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Pediatric Brain Tumor Research

Research into two rare and aggressive childhood brain tumors unveils discoveries that may be applicable well beyond those pediatric cancers.

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Background

Diffuse intrinsic pontine glioma (DIPG) and atypical teratoid/rhabdoid tumor (AT/RT), a subset of rhabdoid tumors (RT) that occur in the brain, are both rare and lethal childhood cancers. Approximately 300 children are diagnosed with DIPG each year, typically around age six. Radiation reverses the symptoms, but only temporarily and within six months to a year, symptoms return. Approximately 100 new cases of AT/RT occur each year, typically in children under three years of age, though AT/RT also occurs in adults. Treatment varies depending on many factors. Less than twenty percent of children survive, most with significant life-long side effects from therapy.

For a long time, little was understood about these pediatric cancers, but recent and ongoing research at Dana-Farber Cancer Institute has led to important discoveries that are pointing the way to new therapies for these diseases — and for cancer in general.

For the past fifty years, the families of children with DIPG had little hope because no one had ever been able to thoroughly study the disease. Other forms of cancer have been examined extensively through the analysis of tissue biopsies to determine the biology driving the tumor growth. But DIPG tumors grow in the brain stem, the central relay station for the nervous system. No one thought that they could be safely biopsied.

Early DIPG Research

In 2002, Mark Kieran, MD, PhD, director of pediatric medical neuro-oncology at Dana-Farber/Boston Children's Cancer and Blood Disorders Center, began a campaign to biopsy patients with newly diagnosed DIPG. Surgical techniques were advanced enough to do it safely, he argued, and molecular biology had become sophisticated enough to make good use of the tiny, hair-thin thread of tissue the surgeons could safely collect. Kieran's efforts were met with resistance in the United States. But in 2007, a French medical team started to perform the procedure.

With proof that DIPG biopsies could be done safely, Kieran was able to convince surgeons in the United States to begin. He launched a clinical trial

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in 2010 at 25 sites across the country, all following the same surgical protocol with strict regulatory oversight. The trial is still recruiting patients, with results expected in late 2015.

The Molecular Drivers of DIPG

The French surgeons collaborated with Kieran to perform a molecular analysis of their first twenty samples at the Broad Institute of Harvard and MIT. That analysis, which searched for a set of genes with known links to cancer, identified the first clues about which genetic mutations might be causing DIPG. The list included PI3KCA, found in fifteen percent of samples, TP53 in forty percent of samples, and ATM/MPL in five percent of samples (Grill 2012). PI3KCA was of particular interest since PI3K inhibitors were already in trials in other forms of cancer.

As US biopsies proceeded, Kieran worked with a team of molecular biologists in Montreal, Canada, to perform whole exome sequencing on the first twelve samples collected. Whole exome sequencing looks at every protein-coding gene in the genome, allowing for a more complete picture of the genetic profile of these tumors. In 2014, Kieran and collaborators reported the discovery of ACVR1 mutations in DIPG biopsies (Fontebasso 2014). This gene mutation had never been seen before in cancer.

Other research teams have analyzed post-mortem DIPG samples. These studies have found that the PDGF receptor is overexpressed in DIPG. In addition, histone mutations are present, suggesting that errors in gene regulation may contribute to DIPG. But since these analyses are done on tissue after treatment with radiation and cancer progression, it is difficult to know if the mutations in the samples are present at diagnosis or if they were induced by therapy later.

Advancing Understanding and Care for DIPG

The discoveries of Kieran and colleagues have built a foundation for developing scientifically grounded therapeutics for DIPG. Kieran is currently developing clinical trials for DIPG patients with existing drugs that target PI3K and PDGFR. Challenges include finding a PDGFR inhibitor that effectively crosses the blood-brain barrier and finding an appropriate combination therapy for a PI3K inhibitor, since these inhibitors may be ineffective alone. He and others are also working to understand the newly discovered ACVR1 mutation and to begin to look for therapeutics that target it.

Researchers, including Kieran, are also working to model DIPG in cellular culture and in mice to facilitate research, understand if DIPG is a single disease or many different diseases, and to test potential therapies. This effort includes pursuing the hypothesis that a certain set of neural precursor cells in the brain may be the source of DIPG. These cells are supposed to peak in growth before birth, but instead may continue to grow because of histone mutations. The addition of a gene mutation that accelerates growth may create a perfect storm that leads to DIPG (Robinson 2014).

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AT/RT: Discovering a New Cancer Lineage with Broad Implications

As a post-doctoral fellow at Dana-Farber, Charles Roberts, MD, PhD, now associate professor of pediatric oncology at Dana-Farber/Boston Children's Cancer and Blood Disorders Center followed up on a discovery that a gene called INI1 was mutated in pediatric rhabdoid tumors (RT). Earlier research suggested that genes that drive early onset childhood cancers often have wider relevance.

Roberts began by examining the effects of loss of INI1 in mice. Complete knock-out of the gene was lethal for embryos. Those with INI1 knocked out in just one of two chromosomes survived, only to develop cancer shortly after birth. (Roberts 2000) Roberts then developed a method for selectively turning off the gene without immediate lethality. In this study, mice survived to birth, but all of them developed cancer, with a median onset of only eleven weeks. This was among the fastest onsets of cancer ever reported from the loss of a single gene (Roberts 2002).

Based on the conventional understanding of cancer biology, Roberts assumed that such rapid onset was the result of genomic instability, a rapid accumulation of mutations resulting in aggressive cancer growth. But when he looked for other mutations in human rhabdoid tumor samples, he found none. The findings led to the conclusion that INI1 mutations led to remarkably genetically simple but rapidly developing cancer (Lee 2012).

An explanation of this counterintuitive finding comes from an understanding of the role of INI1, which is also called SNF5, SMARCB1, and BAF47 in the literature since it has been studied in different contexts. INI1 codes for one of fifteen proteins that together form a large molecular machine that packages DNA inside cells, a process called chromatin remodeling that resembles the spooling of thread. The machine, called the SWI/SNF complex, winds the two meters of DNA inside each cell around nucleosome spools for compact storage. The spooling also plays a regulatory role. Spooled genes are turned off, while unspooled genes may be turned on.

The SWI/SNF complex acts as the master regulator of master regulators by determining which genes can be expressed in a given cell. Roberts discovered that defects in this machine lead on their own to aggressive cancers, defining a new paradigm for the development of AT/RT tumors.

Beyond AT/RT: A New Lineage for Cancer

Meanwhile, genome-wide association studies were uncovering defects in other proteins in the same spooling machinery in other forms of cancer, including ovarian, lung, bladder, colorectal, and more. A 2010 study mined all of this data and found that twenty percent of all cancers had SWI/SNF mutations, suggesting that this machinery plays a significant role in the formation of cancer in general.

Roberts and other researchers are now working to better understand how this machinery works normally as a means to provide insights into how to repair it when it malfunctions. For instance, Roberts likens the spooling machine to a car and a

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defect in INI1 to a cruise control mechanism stuck at highway speeds. Rather than fix the cruise control, he is searching for ways to disable it so the car can function normally.

In Search of New Therapeutics

While Roberts was working to understand how AT/RT develops, he was also looking for potential therapeutics to address the INI1 defect. Since INI1 is completely lost, it cannot be targeted with a drug, so Roberts looked for alternatives. He found EZH2. In the context of the spooling machine, the EZH2 protein spools DNA, while INI1 unspools it. Roberts tried inhibiting EZH2 as a way to bring the process back into balance. He found that the inhibitor blocked tumor formation in mice (Wilson 2012).

In response to these findings, the biopharmaceutical company Epizyme became interested in testing their newly developed EZH2 inhibitor. They found that it worked well against AT/RT mouse models (Knutson 2013). A recent test in humans included an adult with RT who responded to the drug.

The company is working closely with Susan Chi, MD, pediatric oncologist at Dana-Farber/Boston Children's, to launch a phase 1 clinical trial of the inhibitor in children with recurrent RT and solid tumors. Chi is also developing a trial for children with newly diagnosed RT that is also expected to open later this year. The trial will test a protocol that produced good survival rates in a 2009 trial she ran, but will add a biologic agent to the treatment.

Recently, Roberts found two more leads pointing to potential targets for repair of a defective spooling machine. One points to ARID1B as a potential therapeutic target in ARID1A-mutated cancers, such as a percentage of ovarian, gastric, bladder, colorectal, lung, and hepatocellular (Helming 2014). Another points to SMARCA2 as a potential target in SMARCA4-mutant cancers including a percentage of non-small cell lung cancer, Burkitt's lymphoma, childhood medulloblastoma, and others (Wilson 2014). ARID1B and SMARCA2 are both part of the SWI/SNF complex.

Roberts is also screening existing drugs to find candidates that target the SWI/SNF complex that could be brought rapidly to clinical trials. One promising drug has already been found using this approach, and Roberts and Chi are working to advance it to clinical trials.

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