The Biological Concepts and Treatment Therapies of Rhabdoid Tumors

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BRIEFS. This review will explore the biology behind rhabdoid tumors and current/potential future treatment therapies.

ABSTRACT. Rhabdoid tumors are a type of soft tissue sarcoma that primarily affects children under the age of 2. These tumors are often defined by genomic alterations in the SMARCB1 gene, which normally encodes SNF5, a tumor suppressive protein. As a subunit of the SWItch/Sucrose NonFermentable chromatin remodeling complex (SWI/SNF), SNF5 is centrally involved in the regulation of the oncogene MYC. The integrity of the SWI/SNF complex is greatly compromised by the loss of the SNF5 protein, enabling MYC to bind chromatin and promote expression of genes involved in cell replication, a driver of cancer. On the other hand, when SNF5 is present as the complex is functioning normally, it binds MYC, modulates MYC's interaction with chromatin, represses the transcription of MYC-target genes and prevents tumorigenesis. The current treatment strategies are not particularly effective as they include just standard cancer therapies: chemotherapy, radiation, and surgery. Since the patients are so young, the outlook for these therapies has not been very positive. Currently, there are more treatments with potential undergoing evaluation.

INTRODUCTION.

Recent advances in biomedical technology have spurred new treatment opportunities for rare cancers. More specifically, progress in bioinformatics coupled with gene targeting and cell and tissue engineering have improved tremendously allowing scientists to shift their view on the mechanisms of cancer from a descriptive classification system to a more precise genetic profile of cancer. As a result, the treatment opportunities for cancer patients have expanded, providing optimism for progress in therapeutic development for rare cancers, including rhabdoid tumors *(1)*.

Rhabdoid tumors are aggressive pediatric soft tissue sarcoma that arises in the central nervous system (CNS), peripheral nerve roots, and a variety of soft tissue of the body including kidneys, paravertebral muscles, liver, and heart. *(2)*. Rhabdoid tumors arising in the CNS, are classified as atypical teratoid rhabdoid tumors (AT/RT) whereas when they arise in soft tissue outside of the brain, they are known as malignant rhabdoid tumors (MRT). These tumors are often defined by genomic alterations in SMARCB1, a subunit of the SWItch/Sucrose NonFermentable chromatin remodeling complex (SWI/SNF). To a lesser extent (only about 5%), alterations in SMARCA4 (BRG1) subunit also cause rhabdoid tumors. >98% of MRT exhibit mutations which result in biallelic SMARCB1 inactivation. This is a complete loss of expression *(3,4)*. This definition of genomic alterations in SMARCB1 provides certainty for identification, making it very important.

The Biology of Rhabdoid Tumors.

The SMARCB1 gene, mating-type switching/SNF-related matrix-associated actin-dependent regulator of chromatin, subfamily B, member 1 (also named INI1 or BAF47), encodes an epigenetic tumor suppressor, a protein that help control cell growth – SNF5 *(3,4)*. It was discovered early on that SMARCB1 was linked to cancer, but it was after systematic cancer genome-sequencing studies that the mutations were more clearly recognized *(5)*. The key importance of the SMARCB1 gene is that when it is lost or inactivated, it drives rhabdoid tumor formation. In patients diagnosed with AT/RT, 25%-35% were noted to have mutations of SMARCB1 in the germline, meaning that they were born with it. *(1)*. SNF5 is important for suppressing tumorigenesis, and functions by downregulating cell proliferation through interactions with cell cycle genes such as p16, pRb, and HDAC1. SNF5 is a core component of the SWI/SNF complex.

The SWI/SNF complex, also known as BRG1/BRM-associated factor (BAF) complex, plays a crucial role in RTs. This multi-subunit chromatin remodeling complex is known by a diverse set of nomenclature that was derived from original discoveries in yeast. Researchers found in yeast, orthologous complexes, which are genes that evolved from a common genetic ancestor developed by speciation that usually have similar functions in different species *(6)*. They discovered the mating-type switching and its function of sucrose fermentation which explains the origins of its particular nomenclature. Mutations affecting the complex lead to a sucrose non-fermenting phenotype *(5)*. The SWI/SNF complex is mutated in about 20% of cancers *(7)*. Most of these mutations are a loss of function, meaning that the gene is inactivated, resulting in non-functioning proteins with reduced or no activity. The integrity of the SWI/SNF is greatly compromised by the loss of the SNF5 protein as the loss causes widespread collapse of enhancers that regulate differentiation – the process by which a less specialized cell mature to change function, usually to become more specialized. The loss of SNF5 also mobilizes the residual SWI/SNF complexes to become super-enhancers that promote tumorigenesis and maintenance of tumor cells *(5)*.

Mammalian SWI/SNF complexes (mSWI/SNF) are ATP dependent chromatin remodelers. They have many roles in transcriptional regulation including modulating genomic architecture and mediating cell differentiation. mSWI/SNF complexes are combined from 29 genes and divided into three broad subfamilies: canonical BAF (cBAF), polybromo-associated BAF (PBAF), and non-canonical BAF (ncBAF). Each subfamily shares multiple subunits as all three contain the core subunits: SMARCC1, SMARCC2, SMARCD1 and either of the ATPases SMARCA4 or SMARCA2 *(6-8)*. The SMARCB1 subunit, implicated in RT, is a member of the BAF and PBAF complexes. There are specific subunits to provide their distinct identity in each with ARID1A/ARID1B and DPF2 in cBAF, PBRM1, ARID2 and BRD7 in PBAF, and GLTSCR1L and BRD9 in ncBAF complexes (giving ncBAF the extra nomenclature of GLTSCR1L- containing and BRD9- containing (GBAF) complex). Further, *Mittal and Roberts* determined that MRT are dependent on ncBAF complexes for maintenance of the tumorigenic state *(8)*.

SNF5, the protein product of the SMARCB1 gene, is also known to bind c-MYC, an oncoprotein transcription factor *(7)*. SNF5 controls the ability of c-MYC to bind chromatin. When SNF5 is not present, as the case in RTs, c-MYC is able to bind to chromatin and promote gene expression, which helps maintain the tumor. In normal tissue, when SNF5 is present, it binds to c-MYC and can modulate c-MYC's interaction with chromatin to prevent overexpression. It represses the transcription of c-MYC-target genes *(7)*.

CLINICAL INFORMATION AND TREATMENTS.

Current Statistics.

Rhabdoid Tumors are a rare cancer with only approximately 20 to 25 new cases diagnosed each year in the United States *(4)*. They are early onset, occurring in children 1-2 years old with the median age of onset being 18 months *(4)*. Because they have the capability of metastasizing widely, patients' 3-year event-free survival rates range from 31%–38% while the 5-year overall survival rates range from 15%–36% *(4)*. There is a reported male dominance with a 1.3 to 1.5 male to female ratio. AT/RTs account for 40%–50% of all embryonal CNS tumors in the first year of life. They are the most common malignant CNS tumor in children below 2 year of age *(5)*.

Histology.

Most rhabdoid tumors are made of pattern-less sheets of discohesive polygonal cells commonly with necrosis, or cell death, in the background. The key feature of RT is "rhabdoid" cells, which are shaped like epithelioid cells as they are large and polygonal. They have vesicular (small, fluid-filled) nuclei with large, very prominent, inclusion-like nucleoli *(4)*. These cells also have abundant cytoplasm as they usually have high mitotic activity. Electron microscopy revealed major cytoplasmic swirls of filaments and like light microscopy, large nucleoli. Occasionally, they form a nested pattern with a more central discohesion. Others take a more rigid shape. Although most RT take a polygonal shape, some show mild spindling *(4)*.

Some instances of RT are made up of smaller cells. This makes them almost histologically indistinguishable from Ewing sarcoma, a cancer that mostly occurs in and around the bones. Because of this histological similarity, the lack of classic "rhabdoid" cells does not exclude the possibility of RT, especially in small biopsy samples, so even if the cells don't look like typical RT, the tumors may still be RT, which is why the diagnosis procedure is very specific *(4)*.

Symptoms and Diagnosis.

RT is hard to identify by symptoms alone as the symptoms aren't particularly obvious. Infants and toddlers may experience pain expressed as fussiness, blood in the urine, a large mass in the abdomen, or high blood pressure. There may be signs of abnormal function of extremities in the brain – problems with motor coordination, and changes in level of consciousness such as lethargy *(9)*. A diagnosis can be strongly suspected based on hematoxylin and eosin morphology (H&E stain). However, it is necessary to have a nuclear SMARCB1 stain to identify a lack of SMARCB1 as confirmatory immunohistochemistry in all cases for diagnosis *(8)*.

CURRENT TREATMENTS.

Treatment Options.

The current treatment strategies are not particularly effective. In general, the patient undergoes surgery to remove the tumor. They are then treated with chemotherapy with or without radiation. For brain tumors in particular, radiation therapy is more crucial for management of the disease. Under the pretense of a successful surgery, the patient may also need a stem cell transplant to allow them to restore healthy blood cells. Most children diagnosed with RT die quickly from metastasis despite treatment *(6)*. Most of the ongoing clinical trials reflect the trend of limited current treatment options but also explore many potential drugs as seen in Table 1.

Radiation Therapy.

Radiotherapy, using high doses of radiation to kill cancer cells and shrink tumors, at any time during treatment was found to significantly influenced survival (median survival 17.8 mo vs 14 mo; P=0.64) *(5)*. In a sample of 28 patients, the Children's Cancer Group found a 1.5-fold lower risk of death in infants with AT/RT if they were treated with radiotherapy *(5)*. Researchers are looking for ways to minimize the harmful side effects of radiotherapy. One possible method is to treat such as by treating patients with a proton beam. This treatment was used to treat 31 patients at MD Anderson *(5)*. The Massachusetts General Hospital's treated 10 patients with proton therapy, successfully avoiding damage of at-risk organs such as the hypothalamus and cochlea *(5)*.

Table 1. Ongoing clinical trials for rhabdoid tumors.

Trial Name	Interventions	Phase
Combination Chemotherapy, Radiation Therapy, and an Autologous Peripheral Blood Stem Cell Transplant in Treating Young Patients With Atypical Teratoid/Rhabdoid Tumor of the Central Nervous System	3-Dimensional Conformal Radiation Therapy Autologous Hematopoietic Stem Cell Transplantation	III
Antineoplaston Therapy in Treating Children With Rhabdoid Tumor of the Central Nervous System	Antineoplaston therapy (Atengenal + Astugenal)	II
Treatment of Patients With Newly Diagnosed Medulloblastoma, Supratentorial Primitive Neuroectodermal Tumor, or Atypical Teratoid Rhabdoid Tumor	filgrastim cisplatin, cyclophosphamide, vincristine autologous hematopoietic stem cell transplantation radiation therapy	III
Modified Measles Virus (MV-NIS) for Children and Young Adults With Recurrent Medulloblastoma or Recurrent ATRT	Modified Measles Virus Modified Measles Virus Lumbar Puncture	I

It has been found that patients above the age of 4 experience less benefits from radiation therapy when compared to younger patients *(5)*. Infants who were treated with radiation therapy were at high risk for leukoencephalopathy, disorders of brain white matter, or radio necrosis as their CNS are not well-developed. This concern lead to exploration of alternative treatments such as high-dose chemotherapy (HDCT).

High Dose Chemotherapy.

High-dose chemotherapy (HDCT) is an intensive drug treatment to kill cancer cells. It is characterized by severe side effects, including destruction of rapidly dividing cells in the bone marrow *(10)*. In 1998, a cohort of RT patients underwent HDCT. One of the young patients remained alive for 46 months after treatment. The overall 3-year EFS was 43%±19% in this study *(5)*. In another study done on a group of 19 children, only 4 completed induction chemotherapy, the first line treatment of cancer with a chemotherapeutic drug to induce a remission. The survivors were noted at 40, 42, 46, and 79 months. These results were not all positive as there were 5 toxic deaths. The 3-year EFS was rather insufficient at 21±9%. From 2003 to 2008, six patients were treated with HDCT in a study in Toronto; four of the six patients survived with no signs of diseased after 52 months (median follow up). In another study, HDCT was used with thiotepa plus carmustine (BCNU) for the first course and thiotepa plus carboplatin in the second. This phase I trial was performed on 2 patients with AT/RT. Both patients were alive more than 7 years after HDCT treatment *(5)*.

Innovative Treatments.

Researchers have been studying multiple compound-driven approaches. Lunenburger *et al*. used *in vitro* proliferation assays to identify the *in vitro* activity of vinorelbine, sorafenib, rapamycin, and curcumin, an herbal compound *(11)*. These compounds were selected as vinorelbine is commonly used to treat breast cancer and non-small cell lung cancer; sorafenib is used to treat kidney, liver, and thyroid cancer;

rapamycin is used to prevent organ rejection after a kidney transplant; and curcumin is often used to treat pain and inflammation. D'Cunja *et al*. used antisense oligonucleotides against insulin like growth factor 1 receptor. They tried to establish chemotherapy sensitization against cytostatics, drugs that inhibit cell growth, such as cisplatin and doxorubicin. These tests suffer major drawbacks as the drug testing process is rather inefficient due to heavy dependence on cell lines and xenografts which takes a long time to properly select *(5)*.

Directly Targeting SWI/SNF Complexes.

One potential target in RT is BRD9 as it is a component of ncBAF, which RT are dependent on for maintenance of the tumorigenic state. There is particular interest on small-molecule inhibitors of BRD9 (such as BI-7273 and I-BRD9), and some of these molecules have been tested in RT and synovial sarcoma cell lines. In these studies, knockdown or deletion of BRD9 successfully impaired cell proliferation. The inhibitors that were bound to BRD9 bromodomain had no effect. On the other hand, introduction of a "degron" compound induced the opposite effect. The "degron" compound causes the degradation of BRD9 (dBRD9). These findings indicate that degradation of BRD9 is likely to be required in RTs and possible structural disruption of ncBAF as well *(1)*.

Targeting Cell Cycle Regulators in Rhabdoid Tumors.

In AT/RT in infants, researchers found an overexpression of cyclin D1, a protein that mediates cell proliferation, in AT/RT. They experimentally reintroduced SMARB1 in cell lines. This resulted in G0/G1 cell cycle arrest and importantly, a repression of cyclin D1. It also resulted in the induction of p16INK4A, a cell cycle regulator, and hypophosphorylation of retinoblastoma, which is a sign the cell is in late G1 phase resulting in halted proliferation *(13)*. In a clinical trial, researchers tested the CDK4/6 inhibitor ribociclib in patients with RT, neuroblastomas, and CDK4-amplified malignancies. This compound may be used in the future as combination with conventional chemotherapy *(5)*.

CONCLUSION.

Rhabdoid Tumors are a rare but deadly pediatric cancer. They are defined by genomic alterations in SMARCB1, a gene that produces the tumor suppressive protein SNF5 on the SWI/SNF complex. Current treatment therapies include general cancer treatments of chemotherapy with or without radiation and surgery. The current available treatments are not very effective but with more advanced cancer technologies and drugs, there is the potential for better treatment options for patients with these tumors.

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