Insights into ependymoma

By Tracey Baas, Senior Editor

Two independent studies have illuminated the fine molecular details of ependymoma tumors and identified targets for a previously intractable disease. A **St. Jude Children's Research Hospital** team focused on forebrain ependymomas and identified a fusion protein as a potential target,[1] whereas researchers at **The Hospital for Sick Children** and the **German Cancer Research Center** focused on hindbrain ependymomas and uncovered epigenetic modifiers.[2]

In both cases, companies think follow-up studies will be necessary to determine the therapeutic potential of the targets.

Ependymomas are tumors of the brain and spinal cord. Each anatomical compartment—supratentorial (forebrain), posterior fossa (hindbrain) or spinal—can house additional molecular subtypes of ependymoma.[3–5]

Surgery and radiation are standard care as chemotherapy is ineffective in most patients. Indeed, up to 40% of ependymomas are incurable.[6]

Part of the problem is that although ependymoma tumors are histologically similar to each other, they exhibit variable transcriptional profiles and DNA copy number alterations.

In the new studies, both teams first used whole-genome sequencing to characterize genetic alterations in samples from patients with ependymoma in hopes of finding unique genetic signatures for each molecular subtype. However, both teams found very few single nucleotide variations, insertions, deletions or focal copy number variations.

The teams thus dug deeper to characterize the genomic makeup of ependymoma subgroups and identify targets that could provide therapeutic leads.

The St. Jude team saw structural differences in supratentorial ependymoma tumors and identified a fusion protein with oncogenic properties.

The Hospital for Sick Children and the German Cancer Research Center team saw differences in epigenetic patterns and identified a specific methylation phenotype associated with poor prognosis.

Finding the fusion

The St. Jude team used an algorithm called CREST (clipping reveals structure)[7] to map genomic structural variations. The group studied nine supratentorial ependymomas and in all cases found structural variations that clustered within chromosome 11.q12.1–11q13.3.

Using whole-genome sequencing, transcriptional analysis and fluorescence *in situ* hybridization (FISH), the team determined that eight of the nine cases involved a fusion between *chromosome 11 open reading frame 95* (*C11orf95*) and *v-rel reticuloendotheliosis viral oncogene homolog A* (*RELA*; *p65*).

These genes normally are separated by 1.9 Mb of sequence that contains 73 genes.

By extending the study to include an additional 85 formalin-fixed, paraffin-embedded tumor samples, the group was able to show that the *C11orf95-RELA* translocation occurs in 70% (*n*=29/41) of ependymomas but never in posterior fossa tumors.

The analysis also identified two other fusion transcripts in ependymomas that lacked the *C11orf95-RELA* translocation. The fusions were *C11orf95-YAP1* (*yes-associated protein 1*) and *C11orf95-MAML2* (*mastermind-like 2*).

RELA is the principal effector of NF-κB signaling. Thus, the team next examined if C11orf95-RELA fusion proteins induced aberrant NF-κB signaling, which is a known driver of solid tumors.

Indeed, in mouse forebrain neural stem cells, expression of *C11orf95-RELA* upregulated NF-κB target genes, whereas *RELA* alone did not.

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The final step was examining the oncogenic properties of the fusion protein. In nude mice, implants of neural stem cells expressing C11orf95-RELA led to brain tumors and death in all animals within 20 days. Animals receiving implants of neural stem cells expressing the individual wild-type proteins, C11orf95 or RELA, showed no tumor growth and survived.

Implantation of neural stem cells expressing C11orf95-YAP1 also formed brain tumors and killed all mice within 35 days.

Results were published in *Nature*.

Cold fusion

Team leader Richard Gilbertson, director and chair of the Comprehensive Cancer Center at St. Jude, thinks that the findings have diagnostic and therapeutic implications.

"The new fusion protein is the most frequent alteration detected to date in ependymoma and clearly divides patients into two subgroups—those with or without the fusion," he said. "Since we have developed immunohistochemical and FISH assays that work on routine formalin-fixed, paraffin-embedded material, we can use the presence of the fusion as a diagnostic tool that may have prognostic or treatment relevance."

"It seems that the *C11orf95* gene product plays a key aberrant role in ependymomas because it is present in most of the detected fusions," said Patrick Trojer, senior director at **Constellation Pharmaceuticals Inc.** "From the current findings it is still quite a way to go before one

can devise novel therapies. One needs to identify druggable regulatory intervention points that are critical for the fusion protein–driven ependymoma."

"The description provided of the fusion protein does not immediately suggest that there is a suitable locus for drug binding on the fusion protein per se," noted Robert Copeland, EVP and CSO at **Epizyme Inc.** "Perhaps druggable targets along the NF-κB pathway could be commandeered for this purpose."

One avenue, said Trojer, could be to test inhibitors of B cell lymphoma 2 (BCL-2; BCL2) in the indication because the target was significantly induced by the C11orf95-RELA fusion protein.

At least seven companies have BCL2 inhibitors in development stages ranging from preclinical to Phase II trials for multiple cancers.

Gilbertson said that the group is running high throughput screens of its mouse models and human fusion-positive cells. The goal, said Gilbertson, is to "detect novel therapeutics that may target the fusion or its downstream oncogenic signals. This may well unmask new treatments."

The researchers also want to tease out the precise mechanism by which the fusion protein induces malignant transformation.

"We have several potential leads, such as the fusion protein escaping normal NFKBIA [nuclear factor κ light polypeptide gene enhancer in B cells inhibitor-α] sequestration; altering the dynamics of *RELA* DNA binding to NF-κB target genes; binding promiscuously to new, inappropriate gene targets; and/or binding aberrant protein partners," said Gilbertson.

Ependymoma epigenetics

The Hospital for Sick Children and German Cancer Research Center team took a different tack after finding a dearth of recurrent genetic events in its initial analyses. The group used profile analyses on methylation microarray and mass spectrometry data to divide the cancer into three distinct subgroups: supratentorial, posterior fossa, and mixed spinal and posterior fossa tumors.

The posterior fossa group was further segregated into group A, which occurs primarily in infants and is associated with poor prognosis, and group B, which occurs in older children and adults and is associated with very good prognosis.

The team determined that group A had a greater extent of CpG island methylation, which they termed a CpG island methylator or CIMP phenotype.

Drilling down, the group identified CpG methylation on three genes in most posterior fossa group A tumors and none of the posterior fossa group B tumors. The genes were *cysteine-rich protein 1* (*CRIP1*), *cytochrome P450 26C1* (*CYP26C1*), and *plakophilin 1 (ectodermal dysplasia/skin fragility syndrome)* (*PKP1*).

Pathway analyses suggested that a large portion of the methylated gene targets were silenced by polycomb repressive complex 2 (PRC2). The complex contains the histone methylase enhancer of zeste homolog 2 (EZH2), which trimethylates H3K27 to drive gene silencing.

Chromatin immunoprecipitation followed by massive parallel sequencing showed that distinct H3K27me3 signatures could be used to diagnose group A tumors from group B tumors, suggesting that PRC2 or EZH2 could be potential targets.

To evaluate the role of methylation in tumor maintenance, the team established short-term, patient-derived ependymoma cell cultures: two posterior fossa group A cultures with a CIMP phenotype and two supratentorial cultures. The team was unable to generate posterior fossa group B ependymoma cultures for comparison.

In the patient-derived cell cultures, the DNA-demethylating agent Dacogen decitabine significantly increased antineoplastic effects in the group A cell cultures compared with in the supratentorial cell cultures (p=0.05).

Otsuka Pharmaceutical Co. Ltd.'s Dacogen is approved to treat myelodysplastic syndrome (MDS).

Similar significant effects were seen with 3-deazaneplanocin A (DZNep), which is a research reagent that targets PRC2. In mice with group A tumors, DZNep decreased tumor volume and increased survival compared with vehicle.

In group A cell cultures, the EZH2-targeting small molecule inhibitor GSK343 significantly derepressed *PRC2* gene targets compared with controls (p=0.001). The molecule also diminished levels of H3K27me3 and showed potent antineoplastic effects.

GlaxoSmithKline plc's GSK343 is available as a chemical probe through the **Structural Genomics Consortium**.

In patient-derived cell lines, combinations of small molecules produced even better results. For example, additive effects were seen with Dacogen plus DZNep, Dacogen plus the histone deacetylase (HDAC) inhibitor Zolinza vorinostat and DZNep plus vorinostat.

Merck & Co. Inc. markets Zolinza to treat cutaneous T cell lymphoma (CTCL). The drug is in Phase I trials to treat brain cancer.

Results were published in *Nature*.

Coauthor Stephen Mack said that the take-home message is that genomically quiet ependymomas—especially group A—exhibit widespread epigenomic alterations and are highly sensitive to agents that target DNA and H3K27 methylation.

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> ***—Stephen Mack, The Hospital for Sick Children***

Mack is a graduate student and project manager in the laboratory of Michael Taylor, who is an associate professor of surgery, laboratory medicine and pathobiology in The Hospital for Sick Children.

"These findings need to be expanded and independently validated in additional studies in order to fully evaluate the degree of subgroup sensitivity of ependymomas to epigenetic modulation," noted Mack. "The safety of EZH2 inhibitors for use as cancer therapy also needs to be thoroughly assessed in clinical trials, particularly in the pediatric population."

"DNA methylation inhibitors, such as FDA-approved decitabine, could be rapidly repurposed for potential treatment of group A ependymoma patients, who currently have no effective chemotherapies available," added Mack.

Hendrik Witt, one of the first authors of the study, said that the next step could be a clinical trial of patients with group A ependymoma. Witt is a physician scientist in the laboratory of Stefan Pfister, who is

a professor of pediatrics and head of pediatric neuro-oncology at the German Cancer Research Center.

Copeland wanted to see the animal results reproduced using a more selective EZH2 inhibitor.

"I was disappointed that the only *in vivo* studies reported by the epigenetics team were with the nonselective compound DZNep. This compound is not even a histone methyltransferase inhibitor but rather has broad, indirect effects," he said.

Selective EZH2 inhibitors in development include E7438 from Epizyme and GSK2816126 from GSK. The molecules are in Phase I/II testing to treat lymphomas. Constellation, GSK and **Novartis AG** also have EZH2 discovery programs.

"I would also like to see more solid data that supports the team's idea that there is a convergence of gene targets found within the DNA methylation and PRC2-induced H3K27me3 pathways," said Trojer. "The gene overlap found with their pathway analysis of the methylation microarray data is at best modest, about 43 genes. To assume these 43 genes, and not other PRC2 targets, drive group A type ependymoma is rather arbitrary."

Mack agreed that DZNep was not a very specific EZH2 inhibitor. They would like to use more specific EZH2 inhibitors when they expand their preclinical studies for ependymoma.

Regardless of the details, Trojer said that the data suggest that targeting chromatin modifiers could be useful in ependymomas.

"The data obtained with GSK343, a true EZH2 inhibitor that suppresses H3K27me3 levels, is indeed intriguing," said Trojer. "The GSK343 data suggest that application of EZH2 inhibitors in ependymomas of the posterior fossa group A type could be beneficial."

Mack said that both teams' findings should immediately start steering clinical trials.

"Moving forward, future ependymoma clinical trials will need to consider supratentorial, posterior fossa and spinal ependymomas and their associated subgroups as distinct clinical entities," he said. "At the very least, the trials should ensure that tumor tissue is collected such that patient outcomes can be examined in a manner guided by molecular subgroups and [have the] potential to be used to inform future decisions regarding therapies."

The findings by both independent teams are not patented.

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   Contact: Richard J. Gilbertson, St. Jude Children's Research Hospital, Memphis, Tenn.
   e-mail: richard.gilbertson@stjude.org
   Contact: David W. Ellison, same affiliation as above
   e-mail: david.ellison@stjude.org
   Contact: Jinghui Zhang, same affiliation as above
   e-mail: jinghui.zhang@stjude.org
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   Contact: Michael D. Taylor, The Hospital for Sick Children, Toronto, Ontario, Canada
   e-mail: mdtaylor@sickkids.ca
   Contact: Andrey Korshunov, German Consortium for Translational Cancer Research, Heidelberg, Germany
   e-mail: andrey.korshunov@med.uni-heidelberg.de
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COMPANIES AND INSTITUTIONS MENTIONED

Constellation Pharmaceuticals Inc., Cambridge, Mass.
Epizyme Inc. (NASDAQ:EPZM), Cambridge, Mass.
German Cancer Research Center, Heidelberg, Germany
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
The Hospital for Sick Children, Toronto, Ontario, Canada
Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan
St. Jude Children's Research Hospital, Nashville, Tenn.
Structural Genomics Consortium, Oxford, U.K.