Thyroid cancer: In a flourish of subtypes, genes, and drivers

Karen Titus

January 2015—Dark matter is shrinking—at least in the thyroid cancer part of the universe.

Until recently, the percentage of papillary thyroid carcinomas with no known oncogenic drivers hovered around 25 percent. Now, with the publication of the most recent research effort by The Cancer Genome Atlas project, that number has shrunk considerably, to about 3.5 percent.

Questions about PTCs are tantalizingly close to being answered, thanks to the TCGA effort (*Cell.* 2014;159:676-690). "We've expanded the somatic mutational landscape of the most common type of thyroid cancer [PTC]," says co-lead author Thomas Giordano, MD, PhD, professor of pathology and internal medicine at the University of Michigan.

Dr. Thomas Giordano, here with research associate Michelle Vinco, co-led the three-part analysis of 496 papillary thyroid carcinomas. Perhaps the time has come, the study says, to revise the classification of thyroid cancer.

That, in turn, has direct bearing on the molecular testing of thyroid nodules, he says, and improves the performance of those assays. The eventual clinical implications could be impressive—pathologists eventually might be able to detect up to 95 percent of papillary carcinoma by FNA and reliably classify them as benign or malignant without additional surgery.

"We know that thyroid cancer is overall a relatively indolent disease," says TCGA project member Yuri Nikiforov, MD, PhD, professor of pathology, vice chair for molecular pathology, and director, Division of Molecular and Genomic Pathology, Department of Pathology, University of Pittsburgh Medical Center. Five-year survival for well-differentiated thyroid cancer is 95 percent. "So we typically overtreat most of the patients because we cannot discriminate which cancers will be aggressive and which will not."

Having more granularity can only help endocrinologists, says project member Robert Smallridge, MD, who is the current president of the American Thyroid Association. "The cases we wrestle with on a daily basis are the ones suspicious for follicular neoplasm, or what used to be indeterminants but now get into this classification of atypia of

unknown significance-type," says Dr. Smallridge, deputy director of the Mayo Clinic Cancer Center and the Alfred D. and Audrey M. Petersen professor of cancer research, Jacksonville, Fla. "We are stuck with telling the patient the odds remain high that the lesion is benign, but we can't do anything further without taking you to surgery and removing all or some of your thyroid. That can be frustrating when the odds are only 15 to 20 percent it's cancer."

Such conundrums lay at the heart of TCGA, a study Dr. Giordano calls "exhaustive and huge." It's not an exaggeration. The study looked at 496 PTCs in a three-part analysis. As the paper explains, the researchers identified somatic mutations, including single nucleotide variants, small insertions and deletions, gene fusions, and copy-number alterations, which allowed them to characterize the genomic landscape of PTC. This let them identify previously unknown drivers, that so-called dark matter.

Dr.Giordano

They then developed a gene expression signature of samples containing the mutations, characterizing the tumors based on this signature. This was followed by determining, via protein and mRNA expression data, the signaling impacts of the *BRAF* V600E and *RAS* mutations. As it turns out, the two pathways are strikingly different.

After obtaining the molecular data, the researchers developed molecular classifications of PTC and integrated them with data related to genotype, signaling, differentiation, and risk.

It could easily be said that the study was also exhausting. Recalls Dr. Giordano: "It took four years." Initially the researchers analyzed data from 200 tumors—and decided not to publish. "We wanted to do more." After 350 tumors, they again decided to hold off, "and pushed ourselves to do 500. I think it was a good decision, but it made us a little nervous," he says, noting that the researchers were well past their embargo date. "So some of our results started showing up in other papers. But if enough people cherry-pick your best results, it gets a little harder to publish."

Dr. Giordano, who led the team effort jointly with Gad Getz, PhD, of Massachusetts General Hospital and the Broad Institute of MIT and Harvard, credits the editors of Cell for allowing him and his colleagues "to tell the whole story in all its glory. Cell actually allowed us to bend their rules on both length of the paper and number of supplemental figures. They were fabulous to work with."

For all the hard data, the study ends on an almost philosophical note: Might it be time to revise the classification of thyroid cancers?

That's not a debate that will be settled anytime soon. But the Cell paper does settle other debates, says TCGA project member James Fagin, MD, chief of the endocrinology service and member of the Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center. The level of detail emerging from TCGA means "nobody will be able to think about papillary thyroid cancer in the same way after this study."

Fresh thought may be in order, especially since, as the researchers note, thyroid cancer therapy is entering

the realm of precision medicine.

From the perspective of endocrinologists, Dr. Fagin says, “We had a fairly simplistic perspective of this prior to the TCGA.” *BRAF* and *RAS* mutations aren’t new, and TCGA data recapitulate their importance. “But with much more profound nuances,” Dr. Fagin says. “Within each of the cohorts of tumors associated with the different drivers, there was some interesting heterogeneity that has real potential clinical interest.”

Thyroid nodules are common and, most of the time, benign. Nevertheless, a substantial portion—about a third—of cases come back indeterminate on fine-needle aspirations. “You can repeat the FNA, but if it keeps coming back indeterminate, most people eventually go to surgery,” says Dr. Giordano.

Therein lies the potential for molecular testing. If a nodule has a driver oncogenic mutation, the correlation with malignancy (depending on the mutation) is quite high. “That can give surgeons the confidence that they’re doing the right thing in proceeding to surgery,” says Dr. Giordano.

Such confidence could have widespread impact. Anywhere from 100,000 to 150,000 patients with thyroid nodules annually in the U.S. receive an indeterminate cytologic diagnosis on their nodules.

Pathologists already have good tools at hand to enhance cytology’s diagnostic yield, says Dr. Nikiforov, including the Afirma (Veracyte) gene expression assay, which uses genes with a high negative predictive value.

The other approach, direct genotyping of DNA and RNA isolated from the cells collected during FNA, is being led by Dr. Nikiforov, who is also codirector, Multidisciplinary Thyroid Center, UPMC. He launched his first panel with seven genes: *BRAF*, NRAS, HRAS, and KRAS, plus RET/PTC1 and RET/PTC3 and PAX8-PPARG rearrangement. These covered about 65 to 70 percent of thyroid cancers. When next-generation sequencing became available, the test panel jumped to 15 genes and included prognostic as well as diagnostic markers, such as TP53 and PIK3CA; sensitivity rose to 80 percent. The latest version, ThyroSeq v.2, has 60 genes and 90 percent sensitivity, says Dr. Nikiforov. With the new data from TCGA, “we will probably be able to package an even broader panel that will cover 95 to 97 percent of papillary carcinoma.”

Another potentially big impact from the paper, from a clinical perspective, is the demarcation of *BRAF* V600E and *RAS* tumors. Assessing the genomic data associated with each type, the researchers could see that the biologies differed significantly. That leads to the intriguing potential of tumor reclassification: Do they truly belong under the umbrella of papillary thyroid cancer?

Decades ago, the follicular variant of papillary carcinoma was essentially unrecognized—they were considered to be follicular carcinomas. The Cell paper suggests that it may be time to reunite them again. “So what’s nice about TCGA is that it will catalyze a very serious discussion on the proper classification of thyroid cancer. And that strikes at the most fundamental level of tumor pathology,” says Dr. Giordano.

Along those lines, Dr. Nikiforov is organizing an international conference just prior to the USCAP annual meeting in Boston in March to reexamine the histopathologic features and long-term follow-up for patients with encapsulated follicular variant PTC. No one’s making any grand predictions. In fact, for the pathologists on the TCGA paper, “Just getting us to agree what that classification might look like was not trivial,” Dr. Giordano says. “It’s going to be an interesting time,” he added, echoing the apocryphal Chinese curse: May you live in interesting times.

Dr. LiVolsi

A little molecular 101 might be in order here. The great majority of papillary thyroid carcinomas, the so-called classic type, have some degree of follicle formation within the tumor, says TCGA member Virginia LiVolsi, MD, professor of pathology and laboratory medicine, professor of surgical pathology in surgery, and professor of otorhinolaryngology head and neck surgery, University of Pennsylvania Perelman School of Medicine. The diagnosis is predicated on the nuclear characteristics of this particular type of tumor. “They have been called—and it’s a very spectacular description—Orphan Annie nuclei,” she says, describing the similarity to the cartoon character’s eyes: “oval, rather large, very clear in the middle, with a rather thick, black outline.”

Classic PTC is, for the most part, unencapsulated, infiltrative into the thyroid, and spreads through the lymphatics, says Dr. LiVolsi, who is also director of strategic initiatives and quality improvement, anatomic pathology. It may be multifocal in the gland and has a very high propensity at the time of diagnosis to have lymph node metastases in the neck. It’s unusual, Dr. LiVolsi says, for ordinary, classic papillary carcinoma to spread beyond the neck. Survival rate, with good quality of life, is over 90 percent.

The second most common type, “as we know it now,” says Dr. LiVolsi, is the follicular variant of papillary carcinoma. “This is where not only the general practitioner of pathology but also the so-called experts have a great deal of disagreement,” she says. One variety grows similarly to classic papillary carcinoma. Known as the infiltrative type of follicular variant of papillary carcinoma, “lo and behold, it appears to behave almost identically with classic papillary carcinoma.” The nuclei in this subtype are very much like classic papillary carcinoma nuclei.

Some follicular variants of PTC are encapsulated. If they invade the capsule, most pathologists will call them cancers, she says. But because in many examples of this type of tumor the nuclei are not absolutely perfect, some pathologists prefer not to call them papillary carcinomas. They may call them well differentiated, and they may or may not place them in the follicular carcinoma group.

Fast forward to TCGA. The classic papillary carcinomas tend to have molecular changes along the *BRAF* pathway; the follicular variant ones tend to be in the *RAS* mutation family.

“So what I think TCGA is saying is that histopathologists and cytopathologists have been right in making diagnoses of classic papillary carcinoma for many years, all the way up to modern molecular analysis,” says Dr. LiVolsi. “In addition to the cytology, the histopathology, and the clinical, it shows that at a molecular level, it’s a family.”

The follicular variant may not deserve the same designation. “What we as pathologists are going to have to admit is that we pigeonholed this follicular variant as papillary carcinoma because there was some nuclear change. And maybe we were wrong,” says Dr. LiVolsi. “Maybe that group of tumors really is either in the follicular carcinoma family, or is somewhere in between. But from a molecular point of view, it’s more closely aligned to follicular carcinoma.”

It’s possible, she says, that the follicular variant of papillary carcinoma diagnosis will eventually become more restrictive, used only for the subtype that is follicular in pattern but infiltrative in its growth, while the encapsulated ones will be given a new name. “I personally think they need to be distinguished from classic follicular carcinoma, because their nuclei are different, and because every now and then one of these encapsulated ones will go into a lymph node,” she says. “There’s something a little bit different. Maybe they’re hybrids—I’ve been guilty of using that term.”

“This has been a murky area,” Dr. Fagin agrees. “Pathologists have differed in terms of how they classify these types of tumors, even histologically, and there’s been concern that some of the tumors that in the past were called follicular carcinomas may have actually been follicular variant of PTC.” The RAS-mutant follicular variant of PTCs tend to have few nodal metastases, and when they do metastasize it tends to be more frequently and at a distance. “Which is what follicular carcinomas do as well.”

Given that, Dr. Fagin says, “I’m totally in favor of the idea of thinking of the disease in terms of genetic drivers, and that being the dominant mechanism of classification. I know when I see patients, I think much more of the underlying driver mutation than anything else, and that colors how I would monitor that patient.”

The pre-USCAP conference may be the first to nudge pathologists toward a better answer, though. “My gut tells me there will not be a lot of consensus,” says Dr. LiVolsi. The need for such discussion seems apparent, based on how physicians respond in their own practices to ambiguities. Dr. Giordano talks about one endocrinologist who makes a point of converting a diagnosis of follicular variant of papillary carcinoma, when it’s noninvasive, into a follicular carcinoma in situ diagnosis. “Which doesn’t really exist,” Dr. Giordano says.

The researchers also looked at a cohort of patients with *BRAF* V600E-mutant PTCs. In the past, the literature has treated these as a very homogeneous type of tumor, Dr. Giordano says. TCGA, however, shows there are at least four molecular subtypes in the *BRAF* V600E category. “This has implications for clinical trials and pathologic studies,” Dr. Giordano says. One category for *BRAF* V600E might no longer be appropriate. “You have to get down to a finer level to try to capture some of this genetic diversity within this tumor group.”

One subset of tumors in the cohort, about 15 to 20 percent, retain, to a significant extent, the ability to express the genes involved in iodine metabolism. “That was a surprise,” Dr. Fagin says.

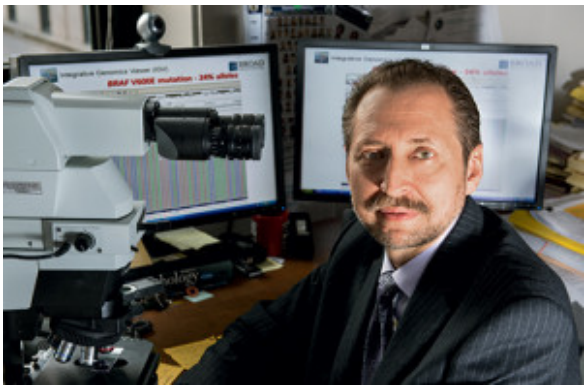
“It might tell us two things,” he continues. One is that while current thinking is that radioactive iodine might be ineffective or less effective in *BRAF*-mutant tumors, it may not be true for all of them. “There’s a subset that is biologically different, for reasons that are not entirely clear, but they’re associated with some interesting changes in microRNA expression, for instance.” If that subset can be reliably identified, endocrinologists might be able to more rationally predict whether a tumor will benefit from adjuvant radioactive iodine, for example.

On a related note, he says, many patients are treated with high doses of thyroid hormone to suppress expression of TSH, which is a growth factor for thyroid cells. Since the typical *BRAF* tumor does not express the TSH receptor, there may be no need to make patients undergo this treatment. “It has significant side effects,” Dr. Fagin says, including osteoporosis and increased risk of arrhythmias. Clearly, it would be an advantage to provide precision therapy in the early stages of the disease.

Sums up Dr. Giordano, in what could be the tag-line if someone ever markets thyroid cancer: “*BRAF*: It’s just too common to be the whole story.”

There are times when TCGA researchers sound like members of the Senate Judiciary Committee. Just as valuable as the new information, they say, is the confirmatory power of the study.

“The great thing about TCGA is that it confirmed virtually everything about classic papillary carcinoma,” Dr. LiVolsi says. “A lot of what we knew about papillary carcinoma and thought about it was correct.”



Dr. Nikoforov

Adds Dr. Nikiforov: “In no way do I want to diminish the importance of this paper,” he says, “but all the major classes of changes we knew already, from multiple, multiple studies. The value of this paper is that it looks at everything, including alterations in DNA, methylation, gene expression, and pathway activation, and connects and

compares them."

TERT mutation had been discovered a year prior to TCGA, for example, but this paper confirms its link with a more aggressive presentation of papillary carcinoma.

It also bolsters current thinking about the increased incidence of thyroid cancer. Several good observations have already shown that radiation-induced thyroid cancer is associated mainly with gene fusions, says Dr. Nikiforov, whereas sporadic cancer likely develops through point mutations. TCGA shows that about 75 percent of papillary thyroid carcinomas have point mutations, not gene fusions. "So it supported the notion that we thought before, that the increase in cancer is very unlikely due to radiation exposure," which is the only well-known risk factor currently. Put another way, the molecular signature favors some kind of spontaneous cancer that occurs due to chemical carcinogenesis.

The TCGA study had the benefit of looking at a disease with a relatively quiet genome, says Dr. Fagin. The mutation rate is low—perhaps the lowest of any of the cancers studied by TCGA so far. ("That fits quite nicely with the notion that these are relatively indolent tumors," he says, though the study did observe that the older the patient, the higher the mutation rate, and the higher the risk of the disease being somewhat more aggressive.) But because there is very little else happening in the genome apart from the driver mutations, the researchers could look at the impact of those in terms of functional mutations, that is, gene expression changes, in a relatively unobstructed way. In other cancers, such as melanoma, the impact of a driver mutation can be countered by many other genetic changes. "So this was a crystal clear picture of what's going on in the biology of the cell."

And for all the enthusiasm about TCGA, Dr. Nikiforov realizes that caution is in order. "I cannot say there is a direct link between this paper and the immediate effect on the practice of pathology. It's obvious, after such a very large study, it will take time for this to be digested and to trickle down into clinical practice."

Dr. LiVolsi, too, is cautious. "The clinical people have become extremely interested in molecular features," says Dr. LiVolsi. At her institution, "We talk about it all the time. But we're trying not to say this is the greatest thing since French toast, and that we should do molecular testing on everybody." *BRAF* testing can provide a definitive diagnosis of papillary carcinoma on FNA specimens, but only 40 to 45 percent of classic papillary carcinomas are *BRAF* mutated.

There may even be some who respond to the study with a yawn. "Some people would say—simplistically, in my view—'Who cares?'" Dr. Fagin says. Very few people die from this disease (goes this line of thinking), and surgery, radioactive iodine, and thyroid hormone-suppressive therapy get the job done. "But I think in time people will grow to understand that each of these approaches needs to be individualized, the way we do for advanced disease. Even for indolent disease there are real opportunities for sharpening the way we do our treatment. What we do now alters peoples' lives in significant ways."

What lies ahead?

The paper makes note of new drivers, all of which will need to be explored further. One of the more exciting possibilities is EIF1AX, because it's relatively common, says Dr. Nikiforov, present in maybe two to four percent of papillary carcinomas. CHEK2 also looks compelling. TCGA also identified new isoforms of *BRAF*, *RAS*, *RET, NTRK3,* and *ALK* gene fusions. "Those will definitely have scientific novelty," says Dr. Nikiforov.

"It definitely enriches our overall understanding of the spectrum of drivers involved in the disease," says Dr. Fagin.

Dr. Giordano would like to see a "big gene hunt" to identify the driver genes in cases without point mutations or fusions (they're mutually exclusive), specifically those that may instead be driven by large copy-number alterations. "It's not a trivial task," he cautions. Then there's the need to "fill out the landscape," as he puts it, to identify the drivers of poorly differentiated and anaplastic, or undifferentiated, thyroid cancers. These highly aggressive tumors were not part of TCGA.

The new mutations and fusions bring with them a host of questions, Dr. Smallridge says. Are they alone driving the cancer? Will there be other epigenetic or other abnormalities at the RNA or protein level that modify the expression that accounts for differences in aggressive behavior? While the paper doesn't answer these questions—long-term outcomes are needed—it does give a broader field of potential abnormalities to explore.

"We need a broader signature of identifying positive genetic mutations or changes that will, with high predictive value, tell us, This is *cancer*," says Dr. Smallridge. "I think that perhaps will ultimately be one of the strengths coming out of the *Cell* paper."

Dr.Smallridge

It's unclear if the findings and confirmations of this study will translate to the more aggressive types of tumor. Dr. Fagin is using a next-generation panel—soon to contain 400-plus genes—to study more than 100 cases of anaplastic and well-differentiated thyroid cancers as well as more than 40 thyroid cancer cell lines. TCGA information, he says, will likely help build on work to study more aggressive disease. It's been suggested that the drivers occur early in disease. Some are preferentially present in different subtypes of advanced forms of thyroid cancer; while they also dictate the biology of the tumors, they're associated with many additional genetic changes that are absent in early stages. TCGA thus should provide a useful comparator to see what changes occur from early to late-stage disease.

In terms of treatment, in addition to perhaps identifying patients who will benefit from radioiodine treatment, TCGA "is maybe telling us there are other opportunities that we should be exploring more carefully," says Dr. Fagin. There are thyroid cancers with oncoproteins that can be targeted directly, for example.

The work and the questions never end, even after four years and nearly 500 tumors. Dr. LiVolsi is fine with that. For all that the *Cell* paper contains, she says, what it lacks might be just as important.

"It tells us about the state of non-knowledge we have at this point in time," she says. "It tells us that we should be humble, because we don't have all the answers."

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