*blue Xs*) and re-epithelialization is enhanced (*blue arrow*).

Radiation therapy often induces a pathological fibrotic response. The subsequent scarring compromises patients' quality of life and complicates later surgical intervention. Ionizing irradiation damages skin by inducing an inflammatory response accompanied by increases in TGF-β1 concentration, thereby initiating an injury repair response and resulting in increased inflammation and ECM production. In certain cases this fibrotic process does not resolve, which leads to clinical complications. We found that Smad3-null mice have a reduced cutaneous response following ionizing irradiation (Flanders KC et al. *Am J Pathol* 160: 1057-68, 2002): 6 weeks after exposure to high-dose irradiation, the skin of Smad3-null mice showed significantly less epidermal hyperplasia; dermal influx of mast cells, neutrophils, and macrophages; and expression of TGF-β1 than did the skin of wild-type littermates. Furthermore, skin from irradiated Smad3-null mice contained few activated fibroblasts and showed relatively normal collagen architecture compared with skin from irradiated wild-type mice, which was severely scarred.

Our most recent work shows that, even in previously irradiated skin, incisional wounds are still able to heal more rapidly in Smad3-null mice than in wild-type littermates. Five weeks post-irradiation, when the resulting skin lesions had healed, we made incisions in the irradiated field. The wounds were narrower, had a smaller area, and re-epithelialized two to three times more quickly in Smad3-null mice than in wild-type littermates. Wounds in Smad3-heterozygous mice healed at an intermediate rate. Compared with wound beds in irradiated skin of wild-type littermates, wound beds in irradiated skin of Smad3-null mice showed a lower TGF-β1 concentration and fewer neutrophils and activated fibroblasts. The decreased number of activated fibroblasts resulted from the inability of Smad3-null fibroblasts to migrate in response to TGF-β1, even though these cells could still be activated by TGF-β1 to produce the contractile protein α-smooth muscle actin. Wounds in irradiated Smad3-null mice also expressed less connective tissue growth factor, which acts with TGF-β1 to induce ECM expression. When dermal fibroblasts from wild-type littermates were exposed to irradiation plus TGF-β1 *in vitro*, we observed a synergistic induction of both TGF-β1 and connective tissue

growth factor that did not occur in Smad3-null fibroblasts. Lower levels of these fibrogenic factors contributed to the decreased scarring in Smad3-null mice. Even though the wound beds of irradiated Smad3-null mice showed decreased cellularity and scarring, the tensile strength of the healed wounds was similar to that in irradiated wild-type littermates.

Using a transgenic mouse model, we identified a therapeutic intervention that may improve wound healing in irradiated skin. The more rapid wound closure with reduced inflammation and scarring in irradiated skin of Smad3-null mice suggests that small-molecule inhibitors of Smad3 may enhance the healing of surgical wounds in previously irradiated tissues and protect against radiation injury itself. Because mice heterozygous for Smad3 also show improved healing, even a partial reduction of Smad3 levels might have a favorable clinical effect.

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FROM THE DIRECTOR'S OFFICE

A Comparative Molecular Pathology Research Training Program Is Successfully Launched

To provide a strong foundation supporting future translational research, the CCR has established a novel graduate research training program in comparative molecular pathology open to individuals with doctorates in veterinary medicine (DVM). The program will prepare comparative pathologists for careers as experimentalists and comprehensively trained scientific collaborators. Training will focus on molecular derangements in the cancer cell within the context of whole-animal systems biology, which necessitates multidisciplinary and transdisciplinary approaches to understanding the functioning biosystem.

Jonathan Wiest, PhD, Associate Director of the CCR Office of Training and Education, has established a university-NCI graduate partnership consortium consisting of four US colleges of veterinary medicine: North Carolina State University; the University of Illinois, Urbana-Champaign; Michigan State University; and the University of Maryland. Other schools may eventually be added. The program is part of an NIH-wide initiative to increase opportunities for graduate education organized by the NIH Graduate Partnership Program Office, headed by Mary DeLong, PhD. It offers a residency in comparative pathology combined with doctoral-level human cancer biology research training for graduate veterinarians that will stress the translation of findings made through the study of animal models to the clinical setting. Investigators educated through this novel approach will unite a clinical perspective gained from training in veterinary medicine and comparative pathology with expertise gained from basic research in the molecular mechanisms of human disease. The transdisciplinary program will provide trainees with experience in animal clinical medicine, diagnostic veterinary pathology, human cancer pathology, and molecular biology, as well as other branches of biomedical research.

Participants will complete 5 years of combined training in pathology, first as a university teaching hospital diagnostic pathology resident and then as a researcher working in the laboratory of a CCR principal investigator (PI). PIs will provide mentored research experiences designed to satisfy the dissertation research requirements defined by partnering universities. Those completing the program will obtain a PhD from their universities and will be eligible to become board certified as medical specialists in veterinary pathology. The first two DVMs entered the program in July 2003; two additional trainees will be enrolled annually during the initial phase of the program.

The program offers a residency in comparative pathology combined with doctoral-level human cancer biology research training for graduate veterinarians that will stress the translation of findings made through the study of animal models to the clinical setting.

Including veterinary pathologists as interdisciplinary research trainees will provide substantial benefits both to the intramural research program as a whole and to participating PIs. The CCR will gain access to the comparative pathology faculties of participating universities, opening the door to new collaborations, and to a student body made up of highly motivated veterinarians with 2 to 3 years of postgraduate pathology experience who also have received training in the CCR molecular pathology curriculum. Potential collaborations between intramural PIs and university faculty members may include project grant development or scientific investigation. Additionally, intramural PIs will be offered the opportunity to participate as university adjunct

faculty. PIs will be expected to provide trainees with CRTA support and benefits, while the CCR Director's Office will be responsible for university-related costs.

Thirty-five intramural PIs are currently affiliated with the training program. When fully operational, the program will link together CCR PIs, university faculties, and trainees to further research into diagnostic pathology, molecular pathology, *in vivo* modeling, and the basic biology of cancer.

The molecular pathology research training program is presently administered through the Molecular Pathology Unit (MPU) headed by R. Mark Simpson, DVM, PhD. Dr. Simpson is board certified by the American College of Veterinary Pathologists. The MPU was established to foster a better understanding of the similarities between cancer in animal models and human cancers. It has developed a molecular pathology curriculum that includes a molecular biology and pathology workshop, diagnostic histopathology seminars, a rodent necropsy training module, human cancer pathology seminars hosted by the CCR Laboratory of Pathology, and clerkships in the research core technology laboratories.

MPU initiatives include applied research and collaborations aimed at developing new reagents, methods, or technologies in preclinical diagnostic medicine, and enhancing researchers' abilities to integrate molecular and systemic aspects of disease mechanisms. The MPU takes a lead in developing, applying, and facilitating use of such methods as noninvasive whole animal imaging and proteomic technologies for mouse models inside the CCR. Anticipated synergies between the MPU and a newly organized Comparative Oncology program, directed by Chand Khanna, DVM, PhD, will enhance CCR preclinical research by encompassing studies of experimental diseases in rodents and spontaneous cancers in large animals. Comparisons across a spectrum of model systems will enable both CCR researchers and the veterinary clinician-scientists trained through the new comparative molecular pathology program to better translate findings derived from the study of animal models to the understanding of human disease.

■ R. Mark Simpson, DVM, PhD

■ CANCER AND CELL BIOLOGY

Telomerase Repression as a Tumor Suppressor Function

Horikawa I, Cable PL, Mazur SJ, Appella E, Afshari CA, and Barrett JC. Downstream E-box–mediated regulation of the human telomerase reverse transcriptase (hTERT) gene transcription: evidence for an endogenous mechanism of transcriptional repression. *Mol Biol Cell* 13: 2585-97, 2002.

Normal human somatic cells are destined to divide only a limited number of times *in vitro* and *in vivo* and eventually enter into a permanent growth arrest called cellular senescence. In contrast, cancer cells can divide indefinitely (or at least many more times than normal cells do). Thus, the cellular senescence program can be a tumor-suppressive mechanism, and escape from it—immortality—is a hallmark of human cancers.

What mechanism counts the number of divisions that normal human cells undergo? This question is explained largely by the telomeres, tandemly repeated DNA sequences at each chromosome end. Because conventional DNA polymerases are unable to completely replicate the ends of linear DNA molecules, the telomere repeat sequences are progressively lost with each cell division cycle. Eventually, in normal human somatic cells, critically short, dysfunctional telomeres induce the cells to stop dividing, likely by initiating DNA damage responses. Most cancer cells, in contrast, maintain their telomere length. Telomerase, a ribonucleoprotein enzyme that synthesizes telomeric repeat DNA, is responsible for this maintenance in about 85 percent of human cancers.

What mechanisms control telomerase activity? The simplest and most direct mechanism controls enzyme activity, namely the expression level of the catalytic protein subunit human telomerase reverse transcriptase (hTERT). hTERT expression at mRNA and protein levels correlates very well with telomerase activity. Various cellular and viral factors act on the hTERT promoter to regulate its transcriptional activity (Figure 1, *top*) (Horikawa I and Barrett JC. *Carcinogenesis* 24: 1167-76, 2003). However, current knowledge of hTERT transcriptional regulation is largely based on data from experimental settings

The cellular senescence program can be a tumor-suppressive mechanism, and escape from it—immortality—is a hallmark of human cancers.

of overexpression or ectopic expression. It remains to be examined whether inactivation of a tumor suppressor gene (by genetic alteration via mutation or deletion) plays a primary role in telomerase activation. Chromosome band 3p21 carries tumor suppressor genes in various types of cancers, including renal cell carcinoma. Our investigation of a physiological role of a 3p21 tumor suppressor gene for renal cell carcinoma has illuminated the endogenous and physiological mechanisms of human telomerase regulation.

We used a pair of cell lines that have similar genetic backgrounds but differ in telomerase activity: a telomerase-positive renal cell carcinoma (RCC23), and its telomerase-negative counterpart

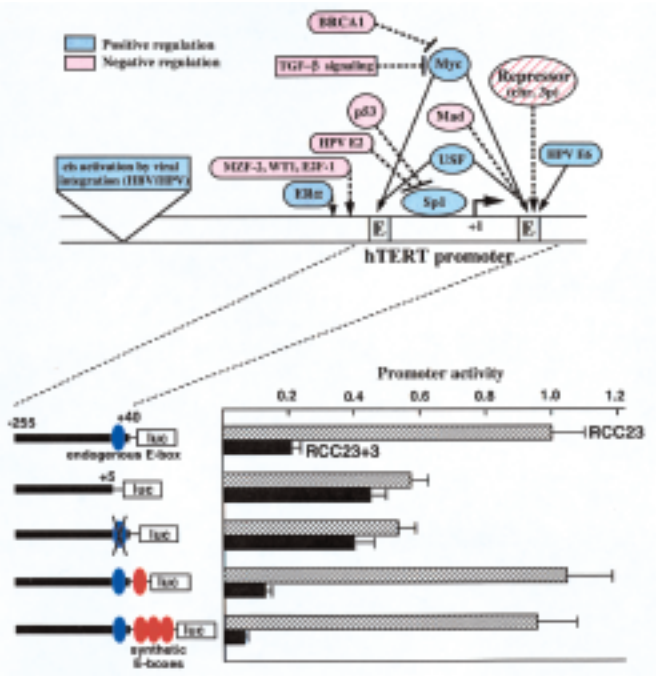

Figure 1. *Top:* Candidate factors and mechanisms to regulate positively (light blue) or negatively (pink) the activity of the human telomerase reverse transcriptase (hTERT) gene promoter. A variety of cellular and viral transcription factors associated with carcinogenesis can affect the promoter activity, including oncogene products (e.g., c-Myc and human papillomavirus E6 [HPV E6]) and tumor suppressor gene products (e.g., WT1, p53, and BRCA1). An endogenous repressor of our interest (encoded or regulated by a tumor suppressor gene on chromosome 3p21 and yet to be identified) is highlighted by pink diagonals. E, canonical E-box elements (CACGTG). *Bottom:* The downstream E-box (blue oval) is responsible for the differential hTERT promoter activity between the telomerase-expressing RCC23 cells and the telomerase-negative RCC23+3 cells. Deletion (*lane 2*) or mutation (*lane 3*) of the E-box abrogated the repression observed with the wild-type promoter in RCC23+3 cells (*lane 1*). The hTERT promoter fragments with one copy (*lane 4*) or three copies (*lane 5*) of synthetic E-box sequences (red ovals) showed a copy number-dependent enhancement of the repression in RCC23+3 cells but not in RCC23 cells.

(RCC23+3) generated by microcell-mediated transfer of a normal human chromosome 3. This transfer allowed us to investigate how a gene on a particular chromosome (even if it's not cloned) functions under its physiological control (i.e., expression from the natural regulatory sequences rather than forced expression from a heterologous promoter). We found that an unidentified tumor suppressor gene on chromosome 3p21 represses telomerase activity, leading to progressive shortening of telomeres and induction of cellular senescence (Horikawa I et al. *Mol Carcinog* 22: 65-72, 1998), just as occurs in normal human somatic cells. The telomerase repression by chromosome 3 transfer was due to the marked down regulation of hTERT mRNA expression. With the promoter region of the hTERT gene in our hands (Horikawa I et al. *Cancer Res* 59: 826-30, 1999), the repression of hTERT mRNA in RCC23+3 was attributed to the transcriptional repression of the hTERT gene.

Examination of deleted and mutated hTERT promoter fragments (Figure 1, *bottom*) identified the E-box sequence downstream of the transcription initiation site as a DNA element critical to the differential hTERT transcription in RCC23 and RCC23+3. Deletion or mutation of this sequence abrogated the repression observed with the wild-type promoter in RCC23+3. The hTERT promoter fragments with one or three copies of synthetic E-box sequences showed a copy number-dependent enhancement of the repression in RCC23+3 but not RCC23, supporting the existence of an E-box–mediated repressive mechanism that actively functions in RCC23+3 cells and is defective in RCC23 cells. No evidence has been obtained that endogenously expressed c-Myc or Mad is responsible for this mechanism. Instead, we detected a novel E-box binding factor specific to RCC23+3 cells (distinct from the Myc/Mad family and currently of unknown identity).

The downstream E-box–mediated mechanism for hTERT repression is also active in various types of normal human cells. It will be interesting to examine whether inactivation of this mechanism is also responsible for the telomerase activation in other types of cancers with chromosome 3p21 deletion.

In conclusion, downstream E-box–mediated repression of hTERT transcription is a function of a tumor suppressor gene, and inactivation of this gene plays an important role in human renal cell immortalization and carcinogenesis. Our biggest interest now is identifying the E-box binding factor specific to the telomerase-negative cells. The modified hTERT promoter containing synthetic E-box elements will be an excellent tool to drive cancer-specific expression of cytotoxic genes in cancer gene therapy.

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Jeff Gildersleeve, PhD

Jeff Gildersleeve began his professional training in sunny southern California at the University of California at San Diego. He graduated in 1993 with a BS in biology and then drove 3,000 miles to New Jersey to pursue a PhD in organic chemistry at Princeton University. Under the guidance of Professor Dan Kahne, Dr. Gildersleeve's graduate training focused on mechanistic organic chemistry, carbohydrate chemistry, and chemical biology. After completing his PhD, he drove back to southern California where he worked with Professor Peter Schultz at the Scripps Research Institute. Dr. Gildersleeve's postdoctoral work focused on directed evolution of protein catalysts and the development of high-throughput screens for catalytic antibodies. In the summer of 2003, he completed one last 3,000-mile shot across the heartland and began his current position as a tenure-track principal investigator in CCR's Laboratory for Medicinal Chemistry.

Dr. Gildersleeve's research program specializes in chemical glycobiology. A combination of organic synthesis, protein chemistry, and protein engineering is used to address fundamental issues of glycobiology. One long-term objective is to develop reliable methods for generating strong, specific antibody responses to carbohydrate tumor antigens. Antibodies are one of the primary weapons our immune system uses to defend against invading pathogens, are key elements in the development of vaccines, and are extremely useful research tools. Unfortunately, existing methods produce weak, non-specific antibody responses toward carbohydrate tumor antigens. Dr. Gildersleeve's research group is developing new strategies to improve and direct the immune response toward carbohydrate antigens as well as better methods for evaluating the response. Since most carbohydrates of interest are not commercially available, the research group relies heavily on chemical synthesis to obtain biologically relevant carbohydrates.

Dr. Gildersleeve

A second objective of the research group is to develop a method to rapidly monitor the expression of carbohydrate epitopes/antigens in a high-throughput fashion. By comparing expression levels of many carbohydrate epitopes at different stages of development, at different stages of disease progression, or from different types of organs, one could rapidly identify specific carbohydrate epitopes differentially expressed in a given state. In addition, one could rapidly screen for small molecules that disrupt the biosynthesis of oligosaccharides.

Dr. Gildersleeve is married, has two children ages 2 and 4, and two black Labs. He enjoys goofing around with his kids, plays soccer, and is an avid Lakers fan.