

Pediatric Tumors Made Personal

A mixed collection of relatively rare but often deadly pediatric tumors are collectively known as small round blue cell tumors (SRBCT) for precisely the reason one might imagine. Examined under a microscope after routine processing, bone marrow biopsies from cancers including neuroblastoma, Ewing sarcoma, rhabdomyosarcoma, and lymphoma appear as small, blue, and round cells. Despite some distinguishing molecular markers to guide them, oncologists can, on occasion, find it hard to diagnose these tumors specifically. Javed Khan, M.D., Head of the Oncogenomics Section of CCR's Pediatric Oncology Branch, has been using genomic approaches to study pediatric cancers for several years. He is now poised to launch an ambitious multicenter project to use comprehensive genomic data to guide the individualized treatment of children with advanced solid tumors.

Tapping Gene Expression

Khan is a strong believer in the power of genomic information to guide solutions to the riddles of cancer. A pediatric oncologist who trained in Cambridge, England, Khan first came to the NIH on a hematology/oncology fellowship that involved translational research at the National Human Genome Research Institute (NHGRI). Jun Wei, Ph.D., was also at the NHGRI and moved with Khan to CCR when he became Head of the Oncogenomics Section in 2001. At the time, the NHGRI was heavily involved in developing microarray technology to analyze gene expression. "Those were very heady, exciting days," remembered Khan. "Working with

pediatric solid tumors, we were one of the first to use microarrays to find a cancer diagnostic."

In 2001, Khan, Wei, and their colleagues published a paper in *Nature Medicine* in which they demonstrated that relatively small numbers of genes could be used to distinguish four different SRBCTs. In the paper, they used artificial neural networks, a computational technique in which the correct method for finding a solution evolves through a training process. A set of microarray data from identified tumors is used to train the network to recognize patterns in the data that uniquely correspond to each tumor type. Once the network is trained in this way, it

can use the rules it learns to predict new cases.

"The advantage of our method," explained Khan, "is that it allows you to analyze multiple cancers and generate a score that reflects confidence in any particular diagnosis." It is, for example, easily adaptable to a Web site format so that physicians could load microarray or other gene expression data from their own patients to obtain diagnostic information. In fact, Khan and his colleagues have a patent on their method, which a San Diego-based diagnostic company, AltheaDx, is developing into just such a product for pediatric cancers.

Reading the Whole Genome

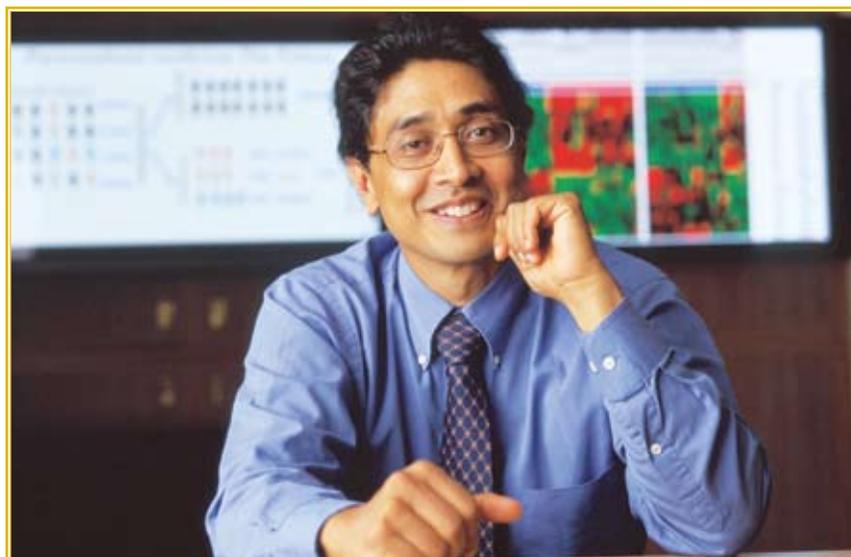
“The end game for me is personalized therapy,” said Khan, “in other words, being able to use genomics to diagnose cancers and to distinguish those who will survive on existing therapies (prognostication). And in the midst of studying all those genetic alterations, find ones that are the key targets for therapeutic intervention in advanced disease.” To search for genetic changes that might be driving these cancers, Khan and his team rely on multiple strategies.

Microarrays measure the expression of genes that are being actively transcribed from only a subset of the entire genome—the transcriptome. These data give you important information about changes that occur during RNA transcription and processing. Although he has firsthand experience with the diagnostic value of gene expression data, when it comes to stratifying disease progression, defining targets, and predicting outcomes, Khan’s first bet is on looking at the DNA directly. DNA sequence information does not tell you which genes are expressed at a given time, but it does tell you directly which genes have been mutated.

“To distinguish one cancer from another, the differences [in gene expression] are quite large,” explained Khan. “But to distinguish survival outcomes for one type of cancer, the differences are often much smaller. So it becomes much more of a challenge to distinguish prognostic signatures using gene expression data.”

The problem with RNA is largely a practical one. The molecules

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(Photo: R. Baer)

Javed Khan, M.D.

themselves are simply much more dynamic. “If you take a tumor sample out and you don’t freeze it immediately and then wait an hour, the expression profile can be profoundly altered. Also, tumor cells that are hypoxic at the center of a tumor may have a very different profile from cells in the periphery of the mass. DNA doesn’t change. RNA does.” Khan noted that although there are several published prognostic gene expression signatures for breast cancer or neuroblastoma, for example, there is very little overlap between each of the gene sets for a given cancer. Thus, to validate these signatures for prognostic purposes requires prospective clinical trials in which sample handling and analysis are stringently controlled with standard operating procedures.

As a result of incredible advances in DNA sequencing technology over the last decade, it is no longer impossible to think about sequencing the whole cancer genome of an individual cancer. “Where it’s going is next generation sequencing,” said Khan. “The human genome project sequenced the first human genome in 15 years. Now you can do a whole genome in about a month, which is still too long in terms of using it to make therapy decisions. But, you

can sequence all the protein-coding genes—the exome—within a week.”

With exome sequences in hand, it is still a long and laborious process to identify the mutations that might be critical to tumor growth and survival. The sequence from the tumor must be compared to the patient’s germline DNA and also to published sequence data to find mutations that are specific to the cancer. From one tumor, a hundred functional mutations might emerge and many of these are probably passenger mutations resulting from an unstable genome that are not critical to cancer progression. Comparing mutations across tumors can help to narrow the field, as can analyzing the pathways that might be compromised by individual mutations.

In addition to the transcriptome and the exome and whole genome sequencing, Khan and his colleagues are also interested in applying next generation sequencing to analyzing epigenetic changes in the DNA (methylation) and miRNA profiles. Both have been shown to be important in different models of cancer, and drugs have been developed that specifically impact epigenetic states (e.g., HDAC inhibitors); however, therapeutic strategies to target miRNA changes are still in their infancy.

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Hitting the Target

James Taylor, M.D., currently serves as a Staff Physician and Postdoctoral Fellow at the National Heart Lung and Blood Institute (NHLBI). He began collaborating with Khan's team when he was a CCR Fellow in a laboratory just down the hall. Although his primary research interest these days is in monogenic diseases and sickle cell anemia in particular, as a hematologist, he has seen plenty of SRBCTs.

"When I am on clinical service in hematology, I look at bone marrows all the time and am often called upon to make the diagnosis in the middle of the night." So, Taylor knows first hand how difficult a diagnosis of rhabdomyosarcoma or neuroblastoma can be when based only on what you can observe under a microscope. "That [*Nature Medicine*] paper was really important from a diagnostic standpoint," he noted.

But what was of mutual interest to him and Khan and subsequently

became the subject of their collaboration was one gene in particular, among the many that showed altered expression patterns predictive of disease. "One of the big hits in that paper was the discovery of high expression of *FGFR4* in rhabdomyosarcoma." *FGFR4* codes for a particular receptor subtype of fibroblast growth factor (FGF). When FGF activates its receptors, it activates a molecular signaling cascade within the cell that ultimately stimulates growth. Khan and his colleagues have shown that *FGFR4* is overexpressed in rhabdomyosarcoma and that it is particularly highly expressed in an aggressive subtype called alveolar rhabdomyosarcoma. "So from a clinical standpoint, it made sense that *FGFR4* might be a good diagnostic marker," noted Taylor. "But what nobody knew is whether this gene did anything [to promote disease]."

Taylor and Adam Cheuk, Ph.D., a Postdoctoral Fellow in Khan's laboratory, led a study to analyze the

role of *FGFR4* in rhabdomyosarcoma. They sequenced the gene in available tumor samples and discovered that the gene was mutated and that the mutations seemed to cluster at a site on the molecule that was critical to its function as an enzyme. They were then able to show that the mutation actually enhanced the activity of *FGFR4* in cells. "I think that's the most exciting part of this," said Taylor. "A lot of genetic studies report mutations, but Javed and his group went into the lab to prove that these were functional mutations."

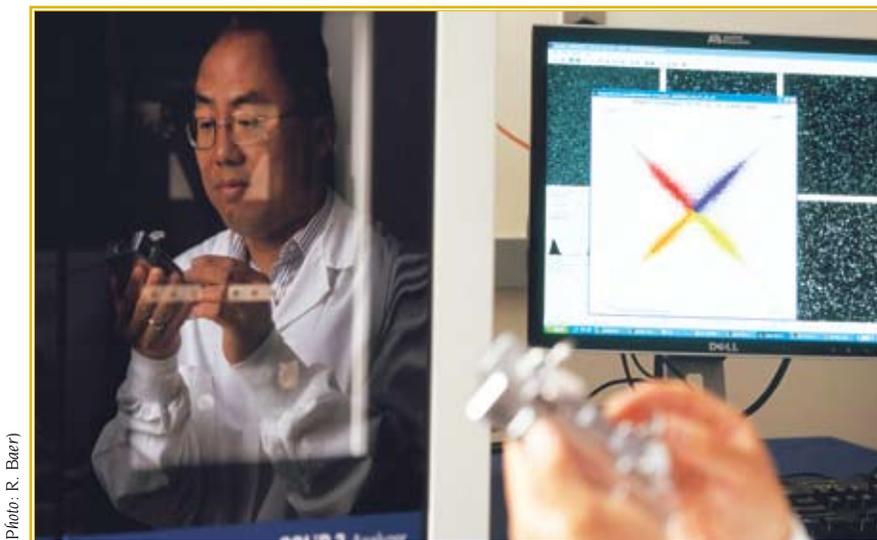
But that is far from the end. The next step for Khan is to bring FGFR inhibitors to patients with these mutations. "There actually is an FGFR inhibitor in Phase 2 clinical trials—the company has contacted us and we are getting hold of the drug. We're also making a therapeutic antibody against the protein."

A Protocol for Personalized Medicine

"That's the paradigm," said Khan about the *FGFR4* work. "First, find a gene that seems key to the particular cancer, then find the mutations. Establish that the mutations promote the cancer phenotype by activating the gene to promote growth or metastasis in cellular models. And then find—or make—an inhibitor to administer with chemotherapy."

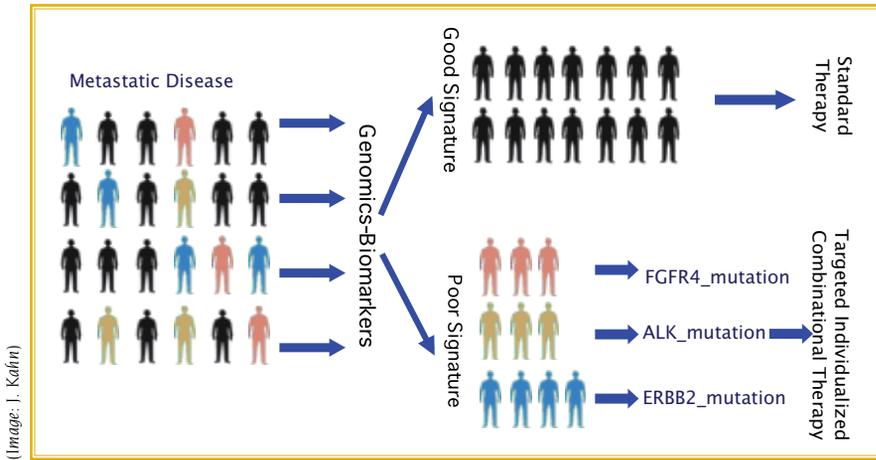
Khan predicts that each gene like *FGFR4* that is discovered for a particular tumor type will only be responsible for a smaller fraction, say 10-20 percent of the cases. "This is where personalized medicine will come in."

In a multicenter collaboration that includes the Translational Genomics Research Institute (TGen), Helen DeVos Children's Hospital, and the Vermont Cancer Center, Khan is developing a protocol that will make personalized medicine for pediatric tumors a reality. In the first phase, all admitted patients



(Photo: R. Baer)

Jun Wei, Ph.D.



(Image: J. Kohn)

The goal of personalized medicine is to treat each patient with the best possible therapy.

will have samples taken before initial standard-of-care treatment. If they relapse, the patients will have new biopsies taken. “Often, when they relapse, it’s because the cancer has changed and evolved. The relapsed cancer is not the same cancer they started with,” explained Khan.

The researchers will then do a comprehensive analysis of each cancer genome including gene expression microarrays, look for increased expression of certain proteins, and sequence the exome and the transcriptome to see whether they can identify a molecular therapeutic target. If a particular target is found and there is an ongoing clinical trial that involves an inhibitor of that target, the patient will be enrolled into that trial. Otherwise, the researchers will investigate whether there are any FDA-approved drugs active against the identified target that might be effective.

“We know 60-70 percent of these patients with high-stage disease will relapse after standard treatment,” explained Khan. Normally, after relapse, without molecular markers to guide them, choice of clinical trial for advanced disease is something of a shot in the dark. “This is a way of personalizing the choice of clinical trial.”

The Need for Drugs

Khan is optimistic about the timeframe for developing the individualized analysis of cancer at a molecular

level. “Within the next couple of years, researchers will have catalogued all the mutations. There are groups around the world doing this for all kinds of cancers.” He believes the genomic analysis of individual tumors will be standard practice in clinical trials within five years. Where he is more cautious, however, is in the timeframe for delivering personalized cures. “The biggest problem is that there are only approximately 260 FDA-approved drugs that target a known human protein. So you’re not necessarily going to have the drugs even when you know which mutations to target.”

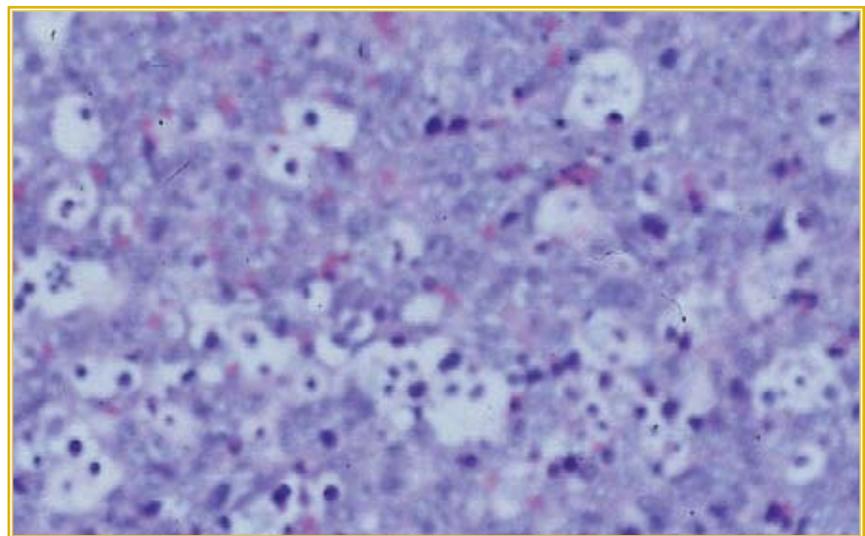
Khan wonders if federally funded programs to produce anti-gene inhibitors rapidly, using antibody or

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cell-based technologies, might be one answer. Not one to sit idly by while others solve the next problem, Khan is deploying some of his own resources towards developing therapies. He has a small group in his laboratory working on a class of inhibitors called peptide nucleic acids that can bind to DNA or RNA and stop transcription or translation. He also has a postdoctoral fellow working on aptamers—molecules that may be able to target specific markers on cancer cells and deliver chemotherapeutic agents directly to them.

“Developing those inhibitors for known mutations—that’s going to be on my 10-year plan.”

To learn more about Dr. Khan’s research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=khan>.



(Image: J. Kohn)

Small round blue cell tumors can be difficult to diagnose. This case of rhabdomyosarcoma was originally diagnosed as lymphoma.