

Combined physical and genetic map finds cancer's 'ignition key'

Whole-organ maps that superimpose genetic information over the terrain of cancerous bladders chart the molecular journey from normal cell to invasive cancer, an international research team led by scientists at The University of Texas M. D. Anderson Cancer Center reports online at the journal *Laboratory Investigation*, a member of the Nature Publishing Group.

By geographically relating an organ's varied tissues – normal, precancerous and malignant – to their underlying genetic variation or regulation, the team also identified a crucial new category of genes that launches the process of cancer development.

“These ‘forerunner genes’ are the ignition key that starts the engine of carcinogenesis,” said senior author Bogdan Czerniak, M.D., Ph.D., professor in M. D. Anderson’s Department of Pathology.

“Discovery of forerunner genes opens an entirely new field of investigation to identify biomarkers for the early detection and prevention of cancer,” Czerniak said. “Inactivation of these genes occurs during cancer’s invisible stage, when it is undetectable by traditional means.”

The team reports that forerunner genes must be shut down before a major tumor-suppressing gene called RB1 is silenced, paving the way for invasive cancer. By characterizing the genetic aspects of all tissue types in the organ, the researchers illuminated the sequence of events that carries a normal cell through various stages to invasive cancer. In the case of bladder cancer, they identified three “waves” of genetic hits that drive the process.

Czerniak’s unique approach is called whole organ histologic and genetic mapping, which combines genetic information with microscopic study of the organ tissue, or histology.

Biomarkers for detection, prevention, treatment

Czerniak’s mapping techniques and the team’s findings are seminal work, said T. Sudhir Srivastava, Ph.D., chief of the Cancer Biomarkers Research Group, Division of Cancer Prevention of the National Cancer Institute.

“Identifying genes involved in precancerous development has been an arduous task, primarily for lack of a systematic approach to discovering them and the non-availability of quality tumor specimens,” Srivastava said. “Dr. Czerniak has overcome these difficulties by utilizing the resources available at M. D. Anderson and employing the gene-mapping expertise of his group to uniquely characterize chromosomal regions involved in genomic imbalances, particularly those involved in progression of precancerous conditions to clinically aggressive bladder cancer.

“These findings will accelerate the development of clinically useful biomarkers for the early detection, surveillance, and clinical management of bladder cancer,” said Srivastava, who leads the NCI’s Early Detection Research Network, which partially funds Czerniak’s work.

Bladder cancer is the fifth most common cancer in the United States and accounts for 3 percent of cancer deaths annually. It is strongly associated with smoking, which studies show is a factor in half of cases

The research model can be used to study other cancers of the epithelium -- the tissue that lines the surfaces and cavities of the body's organs. Epithelial cancers, or carcinomas, make up 80 percent of all cancers.

The researchers report that silenced forerunner genes involved in early development of bladder cancer also are silenced to varying degrees in lung, breast, blood and common pediatric malignancies.

Map leads way to six chromosomal regions

The 29-page paper, which covers multi-step genetic screening, validation studies of initial findings, studies of gene expression, gene sequencing and a regulatory process known as methylation, functional studies of candidate genes and epidemiological analysis, takes up half of Laboratory Investigation's space for original research in its July issue.

"We've basically cleared out the issue for this paper," said the journal's editor, James M. Crawford, M.D., Ph.D., professor and chair of the University of Florida College of Medicine Department of Pathology, Immunology and Laboratory Medicine. Devoting that much space to a single paper is highly unusual, but Crawford is convinced it's worthwhile. "A person should be able to read this paper and know this entire, important story," Crawford said.

The paper documents three waves of genetic hits, mainly involving genetic deletions, that drive cells from normal to precancerous states. The first wave leads to widespread expansion of urothelial cells that harbor genetic changes but otherwise appear normal under microscopic analysis.

The second wave provides a growth advantage to cells that now have recognizable outward features of dysplasia – precancerous cellular abnormalities. The third wave fully transforms the cell's appearance and features the onset of severe dysplasia or carcinoma in situ, noninvasive cancer still limited to its tissue of origin, in this case the urothelium. Carcinoma in situ and dysplasia advance to invasive cancer.

The team mapped the tissue of five cancerous bladders. Next, they employed 787 DNA markers to identify chromosomal regions that display genetic deletions. By superimposing the low-resolution map of genetic variation over the geographic map of the organ's tissue, they identified regions associated with both first-wave and second-wave cells.

Additional analysis narrowed the chromosomal regions to portions of six chromosomes. These six sites were confirmed by testing multiple markers of genetic loss in those chromosomal regions in the urine and blood of 32 bladder cancer patients and 31 disease-free patients with a history of bladder cancer. Genetic losses from at least one of the six regions were found in 98 percent of patients. In 82 percent of the cases, two to five chromosomal regions were involved.

Silenced forerunners stifle a tumor-suppressor

The team chose the 13q14 region on chromosome 13, which they knew harbored the tumor-suppressor RB1, for high-resolution genetic analysis to identify candidate genes affecting RB1 by using single-nucleotide polymorphisms (SNPs) as markers. SNPs are points in the genome that vary by a single DNA chemical building block or nucleotide.

They examined 92 SNPs mapping to a region around the RB1 gene in 84 paired samples of bladder tumors and blood DNA. This high-resolution genetic analysis pointed to the same section of the chromosome that whole organ histologic and genetic mapping had identified with expansion of abnormal cells.

Two genes in the region were found to give cells an initial minimum genetic advantage needed to grow into cancer.

A neighboring gene called ITM2B was found to be silenced by methylation, which is the connection of a methyl chemical group to portions of the gene that shuts it down. In bladder cancer tumors and cancer cell lines, this gene was methylated 40 percent of the time.

A gene known as P2RY5 located inside a portion of the RB1 gene was affected by a number of single-nucleotide changes. A case-control study of one of the gene's variant forms was conducted using blood DNA from 790 bladder cancer patients and 712 controls matched for age and gender. The specific variation was present in 2.78 percent of patients and every patient with the variation who also smoked developed bladder cancer.

The forerunner genes identified in bladder cancer were analyzed for their expression, methylation and sequence in 62 cell lines derived from major groups of common cancers. Of the cell lines tested, forerunner gene expression was reduced in 63 percent of the cases and ITM2B was methylated in 42 percent.

While forerunner genes were downregulated in lung, breast, blood and pediatric malignancies, they were strongly expressed in colon and liver cancers. This makes sense, the researchers note, because those two cancers do not rely on inactivation of RB1 in order to thrive.

Research continues. "It took us 10 years to get where we are now. With the new high-throughput technology now available, we will complete a high-resolution genetic map of the entire genome for bladder cancer in the next two or three years," Czerniak said.

Source: University of Texas M. D. Anderson Cancer Center

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