Translating Science into Survival
Behind the Cover
The scientific image on the cover is a scanning electron microscopic image of a living T lymphocyte. Once cells have served their biological function, they are destined to die and be replaced by new cells to ensure the continued well-being of the organism. The recognition and removal of dying or dead cells are regulated through multiple cell death pathways that have evolved to provide a survival advantage to organisms. If one cell death pathway is blocked, another will take its place to remove the debris and ensure the health of surrounding tissues. Douglas R. Green, PhD (Immunology), and his colleagues are investigating the molecular events that drive various types of cell death. Their goal is to characterize the mechanisms underlying different forms of cell death to determine their significance during normal development and disease.
AT ST. JUDE, SCIENCE MATTERS. FROM MOLECULAR BREAKTHROUGHS TO INNOVATIVE THERAPIES, OUR RESEARCHERS ARE DISCOVERING THE CURES OF TOMORROW AND SAVING CHILDREN’S LIVES TODAY.
TRANSLATING SCIENCE INTO SURVIVAL

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When St. Jude Children’s Research Hospital opened its doors 55 years ago, the word “cure” was not part of the conversation. However, today we are doing what others cannot—we are leading the world in treating and curing childhood cancers and other catastrophic diseases. We have developed curative treatments for leukemia and medulloblastoma and increased survival rates for patients with other cancers or life-threatening blood disorders. Initiatives such as the Pediatric Cancer Genome Project have also helped pave the way for the development of new precision-medicine approaches.

In this Scientific Report, we describe the medical and scientific advances made last year. In the first article, we describe recent work by immunology researchers who are deciphering the mechanisms that underlie cell death. This process, essential for normal development and maintenance of organisms, could also be harnessed to fight cancer. The second feature presents recent discoveries of new pediatric brain tumor subtypes. Designing molecularly targeted treatments specifically for these subtypes holds promise for improving patient outcomes and decreasing long-term, adverse side effects. The third story describes the role of transcription factor deregulation in the development of pediatric leukemia and solid tumors. The fourth feature conveys exciting breakthroughs in gene therapy research. Hematology researchers are working with scientists in the Children’s GMP, LLC, at St. Jude to develop safe, effective gene therapy to permanently repair single-gene mutations that cause catastrophic diseases such as severe combined immunodeficiency syndrome and sickle cell disease.

Beyond the work highlighted in this report, the institution saw progress last year in clinical, research, and administrative operations. On the clinical front, the St. Jude Red Frog Events Proton Therapy Center marked a milestone—treating 125 patients with brain or other solid tumors. We advanced the standards for delivering pediatric care by opening three state-of-the-art inpatient units in the Kay Research and Care Center. The units ensure a seamless delivery of care and provide families with comforting, supportive accommodations. In addition, we created the Patient and Family Experience Office, which focuses on optimizing families’ experiences in the hospital and our housing facilities. Our eighth St. Jude Affiliate Clinic opened at The Children’s Hospital at Saint Francis in Tulsa, Oklahoma, thereby extending St. Jude’s national reach and offering more children care closer to their homes. St. Jude also bolstered its international reach by recruiting new faculty and staff to the Department of Global Pediatric Medicine and laying the groundwork for St. Jude Global, a new program aimed at increasing survival rates for all children with cancer or nonmalignant hematologic disorders, regardless of where they live.

Garnering the spotlight for St. Jude on the national research stage, Leslie Robison, PhD (Epidemiology & Cancer Control), was awarded the 2016 American Cancer Society Medal of Honor; Paul Northcott, PhD (Developmental Neurobiology), was named a Pew-Stewart Scholar for Cancer Research; and I served on the Blue Ribbon Panel advising Vice President Joe Biden’s Cancer Moonshot Initiative through the National Cancer Institute. The hospital also welcomed the following new leaders: James Morgan, PhD, scientific director; Ellis Neufeld, MD, PhD, clinical director and physician-in-chief; and Michael Dyer, PhD, chair of the Department of Developmental Neurobiology.

We began construction of a three-story, 55,000-square foot data center that will house the institution’s advanced scientific computing infrastructure and support resources. This innovative facility will be completed later this year. We accepted applications to the St. Jude Graduate School in Biomedical Sciences. The inaugural class, representing the next generation of scientists who will aid in the discovery of improved treatments and cures for pediatric catastrophic diseases, will begin their studies in August 2017.

Finally, St. Jude was again ranked one of Fortune magazine’s “100 Best Companies to Work For.” Although the past year gave us much to celebrate, our work is not done. Looking forward to the next 55 years, St. Jude will continue to be bold and ambitious. We will chase big dreams, and we will pursue scientific and medical excellence. It is not only our legacy but also our future.
THE LIVES AND MANY DEATHS OF CELLS

Like all living things, cells die. The timely death of cells is necessary for the normal development and functioning of organisms. Aged cells are replaced with new ones, nonfunctional cells are eliminated, and wayward cells are destroyed before they can become cancerous.

The laboratories of Douglas R. Green, PhD (Immunology), and others at St. Jude are deciphering the core mechanisms underlying various forms of cell death and clarifying how the way in which a cell dies leaves a lasting influence on the living cells and tissues that surrounded it.

Douglas R. Green, PhD; Larissa Dias da Cunha, PhD
BASIC MECHANISMS OF PROGRAMMED CELL DEATH

In multicellular organisms, cells die for a variety of reasons. Dying cells are typically replaced by new cells to ensure the development, maturation, and continued function of the organism. Different forms of cell death have evolved. If one pathway is blocked, a different pathway can take its place, providing a survival advantage for the organism. However, different types of cell death also play different roles in the life of an organism. These modes of cell death are characterized by changes in a cell’s morphology and involve distinct and characteristic molecular pathways that provoke a cell’s demise.

Apoptosis, the most thoroughly studied form of programmed cell death, occurs by different mechanisms that converge on a set of enzymes that prompt a series of morphologic events, including cell shrinkage, chromatin condensation, and fragmentation into apoptotic bodies. These cell fragments are engulfed and removed by phagocytes (i.e., cells that “eat”).

 Necrosis is often an unregulated form of cell death caused by injury or disease, but it can also exist in regulated forms. Necroptosis resembles necrosis, but it is induced by receptor signaling. Receptor-interacting protein kinase-3 (RIPK3) is activated and initiates necroptosis. This promotes the formation of a “necrosome” complex that is essential for this form of cell death. Necroptosis plays a key role during viral infection by killing infected host cells and minimizing the severity of infection.

Pyroptosis, or caspase-1–mediated cell death, is believed to play a central role in local and systemic inflammatory responses. During pyroptosis, the cell swells rapidly and its outer membrane ruptures. This releases fever-inducing and other cytokines into the extracellular environment.

Autophagy has a key role in cell survival. During nutrient deprivation, a starving cell forms a large cytoplasmic vacuole in which it digests, in a controlled manner, some of its own cytoplasmic contents. Some evidence indicates that autophagy can promote cell death in specific settings, though it predominantly supports cell survival.

Figure. Scanning electron microscopy images of a living T lymphocyte (left) and a T lymphocyte undergoing apoptosis (right).

BOK-DEPENDENT APOPTOSIS MAY PROVIDE A NEW TARGET FOR KILLING CANCER CELLS

Apoptosis occurs primarily through a pathway that is dependent on mitochondria, the primary energy factories in cells. In the mitochondrial pathway of apoptosis, signals that trigger cell death converge on this organelle. These signals cause the mitochondria’s outer membranes to become leaky. Mitochondrial outer membrane permeabilization (MOMP) releases mitochondrial proteins, such as cytochrome c, that activate a set of enzymes called caspases. Caspases are proteases that, in turn, cleave hundreds of other proteins, thereby provoking cell death. Although caspases are the terminal effector molecules for apoptosis, the upstream process of MOMP is highly regulated and controlled by proteins of the BCL-2 family. BCL-2 family proteins are either proapoptotic or antiapoptotic, alternatively promoting or inhibiting MOMP and apoptosis.

Conventionally, MOMP has been thought to require one of two proapoptotic BCL-2 proteins, BAK or BAX. Fabien Llambi, PhD, a postdoctoral fellow working in Dr. Green’s laboratory, discovered that BAK and BAX are not always needed. The team described a new mechanism of MOMP and apoptosis that functions in the absence of these molecules. In a paper published in Cell, the investigators showed that many cells express another little-studied BCL-2 protein called BOK (BCL-2 ovarian killer). BOK can function independently of other BCL-2 proteins and, under normal circumstances, is rapidly degraded before it can bring about MOMP. However, signals that disrupt this degradation can induce MOMP and promote BOK-dependent apoptosis in the absence of BAK and BAX. Furthermore, BOK activity is not inhibited by the antiapoptotic BCL-2 proteins (BCL-2, BCL-xL, or MCL-1) that normally protect a cell from apoptotic signaling. Instead, BOK is regulated by an alternative set of proteins, VCP and gp78, that are involved in the endoplasmic reticulum–associated degradation pathway.

In many human cancers, mechanisms that would typically regulate and promote MOMP are blocked. Dysregulated malignant cells can thereby avoid apoptotic death, allowing them to persist and grow in the presence of cellular signals that normally would kill them. Because BOK is regulated independently of classical regulatory mechanisms, activating BOK or preventing its degradation should bypass the inhibition of MOMP and induce the death of otherwise protected cancer cells.

Figure. Illustration of canonical and noncanonical MOMP-mediated apoptosis. Reprinted from Cell,165, Llambi F et al, BOK is a non-canonical BCL-2 family effector of apoptosis regulated by ER-associated degradation, 421–33, © 2016, with permission from Elsevier.
SEQUENTIAL STRUCTURAL CHANGES IN MLKL INDUCE NECROPTOSIS

Necroptosis is a type of regulated cell death that contributes to host defense during viral infections and is distinct from apoptosis. During necroptosis, signals converge on the MLKL (mixed-lineage kinase domain–like) pseudo-kinase protein. Once activated, MLKL directly binds to and ruptures the plasma membrane, ultimately killing the cell. Giovanni Quarato, PhD, a postdoctoral fellow working in Dr. Green’s lab, collaborated with the laboratory of Tudor Moldoveanu, PhD (Structural Biology, Chemical Biology & Therapeutics), to explore how MLKL interacts with the cell membrane to induce necroptosis.

In the journal Molecular Cell, the authors reported the precise sequence of events that MLKL must undergo. Earlier structural studies had shown that MLKL contains a brace region that separates its two terminal domains. This brace mediates the oligomerization (or linking) of multiple MLKL molecules into a larger complex, which recruits more MLKL to the plasma membrane. The oligomerized MLKL then rolls to allow binding sites on the N-terminal domain to tightly interact with specific lipids and rupture the cell membrane.

Although MLKL is now recognized as the key mediator of the necroptosis pathway in isolated cells, its role in living organisms has remained unclear. Further studies by Christopher Dillon, PhD, another postdoctoral fellow in Dr. Green’s laboratory, in collaboration with the laboratory of Dr. Andreas Strasser at the Walter and Eliza Hall Institute of Medical Research (Melbourne, Australia), analyzed genetic defects that can cause necroptosis in developing mouse embryos. This work, published in Immunity, indicates that the necroptosis-associated kinase RIPK3, in addition to activating MLKL to cause cell death, has other functions. These include promoting lymphadenopathy (enlargement of lymph nodes and spleen) and immune pathways that can provoke autoimmune disease.
ZBP1 TRIGGERS THREE FORMS OF CELL DEATH AND THE INFLAMMATORY RESPONSE UPON INFECTION

Although initially identified as a DNA sensor that induces innate immune responses, the identification of ZBP1 (Z-DNA–binding protein 1, also known as DAI) as an innate sensor of influenza virus infection has been the subject of debate. Teneema Kuriakose, PhD, a postdoctoral fellow working in the laboratory of Thirumala-Devi Kanneganti, PhD (Immunology), investigated how ZBP1 functions during influenza virus infection by using cells isolated from wild-type mice or mutant mice lacking ZBP1.

In an article published in *Science Immunology*, the authors showed that ZBP1 actually senses the presence not of DNA but of two influenza virus proteins (NP and PB1). This binding activates the NLRP3 (NLR family, pyrin domain containing 3) inflammasome within the cell and prompts three distinct forms of cell death: apoptosis, necroptosis, and pyroptosis. Apoptosis and necroptosis kill host cells containing the pathogen, thereby destroying the virus’ ability to replicate and spread. In contrast, pyroptosis is activated by the inflammasome and functions in a protective manner during influenza virus infection.

RIPK3 ACTIVATES MULTIPLE CELL DEATH PATHWAYS TO PROTECT AGAINST INFLUENZA VIRUS INFECTION

Additional studies led by Dr. Dillon, in collaboration with Dr. Siddhartha Balachandran at the Fox Chase Cancer Center, Temple University Health System (Philadelphia, PA), examined the cellular mechanisms of host defense against influenza infection. In a paper published in *Cell Host & Microbe*, the authors showed that the kinase RIPK3 can activate both necroptosis and apoptosis to prevent the spread of viral infection and limit immunopathology within a host.

A defining feature of RIPK3-mediated cell death is the formation of a protein complex called the necrosome. Necrosome formation is initiated by the association of RIPK3 with RIPK1. During influenza A infection, the necrosome’s two remaining components, MLKL and the adaptor protein FADD (Fas-associating death domain), are recruited. Once all four of the components are assembled, RIPK3 activates both MLKL-induced necroptosis and FADD-dependent apoptosis. These mechanisms work in conjunction to kill host cells that are infected by influenza A virus.

The timing and magnitude of the host’s immune response to influenza A virus infection and the mode(s) of cell death that are engaged influence the outcome of the disease. Although both forms of cell death limit the infection by minimizing virus spread, apoptosis appears to be the predominant route to prevent host immunopathology during the early stages of infection. Necroptosis, in contrast, can cause severe damage to the respiratory epithelium, diminished lung function, and in some cases death.
This study showed that ZBP1 is an upstream sensor and regulator that acts through RIPK3 to activate the inflammasome and programmed cell death pathways and through RIPK1 to initiate proinflammatory responses in mice. The researchers have proposed further studies to determine the role of ZBP1 in human influenza virus infection, including the identification of ZBP1 genetic mutations in patient populations infected with influenza virus.

Figure. ZBP1 mediates cell death in response to influenza A virus infection. (A) Images of wild-type (WT) and Zbp1−/− (Red arrows indicate dead cells. (B) Quantification of cell death in A. From Karakoc T, et al. ZBP1 is an innate sensor of influenza virus triggering the NLRP3 inflammasome and programmed cell death pathways. Sci Immunol 1:aag2045, 2016. Reprinted with permission from AAAS.

A PUTATIVE CELL DEATH PROTEIN CONTROLS LYMPHOCYTE METABOLISM
Apoptosis-inducing factor (AIF) is a protein present in the mitochondrial intermembrane space. During MOMP, AIF is released and translocates to the nucleus, where it can promote breaks in double-stranded DNA. It was initially thought that AIF is an effector molecule that induces cell death. Sandra Milasta, PhD, a postdoctoral fellow working in Dr. Green’s laboratory, investigated the function of AIF in immune cells. However, she was not able to identify a role for AIF in cell death.

In the journal Immunity, the authors showed that AIF acts to control energy production by mitochondria in lymphocytes. The protein is required for normal proliferation and maintenance of T lymphocytes but is dispensable for that of B lymphocytes. The team examined the metabolic requirements of T cells and B cells to explain this difference. They found that T cells depend on oxidative phosphorylation, a pathway dependent on mitochondria, to fulfill their energy needs, but B cells primarily depend on glycolysis, which does not require mitochondrial function.

This study disproved the idea that AIF has a key role in cell death mechanisms. The primary function of AIF, instead, appears to be metabolic. AIF ensures the assembly and proper function of electron transport chain components, thereby ensuring the health of T lymphocytes and other cells dependent on mitochondria for energy production.
DEFECTIVE PHAGOCYTOSIS OF DEAD CELLS MAY CONTRIBUTE TO THE PATHOGENESIS OF LUPUS

After a cell dies, regardless of the mechanism involved, it is rapidly removed via a process called phagocytosis (or “cell eating”). Phagocytosis is a key function of macrophages and other cells. Ten years ago, Dr. Green’s laboratory discovered LC3-associated phagocytosis (LAP), which is distinct from classic phagocytosis. During LAP, a set of small proteins (LC3) is recruited directly onto the phagosome, a vesicle containing ingested material that is formed through phagocytosis. The placement of these proteins involves proteins that are normally associated with autophagy.

Autophagy is a survival mechanism that is activated to keep cells alive when nutrients are limited. However, the role of autophagy proteins during LAP is distinct from that during classic autophagy. Furthermore, other proteins not involved in autophagy, such as NOD2 and RUBCN, are also required for LAP.

Jennifer Martinez, PhD, and Larissa Dias da Cunha, PhD, two postdoctoral fellows working in Dr. Green’s laboratory, studied how the disruption of LAP in macrophages affects health. Publishing in the journal Nature, the team reported that when LAP is defective, mice increase their production of inflammatory cytokines and develop a condition resembling the human autoimmune disease systemic lupus erythematosus. The mice fail to gain weight, their kidney function is compromised, and their peripheral blood shows high circulating levels of antibodies against double-stranded DNA and nuclear proteins. Dying cells are not effectively digested in the macrophages in the absence of LAP. This leads to an inappropriate immune response to the individual’s own cells, the development of autoantibodies, and autoimmune disease. The findings from this study implicate LAP in inflammatory autoimmune diseases. Lupus may develop in individuals who cannot effectively clear dead or dying cells, and the noncanonical autophagy process of LAP may be involved.

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For centuries, an enduring tenet of basic cell biology was that when a cell divides, two identical daughter cells are generated. In a paper published in Nature, Dr. Green’s team reported that as an activated T cell divides, c-Myc preferentially sorts into one of the two daughter cells. This observation was puzzling because a c-Myc molecule has a very short lifespan—it lasts for only tens of minutes in a cell. How then does one daughter cell receive the lion’s share of c-Myc?

The investigators showed that c-Myc distribution depends on a metabolic pathway involving molecules on the cell surface that transport amino acids into the cell. These transporters generate signals that are responsible for the unequal distribution of c-Myc. As a consequence, c-Myc helps direct the distinct fates of the two daughter cells. The daughter cell containing high levels of c-Myc and amino acids is destined to become an effector-like T cell, which generates the adaptive immune response to a pathogen. Effector cells are highly active, but they are also highly susceptible to programmed cell death pathways and die as immune stimuli necessary for their maintenance recede. The daughter cell containing low levels of c-Myc and amino acids differentiates into a memory-like T cell. This cell is long-lived and remains quiescent but ready to rapidly launch a protective immune response should this be necessary.

The results of this landmark study show that metabolic pathways and transcription programs foster asymmetry during cell division, and this asymmetry sends the progeny of the dividing cell along different differentiation paths. Furthermore, c-Myc, an oncogene whose overexpression is necessary for the development and maintenance of several types of cancer, is responsible for this fate decision. A better understanding of the asymmetric division of T cells may shed light on the role of c-MYC in lymphomas that arise from mature T cells.

ASYMMETRIC CELL DIVISION GIVES RISE TO DAUGHTER CELLS WITH DIFFERENT FATES

Cells require nutrition to survive. Different types of cells have different nutritional and metabolic needs. Furthermore, a single cell’s metabolic needs change, depending on its differentiation and activation state and its environment. When a T lymphocyte is activated to initiate an immune response, it changes the way in which it metabolizes nutrients. Several years ago, Dr. Green and his colleagues showed that the protein c-Myc is important for reprogramming the metabolism of T cells when they are activated. Last year, Katherine Verbist, PhD, and Swantje Ledmann, PhD, two postdoctoral fellows working in Dr. Green’s laboratory, made a remarkable discovery about c-Myc in activated T cells.

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Figure. (A) Representative image of a dividing T cell and (B) quantification of the mean fluorescence intensity (MFI) of c-Myc in the two daughter cells. The c-Myc appears green in the image. Original magnification = 100X. © 2016 Verbist KC et al.
CONCLUSION

Understanding how cells live and the many ways in which they die is crucial to deciphering how our immune system and organs function and how their biology is altered to initiate autoimmunity, cancer, and other catastrophic diseases. St. Jude researchers who study cell death are ultimately pursuing discoveries that will teach us how to preserve life.
Brain tumors are the leading cause of death due to cancer in children, and medulloblastoma is the most common form of malignant pediatric brain tumor, accounting for approximately 20% of all cases. Nearly 1000 new cases of pediatric medulloblastoma are diagnosed each year globally.
Medulloblastoma was at one time considered a form of primitive neuroectodermal tumor (PNET) because the two diseases are histologically and morphologically similar. However, it is now considered a distinct disease entity. Medulloblastoma is an embryonal tumor of the cerebellum, which unlike the rest of the brain, develops primarily after birth. Within the last decade, four subgroups of medulloblastoma have been recognized. PNET is another form of rare embryonal brain tumor whose appearance and protein-expression patterns suggest that it originates from primitive neuronal cells. PNETs more often arise in the cerebrum and behave more aggressively than medulloblastoma. Using advanced molecular technologies, St. Jude scientists have now defined four classes of PNETs.

RECOGNITION OF FOUR DISTINCT MEDULLOBLASTOMA SUBTYPES

Over the past decade, genomics has revolutionized our understanding of medulloblastoma and revealed distinct molecular subgroups characterized by disparate biological and clinical features. In 2012, medulloblastoma researchers proposed the recognition of four consensus subgroups: Wingless (WNT), Sonic hedgehog (SHH), Group 3, and Group 4. The definition of these subgroups has altered how medulloblastoma is studied in the laboratory and approached in the clinic. Next-generation sequencing (NGS) has enabled scientists to catalog the mutational landscapes of medulloblastoma subgroups and elucidate the pathways and genes that drive oncogenesis. Moreover, in 2016, the World Health Organization (WHO) published an updated Classification of Tumors of the Central Nervous System that for the first time recognized medulloblastoma subgroups as discrete diagnostic entities. This represented a major advance in pediatric neuro-oncology. Prospective clinical trials for medulloblastoma, such as St. Jude’s current SJMB12 trial, are now incorporating these subgroups into their risk-stratification schema and specifically tailoring treatment protocols based on tumor subgroup.

Scientists have further used NGS data to identify a limited number of candidate driver genes believed to be responsible for medulloblastoma initiation, maintenance, and progression. Some involve expected developmental signaling pathways, such as CTNNB1 in the WNT subgroup and PTOH1, SMO, and SUFU in the SHH subgroup. However, the most consistent and prominent theme to emerge from medulloblastoma genomes relates to somatic alterations, including mutations and copy-number changes, targeting epigenetic regulators. Several recurrently altered chromatin-modifying genes have been reported, including KDM6A, SMARCA4, MLL2 (KMT2D), and MLL3 (KMT2C). Although functional studies validating these candidate driver genes in the context of medulloblastoma are mostly lacking, mutations in the epigenetic machinery appear to contribute to at least half of all medulloblastomas, irrespective of subgroup. This finding suggests that deregulation of the epigenome is fundamental to medulloblastoma pathogenesis.

Treatment for medulloblastoma and PNET includes surgical resection, craniospinal irradiation, and cytotoxic chemotherapy, a combination that has lasting, detrimental effects on a developing child. Recently, molecularly targeted therapies for these embryonal brain tumors have been tested, opening up the possibility of decreasing treatment-related toxicity and its long-term consequences. New insights into the genes, pathways, and molecular processes underlying the pathogenesis of medulloblastoma and PNET are needed if we are to improve the diagnosis, treatment, and survival of children with these devastating tumors and minimize the late effects of therapy.

Paul A. Northcott, PhD; Yiai Tong, PhD
UNRAVELING THE CELLULAR ORIGINS OF MEDULLOBLASTOMA THROUGH EPIGENOMICS

The consequences of dysregulated chromatin-modifier function in medulloblastoma remain largely unknown, including how the mutations affect epigenetic states and gene regulation. Paul A. Northcott, PhD (Developmental Neurobiology), recently collaborated with investigators at the German Cancer Research Center (Heidelberg, Germany) and the Dana-Farber Cancer Institute (Boston, MA) to systematically investigate the epigenetic landscape of medulloblastoma.

In Nature, the team reported their evaluation of the epigenetic and genetic profiles of primary medulloblastoma samples. They used a combination of histone and transcription factor (TF) chromatin immunoprecipitation coupled with NGS (ChIP-seq) and sample-matched transcriptome profiling (RNA-seq) to examine regulatory elements in the tumor tissue. They focused on active enhancers (i.e., DNA sequences that increase the transcription of particular genes), given the importance of these molecular “switches” in defining the gene-expression programs of the cell. The data they acquired were particularly unique, because the ChIP-seq for markers of active enhancers, including H3K36me3, H3K4me1, and H3K27ac, was conducted using frozen tumor samples. Most ChIP-seq analyses of other cancers have been restricted to immortalized, high-passage tumor cell lines. Through application of a series of advanced computational analyses, Dr. Northcott and his colleagues identified 78,516 active enhancers, including nearly 20,000 that had not been previously annotated by the ENCODE Consortium or the Roadmap Epigenomics Project.

Focusing on tumor subgroup-specific enhancers, the authors identified and annotated clustered regions of disproportionately active enhancers known as super-enhancers. Super-enhancers are important due to their role in the regulation of genes associated with cell identity (i.e., master regulators) and oncogenesis (e.g., MYC family oncogenes). Each medulloblastoma subgroup harbors approximately 550 to 1100 super-enhancers, a considerable proportion of which are subgroup specific.

Using their highly integrative epigenomic/transcriptomic dataset, the authors inferred super-enhancer–associated gene targets and identified new candidate genes of potential relevance to medulloblastoma subgroup biology. Although these analyses verified known medulloblastoma oncogenes such as GLI2 (SHH-subgroup), MYC (Group 3), and OTX2 (Group 3 and Group 4), the most provocative and compelling result was the identification of lineage-specific neuronal TFs that appeared to be intimately linked to subgroup-specific regulatory elements. The epigenetic landscape of medulloblastoma subgroups appeared to be more informative of developmental origins than oncogenesis or epigenetic deregulation.

Through computational reconstruction of regulatory networks derived from the enhancer landscape defined in this study, the authors implicated a number of master TFs responsible for subgroup identity. Some TFs demonstrated spatiotemporal-restricted expression and activity in the developing mouse hindbrain. Most notable among these were Lmx1a, Eomes, and Lhx2, all of which showed highly specific activity in Group 4 medulloblastoma and are believed to regulate a substantial proportion of the Group 4 transcriptional program. These TFs play essential roles during early cerebellar development, and in this study Dr. Northcott’s team showed that their mouse homologs (i.e., Lmx1a, Eomes, and Lhx2) are coexpressed in upper rhombic lip progenitors and deep cerebellar nuclei of the nuclear transitory zone, the latter being derived from migratory upper rhombic lip progenitors. These observations strongly suggest that upper rhombic lip progenitors are plausible cells of origin for Group 4 medulloblastoma.

Deciphering the cellular origins of medulloblastoma has broad implications for understanding and treating this malignancy more effectively. Previous studies, including those conducted at St. Jude, have provided insight into the disparate cellular origins of the WNT and SHH medulloblastoma subgroups. However, the cellular origins of Group 3 and Group 4 medulloblastoma remain unconfirmed. To follow up on these findings reported in Nature, Dr. Northcott and his team are collaborating with colleagues in the Department of Computational Biology to identify the cellular origins of Group 3 and Group 4 medulloblastomas via single-cell genomics, cutting-edge bioinformatics, and molecularly informed functional approaches. Collectively, these advances will be fundamental toward gaining a better understanding of the pathobiology of medulloblastoma subgroups, which will advance the development and implementation of more effective treatments.

Figure. (A) Ranked enhancer plot of the H3K27ac landscape in Group 4 medulloblastoma. Genes associated with super-enhancers (SEs) are noted. (B) Zebrafish reporter assay for the Mash1 enhancer (green) in medulloblastoma. The HIF signal is restricted to the hindbrain of the central nervous system (CNS). (C) Transcription factor (TF) and H3K27ac ChIP-seq meta tracks for the super-enhancer–regulated TFs Lmx1a, Lhx2, Eomes, and EOMES. (D) Subgroup-specific regulatory circuitry for Group 3 and Group 4 medulloblastomas. (E) Expression of Lmx1a, Eomes, Lhx2, and TFs in the embryonic day 13.5 mouse cerebellum is indicated by red arrows. © 2016 Lin CY, et al
MOLECULAR CLASSIFICATION OF PRIMITIVE NEUROECTODERMAL TUMORS IDENTIFIES NEW BRAIN TUMORS

PNETs have been the subject of significant controversy in the fields of neuropathology and neuro-oncology. They are malignant neuroepithelial tumors with a propensity for both glial and neuronal differentiation. Because PNets show histologic overlap with other brain tumors (e.g., medulloblastomas) at the microscopic level and lack specific biomarkers, distinguishing them from other high-grade brain tumors is a significant challenge. In particular, diagnostically discriminating PNets from high-grade gliomas is especially difficult. This distinction is clinically important because high-grade gliomas and PNets are treated with different regimens of chemotherapy and radiotherapy. Unlike high-grade gliomas, PNets are treated with craniospinal irradiation, which can result in substantial long-term treatment-related morbidity.

In a study co-led by Brent A. Orr, MD, PhD, and David W. Ellison, MD, PhD (both of Pathology), and colleagues at the German Cancer Research Center, a novel approach was used to resolve long-standing questions about the classification of PNets. In this work published in Cell, the authors relied on an epigenetic mark in the tumor DNA called CpG methylation to molecularly classify PNets based on their genome-wide methylation signature. The team compared the methylation signatures of 523 tumors originally diagnosed as PNets to a reference library of methylation signatures from other known brain tumor types. Using this method, they found that most of the tumors diagnosed as PNets could be reclassified as another more specific brain tumor type. Many of the reclassified tumors had additional defining genomic abnormalities that validated their rearrangement.

Among the tumors that could not be reclassified as another known brain tumor, the researchers identified four novel brain tumor types, each of which was defined by recurrent gene fusions or intragenic gene duplications. These new brain tumor types were designated as central nervous system (CNS) neuroblastoma with FOXR2 activation (CNS NB-FOXR2), high-grade neuroepithelial tumor with BCOR alteration (CNS HGNET-BCOR), high-grade neuroepithelial tumor with MVI1 alteration (CNS HGNET-MVI1), and CNS Ewing family of tumors with CIC fusion (CNS EFT-CIC). Limited clinical correlation suggested that these new tumor classes have substantial clinicopathologic differences and most likely demonstrate different responses to therapy.

The results of this study suggest that although histomorphologic overlap exists in tumors designated as PNets, this diagnostic category represents a heterogeneous group of tumors. Largely on the basis of this study, the WHO removed PNET as a diagnostic entity from its 2016 update of the Classification of Tumors of the Central Nervous System. Although more research is necessary to fully define the four novel brain tumor types identified in this study, especially with regard to prognosis and treatment, these tumors will most likely be added to future updates of the WHO classification system. The genomic drivers of these new brain tumors were elucidated here and will facilitate future studies of their underlying biology and mechanisms of tumorigenesis. These findings will pave the way for more specific diagnoses of pediatric brain tumors and eventually enable us to target these tumors with greater precision.
INTERACTIONS BETWEEN MYC PROTEINS AND MIZ1 DETERMINE MEDULLOBLASTOMA SUBGROUPS

The MYC family is composed of three proto-oncogenes: MYC, MYCN, and MYCL. Each gene is located on a different chromosome and is expressed in different tissues during development and adulthood. MYC proteins are TFs that regulate several processes, including cell proliferation, differentiation, cell death, and cancer. All three proteins share similar structures, including a C-terminal basic helix-loop-helix domain, that enable them to interact with their protein partner MAX and bind specific DNA sequences called E-boxes. In addition, three amino acid motifs in the N terminus and body of the protein, called MYC boxes, permit the recruitment of other complexes to regulate gene expression.

Despite their differential expression patterns, all MYC members bind to the same DNA sequence. This has led to the assumption that MYC proteins are functionally interchangeable, an idea that was solidified by a genetic experiment in which MYC was replaced by MYCN in the mouse genome, thereby forcing all cell types to rely on MYCN to regulate gene expression. That experiment showed that “MYCN-only” mice develop normally. More recent investigations from the laboratory of Martine F. Roussel, PhD (Tumor Cell Biology), found that the cerebrum and cerebellum of MYCN-only mice appear unaffected by the protein substitution. Together, these results indicate that under normal physiological conditions, MYCN can substitute for MYC in many tissues, including the CNS, without disrupting development or function.
The four subgroups of medulloblastoma (WNT, SHH, Group 3, and Group 4) have different gene-expression profiles and express different MYC proteins, which affect prognosis. WNT and Group 3 medulloblastomas express MYC, whereas SHH and Group 4 express MYCN. Dr. Roussel’s team further observed that different medulloblastoma subgroups are induced under conditions in which supraphysiologic mitogenic stimuli, such as MYC and MYCN, are overexpressed in neuronal progenitors through gene amplification or other unknown mechanisms. A recent report published in Cancer Cell, Dr. Roussel and her colleagues evaluated the overexpression of MYC and MYCN in cerebellar granule neuron progenitors to determine how the proteins dictate which subgroup of medulloblastoma will arise. The experiments were influenced by earlier studies from their collaborator Dr. Martin Eilers (University of Würzburg, Germany) showing that high levels of MYC and MAX recruit MIZ1, a POX virus and zinc-finger (POZ)-domain TF that is expressed in all cells and activates transcription upon binding to a specific DNA-binding sequence different from E-boxes. Dr. Eilers’ group also showed that the MYC-MAX-MIZ1 complex represses transcription rather than activates it.

To understand the differences between MYC and MYCN, Dr. Roussel’s team first performed immunoprecipitation experiments. They found that the MYC-MAX complex interacts with MIZ1 with much higher affinity than does the MYCN-MAX complex, and this resulted in differential repression of certain genes. Furthermore, this repression of gene transcription was crucial for Group 3 medulloblastoma to develop. When the interaction between MYC and MIZ1 was prevented using a MYC mutant that does not bind to MIZ1, Group 3 medulloblastoma development was inhibited. The researchers found that the genes repressed by the MYC-MIZ1 complex included those required for the formation of primary cilia, organelles expressed at the surface of tumor cells that are required for SHH signaling. The absence of cilia leads to the reprogramming of the transcriptome of SHH-dependent neuronal progenitors into Group 3 tumors that now express markers of stemness.

In addition to elucidating how MYC and MYCN mediate the formation of different medulloblastoma subtypes, findings from this work have potential clinical implications. For instance, inhibitors of the interaction between MYC or MYCN and MIZ1 might be therapeutically advantageous for treating Group 3 and SHH medulloblastoma, respectively. Studies of how the disruption of this pathway affects medulloblastoma biology are now ongoing in collaboration with Dr. Eilers’ group and the laboratory of Richard J. Kriwacki, PhD (Structural Biology).

As a part of the St. Jude Lifetime Cohort Study, Tara M. Brinkman, PhD (Epidemiology & Cancer Control, Psychology), and her colleagues evaluated the prevalence and severity of long-term cognitive and social morbidities in more than 200 adult survivors of childhood brain tumors who were treated nearly two decades earlier. In the Journal of Clinical Oncology, Dr. Brinkman’s team reported that 20% to 30% of the survivors demonstrated severe neurocognitive impairment on tests of intelligence, memory, and executive function (e.g., planning, organization, and flexibility). Among adults in the general population, the expected cognitive impairment rate is 2%. Survivors who underwent irradiation to their entire brain were 1.5 to 3 times more likely to have severe neurocognitive impairment than were survivors who did not receive any cranial irradiation. Approximately 50% of the survivors did not graduate from college, were unemployed, and were not living independently as adults. The presence of severe neurocognitive impairment substantially increased the risk of survivors not achieving expected adult outcomes.

The results of this study suggest that the delivery parameters of contemporary radiation therapy, which are designed to reduce the amount of radiation delivered to the healthy brain, may reduce the risk of long-term neurocognitive impairment. However, additional follow-up data are necessary to confirm these findings. Beyond changes to frontline therapies, prophylactic cognitive interventions during therapy and remedial approaches may reduce the severity and functional impact of neurocognitive impairment in survivors of pediatric brain tumors.

Research elucidating the biology of pediatric brain tumors, including distinct molecular subtypes of medulloblastoma, has resulted in the development of a clinical and molecular risk-directed therapy for newly diagnosed medulloblastoma at St. Jude. The SJMB12 clinical trial aims to evaluate whether therapeutic modifications (e.g., reduced-dose craniospinal irradiation in patients with low-risk WNT medulloblastoma) can result in improved outcomes. This study will also evaluate the effectiveness of an aerobic training and neurocognitive intervention designed to prevent and/or mitigate the neurocognitive morbidities often experienced in this patient population.
CONCLUSION

St. Jude researchers are elucidating the molecular processes underlying the pathogenesis of pediatric brain tumors. While doing so, they are identifying new disease entities, ensuring accurate diagnoses, developing optimized treatments, and improving the long-term survival of children with these catastrophic diseases.
THE MANY ROADS TO TRANSCRIPTION FACTOR DEREGLULATION IN PEDIATRIC CANCER

All cancers are diseases of the genome. Genetic mutations activate, impair, or misdirect key cellular pathways to transform a normal cell into a malignant cell. Tumors differ in their number and types of genetic alterations, the order in which these alterations are acquired, and the growth and survival pathways that they disrupt.

Studies such as the Pediatric Cancer Genome Project have shown that a comprehensive analysis of the genetic changes within a tumor can provide important insights about basic mechanisms of the disease and new opportunities for therapeutic intervention. Unlike many adult tumors, pediatric tumors often have relatively few genetic alterations. This low mutation burden facilitates the dissection of how individual changes contribute to and collaborate in tumor formation. Several studies led by St. Jude investigators have provided examples of the various ways in which the “quiet” mutational landscapes of pediatric leukemia and solid tumors drive tumor development and growth.
EPOR REARRANGEMENTS INDUCE PHILADELPHIA CHROMOSOME–LIKE ACUTE LYMPHOBlastic LEUKEMIA

Studies from the laboratory of Charles G. Mullighan, MBBS(Hons), MSc, MD (Pathology), examined the genomic basis of a rare form of childhood acute lymphoblastic leukemia (ALL) termed Philadelphia chromosome–like ALL (Ph-like ALL). The term “Ph-like” in the name of this disease is derived from the observation that these ALL cases exhibit a gene-expression profile similar to that of ALL driven by the Philadelphia chromosome. The Philadelphia chromosome encodes BCR–ABL1, a chimeric protein with constitutively active tyrosine kinase activity, and the activation of kinase signaling in leukemic cells can be blocked with currently available tyrosine kinase inhibitors (TKIs).

Dr. Mullighan and his colleagues identified a diverse range of chromosomal rearrangements and DNA-sequence mutations and deletions in Ph-like ALL that activate several cytokine receptor– and kinase-signaling pathways. These findings attracted great clinical interest, because patients with Ph-like ALL have poorer treatment outcomes than do patients with other types of childhood ALL. Clinical trials of ALL at St. Jude and around the world now include the detection of genomic alterations in Ph-like ALL to determine the benefit of treatment with TKIs targeting deregulated pathways.

In a recent study from Dr. Mullighan’s laboratory, investigators examined how a chromosomal rearrangement of the erythropoietin receptor gene (EPOR) drives the development of a subset of Ph-like ALL cases. As many as 10% of Ph-like ALL cases have rearrangements of the EPOR gene. Erythropoietin is a cytokine that is essential for the normal growth and development of red blood cells but is not considered important for the growth of lymphocytes.

Ilaria Iacobucci, PhD, a staff scientist in Dr Mullighan’s laboratory, sought to characterize the genetic alterations of EPOR and determine how those changes contribute to leukemogenesis. In Cancer Cell, the team reported several unique features of EPOR rearrangements. These aberrations involved multiple partner chromosomes, but in each case the EPOR gene was positioned adjacent to strong enhancers that stimulate high expression levels of EPOR in leukemic cells. Most of these rearrangements are not apparent by conventional clinical genetic analysis. Rather, they are most reliably detected by whole-genome sequencing, which has been integrated into the clinical standard of care for patients with ALL treated at St Jude.
EPOR alterations demonstrate a unique twist to the way in which genetic rearrangements drive the activation of signaling pathways in ALL. For many rearrangements in ALL, inappropriate activation, or “hijacking,” of gene expression in a lymphoid cell is sufficient to activate downstream signaling pathways and cell proliferation. In contrast, EPOR rearrangements not only activate gene expression but also truncate the cytoplasmic tail of the receptor. This portion of the receptor can both activate signaling and limit it by the receptor’s subsequent inhibition and degradation. Phosphorylation of a series of eight tyrosine residues in the cytoplasmic tail regulates this process. The first tyrosine is indispensable for activation, and the remaining residues mediate negative regulation. All EPOR rearrangements result in preservation of the activating tyrosine and removal of the distal residues. This suggests that the alterations do not simply activate the receptors. Instead, EPOR rearrangements may induce a more subtle mechanism in which rearrangements influence the degree and duration of signaling through the receptor. The investigators used various experimental approaches, including expressing wild-type and mutant receptors in cell lines, examining the level and duration of receptor expression on exposure to ligand, and measuring the intensity of activation of downstream signaling pathways to confirm this hypothesis.

In contrast to cells expressing normal EPOR, cells expressing a truncated receptor did not downregulate receptor expression after stimulation with erythropoietin and exhibited a sustained, intense burst of signaling. Moreover, expression of the truncated receptor in isolated bone marrow cells resulted in leukemia development, indicating that EPOR rearrangement is sufficient to promote leukemogenesis. Finally, modeling of treatment using isolated leukemic cells showed potent synergy between a TKI that inhibits EPOR signaling and conventional chemotherapy. Together, these results provide insight into the unique mechanisms by which genomic alterations in EPOR promote leukemogenesis and a compelling rationale for genome sequencing of leukemic cells to facilitate accurate diagnosis and administration of appropriate TKIs to patients with Ph-like ALL. These approaches have been implemented in Total Therapy XVII, the current frontline clinical trial of ALL at St. Jude.

In a second study, Dr. Mullighan collaborated with Jinghui Zhang, PhD (Computational Biology), and colleagues to elucidate a distinct mechanism through which a sequence of genomic alterations drive the development of B-cell progenitor ALL (B-ALL). In this disease, the presence of specific leukemia-initiating gene rearrangements is associated with a favorable outcome in patients receiving conventional treatment.

Previous research led by James R. Downing, MD (Pathology), showed that many subtypes of B-ALL with different chromosomal translocations exhibit distinct gene-expression profiles. Those studies also identified a separate group of B-ALL cases that lacked a known leukemia-initiating chromosomal rearrangement and had a distinct gene-expression profile. Such cases commonly, but not universally, also have deletions of the gene ERG, which is a member of the ETS family of transcription factors. In a study reported in Nature Genetics, Drs. Mullighan and Zhang and their teams examined data from more than 1900 cases of childhood ALL. By integrating gene-expression, whole-genome, and transcriptome-sequencing data to define the genomic alterations that occur in B-ALL, they were able to decipher a novel and indirect mechanism for ERG downregulation and leukemogenesis.

The researchers identified rearrangements of the DUX4 gene, which encodes a homeobox transcription factor, as a universal event in this B-ALL subtype with a distinct gene-expression profile. DUX4 and ERG alterations are associated with a favorable outcome in acute lymphoblastic leukemia (ALL). In this disease, the presence of specific leukemia-initiating gene rearrangements is associated with a favorable outcome in patients receiving conventional treatment.

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The researchers identified rearrangements of the DUX4 gene, which encodes a homeobox transcription factor, as a universal event in this B-ALL subtype with a distinct gene-expression profile. DUX4 rearrangements usually involve juxtaposition of the gene to a strong enhancer element, such as the immunoglobulin heavy chain (IGH) gene or similar locus. This results in overexpression of DUX4 and truncation of the DUX4 protein. The research team demonstrated that deregulation of DUX4 exerts leukemogenic effects, in part, by deregulating ERG.
Figure. Illustration of the sequential deregulation of the transcription factors DUX4 and ERG in B-ALL. 

Influences gene expression.

 Alterations can also indirectly alter transcriptional regulation by remodeling chromatin and modifying the DNA that transcription factors that are key to leukemia development. In contrast, the following studies explore how genetic alterations directly and indirectly deregulate the expression and function of transcription factors that are key to leukemia development. This study is also important for diagnosis and management of childhood B-ALL.

This study has important biological and clinical implications. It demonstrates that deregulation of expression of a transcription factor, in this case DUX4 by genetic rearrangement, can promote tumorigenesis in an unusually indirect manner. The expression of a second key hematopoietic transcription factor, ERG, is dysregulated, which leads to RAG-mediated deletion, resulting in the ERG deletions observed in many patients with rearrangement of DUX4 and providing an additional mechanism for loss of activity of wild-type ERG. The expression of truncated DUX4 in normal human cord blood cells and leukemia cell lines confirmed that DUX4 directly deregulates ERG and induces the expression of ERGalt. Furthermore, expression of ERGalt in a mouse showed that this abnormal ERG isoform is sufficient to promote the development of a leukemia subtype that recapitulates human B-ALL.

Using chromatin immunoprecipitation and sequencing, the team showed that DUX4 binds to an intrinsic region of ERG, thereby inducing aberrant transcription and expression of a truncated C-terminal fragment of ERG called “ERGalt.” ERGalt retains DNA-binding activity but lacks N-terminal domains and acts as a competitive inhibitor of wild-type full-length ERG. This transcriptional deregulation of ERG also renders the genomic locus susceptible to RAG-mediated deletion, resulting in the ERG deletions observed in many patients with rearrangement of DUX4 and providing an additional mechanism for loss of activity of wild-type ERG. The expression of truncated DUX4 in normal human cord blood cells and leukemia cell lines confirmed that DUX4 directly deregulates ERG and induces the expression of ERGalt. Furthermore, expression of ERGalt in a mouse showed that this abnormal ERG isoform is sufficient to promote the development of a leukemia subtype that recapitulates human B-ALL.

This study is also important for diagnosis and management of childhood B-ALL associated with favorable outcome. Thus, accurate detection of these founding genetic alterations and DUX4 overexpression via a “total-sequencing” approach at the time of diagnosis is essential to guide appropriate treatment. This has been implemented in current St. Jude treatment protocols.

These studies illustrate how genetic alterations directly and indirectly deregulate the expression and function of transcription factors that are key to leukemia development. In contrast, the following studies explore how genetic alterations can also indirectly alter transcriptional regulation by remodeling chromatin and modifying the DNA that influences gene expression.

SMARCB1 ALTERATIONS IMPAIR ENHANCERS AND DRIVE RHABDOID TUMOR DEVELOPMENT

Childhood rhabdoid tumors are aggressive cancers that arise in the brain, kidney, or soft tissues. Although rhabdoid tumors harbor few genetic changes, alterations in the SMARCB1 gene is a hallmark of the disease. SMARCB1 is a component of a large multiprotein complex known as the SWI/SNF chromatin-remodeling complex. This complex serves important roles in the regulation of gene expression, but the mechanisms through which it influences tumor formation have been poorly understood.

Charles W. M. Roberts, MD, PhD (Oncology), and his collaborators at Dana-Farber Cancer Institute (Boston, MA) sought to understand the function of SMARCB1 in rhabdoid tumor formation. In Nature Genetics, the investigators used a system in which expression of SMARCB1 could be tightly controlled in rhabdoid tumor cell lines that lack endogenous SMARCB1 expression. Re-establishment of SMARCB1 expression was accompanied by increased expression of other components of the SWI/SNF complex, such as SMARCC1, SMARCA4, and ARID1A. In parallel, controlled deletion of Smarcb1 in nontumor mouse cells was accomplished by reduced levels of multiple SWI/SNF complex proteins, demonstrating the central role of SMARCB1 in maintaining the integrity of this large multiprotein complex. By integrating their analysis of the sequencing of chromatin marks and sites of SMARCA4/SMARCC1 binding, the team showed that the SWI/SNF complex binds predominantly at enhancer regions.

Enhancers are regulatory regions of DNA that control when genes are active or silent. The expression of SMARCB1 was accompanied by increased expression of many genes with central roles in the development and differentiation of the tissue types studied. The loss of SMARCB1 affected the presence of the SWI/SNF complex at enhancers of genes required for cellular differentiation, while leaving super-enhancers unscathed and functional. Such super-enhancers mark a small subset of genes that are essential for the maintenance of the current cell fate.

On the basis of these findings, Dr. Roberts and his colleagues developed a model in which the SWI/SNF complex— with SMARCB1 at its heart—has distinct roles in two types of enhancers, typical enhancers and super-enhancers. Inactivation of SMARCB1 primarily affects typical enhancers, which prevents the highly proliferative progenitor cells in which SMARCB1 has been lost from differentiating. In contrast, the loss of SMARCB1 does not affect SWI/SNF complex formation at super-enhancers, thus locking in the proliferation program of the progenitor cell and driving the malignant state.
The findings from this study substantially advance our understanding of how the SWI/SNF complex regulates gene expression and development in normal tissues and why mutation of this complex causes cancer growth. They also demonstrate how a single alteration in one gene can induce profound and catastrophic events that cause cancer to develop.

**INACTIVATION OF ARID1A PROMOTES COLON CANCER**

In a second study published in *Nature Genetics*, Dr. Roberts’ laboratory examined the role of a second component of the SWI/SNF complex in cancer. They identified an unexpected role of ARID1A, a component of the complex, in colorectal cancer. ARID1A is the most common target of genetic alteration among the SWI/SNF complex components, which are collectively mutated in approximately 20% of all human cancers. To understand the role of ARID1A in tumorigenesis, Dr. Roberts’ team generated a mouse model in which the Arid1a gene was inactivated in many tissue types. This resulted in the development of colorectal adenocarcinoma. In human colorectal adenocarcinoma, ARID1A is frequently mutated. The tumors were similar to a subset of human colorectal adenocarcinomas with microsatellite instability, a condition of genetic hypermutability resulting from compromised DNA repair. This work established the Arid1a-mutant mouse as a new preclinical model, one that closely matches a form of human colorectal cancer.

To determine how ARID1A loss influences chromatin regulation, the researchers further examined the genome-wide binding of two SWI/SNF proteins, SMARCA4 and SMARCC1, in isolated human colorectal cancer cells with either intact or deficient ARID1A expression. Binding of both proteins was profoundly reduced in cells lacking ARID1A. Changes were accompanied by reduced decoration of enhancers by H3K27 acetylation and reduced expression of genes, including those mediating multiple central pathways that regulate development and differentiation.

Together, these results illustrate the power of detailed modeling of the consequences of inactivating epigenetic regulators commonly mutated in human cancer. They also demonstrate the central role of ARID1A in enhancer-mediated regulation of a broad range of gene-expression programs.

*Figure. Model of defective SWI/SNF targeting and control of enhancer activity in ARID1A-deficient colonic epithelium cells.* © 2017 Mathur R et al
CONCLUSION
By expanding our understanding of genetic mutations and epigenetic events that directly and indirectly regulate tumorigenesis, St. Jude investigators are defining the mechanisms that underlie childhood cancer and paving new paths to its cure.
NOVEL GENE THERAPIES FOR MONOGENIC DISORDERS

The human genome contains approximately 20,000 protein-coding genes. An inherited or spontaneous mutation that alters even one of these genes can have devastating, lifelong effects. Many patients with monogenic (single-gene) disorders suffer greatly and experience premature death. Through gene therapy, investigators aim to replace, repair, or restore faulty genes.
An ideal gene therapy will correct the faulty gene in stem cells and ensure that these stem cells will generate normal progeny throughout the patient's life. In single-gene disorders that impair blood cells, hematopoietic stem cells (HSCs) are an ideal target for gene therapy, given their ability to permanently reconstitute a patient's blood and immune system. However, HSCs naturally resist genetic modification with commonly used approaches. Therefore, new tools are required, such as next-generation, safety-modified lentiviral vectors and genome-editing technologies to reliably modify HSCs. Using these approaches, investigators can harvest HSCs from a patient, alter the cells' genomes in vitro, and then reintroduce corrected cells into the bloodstream, where they home to specific niches in the bone marrow and establish a repaired blood-forming system. Because the HSCs to be targeted are host-derived (autologous), this approach circumvents many complications and toxicities that can occur after bone marrow transplantation (BMT) from a genetically different (allogeneic) donor.

Researchers at St. Jude have attained preclinical and clinical successes toward developing new approaches to cure two devastating pediatric monogenic disorders, X chromosome-linked severe combined immunodeficiency (XSCID) syndrome and sickle cell disease. The goal of these treatments is to restore normal lives to affected patients and their families and negate the need for lifelong, noncurative therapies. As an essential component of this research, the Children's GMP LLC, at St. Jude produces the drug products necessary to perform safe and effective gene therapy. Children's GMP LLC operates using the highest standards for the manufacture of advanced experimental therapeutic products.

**GENE THERAPY FOR XSCID IS CHANGING THE STANDARD OF CARE**

Severe combined immunodeficiency is a collection of monogenic disorders that cause profound immunodeficiency, usually during the first year of life. XSCID, is caused by defects in the common gamma-chain gene IL2RG, which encodes an essential component of multiple cytokine receptors involved in immune cell development and function. Infants with XSCID who do not receive treatment usually die before 1 year of age due to overwhelming opportunistic infections.

The current standard of care for XSCID is allogeneic BMT, preferably from a major histocompatibility antigen-matched sibling donor. However, matched-sibling donors are not available for about two-thirds of patients. In those cases, outcomes are suboptimal. For example, a patient with XSCID who lacks a matched donor may receive a transplant from a parent donor who is matched in only half of their histocompatibility antigens. The child may survive early childhood but will often experience incomplete and temporary restoration of immune function. In particular, B-cell function is usually not restored, thereby necessitating monthly intravenous gamma-globulin infusions, which are inconvenient, expensive, and potentially toxic. These patients may experience clinical complications with progressive loss of immune function, including recurrent pneumonia, gastroenteropathy, chronic viral infections, and failure to thrive. Furthermore, graft-versus-host disease, which can be life-threatening, develops in about 15% of patients.

XSCID was one of the first diseases to be treated by gene therapy. Clinical trials conducted almost 20 years ago using first-generation gamma-retroviral vectors demonstrated the efficacy of this approach. However, although the vectors restored T-cell function, they often did not restore B-cell or natural killer (NK)-cell function, resulting in only partial immune reconstitution. Even more alarming, the vectors used activated the LMCP2 proto-oncogene, which caused T-cell malignancies in about 30% of patients who received gene therapy. This severe complication resulted in an abrupt halt to gene therapy clinical trials for XSCID.

Brian P. Sorrentino, MD (Hematology), and his colleagues did not give up hope of developing gene therapy as a cure for this devastating illness. In collaboration with Drs. Harry Malech and Suk See De Ravin at the National Institute of Allergy and Infectious Diseases/NHI Clinical Center (Bethesda, MD), Dr. Sorrentino led efforts to develop a new generation of safety-modified lentiviral vectors for treating XSCID. Preclinical genotoxicity assays developed at St. Jude showed that the St. Jude–NIH lentiviral vector was much less likely to activate proto-oncogenes than were the gamma-retroviral vectors. The four main safety factors of the new lentiviral vector included its lentiviral backbone, which directs the vector's integration at different genomic locations; removal of all viral enhancers capable of activating proto-oncogenes; use of a cellular promoter that is highly effective in driving common gamma-chain expression; and inclusion of flanking chromatin "insulators" that protect nearby genomic sequences from activation by the integrated vector. The gene therapy vector was generated using a unique, stable lentiviral-packaging cell line developed by John Gray, PhD, and Robert Thom, PhD, two research scientists working in the Hematology Vector Development Laboratory. This stable producer cell line, the first ever to be applied in human gene therapy trials, streamlines good manufacturing practice (GMP) production of therapeutic lentivirus and reduces production costs.

In a study published in Science Translational Medicine, Dr. Sorrentino's and Dr. Malech's groups tested whether the St. Jude–manufactured XSCID vector could be used as salvage therapy in patients who had undergone allogeneic BMT as infants but were experiencing progressive loss of immune function as children or young adults. Previous attempts to restore waning immunity in such patients through gene therapy were unsuccessful. However, the investigators designed a novel treatment approach using Dr. Sorrentino's lentiviral vector and, for the first time, incorporating low-dose busulfan for nonmyeloablative conditioning to facilitate bone marrow engraftment of vector-transduced HSCs.

Five patients (aged 7–23 years) participated in this study, which was conducted at the NIH Clinical Center. The first two patients underwent extensive analysis at 36 and 24 months after completion of treatment. The XSCID gene therapy procedure was associated with an efficient engraftment of genetically modified HSCs that resulted in significant numbers of genetically corrected T cells, B cells, NK cells, and myeloid cells in the peripheral blood. In both patients, gene-corrected autologous T cells emerged gradually as the gene therapy graft slowly replaced allogeneic donor T cells that remained from the previous BMT. This replacement was associated with a significant increase in T-cell function. Moreover, for the first time in XSCID gene therapy, B-cell function was unequivocally corrected, and both patients were able to discontinue immunoglobulin-replacement therapy and mount normal immune responses to vaccination. The other three patients have been followed for less time but are also showing significant immunologic and clinical improvement.
Figure 1. Correction of T-cell and B-cell function in two patients (P1 and P2) who were followed to 36 and 24 months, respectively, after gene therapy for XSCID. (A) T-cell proliferation in response to indicated stimuli. PHA, phytohemagglutinin. (B) Correction of B-cell function is indicated by increasing serum levels of immunoglobin G (blue triangles) and immunoglobin M (red dots) after gene therapy. The dotted lines indicate the normal reference range, and the arrows indicate cessation of supplemental immunoglobin G therapy. (C) Clinical improvement in serum albumin levels in five patients (P1–P5) after gene therapy for XSCID. From De Ravin SS, et al. Lentiviral hematopoietic stem cell gene therapy for X-linked severe combined immunodeficiency. Sci Transl Med 8:335ra57, 2016. Reprinted with permission from AAAS.

Laboratory improvements were accompanied by significant clinical benefits, including resolution of disfiguring warts, cessation of severe protein-losing gastroenteropathy caused by chronic norovirus infection, and restoration of normal growth and body weight. One patient who had severe pulmonary damage and recurrent hemorrhages before gene therapy died of resultant complications more than 2 years after gene therapy. This indicates that gene therapy should be administered to patients with XSCID as early as possible, before severe irreversible organ damage occurs.

The gene therapy protocol, including submyeloablative busulfan conditioning, was well tolerated and is now being adopted by other groups. Whole-genome vector insertion–site analyses revealed a highly polyclonal pattern of hematopoiesis in all five patients. In contrast with previous gene therapy trials, the treatment appears safe, and there is no indication of a pre-leukemic state. Results from this study suggest that lentiviral vector–mediated gene therapy with nonmyeloablative busulfan conditioning is a very promising treatment for XSCID. As of February 2017, eight patients have enrolled in this trial at the NIH Clinical Center.
A NEW XSCID PROTOCOL FOR INFANTS OPENS AT ST. JUDE

Dr. Sorrentino has worked with Ewelina Mamcarz, MD (Bone Marrow Transplantation & Cellular Therapy), to begin enrolling patients in a new St. Jude–based protocol (LVXSCID-ND) for infants with newly diagnosed XSCID. The protocol is open at St. Jude and two collaborating sites—University of California, San Francisco (UCSF), and Seattle Children’s Hospital. Two infants have been treated on the LVXSCID-ND study at St. Jude, and a third was recently treated at UCSF. Initially, the Children’s GMP, LLC, is transducing bone marrow HSCs with the lentiviral gene–replacement vector for all three study sites, under the direction of Michael M. Meagher, PhD (Pathology, Therapeutics Production & Quality). Like the NIH study, this protocol incorporates submyeloablative busulfan conditioning and is open for infants as young as 2 months. Busulfan dosing is adjusted based on pharmacokinetic monitoring to ensure that the lowest possible effective dose is administered.

Although it is too early to make any long term conclusions about the efficacy and safety of gene therapy in the infants treated on LVXSCID-ND, preliminary data show that the treatment is well tolerated and can lead to rapid and broad immune reconstitution. The long-term goal of this project is to develop this gene therapy approach as a first-line treatment for both infants and older patients with XSCID.

THERAPEUTICS PRODUCTION & QUALITY AND CHILDREN’S GMP, LLC, PROVIDE ESSENTIAL SUPPORT TO GENE THERAPY TRIALS

Therapeutics Production & Quality (TPQ) develops the processes and analytical methods used by the Children’s GMP, LLC, to manufacture advanced experimental therapeutics at St. Jude. Development of the XSCID cellular gene therapy product started with the creation of a lentiviral vector by staff in the Department of Hematology’s Clinical Vector Development Core. This vector is the vehicle that transfers the IL2RG gene product into CD34+ HSCs to correct the XSCID defect. The modified HSCs are infused into the patient. TPQ developed processes to both manufacture the lentiviral XSCID vector and purify and transduce autologous CD34+ HSCs from patients with XSCID.

The lentiviral vector is expressed from HERG907/17 cells, a stable, adherent human embryonic kidney cell line. Under the directions of Michael M. Meagher, PhD (VP of TPQ and President of Children’s GMP, LLC), and Timothy Lockey, PhD (director of Process Development), scientists working in the TPQ spent several years devising a novel process using a Wave bioreactor filled with Fibra-Cell® disks to support the growth of large quantities of modified HERG907/17 cells while they produced the lentiviral XSCID vector. The Wave bioreactor keeps the disks suspended in growth medium. The vector is produced continually for 7 days, purified by chromatography, and then formulated for transduction into CD34+ HSCs.

Thasia Leimig, MD, and Suzette Wingo, two senior scientists in TPQ, and Dr. Lockey developed the process to purify and transduce CD34+ HSCs from the bone marrow. The process requires removing red blood cells by hetastarch precipitation, purifying CD34+ cells from other mononuclear cells by using magnetic nanobead–selection technology, and transducing CD34+ cells with the lentiviral XSCID vector. Susan Sleep, PhD (director of Quality Control), and her team developed the analytical methods used to verify the suitability of the lentiviral XSCID vector and the transduced CD34+ cells prior to releasing them for clinical use.

Current Good Manufacturing Practice (cGMP) manufacturing, release testing, and quality oversight of the therapeutic product for XSCID gene therapy is the responsibility of the Children’s GMP, LLC. The cGMP manufacturing and required testing of each batch of lentiviral XSCID vector can take more than 5 months. Manufacturing the transduced CD34+ HSCs from infant marrow for infusion requires approximately 2 weeks. Jennifer Hale and Kim Davis (both of Human Applications Laboratory) assisted in the cGMP manufacturing. All Children’s GMP, LLC, activities are performed under regulatory standards established by the U.S. Food and Drug Administration and are overseen by the Quality Assurance division of the Children’s GMP, LLC.
A GENOME-EDITING APPROACH TO TREATING β-HEMOGLOBINOPATHIES

Genetic mutations that alter the structure or expression of hemoglobin can impair oxygen delivery to tissues, with devastating effects on patients. Fetal hemoglobin (HbF), which is expressed mainly before birth, consists of two α-globin and two γ-globin protein subunits (α2γ2). Postnatally, γ-globin is replaced by β-globin to form adult hemoglobin (HbA, α2β2). Mutations in the HBB gene, which encodes β-globin, cause β-hemoglobinopathies, including sickle cell disease (SCD) and β-thalassemia. Symptoms of these diseases begin to occur after birth, coincident with the γ- to β-globin switch.

In a benign genetic condition termed hereditary persistence of fetal hemoglobin (HPFH), the γ-globin genes HBG1 and HBG2 continue to be expressed postnataally, resulting in permanently elevated levels of HbF in red blood cells. Remarkably, individuals with SCD or β-thalassemia mutations and HPFH are usually asymptomatic because high levels of HbF counteract the effects of the HBB mutation.

The laboratory of Mitchell J. Weiss, MD, PhD (Hematology), is investigating new gene therapy strategies to treat children with β-hemoglobinopathies. They reasoned that if they could recapitulate the mutation causing HPFH in mature blood-producing stem cells from patients with SCD, they should be able to prevent many of the adverse effects of the disease. Elizabeth Traxler and Yu Yao, MD, two scientists in Dr. Weiss’ laboratory, developed an approach to do just this. As a potential therapy for SCD, they used CRISPR-Cas9–mediated genome editing to modify human blood progenitor cells and recapitulate a naturally occurring form of HPFH.

In Nature Medicine, Dr. Weiss’ team reported their use of genome editing to create a 13-nucleotide (nt) deletion in the HGB1 gene promoter. This deletion was shown almost 30 years ago to cause one form of HPFH. The region is presumed to bind repressor proteins that inhibit γ-globin expression postnataally. The investigators expressed Cas9 and one of two guide RNAs (gRNAs) targeting the 13 nt–deleted region in healthy adult CD34+ hematopoietic stem and progenitor cells (HSPCs) and then maintained those cells in culture under conditions that supported their differentiation into red blood cells. Most HSPCs that expressed Cas9 and gRNAs contained the 13-nt deletion or smaller deletions in the same region and produced red blood cells that expressed supranormal HbF levels compared to nonedited cells. They repeated this experiment using HSPCs from patients with SCD. The red blood cell progeny arising from edited HSPCs expressed elevated HbF levels and were resistant to sickling at low oxygen concentrations.

Genome editing to generate HPFH mutations is an attractive possibility for treating β-hemoglobinopathies, because it can be achieved relatively easily in HSPCs by using current technologies. Moreover, HPFH does not cause significant morbidity in people who inherit similar naturally occurring mutations. However, more studies are required to optimize genome editing to create HPFH mutations in HSCs and ensure that no potentially harmful off-target mutations are induced by this approach.
Figure 3. Genome editing of HGB1 and HGB2 to treat SCD. (A) Diagram of the β-globin locus. The sequence of the HGB1 promoter is shown, and the 13-nucleotide deletion associated with HPFH is boxed. (B) Representative flow cytometry plots showing HbF staining in cells 5 days after transduction with a lentiviral vector expressing Cas9, Cas9 and gRNA1, or Cas9 and gRNA2. (C) Images of cells from a patient with SCD. The cells were transduced with a lentiviral vector expressing Cas9 (left) or Cas9 and gRNA1 (right), differentiated into red blood cells, and then maintained in culture. Red arrows indicate sickled cells. Original magnification, 200×; scale bars, 20 µm. (D) Quantification of sickled cells in (C). © 2016 Traxler EA et al.

IMPROVED LENTIVIRAL VECTORS AND GENOME EDITING: THE FUTURE OF GENE THERAPY VIA INTERDEPARTMENTAL AND MULTISITE CONSORTIA

According to Drs. Sorrentino and Weiss, the gene therapy approaches currently being used at St. Jude have the potential to correct many single-gene defects that cause nonmalignant blood diseases. Once gene therapy researchers design a lentiviral vector to correct the aberration, staff in the Clinical Vector Development Core and Children’s GMP, LLC, will optimize the safety and production of that vector. Clinical researchers in the Departments of Hematology and Bone Marrow Transplantation & Cellular Therapy will then design clinical trials to enable patients to receive treatment at St. Jude and collaborating centers. In parallel, St. Jude investigators are developing genome-editing approaches to cure other nonmalignant blood disorders. Drs. Weiss and Sorrentino believe that St. Jude will be a leader in this area by providing scientific expertise, the ability to manufacture complex clinical-grade drug products under cGMP conditions, and outstanding clinical resources, including bone marrow transplantation, for children with devastating blood disorders. Given that many monogenic blood diseases to be targeted by gene therapy are rare, collaborations with other institutions are essential.
CONCLUSION

St. Jude researchers are developing new gene therapy strategies to permanently repair monogenic mutations that cause catastrophic diseases, such as XSCID and β-hemoglobinopathies. Collaborating with scientists in the Children’s GMP, LLC, they are producing safe, effective gene therapy products, with the ultimate goal of making these therapies available to children around the globe.
Reduction in Late Mortality in Long-Term Survivors of Childhood Cancer
Survival of childhood cancer has increased since the 1960s, when the 5-year survival rate was less than 50%, to more than 83% today. However, long-term survivors of childhood cancer remain at risk for severe and life-threatening therapy-related side effects and late mortality, which is defined as death due to any cause occurring more than 5 years after the initial cancer diagnosis. Gregory T. Armstrong, MD, MSCE (Epidemiology & Cancer Control, Oncology), and his colleagues analyzed data from the Childhood Cancer Survivor Study (CCSS), which includes ongoing follow-up of 5-year survivors of childhood cancer whose diagnoses were made from 1970 through 1999. Although all causes of death were evaluated, the goal was to determine whether late mortality due to treatment-related causes (i.e., late effects) has decreased among more modern-day survivors.

In The New England Journal of Medicine, the team reported their findings from a study of 3958 deaths that occurred among the 34,033 survivors in the cohort, with a median follow-up time of 21 years. More than 9000 of the survivors received their initial cancer diagnosis in the 1970s; more than 13,000 in the 1980s; and more than 11,000 in the 1990s. Causes of death, classified according to the criteria listed in the International Classification of Diseases, 9th and 10th Revisions, were categorized into three groups: recurrence or progression of the initial cancer, which accounted for 2022 deaths; external causes (e.g., accident, suicide, or poisoning), which accounted for 338 deaths; and, most importantly, health-related causes (e.g., subsequent cancer, cardiac causes, pulmonary causes, or other treatment-related causes), which accounted for 1618 deaths. Of the 1618 deaths due to health-related causes, 746 were caused by subsequent cancer.

The 15-year mortality rate decreased across decades, both overall and for death due to health-related causes. These reductions were attributed to fewer deaths caused by subsequent neoplasms or by cardiac or pulmonary dysfunction among more modern survivors. The survivors whose initial cancer was treated in earlier eras, when radiation and chemotherapy were more commonly used and at higher dose intensities, had an increased risk for late mortality. Therefore, the authors concluded that reducing radiation and chemotherapy exposure to the lowest dosage that is therapeutically useful has lowered the late-mortality rate of survivors of childhood cancer and extended the lifespans of many survivors. Armstrong GT et al, New Engl J Med 374:833–42, 2016

A Novel Joint E3–E3 Mechanism for Substrate Ubiquitylation
Ubiquitin (UB)-mediated proteolysis plays a central role in regulating several cellular processes by targeting intracellular proteins for degradation, controlling protein interactions, and modulating protein conformation. This process involves adding multiple units of UB to the protein through a cascade involving enzymes E1 (UB-activating enzyme), E2 (UB-conjugating enzyme), and E3 (UB ligases). Cullin-RING E3 ligases (CRLs) represent the largest family of E3 ligases. The activities of CRLs, including numerous tumor suppressors, are modified by the conjugation of the UB-like protein (UBL) Nedd8 through a process called neddylation.

The currently held view of substrate ubiquitylation posits that a trierester-linked E2–UB intermediate interacts with a single E3 to transfer the UB to a target. In a study reported in Cell, Daniel C. Scott, PhD, a research scientist in the laboratory of Brenda A. Schulman, PhD (Structural Biology, Tumor Cell Biology), and collaborators Drs. J. Wade Harper (Harvard Medical School, Boston, MA) and Arno Alpi (University of Dundee, U.K.; Max Planck Institute of Biochemistry, Munich, Germany) and their teams challenged this tenet and provided evidence of a novel E3–E3 to CRL-dependent proteostasis and E3-mediated UB ligation.

The study also raises the possibility of the widespread use of this mechanism by CRLs in not only human cells but also other organisms. Scott DC et al, Cell 168:1198-214, 2016

Figure. Cumulative mortality among 5-year survivors of childhood cancer treated during a 30-year period (1970–1999). The cumulative incidences of death due to any cause (A), disease recurrence or progression (B), and health-related causes (C) are shown. Vertical dashed lines indicate 5-year mortality. From The New England Journal of Medicine, Armstrong GT et al. Reduction in late mortality among 5-year survivors of childhood cancer. 374:833–42. Copyright © 2016 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.
Cranial Radiation Therapy Increases the Risk of Hearing Loss in Pediatric Patients

Sensorineural hearing loss (SNHL), a type of permanent hearing impairment, is caused by damage to the nerve cells in the cochlea that transmit the perception of sounds to the brain. SNHL is a well-characterized adverse effect of cranial radiation therapy (RT) in adults. However, the long-term consequences of this treatment in children are relatively unknown. SNHL risk increases with higher doses of radiation and with exposure of the region of the cranium housing the cochlea. Why some children experience SNHL after cranial RT and others do not is not understood.

In the Journal of Clinical Oncology, Johnnie K. Bass, AuD (Rehabilitation Services), and her colleagues reported a systematic study of SNHL incidence, age of onset, severity, and progression in 235 pediatric patients who received cranial RT for brain tumors. The researchers identified the most highly associated risk factors of SNHL by evaluating patients’ hearing ability over a median of 9 years after cranial RT. Thirty-three (14%) patients experienced SNHL during the follow-up period. The greatest risk factors for SNHL were age at RT exposure, cochlear radiation dose, and the presence of a cerebrospinal fluid shunt. Patients who were younger than 3 years at RT initiation were more than twice as likely to have SNHL, and higher cochlear RT doses incrementally increased the likelihood of SNHL. Although the mechanism by which cerebrospinal fluid shunts increased SNHL risk is not fully understood, the authors postulated that shunt-induced changes in cerebrospinal fluid pressure within the cochlea may have contributed to the risk.

Of the patients who had SNHL, the median time of SNHL onset was 3.6 years after cranial RT. Approximately 75% of patients with mild SNHL at diagnosis experienced worsening of their symptoms and eventually required hearing aids. SNHL impedes speech and language development in children, which may contribute to declines in cognitive ability, academic performance, and quality of life that frequently occur in pediatric cancer survivors. Because early detection of SNHL reduces these declines, long-term follow-up of hearing ability for at least 10 years is particularly important for children who were younger than 3 years at cranial RT initiation, received high cochlear radiation doses, or had a cerebrospinal fluid shunt in place during cranial RT. Bass JK et al, J Clin Oncol 34:1248–55, 2016

Chaperones of the Hsp70 Family Exercise Quality Control by Recognizing Specific Sequences in Proteins

To become active, proteins must fold into specific three-dimensional structures via a process that is guided by multiple molecular chaperones. If a protein fails to fold properly, it must be identified and degraded. Approximately one-third of the proteins encoded by the human genome are processed in the endoplasmic reticulum (ER) of the cell, which is the major site of quality control. Defects in chaperone-guided protein folding in the ER are associated with many human diseases, and some chaperones are involved in ER-associated degradation of misfolded proteins.

Immunoglobulin heavy-chain binding protein BiP is the mammalian ER cognate of the Hsp70 family of chaperones. BiP binds to small hydrophobic sequences on many nascent proteins to assist in their folding. BiP also recognizes proteins that cannot fold and targets them for degradation. These contradictory functions are regulated by co-chaperones, including one of four ER-localized DnaJ co-factors (Erkd3-6) and Grp170. Erkd3 binds to unfolded protein clients and can facilitate pro-folding or pro-degradation functions of BiP, but the substrate-binding preferences of the co-chaperones are not well understood.

In Molecular Cell, Linda M. Hendershot, PhD (Tumor Cell Biology), and her colleagues described how ER chaperones and co-chaperones recognize client proteins and contribute to distinct outcomes for their substrates. The investigators first created an in vivo expression library composed of multiple overlapping peptides covering two model protein clients. Immunoglobulin γ heavy chain and NS-1 κ light chain. This system was used to determine the sequence-specific binding preferences of five members of the Hsp70/72 chaperone system: BiP and its co-chaperones Grp170, Erkd3, Erkd4, and Erkd5. Both the γ heavy chain and κ light chain are natural clients of BiP and Erkd3 but also interact with Erkd4, Erkd5, and Grp170.

By performing in vivo binding studies, the researchers revealed numerous binding sites for BiP and its pro-folding co-chaperones Erkd3 throughout the two clients. These sites were resistant to disruption by mutagenesis, in keeping with low sequence specificity requirements that enable interactions with a variety of sequence-unrelated proteins. In contrast, the pro-degradation co-chaperones Grp170, Erkd4, and Erkd5 recognized a unique type of sequence that was larger, occurred less frequently, and was highly prone to causing protein aggregation. Unlike BiP- and Erkd3-binding sequences, these co-chaperone-binding sites were readily disrupted or introduced by single mutations. The co-chaperone-binding sites were well tolerated in regions of a client protein that folded rapidly, causing the aggregation-prone sequence to be buried. However, if these sites were introduced into a portion of a client protein that was unable to fold, the protein formed large aggregates that were toxic to cells.

The authors concluded that the substrate-binding specificity of members of the Hsp70 system is much more diverse than previously thought. Whereas pro-folding chaperones bind many diverse sequences on their client proteins, pro-degradation chaperones specifically recognize aggregation-prone regions that, if left exposed, represent a potential threat to the cell. By identifying recognition patterns for multiple ER chaperones, some of which could be introduced by mutations associated with human diseases, this study provides a basis for further elucidating the processes by which molecular chaperones control the fate of client proteins. Behnke J et al, Mol Cell 63:739–52, 2016
ProteinPaint can be used to simultaneously visualize genetic lesions and RNA expression from large pediatric datasets. Whereas whole-genome sequencing maps DNA, RNA sequencing (RNA-seq) reveals how this genetic information is transcribed into RNA molecules, which is essential for understanding the expression of mutant genes and developing therapies. To date, ProteinPaint includes information about more than 32,780 mutations or gene fusions from more than 2400 pediatric patients with 37 cancer subtypes. These data were generated by the St. Jude–Washington University Pediatric Cancer Genome Project, National Cancer Institute, German Cancer Research Center, and Shanghai Children’s Medical Center. ProteinPaint offers several advantages over other currently available visualization tools. It employs a novel infographics display that provides an enhanced visualization of individual genes and corresponding proteins. The user interface is designed to maintain legibility while displaying large amounts of data on mutations, showing mutational profiles of the same protein across multiple datasets, and showing expression levels of the same genes in samples in which the mutation was identified. Furthermore, ProteinPaint complements existing cancer genome portals, such as COSMIC, and allows the integration of pediatric and adult cancer data. ProteinPaint is available free of cost to researchers worldwide for data analysis. The overarching goal is to use this web application globally for cancer research, collaboration, and developing effective therapies for pediatric cancer. Zhou X et al, Nat Genet 48:4–6, 2016

**NUDT15 Gene Polymorphisms Alter Thiopurine Metabolism and Hematopoietic Toxicity**

Thiopurines are widely used as anticancer and immunosuppressive agents. One member of this class of drugs, mercaptopurine, is an important component of treatment regimens for acute lymphoblastic leukemia (ALL) but is also associated with common and severe myelosuppression. Precision-medicine approaches are much needed to optimize thiopurine dosing and avoid toxicity in patients with ALL.

Thiopurines are prodrugs and, thus, need to be activated inside a cell to exert their therapeutic effects. Thiopurine metabolites are incorporated into DNA to form DNA-TG, which eventually triggers apoptosis. A number of metabolic pathways can also impede thiopurine activity. For example, thiopurine methyltransferase (TPMT) converts mercaptopurine to an inactive form, and dephosphorylation of thioguanine nucleotides eliminates active metabolites of thiopurines. The extent of thiopurine cytotoxicity is thus determined by competition between the activation vs. inactivation pathways.

Mutations in the TPMT gene cause a loss of TPMT activity, which predisposes patients to hematopoietic toxicity. Pioneered by St. Jude, TPMT-guided thiopurine dose adjustment has substantially reduced this risk without compromising therapeutic efficacy. More recently, groups at St. Jude discovered genetic variation in the NUDT15 gene to be another determinant of thiopurine intolerance in children with ALL. In Nature Genetics, Jun J. Yang, PhD (Pharmaceutical Sciences), and his colleagues reported their characterization of the effects of NUDT15 variants on thiopurine metabolism and clinical tolerance of mercaptopurine toxicity. By sequencing the NUDT15 genes of 270 children with ALL who were enrolled in clinical trials in Guatemala, Singapore, or Japan, the researchers identified four coding variants, all of which changed the amino acid sequence of NUDT15 and greatly diminished its ability to inactivate thiopurine metabolites. This impairment was reflected in a reduced mercaptopurine tolerance among patients carrying these loss-of-function variants.

Dr. Yang’s team also showed that DNA-TG levels were much higher when NUDT15 expression was suppressed, which is consistent with the notion that NUDT15 inactivates thiopurine metabolites and, therefore, directly influences the cytotoxic effects of this class of drugs. In white blood cells obtained from the children in Singapore or Japan who received daily mercaptopurine treatment, DNA-TG levels were strongly correlated with mercaptopurine dosage, and the ratio of DNA-TG levels to drug dosage varied by NUDT15 genotype. Mercaptopurine tolerance was lowest in the Guatemalan children, even in those with wild-type NUDT15; therefore, other genetic variants associated with thiopurine toxicity might exist in that population.

The authors concluded that integrating NUDT15 variants into the thiopurine-dosing algorithm could be particularly valuable for Asian and Hispanic populations, in whom TPMT variants are rare. Furthermore, a polygenic dosing algorithm incorporating NUDT15 and TPMT variants would enable personalized thiopurine therapy in diverse populations worldwide. Moriyama T et al, Nat Genet 48:367–73, 2016
Minimizing the Risk of Low Bone Mineral Density and Frailty in Survivors of Childhood Acute Lymphoblastic Leukemia

Although contemporary treatments have improved survival rates for children with acute lymphoblastic leukemia (ALL), survivors are at high risk of accelerated aging, which is characterized by frailty and deficits in bone mineral density (BMD). In the general population, low BMD is associated with an increased risk of fractures, impaired mobility, and early mortality. Lifestyle factors, such as smoking, alcohol consumption, and physical inactivity, are also established risk factors for low BMD in the general population, but their effect on BMD in survivors of ALL remains to be investigated. Childhood cancer survivors also experience frailty, but its correlation with hormone- and lifestyle-related factors remains unknown.

Lifestyle factors are modifiable, and hormonal imbalances are treatable; thus, a research team led by Carmen L. Wilson, PhD (Epidemiology & Cancer Control), studied the association of lifestyle factors and hormonal deficiencies with the risk of frailty and low BMD. Dr. Wilson and her colleagues found that 30% of survivors had low BMD. After adjusting for body mass index, men who had growth hormone deficiency (GHD) or were current smokers and women who had GHD or consumed moderate levels of alcohol were at increased risk of low BMD. Frailty and prefrailty occurred in 3.6% and 18.6% of survivors, respectively. In both men and women, the most frequent components were low energy expenditure, self-reported exhaustion, and low muscle mass. After adjusting for age, the team found that men who had GHD or were current smokers were at increased risk of prefrailty or frailty. Although no such associations were noted in women, their likelihood of prefrailty or frailty increased in association with greater alcohol use.

This large analysis highlights the need for future studies to focus on the use of growth hormone replacement therapy to improve BMD and physiologic reserve in childhood ALL survivors. Furthermore, counseling on lifestyle changes (e.g., cessation of smoking) and regular screening for hormonal deficits can minimize the risk of frailty and low BMD, thereby improving the quality of life for survivors of childhood ALL.


A Frequent Mutation in Amyotrophic Lateral Sclerosis Impairs Membraneless Organelle Formation and Function

Membraneless organelles are cellular subcompartmental structures that are formed by liquid-liquid phase separation (LLPS) of their constituents. These organelles include the nucleus, the central channel of the nuclear pore, nuclear speckles, Cajal bodies, and stress granules. Low-complexity sequence domains in constituent proteins contribute to the phase separation that leads to the assembly of these organelles.

In earlier work, J. Paul Taylor, MD, PhD (Cell & Molecular Biology), and his colleagues discovered that rare mutations in low-complexity sequence domains play a causal role in the neurodegenerative disorders amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD). However, the most frequent mutation in ALS/FTD is a hexanucleotide (G(4)C(4))-repeat expansion in the C9orf72 gene, which is one of the most common causes of ALS/FTD. This recent study used a proteomics approach to determine the proteins that interact with DPRs in living cells. They found that arginine-rich DPRs preferentially interact with proteins that contain low-complexity sequence domains, including many associated with ALS/FTD. To validate these interactions in vivo, the authors determined the effect of the DPRs on neurodegeneration in fruit flies with synaptic deletion of each interacting protein. Most genetic modifiers of DPR-mediated neurodegeneration were components of membraneless organelles. Arginine-rich DPRs interacted with proteins essential for stress granule formation, leading to stress granule persistence and compromised translation. Expression of arginine-rich DPRs also impaired the assembly and dynamics of nuclear speckles and Cajal bodies, indicating that these DPRs exert a broad adverse effect on all membraneless organelles in the cell.

Because many low-complexity sequence domains interact with arginine-rich motifs or are enriched in arginine-containing proteins, the authors proposed that a common pathogenic mechanism underlying ALS/FTD and other neurodegenerative disorders is the widespread loss of normal function of membraneless organelles through disrupted biophysical interactions required for LLPS.


Figure. (A) Structural illumination microscopy images showing subnucleolar localization of GFP–GR and GFP–PR (green), and GFP–PR (blue), whereas Fibrillarin (cyan), and Fibrillarin (red), all of which exclude the nucleus, in Hela cells. Nuclei were outlined based on DAPI staining (not shown). (B) GR localizes within subnucleolar localization of GFP–PR (green), a protein. Most genetic modifiers of DPR-mediated neurodegeneration are components of membraneless organelles. Arginine-rich DPRs localize to the nucleoli of cells grown in culture and interact with a protein that promotes the liquid-like state of the nucleolus. This interaction accelerated LLPS and decreased nucleolar function. In addition, arginine-rich DPRs interacted with proteins essential for stress granule formation, leading to stress granule persistence and compromised translation. Expression of arginine-rich DPRs also impaired the assembly and dynamics of nuclear speckles and Cajal bodies, indicating that these DPRs exert a broad adverse effect on all membraneless organelles in the cell.
Hedgehog Signaling Promotes the Growth and Folding of the Neocortex

The neocortex, which is the uppermost structure of the mammalian brain, computes high-order sensory, motor, and cognitive processes. During evolution, the neocortex of certain species expanded dramatically and folded, thereby supporting superior sensorimotor and cognitive abilities. Neocortical expansion depends on the number and proliferative capacity of neural progenitor cells. The primary neural progenitors are apical radial glial cells (aRGs). The aRGs generate neurons directly or indirectly via basal radial glial cells (bRGs) or intermediate progenitor cells (IPCs). The expansion of bRGs and IPCs plays key roles in the expansion and folding of the neocortex. In particular, bRGs are rare in species with small/smooth brains but are greatly expanded in species with large/folded brains, especially humans. Little is known about the mechanisms by which those cells expand. Sonic hedgehog (SHH) signaling regulates mammalian neocortical development and may be involved in neocortical expansion, as defective SHH signaling can cause microcephaly, a disorder associated with an unusually small brain size.

Young-Goo Han, PhD (Developmental Neurobiology), and his group are investigating the role of SHH signaling in neocortical expansion and folding in humans and mice. They reported their recent findings in Nature Neuroscience. The team first engineered mice expressing constitutively active Smoothened (SmoM2), an activator of SHH signaling, in aRGs and their progeny cells. They found that elevated SHH signaling in the SmoM2-mutant mice induced neocortical growth and folding in the otherwise smooth mouse neocortex. SmoM2-mutant mice elicited a developmental characteristic that is thought to be necessary and sufficient for the evolution of an expanded and folded neocortex: expansion of both bRGs and IPCs. Conversely, the loss of SHH signaling decreased the number of bRGs and IPCs and the size of the neocortex.

Dr. Han and his colleagues also discovered that SHH signaling is strong in the fetal human neocortex but not in the embryonic mouse neocortex. To investigate whether SHH signaling regulates human bRGs, the team employed a cerebral organoid model, or “minibrain,” grown from human pluripotent stem cells; this model recapitulates human brain development. Blocking SHH signaling in the organoids decreased the number of bRGs and the production of neurons.

Together, these results suggest that SHH signaling in the human fetal neocortex contributes to bRG and IPC expansion and neocortical growth and folding. The SmoM2-mutant mice generated for this study are the first stable, robust mouse model of neocortical expansion and folding; thus, they will be important in future investigations of the mechanisms underlying neocortical development and evolution. Wang L et al, Nat Neurosci 19:888–96, 2016

A B

Figure. Images of Nissl-stained brain sections from wild-type (A) and SmoM2-mutant (B) mice. SmoM2-mutant mice develop a large, folded neocortex compared to that in wild-type mice. Brown areas indicate the singulate cortex, which is part of the cerebral cortex and folded in SmoM2-mutant mice. © 2016 Wang L et al

Age-Dependent Reduction in mIR-338-3p Leads to Auditory Thalamocortical Disruption in Mouse Models of 22q11.2 Deletion Syndrome

In the brain, the auditory cortex processes and interprets signals related to hearing. This auditory processing can be disrupted by genetic disorders such as the 22q11.2 deletion syndrome (22q11DS), which is caused by a deletion of part of chromosome 22. Individuals with 22q11DS have behavioral and communication abnormalities, which are treated by classic antipsychotic agents (e.g., haloperidol). Microarray analysis and in vitro and in vivo experiments showed that the miRNA mIR-338-3p mediated the disruption of TC synaptic transmission, and replenishment of mIR-338-3p in mature mice rescued TC abnormalities and presynaptic neurotransmitter release in 22q11DS mice. Knockout of Mir338 or depletion of mIR-338-3p recapitulated TC disruption in 22q11DS mice by decreasing the probability of glutamate release at TC projections. The deletion of Mir338 or depletion of mIR-338-3p eliminated the age dependency of these deficits and that for sensitivity to antipsychotics.

Together, these results demonstrate that mIR-338-3p is central to disrupting synaptic transmission of TC projections and is responsible for the age-related delay of auditory symptoms in patients with 22q11DS. Current therapies for schizophrenia alleviate the symptoms of psychosis via DRD2 inhibition; however, those agents have multiple adverse effects. The current study has important clinical implications, as it provides evidence that restoring mIR-338-3p in the thalamus is a potentially effective and tolerable approach to alleviating the positive symptoms (e.g., hallucinations, delusions) of 22q11DS and schizophrenia. Chun S et al, Nat Med 23:39–48, 2017

In a study published in Nature Medicine, a research team led by Stanislav S. Zakharenko, MD, PhD (Developmental Neurobiology), determined whether TC disruption follows the same age-dependent trajectory as psychosis in patients with 22q11DS or schizophrenia and analyzed its underlying molecular mechanisms in Df(16)1/+ mice. Whole-cell voltage clamp recordings supported an adult onset of disruption of synaptic transmission in Df(16)1/+ mice. Furthermore, Drd2 mRNA levels were elevated in older mice, making TC projections sensitive to the DRD2 antagonist haloperidol. Microarray analysis and in vitro and in vivo experiments showed that the miRNA mIR-338-3p mediated the disruption of TC synaptic transmission, and replenishment of mIR-338-3p in mature mice rescued TC abnormalities and presynaptic neurotransmitter release in 22q11DS mice. Knockout of Mir338 or depletion of mIR-338-3p recapitulated TC disruption in 22q11DS mice by decreasing the probability of glutamate release at TC projections. The deletion of Mir338 or depletion of mIR-338-3p eliminated the age dependency of these deficits and that for sensitivity to antipsychotics.

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The Differentiation of Follicular Helper T Cells Is Guided by mTOR Signaling and Glucose Metabolism

Follicular helper T (Tfh) cells stimulate B cells in germinal center follicles to produce high-affinity, long-lived immunoglobulins under steady-state conditions and after antigen stimulation. Although the signaling mechanisms that regulate this process were previously unknown, it was well established that T-cell differentiation involves metabolic changes that are regulated by the mechanistic target of rapamycin (mTOR) signaling.

Hongbo Chi, PhD (Immunology), and his colleagues assayed the activities of mTORC1 and mTORC2, the two kinase complexes that form mTOR, during the Tfh-cell response to determine their role and that of glucose metabolism in the differentiation of Tfh cells and promotion of germinal center responses. They also examined the mTORC1 subunit Raptor, the mTORC2 subunit Rictor, and ICOS, a key signaling molecule in Tfh-cell differentiation. In a recent article in Immunity, Dr. Chi and his team reported their results.

The authors compared the gene expression profiles of mice whose T cells were deficient in either Raptor or Rictor. They found that mTORC1 and mTORC2 control overlapping and distinct sets of genes. The group also showed that both molecules are essential for Tfh cell induction in peripheral immune tissue and promotion of Tfh-cell differentiation, germinal center formation, and humoral responses after immunization with foreign antigens. Mechanistic studies in mice and isolated cells with additional key mutations confirmed that a deficiency in Raptor or Rictor reduces ICOS-induced signaling and cellular metabolism. To directly test the metabolic requirements of Tfh cells, the researchers generated Tfh cells in vitro and in vivo with altered expression levels of the glucose transporter Glut1 to specifically isolate the role of glucose uptake. They found that Glut1 promotes Tfh-cell differentiation during steady-state conditions and after foreign antigen exposure.

Together, these results show that mTORC1 and mTORC2 couple ICOS signals to metabolism and transcriptional regulation, thereby facilitating the integration of these signals to regulate Tfh-cell differentiation. Building upon these results may enable the clinical manipulation of Tfh cells via targeting of mTOR or metabolic signaling in the setting of immune-mediated diseases.

The Genomic Landscape of Acute Myeloid Leukemias with Core-binding Factor Rearrangements

Among acute myeloid leukemia (AML) cases, gene rearrangements in the core-binding factor (CBF) transcriptional complex are present in almost 30% of pediatric and 15% of adult cases. CBF-AML typically has a prognosis more favorable than other AML subtypes, with patients having better outcomes overall. Chromosomal abnormalities affecting the RUNX1 and CBFB transcription factors are typical of CBF-AML but are not sufficient to induce leukemia. To gain insight into the pathogenesis and development of CBF-AML by detailing the cancer’s overall genomic landscape, Jinghui Zhang, PhD (Computational Biology & Bioinformatics), Jeffery M. Kico, MD, PhD (Pathology), James R. Downing, MD (Pathology); and other members of the St. Jude Children’s Research Hospital–Washington University Pediatric Cancer Genome Project used whole-genome sequencing and whole-exome sequencing to analyze the genetic code of samples from 87 pediatric and 78 adult CBF-AMLs.

In a Nature Genetics article, Drs. Zhang, Kico, Downing, and colleagues revealed changes common to the RUNX1–RUNX1T1 and CBFB–MYH11 subtypes of CBF-AML. In agreement with previous reports, 66% of the cases had mutations in some combination of Ras pathway constituents NRAS, KIT, FLT3, KRAS, PTPN11, and NFI, with NRAS being the most frequently mutated gene overall. NRAS mutations, in particular the codon 61 mutation, were more common in the CBFB–MYH11 subtype. RUNX1–RUNX1T1 AMLs were enriched with KIT mutations, which were associated with a worse outcome. Mutations in MYC-signaling regulators were found in CBF-AMLs. All seven mutations identified in CBF-AMLs were found in the downstream MYC target, were near a conserved phosphorylation site and resulted in increased protein stability. This pathway may be a novel target in treating CBF-AML.

The group also identified a recurrent alteration in the RNA helicase DHX15 that was unique to the RUNX1–RUNX1T1 cohort. Using RNA interference to knock down the expression of DHX15, mimicking loss of function, the team uncovered differential expression of genes involved in gene splicing and ribosomal biogenesis and increased alternative splicing activity. The RUNX1–RUNX1T1 cohort of CBF-AML was also enriched in mutations in chromatin-modifying genes, which were largely absent in the CBFB–MYH11 cohort, and had unique mutations in several genes encoding proteins responsible for sister chromatid cohesion during mitosis and DNA repair. The identification of loss-of-function mutations in ZBTB7A was consistent with recent reports that this gene acts as a tumor suppressor in RUNX1–RUNX1T1 CBF-AML.

Together, these results indicate that although CBF-AMLs share CBF alterations, a divergent series of mutations may ultimately affect the pathogenic mechanisms driving the RUNX1–RUNX1T1 and CBFB–MYH11 subtypes. Future studies will explore potentially targetable mutations and how they drive leukemogenesis in CBF-AML subtypes. Faber ZJ et al, Nat Genet 48:1551–6, 2016
Regulators of Hematopoietic Stem Cell Repopulation in Mammals

Improving the outcome of hematopoietic stem cell (HSC) transplantation requires that we increase our knowledge of the mechanisms underlying engraftment and repopulation of donor HSCs in the host niche after transfer. The results of previous screens of mouse and human HSCs have identified genes that are central to the self-renewal of hematopoietic stem and progenitor cells (HSPCs) and their maintenance ex vivo. Those studies examined cells that had been maintained in culture for as long as 17 days, which prevents a direct assessment of genes contributing to HSC engraftment. Shannon L. McKinney-Freeman, PhD (Hematology), led a team of researchers to develop a functional screen that enables such an assessment.

The researchers started by analyzing public databases of HSC gene expression. They pursued 51 promising candidates via quantitative RT-PCR and short-hairpin RNA (shRNA)-silencing studies. Of the 15 genes identified (see list below) that serve the regulatory functions listed.

The researchers studied this gene’s role further. Foxa3-null mice had reduced hematopoietic potential and fewer repopulating cells in their bone marrow than did wild-type mice. The team also found evidence of a potential role for Foxa3 in regulating the metabolic and proliferative stress of HSCs, though the gene is not needed for homeostasis. Together, these findings highlight not only a novel role for Foxa3 in HSPC engraftment but also the varied mechanisms involved in HSC repopulation.

This study’s identification of Foxa3 as a positive regulator of repopulation was the first time a Foxa gene was implicated in hematopoiesis. The researchers studied this gene’s role further. Foxa3-null mice had reduced hematopoietic potential and fewer repopulating cells in their bone marrow than did wild-type mice. The team also identified two genes (Gprasp2 and Armcx1) that negatively regulate repopulation. Knocking down the expression of these genes enhanced HSPC repopulation in most mice.

Endoplasmic Reticulum-to-Golgi Trafficking Is Controlled by the Autophagy Proteins ULK1 and ULK2

Autophagy is an inducible program that ensures the proper disposal and recycling of intracellular components, which is necessary for cellular homeostasis. The ULK1 and ULK2 homologs (ULK1/2) are important regulators of many types of autophagy and act as sensors for nutrient depletion, metabolic stress, and intracellular pathogens. Mice lacking Ulk1/2 die soon after birth, ostensibly because of autophagy-associated defects. To study the role of ULK1/2-regulated autophagy in the central nervous system, Mondira Kundu, MD, PhD (Pathology), and her colleagues specifically deleted Ulk1/2 from the neuronal tissues of developing mice.

In Molecular Cell, the researchers reported that progressive degeneration of the pyramidal neurons of the hippocampus occurred in the brains of these mice. This neuronal degeneration was not associated with any overt defects in autophagy. However, the degenerating neurons exhibited expansion of the endoplasmic reticulum (ER), which is the primary site of-secretory protein folding and lipid biosynthesis. ER expansion can be a marker of activation of the unfolded protein response (UPR), which alleviates cellular stress caused by the accumulation of unfolded or misfolded proteins within the ER. The researchers observed that the degenerating neurons of mice with Ulk1/2 deletion revealed that activation of the UPR contributed to the ER stress-mediated death of these neurons.

To elucidate which ULK1/2-protein interactions are specifically required to maintain ER homeostasis, the researchers performed an unbiased proteomics analysis of all proteins that bind ULK1. This study revealed that SEC16A, a protein that localizes to the ER and facilitates ER-to-Golgi trafficking, specifically binds to ULK1. Because defects in ER-to-Golgi trafficking lead to ER stress and activation of the UPR, the researchers next investigated the mechanisms by which ULK1/2 regulates SEC16A. ULK1/2 are kinases that phosphorylate their protein substrates. ULK1/2 phosphorylated SEC16A, which promoted the formation of vesicles required for ER-to-Golgi trafficking in mammalian cells. In contrast, deletion of Ulk1/2 impaired the ER-to-Golgi trafficking of the serotonin transporter protein SERT, which facilitates serotonin uptake.

Expression of a Sec16A construct containing a mutation that mimics constitutive phosphorylation by ULK1/2 restored SERT trafficking in cells lacking Ulk1/2. These findings suggest that the noncanonical role of ULK1/2 in ER-to-Golgi trafficking maintains cellular homeostasis by regulating the intracellular transport of important cargoes, including neurotransmitter transporters. Joo JH et al, Mol Cell 62:491–506, 2016
COMPREHENSIVE CANCER CENTER

The National Cancer Institute (NCI) supports 69 Cancer Centers in the United States. The St. Jude Comprehensive Cancer Center, currently under the direction of Charles W. M. Roberts, MD, PhD, is the first and only NCI-designated Comprehensive Cancer Center solely focused on pediatric cancer. Comprising five programs and 10 Shared Resources, the Comprehensive Cancer Center emphasizes interdisciplinary laboratory-based and clinical research applicable to the understanding, prevention, and treatment of childhood cancer.

CANCER BIOLOGY PROGRAM
Co-leaders: Martine F. Roussel, PhD; Douglas R. Green, PhD
The goals of this program are to define the critical cellular pathways involved in normal cellular regulation and the pathways that are altered in transformed cells. The program is organized into three highly interactive focus groups that provide thematic, complementary, basic science expertise to the other center programs, enhancing the translation of laboratory discoveries to the clinic. The three focus groups are as follows: Cell Stress & Metabolism, Genome Structure & Function, and Signaling Networks & Therapeutics.

CANCER CONTROL & SURVIVORSHIP PROGRAM
Co-leaders: Melissa M. Hudson, MD; Leslie L. Robison, PhD
As treatments for childhood cancers improve, the number of long-term survivors of childhood cancer increases. This multidisciplinary program strives to improve the quality of life of individuals surviving childhood cancer by identifying and reducing treatment sequelae and promoting health-protective behaviors through the conduct of observational, clinical, and interventional research. With the establishment of large national and institutional cohorts of cancer survivors, program members are conducting research on a wide range of health-related and quality-of-life outcomes.

DEVELOPMENTAL BIOLOGY & SOLID TUMOR PROGRAM
Co-leaders: Michael A. Dyer, PhD; Alberto S. Pappo, MD
Some of the most devastating and poorly understood cancers affect children arise in the peripheral nervous system, muscles, and bones. Members of this program are working to understand how the normal development of these tissues goes awry, resulting in malignant diseases such as neuroblastoma, sarcomas, and retinoblastoma. Research in this program extends from basic mechanistic studies of development, to therapeutic studies in preclinical models, and ultimately to testing new anticancer agents in clinical trials.

HEMATOLOGICAL MALIGNANCIES PROGRAM
Co-leaders: Charles G. Mullighan, MBBS(Hons), MSc, MD; Ching-Hon Pui, MD
The overall goal of this program is to improve the cure rates for childhood leukemias and lymphomas, while minimizing treatment-related adverse effects. This program has a distinguished track record in improving the 5-year survival rate of acute leukemias and reducing the use of harmful therapeutic modalities such as cranial irradiation. Most recently, the members of this program have used advanced genetics to identify novel subgroups of leukemias and the mutations that drive these diseases. The same genetic tools are being used to uncover genetic variations that dictate susceptibility to childhood cancers, as well as the response of patients to essential chemotherapies.

NEUROBIOLOGY & BRAIN TUMOR PROGRAM
Co-leaders: Suzanne J. Baker, PhD; Amar J. Gajjar, MD
By integrating the latest genomic and genetic technologies with studies of the developing nervous system, members of this program are efficiently translating laboratory findings into opportunities for new treatments. Recent efforts include the identification of the cells of origin of important pediatric brain tumors and the modeling of some of the most aggressive forms of these tumors, including high-grade gliomas. Close collaboration among the laboratory and clinical members of the program allows the rapid translation of results of high-throughput drug screens in mouse models to clinical trials.

SHARED RESOURCES
Animal Resource Center
Bioinformatics and Biotechnology
Biostatistics
Cell and Tissue Imaging
Cytogenetics
Flow Cytometry and Cell Sorting
Diagnostic Biomarkers
Pharmacokinetics
Protein Production Facility
Transgenic/Gene Knockout
ST. JUDE AFFILIATE PROGRAM

The eight clinics that comprise the St. Jude Affiliate Program contribute to the institution’s mission by enrolling patients in St. Jude protocols and participating in St. Jude treatment and research programs. The clinics also provide patients with the opportunity to receive part of their care at a facility near their home community.

| Location | Clinic Name | Medical Director | Administrative Director | Medical Director | Chair of Pediatrics | Chair of Pediatric Oncology | Medical Director | Medical Director | Medical Director | Medical Director | Medical Director | Medical Director | Medical Director | Medical Director | Medical Director | Medical Director | Medical Director | Medical Director | Medical Director |
|----------|-------------|------------------|-------------------------|------------------|-------------------|------------------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| BATON ROUGE, LA | Our Lady of the Lake Children’s Hospital – Our Lady of the Lake Regional Medical Center | Dr. Russell Gentry | Dr. Lisa McElroy | Dr. Cheryl Alvis | Dr. Sean Jackson | Dr. Michelle A. George | Dr. John D. Thomas | Dr. Kelli Johnson | Dr. Michelle A. George | Dr. Michael J. Phillips | Dr. Jeanne M. Girardin | Dr. Susan C. Hensley | Dr. Sarah M. Pearcy-Black | Dr. Michelle A. George | Dr. Michael J. Phillips | Dr. Jeanne M. Girardin | Dr. Susan C. Hensley | Dr. Sarah M. Pearcy-Black |
| PEORIA, IL | Children’s Hospital of Illinois – OSF Healthcare System | Dr. David O’Leary | Dr. Eric K. Anderson | Dr. Susan A. Weigel | Dr. Benjamin G. Telford | Dr. Tarek A. El-Sayed | Dr. Michael D. Olson | Dr. Eric K. Anderson | Dr. Susan A. Weigel | Dr. Benjamin G. Telford | Dr. Tarek A. El-Sayed | Dr. Michael D. Olson | Dr. Eric K. Anderson | Dr. Susan A. Weigel | Dr. Benjamin G. Telford | Dr. Tarek A. El-Sayed | Dr. Michael D. Olson | Dr. Eric K. Anderson |
| SHREVEPORT, LA | First-Monterey Cancer Center – LSU Health Sciences Center – Shreveport | Dr. Tiffany A. Davis | Dr. Eric L. Sapp | Dr. Richard L. Smith | Dr. Thomas B. Lowery | Dr. Mark A. Holcomb | Dr. Richard L. Smith | Dr. Eric L. Sapp | Dr. Tiffany A. Davis | Dr. Thomas B. Lowery | Dr. Mark A. Holcomb | Dr. Richard L. Smith | Dr. Eric L. Sapp | Dr. Tiffany A. Davis | Dr. Thomas B. Lowery | Dr. Mark A. Holcomb | Dr. Richard L. Smith | Dr. Eric L. Sapp |
| SPRINGFIELD, MO | Mercy Children’s Hospital – Springfield – Mercy Health System | Dr. Joseph P. O’Leary | Dr. Linda L. Kohn | Dr. Wayne L. Smith | Dr. John D. Thomas | Dr. Michael J. Phillips | Dr. Jeanne M. Girardin | Dr. Susan C. Hensley | Dr. Sarah M. Pearcy-Black | Dr. Michael J. Phillips | Dr. Jeanne M. Girardin | Dr. Susan C. Hensley | Dr. Sarah M. Pearcy-Black | Dr. Michael J. Phillips | Dr. Jeanne M. Girardin | Dr. Susan C. Hensley | Dr. Sarah M. Pearcy-Black |
| TULSA, OK | The Children’s Hospital at Saint Francis | Dr. David O’Leary | Dr. Eric K. Anderson | Dr. Susan A. Weigel | Dr. Benjamin G. Telford | Dr. Tarek A. El-Sayed | Dr. Michael D. Olson | Dr. Eric K. Anderson | Dr. Susan A. Weigel | Dr. Benjamin G. Telford | Dr. Tarek A. El-Sayed | Dr. Michael D. Olson | Dr. Eric K. Anderson | Dr. Susan A. Weigel | Dr. Benjamin G. Telford | Dr. Tarek A. El-Sayed | Dr. Michael D. Olson | Dr. Eric K. Anderson |

ST. JUDE GLOBAL

At St. Jude, we believe in giving every child the best chance of a cure. By freely sharing knowledge, technology, and research data, we are saving the lives of children in our clinics and around the world.

St. Jude is committed to addressing the greatest challenge in pediatric hematology and oncology today—ensuring that all children with cancer or a nonmalignant hematologic disorder have access to quality treatment, regardless of where they live. In a high-income country such as the United States, access to modern treatments and robust supportive care has resulted in cure rates of more than 80% for children with cancer. However, for the approximately 90% of children living in low- or middle-income countries (LMICs), where access to quality pediatric oncology care is suboptimal to nonexistent, most children with cancer will die. Children with hematologic disorders, particularly β-hemoglobinopathies such as sickle cell disease, have similar dismal outcomes in LMICs. The vast majority of children with β-hemoglobinopathies live in LMICs, and their diseases are usually not diagnosed due to a paucity of newborn screening and dedicated treatment programs. As a result, hundreds of thousands of children with sickle cell disease die each year from complications that can be prevented.

The unique challenges of delivering treatment for pediatric cancers and nonmalignant hematologic disorders in LMICs represent an opportunity for St. Jude to develop and lead a new field of research in global pediatric catastrophic diseases and, by extension, save countless children’s lives. The overarching goals of the St. Jude Global Program are as follows: (1) Train the local clinical workforce. (2) Develop and strengthen the continuum of care required for children with cancer or hematologic disorders from work at the national health system level to patient-centered care initiatives. (3) Advance knowledge in global pediatric oncology/hematology through translational and scalable research. Work done over the last 25 years through the St. Jude International Outreach Program has planted the seeds for what is the most ambitious initiative ever developed in pediatric oncology/hematology.

In the coming decade, St. Jude–led efforts will strive to improve the survival rates of children with cancer or hematologic disorders worldwide by training experts and developing the tools necessary to provide the highest-quality care that is locally feasible. To meet this goal, we will design and disseminate resource-appropriate, evidence-based interventions to improve patient care and engage stakeholders at the health systems level. We will also build alliances of health centers to maximize local and regional knowledge, support, and leadership to rapidly improve and expand treatment services. We will implement this vision through St. Jude Global and the newly established Department of Global Pediatric Medicine, two distinct but interconnected entities.

The Department of Global Pediatric Medicine and St. Jude Global will seamlessly integrate research, innovation, and education to create a new paradigm in pediatric hematology-oncology. The program will integrate initiatives at the institutional level with the Graduate School of Biomedical Sciences, the Comprehensive Cancer Center, and the Pediatric Hematology/Oncology Fellowship Program and at the national and international levels with collaborators at other academic institutions, governmental agencies (e.g., National Cancer Institute), and nongovernmental organizations, including the World Health Organization and the International Society of Pediatric Oncology. The ultimate goal of St. Jude Global is to impart knowledge and innovative health-delivery models that will enable all children with cancer or a nonmalignant hematologic disorder to receive the best-possible care.
### Academic Departments

#### Epidemiology & Cancer Control
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- MD Anderson Cancer Center
- Cancer Prevention & Control

Members:
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- Gompers, MD
- Tumor Research
- Family Medicine Research: Childhood Cancer Control
- Cancer Control Research: Childhood Cancer Control

#### Global Pediatric Medicine
Chair:
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- Global Pediatric Medicine
- Global Health Research: Childhood Cancer Control
- Global Health Research: Childhood Cancer Care

Members:
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- Pediatric Oncology
- Pediatric Hematology
- Hematology Research: Childhood Cancer Care
- Hematology Research: Childhood Cancer Care
- Cancer Research: Childhood Cancer Care

#### Immunology
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- Immunology
- Immunology Research: Childhood Cancer Care
- Immunology Research: Childhood Cancer Control

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- Immunology Research: Childhood Cancer Control
- Immunology Research: Childhood Cancer Control
- Immunology Research: Childhood Cancer Control

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- Infectious Diseases Research: Childhood Cancer Control

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- Infectious Diseases Research: Childhood Cancer Control
- Infectious Diseases Research: Childhood Cancer Control
- Infectious Diseases Research: Childhood Cancer Control

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- N. Richard Long, MD
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- Hematology Research: Childhood Cancer Control

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- Hematology Research: Childhood Cancer Control

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- Oncology Research: Childhood Cancer Care
- Oncology Research: Childhood Cancer Control

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- Oncology Research: Childhood Cancer Control

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- Genetics Research: Childhood Cancer Control
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- Genetics Research: Childhood Cancer Control

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- Jeffrey W. Miller, MD
- Hematology & Oncology
- Hematology Research: Childhood Cancer Care
- Hematology Research: Childhood Cancer Control

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- Research Associates: Childhood Cancer Care
- Research Associates: Childhood Cancer Control
- Research Associates: Childhood Cancer Control
- Research Associates: Childhood Cancer Control

#### Adjunct Members
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- Adjunct Members: Childhood Cancer Care
- Adjunct Members: Childhood Cancer Control
- Adjunct Members: Childhood Cancer Control
- Adjunct Members: Childhood Cancer Control

#### Research Associates & Adjunct Members
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- Research Associates & Adjunct Members: Childhood Cancer Care
- Research Associates & Adjunct Members: Childhood Cancer Control
- Research Associates & Adjunct Members: Childhood Cancer Control
- Research Associates & Adjunct Members: Childhood Cancer Control

#### Research Associates
- Peter B. Socci, MD
- Research Associates: Childhood Cancer Care
- Research Associates: Childhood Cancer Control
- Research Associates: Childhood Cancer Control
- Research Associates: Childhood Cancer Control

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- Jeffrey W. Miller, MD
- Adjunct Members: Childhood Cancer Care
- Adjunct Members: Childhood Cancer Control
- Adjunct Members: Childhood Cancer Control
- Adjunct Members: Childhood Cancer Control

#### Research Associates
- Charles C. Paint, PhD
- Research Associates: Childhood Cancer Care
- Research Associates: Childhood Cancer Control
- Research Associates: Childhood Cancer Control
- Research Associates: Childhood Cancer Control

#### Adjunct Members
- Robert A. Weinberg, PhD
- Adjunct Members: Childhood Cancer Care
- Adjunct Members: Childhood Cancer Control
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- Tudor Moldoveanu, PhD

**Research Fellow**:
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**Research Program**
- Ependymoma, medulloblastoma, and neuroblastoma

**Secondary Appointment**
- Bhaskar N. Rao, MD

**Primary Appointment**
- William E. Evans, PhD

**Tumor Program**
- Novel treatments for children with brain tumors; high-risk cases

**Clinical Program**
- Preclinical models of infectious diseases & cancer research

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- Kevin R. Krull, PhD
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- Victoria W. Willard, PhD

**Associate Members**:
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- Jennifer A. McArthur, PhD
- Heather M. Conklin, PhD
- R. Ray Morrison, MD

**Research Associates**:
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- A. Peter Vogel, DVM, PhD
- Asya Agulnik, MD

**Tumor Program**
- Novel treatments for children with brain tumors

**Clinical Program**
- Preclinical models of infectious diseases & cancer research

**Secondary Appointment**
- R. Ray Morrison, MD

**Primary Appointment**
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**Tumor Program**
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**Primary Appointment**
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**Clinical Program**
- Preclinical models of infectious diseases & cancer research

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- Victoria W. Willard, PhD

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- R. Ray Morrison, MD

**Research Associates**:
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**Clinical Program**
- Preclinical models of infectious diseases & cancer research

**Secondary Appointment**
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**Primary Appointment**
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**Associate Members**:
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- Jennifer A. McArthur, PhD
- Heather M. Conklin, PhD
- R. Ray Morrison, MD

**Research Associates**:
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- A. Peter Vogel, DVM, PhD
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**Clinical Program**
- Preclinical models of infectious diseases & cancer research

**Secondary Appointment**
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- Victoria W. Willard, PhD

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- R. Ray Morrison, MD

**Research Associates**:
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**Secondary Appointment**
- R. Ray Morrison, MD

**Primary Appointment**
- William E. Evans, PhD

**Tumor Program**
- Novel treatments for children with brain tumors; high-risk cases

**Clinical Program**
- Preclinical models of infectious diseases & cancer research
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Martine F. Roussel, PhD; Endowed Chair in Molecular Oncogenesis • Genes and microRNAs in brain tumors
Brenda A. Schulman, PhD1 • Cellular regulation by ubiquitin-like proteins

Research Associate
Chunliang Li, PhD • Genome editing in cancer development

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<td>Chair, Pediatrics, University of Tennessee Health Science Center Pediatrician-in-Chief, Le Bonheur Children’s Hospital Infectious Diseases</td>
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Boston Children’s Hospital

OPERATIONS & STATISTICS

OPERATIONS

Operating expenses1 $808.8 million
Number of employees2 4,922

RESEARCH STATISTICS

Grant funding1 $98.7 million
Peer-reviewed original research publications 779
Faculty members 261
Postdoctoral fellows 302
Clinical residents and fellows3 205
Graduate research scholars 83

CLINICAL STATISTICS

Number of beds4 66
Outpatient encounters5 308,386
Inpatient admissions 3,959
Total inpatient days 19,188
Patients enrolled on therapeutic trials 1,055
Patients enrolled on nontherapeutic trials 7812
5875 on prospective trials
1937 on tissue-banking protocols

Number of protocols open to accrual in 2016 420
Number of active therapeutic trials 222
Number of active nontherapeutic trials 198
6 prospective trials
192 tissue-banking protocols

Data represents the period July 1, 2015 – June 30, 2016.

1Data is from July 1, 2016.
2Data includes 67 full-time St. Jude fellows and 138 rotating fellows from the University of Tennessee Health Science Center or other medical schools.
3Data represents the number of beds in use. St. Jude is licensed for 80 beds.
4Data represents the total number of ambulatory or ancillary encounters not daily visits.
5Data represents the total number of prospective trials or tissue-banking protocols for any given year.

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