the giovanni ARMENISE HARVARD foundation

## ARMENISE-HARVARD SYMPOSIUM 2004

# CANCER BIOLOGY, GENOMICS, AND POST-GENOMICS

*8th Biennial Symposium*
*May 21-23, 2004, Certosa di Pontignano, Siena, Italy*

### About the Symposium

In 2001, the Giovanni Armenise-Harvard Foundation devoted its 5th Annual Symposium to "Cancer Biology, Genomics, and Post-Genomics." In May 2004, the 8th Annual Symposium revisited this topic and found the landscape much altered. "Using our meeting three years ago as a benchmark, clearly there have been significant advances," said Peter M. Howley, chairman of the Foundation's Scientific Advisory Board and chairman of the HMS Department of Pathology. He co-organized both symposia with Philip Leder, professor and chairman of the Department of Genetics at HMS.

Post-genome technologies are developing so rapidly that some of the experimental results reported at this Symposium could not have been obtained three years ago, the organizers agreed. Now that complete genomes are available for humans and many animal models, researchers can devise "very imaginative and powerful screens which were not possible before," Leder noted. Presentations by Norbert Perrimon of HMS and Enzo Medico of the University of Torino Medical School, for example, illustrate how new technologies can be used to understand the functional genomics of animal models and human tumors.

The Symposium began with a keynote address by Pier Giuseppe Pelicci, who chairs the Department of Experimental Oncology at the European Institute of Oncology and is affiliated with the FIRC Institute, both located in Milan. Pelicci has helped pioneer the understanding of oxidative stress and life span, and his work spotlights the intricate web connecting longevity and cancer risk. Howley predicts that new laboratory and computational tools are going to make this thriving area of inquiry even busier in the coming years.

This year's Symposium was a hub of intellectual activity in an extraordinarily tranquil setting. It took place just outside Siena at the Certosa di Pontignano, a complex of stone buildings dating to the 14th Century. The Certosa was originally a Carthusian monastery, built for members of an order who pursued scholarly, meditative lives inside its quiet, graceful cloisters. Later sold into private hands, it was owned by two different families during the 19th and early 20th Century. When World War II erupted, this rural cloister once again became a refuge; not for monks, but for Jews and other

persecuted peoples. In 1959, it was acquired by the University of Siena and transformed into an exceptionally beautiful conference center. In airy stone chambers where monks once pored over illuminated manuscripts, high-speed internet access is now available.

Nearly 80 scientific participants traveled to the Certosa for the Armenise-Harvard Symposium, which featured 20 invited lectures and 15 poster presentations. In attendance were representatives of Harvard Medical School and two of its affiliated hospitals, 11 Italian universities and research institutions, and two multinational pharmaceutical companies.

The Annual Symposium coincides with the announcement of grants and awards provided by the Armenise-Harvard Foundation. Each year, the Career Development Awards provide seed money for two young Italian scientists who are returning to Italy to launch their own research programs after studying abroad. Molecular biologist Davide Corona is relocating from the University of California at Santa Cruz to DIBIT, at San Raffaele University in Milan. There, he will use genetic and biochemical methods to study ATP-dependent chromatin remodeling, working with Professor Francesco Blasi. Luca Santarelli, who is interested in the biological determinants of mood disorders, augmented his training as a psychiatrist with molecular biology work at Columbia University in New York. The award will help him investigate neuropeptides and adult neurogenesis at Fondazione S. Lucia in Rome, guided by Professor Georgio Bernardi.

The 2004 Junior Faculty Grants are given each year to up-and-coming researchers at HMS. Grace Gill of the Armenise Center for Cancer Biology will use the grant to continue her work on regulation of gene expression in bone cells. David Rudner was honored for his work on regulation of proteolysis and signal transduction in Bacillus subtilis, which he pursues at the Armenise Center for Microbial Pathogenesis and the Host Response.

Symposium participants included winners of this year's Armenise-Harvard Foundation Italian Science Writer Fellowships. Luca Barone, who has a Ph.D. in astronomy and a masters degree in science communication, freelances for RAI, Italy's national public radio network, as well as several prominent newspapers and magazines. Guido Romeo, a trilingual reporter who received his masters in science journalism in France, is a staff editor at Macchina del Tempo, a monthly popular science magazine with a circulation of 90,000 and a spin-off television program. Later this summer, Barone and Romeo will come to HMS to gather material for additional stories. Each year, the fellowship yields significant coverage of Armenise-supported research in the Italian press.

p align=""left"">  p53 – p66shc signal transduction pathway in tumor suppression and life span control

Pier Giuseppe Pelicci

Chair, Department of Experimental Oncology, European Institute of Oncology, and FIRC Institute, Milan

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<p align=""left"">Although stress is obviously harmful to humans and other living creatures, the mechanisms involved are only partly understood. Dr. Pelicci made a key contribution to this field when he discovered the first mammalian stress-regulating gene. After more than a decade of studying how mammalian cells deal with oxidative stress, he and his coworkers have uncovered surprising connections between stress, tumor development, and longevity. The protagonist in this drama is the p66shc gene, which encodes an intracellular signaling protein that looks like others in the Shc family but behaves differently. While other family members serve as couriers, delivering orders to proliferate from tyrosine kinases to Ras, the normal function of p66shc is inducing apoptosis in cells damaged by reactive oxygen species.</p>

There are many similarities between p66 and p53, the archetypal tumor suppressor gene that triggers apoptosis in cells compromised by stress. Experimental manipulations of p53 in mice show that when this gene is highly active, the animals die young but rarely develop tumors. When the gene is inactivated, they live longer but develop many more tumors. In other animal models the correlation between longevity, increased stress-resistance and accumulation of oxidative damage is so strong, Dr. Pelicci said, that he once predicted “it would be impossible to extend life span without accelerating cancer formation, and vice versa.”

Dr. Pelicci and his coworkers were the first to show that deleting p66shc in mice (p66shc-/-) delayed senescence and prolonged life span, an effect they attributed to the cells' increased resistance to apoptosis despite injuries inflicted by reactive oxygen species, ultraviolet light, or other stresses. Would the trade-off for living longer be a higher incidence of cancer? Although this was Dr. Pelicci's forecast, in 2000 he and his colleagues reported in Nature that p66shc -/- mice lived 30% longer than control animals yet were no more susceptible to spontaneous or induced tumors than mice without the deletion.

The most likely explanation, Dr. Pelicci says, is that p66shc works differently from p53 and plays a narrower role in apoptosis signaling. It may be that deleting p66shc leaves p53 function largely intact, so when cells are compromised by stressors other than reactive oxygen species, p53 can pull the plug and keep cancer from developing.

Since about 20% of cytosolic p66Shc is localized in mitochondria, the researchers are pursuing the idea that p66shc shuts down these intracellular energy factories by changing their membrane permeability in response to oxidative damage. Experiments indicate that p66shc does this by activating caspase 3, a cell-death enzyme that induces the collapse of mitochondrial transmembrane potential. When p66shc is absent, however, the damaged cell lives on and can lead to tumor development.

The other known biochemical activity of p66shc is mediating intracellular concentrations of hydrogen peroxide, the most abundant type of reactive oxygen generated by cellular metabolism. Over-expression of p66shc increases intracellular H2O2, while levels drop in p66Shc-/- cells. In vivo, reduced levels of oxidation-damaged macromolecules can be measured in tissues of p66shc-/- mice. It is unclear, however, whether elevated H2O2 causes permeability transition in

mitochondrial membranes, and thus induces apoptosis, or whether mitochondrial damage generates more H2O2. Future experiments in Dr. Pelicci's lab will continue to probe peroxide metabolism.

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<p align=""left"">Remaining Presentations</p>

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<p align=""left""> A Mouse Model of Stem Cell-Derived Liver Cancer

Andrea I. McClatchey

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<p align=""left"">Animal models that parallel the clinical course of human disease are invaluable scientific tools: such models help researchers understand the natural course of disease and

sometimes hold the keys to developing better diagnostic tests and treatments. Such models have been difficult to develop for liver cancer, a major cause of illness and death worldwide, in part because the genetic and molecular bases of hepatic cancer are poorly understood. Although chemical carcinogens can be used to drive tumorigenesis in animals, this is not the same as what happens in human disease.</p>

Dr. McClatchey and her colleagues have found a way to more closely approximate the human condition. They built on earlier work showing that mice carrying a heterozygous inactivating mutation in the neurofibromatosis type 2 (Nf2) tumor suppressor gene develop both hepatocellular carcinoma and cholangiocarcinoma. These mice were of limited use for studying liver and bile duct cancers, however, because their lives were cut short by early and overwhelming osteosarcomas. To avoid this complication, Dr. McClatchey's team used an adenovirus vector to knock out Nf2 only in the livers of fetal mice, creating Alb-Cre Nf2lox/lox animals that appear to be a better model for hepatic carcinogenesis.

Underlying this approach was the knowledge that hepatocytes (liver cells) and cholangiocytes (bile duct epithelial cells) arise from the same bipotential "oval" cells. When Dr. McClatchey and her coworkers deleted Nf2 in the liver only, there was a dramatic and progressive expansion of these progenitor cells. In vitro experiments indicate that unlike normal liver cells, which form stable junctions and stop dividing once they've formed an orderly layer on growth medium, Nf2 -/- cells proliferate wildly and accumulate in untidy heaps. In vivo, this causes mice to be born with lesions, composed mainly of undifferentiated cells, which soon differentiate into cholangiocytes and hepatocytes.

These Nf2-deleted mice eventually develop both hepatocellular carcinoma and cholangiocarcinoma, and their tumors recapitulate the invasive and metastatic nature of human liver tumors. This makes them a valuable model for investigating pathogenesis in humans, and for identifying specific genetic events that might be useful for diagnosis or drug development. Already the researchers have observed that NF2 -/- cells are missing an essential genetic braking system that tells normal cells when to stop growing. Dr. McClatchey's lab also studies Nf2 function in other cell types, which she

believes will provide a framework for understanding how loss of regulation contributes to the development of human cancers.

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<p align=""left""> Invasive Growth: A Genetic Program

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<p align=""left"">Dr. Comoglio has devoted his career to investigating how invasive growth is controlled by complicated genetic instructions that drive cells to proliferate, dissociate from one another, migrate in the extracellular matrix, and ignore signals telling them their time is up. Invasive growth occurs normally during epithelial organ development, angiogenesis, and wound healing; it happens abnormally during malignant transformation and tumor formation. In both cases, HGF (also called Scatter Factor 1) and its receptor (a tyrosine kinase encoded by the MET oncogene) are key players in regulating such growth.</p>

Only a few years ago, the quest for genes turned on or off as a consequence of HGF binding was a slow and labor-intensive process. Today, however, microarrays enable Dr. Comoglio's team to search the entire "transcriptome" for coding and non-coding regulatory genetic elements that turn a normal cell into an invasive one. To accomplish this, the researchers added HGF to cultured hepatocytes, then extracted RNAs and cDNAs while branching morphogenesis (a hallmark of invasive growth) proceeded. Some gene clusters switched on early and stayed on; other clusters turned on and off as the invasive phenotype matured.

Using microarrays, they identified about 1,000 genes turned on or off by HGF's binding with its MET-encoded receptor. About 250 of these genes were cross-validated using other experimental platforms, and the researchers estimate that 20 may have prognostic value for human patients. Meta-analysis on genomic expression datasets obtained from breast and liver carcinomas showed that expression of genes belonging to the MET transcriptional signature is prognostic for cancer aggressiveness (See related presentation, "Functional Onco-Genomics and Tumor Progression," by Enzo Medico.)

Hypoxia causes MET to overexpress and drive cells toward the invasive phenotype, Dr. Comoglio said. There is scant oxygen inside tumors, and he postulates that the cell upregulates MET as a means for escaping a suffocating environment. More MET binds more HGF, giving the cell the mobility needed to search for fresh air. When the cell reaches a more hospitable setting, it may trigger angiogenesis to create a matrix for forming a new tumor.

Dr. Comoglio's team tested this hypothesis in a mouse model, using lentiviral vectors to deliver the MET oncogene to liver cells. Interestingly, these animals exhibited a prodrome much like the disseminated intravascular coagulopathy seen in some cancer patients. His research showed that hemostatis genes are regulated by MET in pre-neoplastic lesions, suggesting that hemostasis

disorders are not a late consequence of cancer progression but an early event that may help drive development of the invasive, metastatic phenotype. Preliminary findings indicate that HGF antagonists delivered via lentiviral vectors block tumor formation in these animals, and Dr. Comoglio's team is pursuing this observation.

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<p align=""left""> Interaction of the Bovine Papillomavirus E2 Protein with Brd4 Tethers the Viral DNA to Host Mitotic Chromosomes

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<p align=""left"">Papillomaviruses (PVs) are unusually talented stowaways. Unlike viruses such as HIV and influenza, which replicate inside a single host cell until it bursts at the seams and dies, PVs maintain multiple discreet copies of their genomes in the nucleus of host cells, then distribute these copies to daughter cells during mitosis. The virus's uncanny ability to cling to host chromosomes amidst the turmoil of cell division helps PV infections persist for years. Long-term infection with

certain strains of human papillomavirus, such as HPV 16 and 18, plays a major role in the pathogenesis of cervical cancer – the second most common cancer in women.</p>

Researchers have spent years trying to understand exactly how viral episomes hang onto host chromosomes during mitosis, because the answer might enable them to develop drugs that would break this tie and stop infections before they cause potentially lethal cervical cancer, as well as treating less severe manifestations of infection such as plantar or genital warts. There are currently no HPV-specific antivirals available.

Dr. Howley and colleagues work with a bovine papillomavirus (BPV) that infects mouse cells and gradually turns them malignant. Since 1997, researchers have known that HPVs use a protein called E2 to tether viral genomes to host chromosomes, but the corresponding protein on the host side was unknown. In the April 30 issue of Cell, Dr. Howley and research fellow Jianxin You described using post-genome technology to identify the bromodomain protein Brd4 as the chromosomal strap seized by viral stowaways. In addition, they have identified an "inhibitor fragment" of Brd4 that loosens the virus's grip, reduces infection, and dramatically slows the cancerous transformation of infected cells.

You used a novel proteomic approach to identify the complex of cellular proteins that viral E2 binds, most of them unrelated to the virus's attachment to mitotic chromosomes. Analysis of E2-associated proteins by mass spectrometry experiments identified Brd4 as the most likely mitotic tether for BPV viral E2. The E2-Brd4 connection appears to be highly conserved across species; the researchers found that a peptide they used to block the attachment kept the PV E2 and the PV DNA from latching onto chromosomes. Dr. Howley's team is now collaborating with Harvard University's Department of Chemistry to identify small molecules that might accomplish this same feat, and thus hold promise as antiviral drugs for HPV.

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<p align=""left"">Mechanisms Coordinating Membrane Traffic, Adhesion, and Cytoskeleton During Cell Motility

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<p align=""left"">Not only are the crawling movements of eukaryotic cells complex and difficult to understand, but it turns out that some of the prime movers are also involved with other processes such as membrane traffic, vesicle formation, and recycling. In 2000, Dr. deCurtis and his colleagues identified P95-APP1, a ubiquitous ArfGAP (GTPase-activating protein) of the GIT family. Like related GIT proteins, this one interacts with various surface receptors and plays multiple roles in intracellular signaling. For example, P95-APP1 plays a role in cytoskeletal organization by interacting with regulators of Rac function, and is needed for protrusion and attachment when cells go on the move.</p>

The paxillin-binding carboxyterminal portion of normal p95-APP1 helps cells form protrusions that are essential for crawling. Defective mutation of the ArfGAP domain, however, causes the protein to accumulate at the recycling endocytic compartment. It is bound there by the Rac exchanging factor

PIX, made available when Rac is activated. Instead of being dynamic and mobile, fibroblasts are quiescent when p95-APP1 is hung up on Rab11-positive recycling endosomes.

In primary neurons, neuritogenesis is strongly inhibited when ArfGAP mutants are expressed and subsequently accumulate at the Rab11 compartment. Endocytic markers crowd into the affected recycling compartment along with ArfGAP mutants. In order for neurite extension to occur, p95-APP1 is required along with a cycling Arf6. Characterization of endogenous proteins indicates that the p95 complex may exist as large oligomers, and that a significant fraction of the endogenous complex is stably associated to cellular membranes. When p95 constructs are labeled with green fluorescent protein, time-lapse analysis shows a clear functional connection between the localization of the PIX/p95-APP1 complex at recycling endosomes and Rac-dependent motility. Dr. deCurtis concludes that by organizing the function of distinct small GTP binding proteins, p95-APP1 coordinates membrane traffic with cytoskeletal dynamics and adhesion.

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<p align=""left"">High-throughput RNAi Screens in Drosophila Cells

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<p align=""left"">Most of what geneticists have learned from Drosophila is derived from studying genes individually or in small clusters. Today, of course, the complete genetic sequence of this venerable model organism has been deciphered, along with those for yeast, C. elegans, mice, humans, and many other creatures. This makes it possible not only to survey whole genomes at once, but also to compare them using the principles of evolutionary conservation and to gain new insights into the functional organization of cellular pathways. In the post-genome era, it is crucial to develop comprehensive methods for analyzing gene functions rapidly and systematically, Dr. Perrimon said.</p>

Last year, Dr. Perrimon launched a new facility that uses RNA interference to make this idea a reality: the Drosophila RNAi Screening Center (DRSC) at Harvard Medical School. Supported by the National Institute of General Medical Sciences, the Howard Hughes Medical Institute, and HMS, the facility can perform genome-wide screens for any researcher in the world at a very low cost, he told symposium participants. (Information about application procedures and data sharing agreements can be found at http://flyrnai.org)

Whole-genome screens are made possible by a library of 21,000 double-stranded RNAs (dsRNAs) that the Harvard team generated in collaboration with researchers at Heidelberg University in Germany. These dsRNAs span all 16,000 Drosophila genes, and each degrades the messenger RNA for a specific gene. Cultured fruit fly cells are arrayed in 384-well plates, the dsRNA library is applied, and it is possible to see what happens to cells when individual proteins are erased. A robotic system makes it possible to complete a full-genome screen in three to five days, Dr. Perrimon said.

So far, the DRSC has performed at least 16 whole genome screens for six different research groups, many seeking to understand signal transduction but others exploring adhesion, cytokinesis, phagocytosis, and other cellular processes. All the screens yielded unexpected functional information. When Dr. Perrimon and his colleagues used the screening system to explore the wingless signaling pathway, it picked up 15 of 17 previously identified components and more than 300 additional genes that may be part of the same chain of events, he reported. Because the human homologue of wingless is Wnt-1 this finding could have important implications for cancer biology.

One of Dr. Perrimon's goals is building a database of functional information that can be used to improve annotation of the Drosophila genome and interpret information in other datasets. Genes with similar RNAi signatures are likely to have related functions, and it may be possible to predict novel gene functions by examining clusters of such genes. His team is also creating dsRNA chips suitable for screening thousands of small molecules as potential drugs.

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<p align=""left"">Haploinsufficiency of the Hmga1 Gene Causes Cardiac Hypertrophy and B-cell Lymphomas in Mice: Tissue-dependent Oncogenic and Anti-oncogenic Effect of Hmga1 

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<p align=""left"">The high mobility group A1 (HMGA1) protein plays important roles in determining chromatin architecture and regulating transcription of several genes. High levels of this protein are hallmarks of rapidly dividing cells in embryonic tissue and many human malignancies, including tumors of the thyroid, colon and rectum, pancreas, and uterus. To show that HMGA1 is a culprit, not an innocent bystander, in malignant transformation, Dr. Fusco's group blocked HMGA1 synthesis in rat thyroid cells. Thus altered, the cells stubbornly resisted transformation when dosed with murine retroviruses that typically cause uncontrolled proliferation.</p>

In separate experiments, the researchers used an adenovirus vector to deliver an antisense version of the Hmga1 gene to anaplastic human thyroid carcinoma cell lines, where it induced apoptotic cell death. The same construct had no effect, however, on normal thyroid cells.

For the past several years, Dr. Fusco's team has been developing transgenic mice with disruptions in Hmga1 gene function as a means for learning more about how the HMGA1 protein functions in embryogenesis and carcinogenesis. They created two types of knockout mice, and both homozygous and heterozygous Hmga1-null mice exhibited cardiac hypertrophy. This suggests that HMGA1 is needed for proper growth and development of the heart, said. Loss of Hmga1 expression also reduced insulin receptor expression in target tissues and dramatically reduced insulin secretion, resulting in a phenotype that closely resembles human type 2 diabetes. A small, separate study found low HMGA1 levels in three type 2 diabetes patients, two with mutations and one with a deletion, Dr. Fusco said.

Hmga1 -/- and Hmga1 +/- mice also developed B-cell lymphomas, indicating that the Hmga1 gene has anti-oncogenic properties and probably plays a crucial role in controlling B-cell proliferation. Conversely, transgenic mice engineered to overexpress Hmga1 developed NK-T cell lymphomas and pituitary adenomas. Taken together, these experiments with knock-out and transgenic mice suggest HMGA1 proteins possess a Dr. Jekyll and Mr. Hyde duality that enables them to promote or suppress malignancy, depending on their cellular context.

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<p align=""left"">A Tumor Host Range Selection Procedure Identifies Sal2 as a Target of the Polyoma Virus Large T Antigen with a Possible Role in Human Ovarian Cancer

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<p align=""left"">Identifying cellular targets that oncogenic viruses use to transform cells is not an academic exercise. On the contrary, drugs that hit these targets may be able to treat cancers that have nothing to do with viral triggers, Dr. Benjamin said. Consider the classic experiments with SV40, which pointed to p53 as a key player in many human cancers. In Dr. Benjamin's lab, years of experience with the highly oncogenic polyoma virus–mouse host system led to a novel method for discovering new cellular proteins that the virus must engage in order to replicate. Although polyoma virus's large T antigen is widely known to bind two major tumor suppressors, Rb and p53, Dr. Benjamin suspected the virus had other ways to promote cell growth and thwart apoptosis.</p>

For the past several years, his team has been using "tumor host range" selection to identify multiple cellular proteins sought by the large T antigen. These include tumor suppressors, cell cycle regulators, effectors of apoptosis, and other factors whose functions are lost or altered in non-viral, human tumors. The Sal2 homeotic transcription factor, p150Sal2 , is one of the most interesting proteins singled out by this method. High levels of Sal2 expression are normally found in nuclei of ovarian epithelial cells, where most human ovarian cancers arise. When Dr. Benjamin's team scrutinized clinical samples supplied by collaborators at Brigham & Women's Hospital and the Dana-Farber Cancer Institute, they found that Sal2 expression is lost in many of these.

In the May 2004 issue of Molecular and Cellular Biology, Dr. Benjamin and his colleagues describe how p150Sal2 affects the growth and survival of ovarian carcinoma (OVCA) cells deficient in this protein, compared with normal human ovarian surface epithelial (HOSE) cells with abundant expression. In OVCA cells deficient in p150Sal2 , inducing transient expression of exogenous p150Sal2 inhibited DNA synthesis and colony formation and boosted apoptosis. Stable transfection of these same cells, which boosted p150Sal2 to physiologic levels, resulted in fewer tumors and higher levels of p21WAF1/CIP1 (p21) and BAX. In normal HOSE cells, however, reducing endogenous levels of p150Sal2 caused p21 expression to fall and DNA synthesis to increase. Dr. Benjamin reported that p150Sal2 binds to the p21 promoter adjacent to the known p53 binding sites and stimulates transcription in the absence of p53. From this, he concludes that p150Sal2 is an independent regulator of growth and survival in some cell types.

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<p align=""left"">Biological Processes and Signaling Pathways involved in Ovarian Carcinoma

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<p align=""left"">Epithelial ovarian cancer (EOC) has a higher death rate than any other gynecologic disease, in part because most cases are not detected until the cancer is advanced. Once detected, the course of disease is variable and clinicians must decide what therapies to recommend for each patient. Unfortunately, these decisions are difficult because traditional histopathology often can't identify tumors that merit more aggressive treatment.</p>

In hopes of improving clinical decision-making, Dr. Schneider's group is using a computer-driven, high-throughput screening approach to classify EOCs based on their molecular signatures. His laboratory collected 81 samples of EOC tissue, 46 from women with stage III-IV tumors. They used cDNA microarrays to see how approximately 10,000 genes are expressed in these samples, then subjected that data to automated class discovery using ISIS, he said. This bioinformatic approach picked out a subset of tumors characterized by expression of gene clusters related to the extracellular matrix (ECM) and its remodeling, and to the fibroblast growth factor 2 (FGF2) signaling pathway.

When this molecular signature was compared with well-studied cell lines derived from ovarian tissue, it matched gene expression patterns typical of mesenchymal-like cells. The ECM-FGF2 signature identified by automated analysis in the Schneider lab match previously published expression profiles for ovarian cancer and for a type of colon cancer, he said. The same signature appears to relate to cellular plasticity and mesochymal maintenance, and Dr. Scheider predicts that additional research will demonstrate its value as a “clinically relevant classifier” for ovarian cancer.

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<p align=""left"">Genetic Analysis of Susceptibility to Infectious Disease

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<p align=""left"">Faced with an organism that is difficult to grow and hard to investigate by studying isolated mutations, researchers must devise other ways of learning how it operates. Chlamydia trachomatis is one of these, an obligate intracellular bacterial pathogen responsible for human pathologies including trachoma, pneumonia, a host of urogenital syndromes (including infertility), and invasive lymphadenopathic disease. It is a major cause of blindness in some parts of the world, and may be the most common sexually transmitted bacterial disease known, Dr. Dietrich said. While effective treatment is available to some people in some places, chlamydial maladies continue to spread and control is unlikely unless safe and effective vaccines and more affordable treatments can be developed.</p>

Dr. Dietrich's research team is investigating how the host immune system defends against chlamydia, why individual susceptibility to infection varies, and what this reveals about the nature of the pathogen. Different inbred strains of mice have heritable differences in resistance and exhibit distinct courses of chlamydial infection, and Dr. Dietrich's lab is using genetic manipulation to explore differences in their innate immunity.

For example, C3H/HeJ (C3H) mice allow greater chlamydial replication and/or survival during the early stages of a systemic infection than C57BL/6J (B6) mice do. Dr. Dietrich's team used quantitative PRC assays to show that within the first several hours of infection, the C3H and B6 mice had about the same bacterial load; after 16 hours the amount of bacteria increased markedly in the C3H animals. In the long term, the C3H animals remained chronically infected for several weeks, while the B6 mice cleared the infection after12 days. These observations suggest that differences in chlamydia resistance are not related to immune responses during initial colonization, but to later reactions to bacterial replication and survival, Dr. Dietrich said.

His team crossed the two strains of mice and found a wide range of susceptibility to chlamydia infection in 169 F2 animals. Mapping studies revealed three quantitative trait loci (QTL) that have high LOD scores (approximately 4.5) and account for an estimated 10% of variance. QTLs on chromosomes 2 and 11 appear to be C3H susceptibility alleles, while chromosome 3 carries a resistance allele from B6 mice. Recent experiments are using positional information as stepping

stones toward a molecular explanation for susceptibility differences, and although interesting hypotheses have taken shape, Dr. Dietrich said, the full story is yet to be told.

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<p align=""left"">Recruitment and Reprogramming of Innate Immunity Cells in Tumors

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<p align=""left"">Two decades ago, the study of inflammation’s role in cancer was accelerated by the seminal observation that many types of tumors actively recruit leukocytes and macrophages as they grow. Further research showed that cytokine levels rise as the population of tumor-associated macrophages (TAM) increases, drawing even more TAMs to the scene. The prognosis for the patient depends on what happens to the macrophages in the tumor microenvironment.</p>

This is what interests Dr. Sica, who is using a genomic approach to study the activities of cytokines and chemokines that not only lure macrophages to tumors, but may also determine where they are positioned and how they may be reprogrammed to favor disease progression. Activated macrophages that would typically process antigens via the MHC Type I pathway are highly plastic cells, and Dr. Sica says that they shift toward a Type II phenotype in response to microenvironmental signals inside the tumor. He bases this claim on whole genome transcriptional analysis using cDNA and RNA chips, which provide a new look at the functional properties of TAMs.

Macrophages flock to oxygen-poor regions of tumors, where hypoxia raises CxCr4 receptor expression, which in turn recruits more macrophages and promotes angiogenesis, Dr. Sica reported. In breast cancers, hypoxia-induced factor (HIF1) increases response to VEGF and promotes tissue remodeling and repair. This not only increases the likelihood that tumor cells will survive, but also spurs development of new blood vessels that nourish the tumor, he said. These events signify a worsening prognosis for the patient.

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<p align=""left"">Errors in Chromosome Segregation Induce Binucleation by Inhibiting a Late Step in Cytokinesis

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<p align=""left"">Mitotic aneuploidy is associated with almost all solid tumors, and many cancers exhibit both numerical and structural changes in chromosomes as well as abnormalities in centrosome number. Although the reasons for aneuploidy in these cells is poorly understood, it is known that the accumulation of extra centrosomes favors the formation of multipolar spindles during mitosis, which in turn makes chromosomes less stable, Dr. King noted.</p>

The main thrust of the King lab's work is developing chemical approaches for studying complex biological phenomena. At the Symposium, he focused on using chemical means to interfere with normal chromosome segregation, in hopes of understanding how errors in the sorting of genetic material might be coupled with subsequent defects in mitosis. The researchers treated HeLa cells and telomerase-immortalized keratinocytes (N-tert1 cells) with a chemical that increases missegregation, then used fluorescent in situ hybridization (FISH) to measure the rate of chromosome missegregation during mitosis, Dr. King said. They also measured the rate of missegregation in spontaneously arising binucleated cells.

Binucleated cells from both cell lines missegregated chromosomes at rates 30- to 50-fold higher than other cells in the same population. Evidence that the two phenomena are closely intertwined comes from experiments where the same chemical boosts rates of missegregation and binucleation in the same cell population. The researchers set up a photomicroscopy experiment that photographed various populations of mitotic cells every 20 minutes for 62 hours. Across the board, measured rates of binucleation and rates of nondisjunction estimated by FISH were almost identical.

Dr. King's time-lapse studies show that the binucleated cells are on track for most of mitosis, and that problems arise in the final phase, when instead of breaking apart at the cleavage furrow and forming daughter cells, the furrow regresses and abscission never occurs. In cells like these, rife with segregation errors, Dr. King speculated that a checkpoint may block abscission and thus keep highly defective daughter cells from being born. The trade-off is that tetraploid cells with an otherwise normal karyotype are formed, and their excess centrosomes may favor formation of multipolar spindles during subsequent mitosis, setting the stage for chromosome instability. Dr. King's findings suggest that even single nondisjunction events could increase the frequency of chromosome instability by stimulating the generation of tetraploid cells.

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<p align=""left"">Prion-like Properties of the Spindle Checkpoint Protein Mad2 are Required for Its Signaling Function

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<p align=""left"">Timing is everything in mitosis, as in many aspects of life. During anaphase, chromosomes pull apart and new chromatids are drawn slowly toward the spindle poles at either end of the cell. It takes time for the chromosomes to segregate properly, and the mitotic spindle checkpoint is a safety device that aims to prevent the cell from leaving anaphase until all the replicated chromosomes are in their proper places. If this safety device fails and anaphase ends prematurely, then aneuploidy and genetic loss can result. Dr. Musacchio's lab uses structural and biochemical approaches to characterize and understand the dynamics of the mitotic spindle checkpoint (MSC). His team is especially interested in the shape-shifting abilities of Mad2, an important MSC protein that circulates in the cytoplasm in an open conformation (O-Mad2), then switches to a closed conformation (C-Mad2) when it binds to its putative receptor, Mad1, or to another key checkpoint protein, Cdc20.</p>

Mad1 has been widely regarded as the Mad2 receptor at the kinetochore. Now, this view is challenged by data from Dr. Musacchio's laboratory showing that an O-Mad2 mutant of Mad2 (Mad2[illegible]C) that is incapable of interacting with Mad1 is nevertheless recruited to the kinetochore and on arrival binds with some previously unknown structure. Because Mad1 and Mad2 form a tight complex that locks Mad2 in the C-Mad2 conformation, Dr. Musacchio asked whether the Mad1-Mad2 complex, rather than Mad1 alone, acted as the O-Mad2 kinetochore receptor.

Results from in vitro and in vivo experiments support this hypothesis. The researchers discovered that O-Mad2 and C-Mad2 form a tight complex that recruits additional O-Mad2 to the kinetochore. Additional experiments showed that in order for the checkpoint signal to be propagated and sustained, both conformationally distinct monomers of Mad2 – the open and closed forms – must be on hand for dimerization. This highly dynamic system also requires Mad1, which acts as a docking site for C-Mad2 at the kinetochore and is essential for formation of the Mad2-Cdc20 complex. Dr. Musacchio also suggested that Mad1-bound C-Mad2 is a structural template for the conversion of cytoplasmic ligand-free O-Mad2 into Cdc20-bound C-Mad2, and that the latter acts as a structural copy of Mad1/Mad2 designed to elicit further conversion of O-Mad2 into C-Mad2 away from kinetochores, leading to signal amplification.

There could be important clinical benefits to understanding this buzz of activity. For example, many aneuploid breast cancers exhibit MSC defects and considerable chromosomal instability, so that two cells from the same tumor may have different numbers of chromosomes. The widely used drug Taxol damages the spindle and arrests mitosis, which causes the cell to die. Sorting out the busy inner life of the MSC could pave the way to new cancer treatments.

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<p align=""left"">Chemical Definition of a Novel Cell Death Pathway

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<p align=""left"">According to the conventional wisdom, brain cells die in one of two ways. The first, apoptosis, eliminates cells at appropriate points in normal development, or kills them in a less beneficial way if the brain is damaged suddenly by trauma or stroke, or slowly by chronic

neurodegenerative disorders such as Alzheimer's. Apoptosis is regulated by highly conserved mechanisms that depend on caspases. The second, necrotic cell death, is a catastrophic reaction to overwhelming stress that is independent of caspace activity. Sometimes the two types of death occur in tandem: in ischemic stroke, necrosis immediately kills cells in the brain region abruptly deprived of blood; apoptosis spends days killing cells surrounding the infarct.</p>

Now, experiments in Dr. Yuan's lab indicate that some brain cells die in a third way that she calls "aponecrosis." She first saw evidence for this when cells were stimulated with a strong apoptotic signal, treated with a reliable caspace inhibitor, and died when she expected them to survive. Her hypothesis is that aponecrosis is a novel type of cell death that occurs only after caspace has been inhibited, either by chemical or natural means.

Dr. Yuan used the resources of the Harvard Institute of Chemistry and Cell Biology (ICCB) to learn more about aponecrosis using a small molecule approach. As a result of screening approximately 100,000 compounds from the ICCB's library, her team identified 9 classes of possible aponecrosis inhibitors. Of these, a chemical known as U4 appeared to block aponecrosis, without having any impact on caspase-dependent apoptosis or on necrosis triggered by oxidative damage. There is preliminary evidence that U4 and two closely related compounds might rescue dying cells by boosting mitochondrial function.

The clinical implications of this work were apparent in experiments Dr. Yuan carried out with long-time collaborators at Massachusetts General Hospital. In a mouse model for stroke that they pioneered more than a decade ago, the most promising compound, U2, delayed ischemic brain damage in a dose-dependent manner when injected directly into the ventricle. Dr. Yuan's team has since developed more than 200 variations of this chemical, in hopes that some will be suitable for development as more easily administered drugs. Doctors would welcome a treatment that gives them more time to dissolve or remove blockages responsible for ischemic stroke.

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<p align=""left"">HIPK2: A New Pro-apoptotic Oncosuppressor?

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<p align=""left"">In his keynote address to the Symposium, Pier Giuseppe Pelicci called attention to HIPK2 as a serine kinase with a newly recognized role in apoptosis. Dr. Soddu told the HIPK2 story in much greater detail. This kinase binds to the p53 oncosuppressor, promoting its acetylation and localization in the nuclear bodies, and phosphorylating Ser46. This enables p53 to promote transcription of other pro-apoptotic genes. HIPK2 also acts on other targets involved in programmed cell death, including p53's relative, p73, the transcriptional co-repressor CtBP, and the p53 inhibitor MDM2.</p>

Because HIPK2 appears so active in apoptosis regulation, Dr. Soddu asked whether creating a shortage of HIPK2, in cells equipped with normal p53, would allow damaged and potentially malignant cells to escape death. This is an important question, because p53-related defects in

apoptosis not only contribute to tumor formation but also can make tumors less responsive to radiation and chemotherapy.

Dr. Soddu used tumors arising from thyroid follicular cells to explore the interplay of HIPK2 and p53. These tumors are usually well-differentiated thyroid carcinomas (WDTC), although a small subset of aggressive, anaplastic carcinomas exhibit mutations in the TP53 gene. As their experiments proceeded, the researchers began to suspect that wtp53 activity is also impaired in less aggressive WDTCs.

Suspicions were aroused by some seemingly paradoxical observations about these tumors. Over-expression of galectin-3, an anti-apoptotic lectin that enhances survival of WDTC cells, is such a reliable marker for these tumors that doctors routinely use it for diagnosis. Given that p53 is known to repress galectin-3 transcription, Dr. Soddu's group was surprised to find both wtp53 and galectin-3 expressed in the cells they were studying.

What did HIPK2 have to do with this? When researchers analyzed clinical samples of WDTC, they found that at least one copy of HIPK2 had been lost in 37.5% of tumors. Further research, including using RNA interference to knock out HIPK2's function, confirmed that this serine kinase plays an important role in tumor suppression. Dr. Soddu concluded that HIPK2 deficiency interrupts a newly identified apoptotic pathway triggered by p53 and involving galectin-3. Her team is presently exploring the possibility that HIPK2 may also be a tumor suppressor on its own, possibly by acting directly on galectin-3.

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<p align=""left"">Cytogenetic Approaches to Gene Discovery in the Genome Era

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<p align=""left"">For many years, the search for chromosomal abnormalities began with extracting metaphase chromosomes from a patient's lymphocytes, arranging the chromosomes in a traditional karyotype on a slide, and then peering through a microscope. This was a painstaking slow process and even the keenest observers sometimes overlooked gene defects that might offer important insights into congenital anomalies, developmental delays, and cancer.</p>

The Human Genome Project has dramatically shortened the interval between realizing that a chromosomal abnormality exists and discovering what genes are responsible for the patient's developmental anomalies or his cancer. Dr. Morton's presentation focused on two efforts that are accelerating the discovery of pathogenic genes by combining genome sequence data with new cytogenetic technology. Both use chromosomal breakpoints to chase down genes: the Developmental Genome Anatomy Project (DGAP, http://dgap.harvard.edu) enrolls patients with congenital abnormalities, while the Tumor Genome Anatomy Project (TGAP) searches out cancer

genes. Both add annotations to the human genome sequence while uncovering pathways that might otherwise have gone unnoticed.

Both DGAP and TGAP use high-throughput methods for processing patient samples: the researchers use FISH (fluorescent in situ hybridization) studies to localize chromosomal breakpoints, then clone these breakpoints and identify candidate genes. The DGAP team uses model organisms to identify the functions of these genes, and applies this information to the interpretation of clinical findings. TGAP takes a slightly different tack, using microarrays to assess copy number and gene expression, then incorporating that data into outcomes correlations.

Both projects are already highly productive, Dr. Morton reported. DGAP has processed samples from 145 patients with congenital abnormalities: about one-third of these have been mapped using FISH, 13 breakpoints have been cloned and 6 have been sequenced. Patients with severe phenotypes often turn out to have small deletions or insertions, many of them in introns. About two dozen of these disruptions occur in known genes, among them DGKD, which is implicated in a seizure phenotype and could be the key to understanding a complex genetic disorder. Knockout mice are being created for functional testing of seven especially promising genes, Dr. Morton said.

Although TGAP is a newer effort than DGAP, its methods have already yielded insights into many cancer-related cytogenetic abnormalities. An important methodological difference between the two is that the microarrays TGAP uses for comparative genomic hybridization (CGH) can pick out tumors that have accumulated high levels of abnormal DNA. This enabled Dr. Morton's team to define a subgroup of chronic lymphocytic leukemia patients who carry a specific deletion and have an especially poor prognosis. This is a promising example of how molecular cytogenetics might be used to target cancer treatments in the future, she said.

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<p align=""left""> Functional Onco-Genomics and Tumor Progression

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<p align=""left"">The traditional approach to biomedical discovery was to start with a gene, then look for its function. This has been a slow process, as evidenced by the fact that 30 years of molecular biology has identified the functions of only about one third of all known genes. The availability of the human genome sequence and the advent of microarrays turns this traditional approach on its head, making it possible to systematically explore the functions of thousands of previously unknown genes at once. Answers are faster to come by, and sometimes they have great clinical potential.</p>

By applying two different microarray technologies to the same set of tumor samples, Dr. Medico has not only identified hundreds of genes involved in human cancers, but also described expression patterns that appear to predict poor outcomes for patients with breast cancer and possibly liver

tumors as well. This sort of information could help physicians do a better job picking the right treatments for individual patients.

This work grew out of Dr. Medico's long association with Dr. Paolo Comoglio (See "Invasive Growth: A Genetic Program."). They have explored how the binding of Hepatocyte Growth Factor (HGF) to receptors in the Met superfamily unleashes a series of events that enables cancer cells to scatter, grow, and metastasize to other parts of the body. In this latest project, Dr. Medico set out to define the full transcriptome of mouse liver cells exposed to HGF and Epidermal Growth Factor (EGF). EGF was chosen for comparison because in these cells it does not elicit the full-blown invasive growth phenotype stimulated by HGF. Dr. Medico employed two commercially available microarray platforms: high-density spotted cDNAs (Incyte) and in situ synthesized oligonucleotides (Affymetrix).

By examining how data from the two platforms overlapped, the researchers pinpointed 257 genes that were likely to be important players in invasive growth, Dr. Medico said. To determine how relevant this invasive signature is to the metastatic propensity of human cancer, they used bioinformatics to compare their results with a breast cancer dataset published in Nature in 2002. This set contains microarray data for primary tumors removed from 78 breast cancer patients, 34 of whom developed metastatic cancer within five years. Dr. Medico's analysis showed that 211 of the HGF signature genes appeared in the breast cancer dataset, but none were individually correlated with poor prognosis. Taken together, however, 20 of the genes that Dr. Medico identified appear to act together as a metagene that defines a subgroup of patients with an 85% probability for metastasis within 2.5 years (p<0.001). More recently, he found evidence that an invasive signature identified in his laboratory correlates well with microarray data that other researchers gathered for 22 primary liver tumors, and this signature may also help predict clinical outcomes.

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<p align=""left"">The Role of Histone H2AX in the DSB Response and in Suppression of Translocations Involving Aberrant V(D)J or Class Switch Recombination

Frederick W. Alt

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<p align=""left"">Histone H2AX is a variant of H2A, one of four core histones in the nucleosome of eukaryotic cells. During the S-phase of the cell cycle, nucleosomes must be assembled at high speed to ensure proper chromatin packaging. In the vicinity of double-strand breaks in DNA, histone H2AX is rapidly phosphorylated by ATM and other kinases during V(D)J recombination. This phosphorylation event facilitates the repair of double-strand breaks (DSBs) via nonhomologous end joining (NHEJ), one of two main mechanisms for repairing such damage. Left untended, DSBs dramatically increase genomic instability and the likelihood that cells will become malignant.</p>

Dr. Alt and his colleagues used a gene-targeting vector to study H2AX. They found that while H2AX-deficient mice exhibited a modest incidence of thymic lymphoma, mice deficient for both H2AX and the p53 tumor suppressor (H2AX/p53-/-) had greatly increased tumor onset, including lymphomas and solid tumors. H2AX/p53-/- thymic lymphomas harbored a variety of chromosomal translocations likely initiated during rapid progenitor expansion. In contrast, several H2AX/p53-/- progenitor-B lymphomas had translocations, linking amplified IgH and c-myc sequences, which are diagnostic of aberrant V(D)J recombination. H2AX-haploinsufficiency in mice, coupled with p53 deficiency, caused genomic instability in normal lymphocytes and increased predisposition to various tumors,

including B cell lymphomas with translocations apparently involving aberrant IgH class switch recombination, Dr. Alt said.

While the precise role of H2AX in suppressing translocations is still speculative, Dr. Alt proposed that this histone variant might suppress S phase-generated translocation for several types of DSBs by holding the broken ends in close proximity until a team of enzymes can knit them together. After being phosphorylated by ATM, H2AX may accomplish this by joining forces with NBS1 (a component of the MRE11/RAD50/NBS1 complex), 53BP1, and other factors downstream of ATM. Supporting the concept of a team effort is the clinical observation that mutations in ATM, NBS1, and MRE11 are often found in patients with lymphoid malignancies characterized by chromosomal translocations. Cytogenetic studies show that H2AX maps to a region that is frequently altered in human tumors, Dr. Alt said, raising the possibility that it may function as a tumor suppressor in humans.

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<p align=""left"">Cell Cycle Inhibition and Cancer Therapy

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<p align=""left"">Many well-known cancer drugs work by arresting the progression of the cell cycle and inducing cell death; among these are taxanes, 5-fluorouracil, and anthracyclines. Unfortunately, these drugs have limited efficacy because tumors often have primary or acquired resistance to their actions. As a result, many laboratories around the world are seeking novel candidate drugs that act on specific regulatory mechanisms of the cell cycle. Dr. Draetta's laboratory is focused on understanding cyclin-dependent kinases and Aurora kinases, both of which are disregulated in cancer. His presentation briefly summarized three basic projects in his lab, all of them related to the quest for better anti-cancer therapies.

Cell cycle regulation depends heavily on epigenetic events such as phosphorylation, acetylation, and sumoylation. Researchers in the Draetta lab have shown that an early gene product made by adenovirus, called Gam 1, inactivates histone deacetylase 1, possibly by acting on the SUMO-1 pathway. They have shown that Gam 1 destroys promyelocitic leukemia bodies, although Dr. Draetta said that there is much more to be learned about why this happens.

A second major project focuses on how inactivation of the Cdc25A phosphatase in S-phase affects checkpoint responses. Cdc25A is an unstable protein, and when DNA damage occurs during mitosis it is hyperphosphorylated by checkpoint kinases and rapidly degraded, which arrests the cell cycle and prevents genome instability. When the researchers used short interfering RNAs to disrupt expression of beta-TrCp1 and beta-TrCp2, two proteins essential for Cdc25A degradation, Cdc25A levels remained high and the cell cycle moved forward. This is part of a signaling cascade that may be disrupted in certain cancer cells, Dr. Draetta said.

His lab is also working to identify specific protein kinases that may be attractive targets for drug development. Dr. Draettta's team screened for protein kinases associated with tumors of colon, breast, and lung, and identified Aurora kinases 1 and 2 as potentially "drugable" targets. RNA interference experiments support this idea, showing that when Aurora 1 and 2 are disabled, caspase 9 is activated and apoptosis occurs. In collaboration with a biotechnology company, his laboratory has identified several candidate Aurora 1 and 2 inhibitors that are being studied now.</p>

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<p align=""left"">Investigation of an Unusual Transcriptional Co-Repressor Complex

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The roster of cellular proteins targeted by viral oncoproteins is a long one, Dr. Shi said by way of introduction. For example, adenovirus E1A targets some proteins involved in the G1- and S-phases of the cell cycle, interacts with others involved in differentiation and proliferation, and induces oncogenic transformation by targeting a number of central transcriptional cofactors including CtBP (C-terminal Binding Protein). CtBP was first discovered more than a decade ago, and since then genetic studies have shown that it has a critical function in animal development as well as a role in tumor formation. CtBP possesses dehydrogenase activity and acts as a transcription co-repressor; the latter due to its ability to interact with the PXDLS motif that many transcription factors contain.

As well known as those features of CtBP have become, mysteries persisted: What exactly takes place after this protein is recruited to DNA? And how does it contribute to tumorigenesis? The answers to those questions, Dr. Shi discovered, are related to a CtBP complex that he says “is as well-equipped as a Swiss Army knife – it has all the tools.” When his lab identified this complex and then began to tease it apart for examination, it turned out to contain the essential components for both gene targeting and coordinated histone modifications, Dr. Shi said. This allows for the effective repression of CtBP target genes.

When the researchers used RNA interference to inhibit expression of CtBP and its associated histone-modifying activities, they observed histone modifications at the promotor of the tumor suppressor gene E-cadherin and increased promoter activity in a reporter assay. These findings identify a novel molecular mechanism by which CtBP mediates transcriptional repression, Dr. Shi said, and provide new insight into CtBP’s participation in.

The CtBP complex is also highly unusual because it contains six potential enzymes, including ones that share significant homology with important metabolic enzymes such as dehydrogenase (CtBP), aminoxidase (nPAO) and enoyl-CoA hydratase (CDYL). CtBP and nPAP can bind NAD and FAD, respectively, which are the two most important electron carriers in catabolic reactions. This suggests that the CtBP complex may be a direct sensor of the cell’s metabolic state, Dr. Shi said. His lab is currently exploring the connection between the CtBP complex and metabolism in normal and pathologic settings.";"