Whole-exome sequencing predicts which bladder cancers and cell lines respond to cisplatin

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Dan Theodorescu, MD, PhD, James Costello, PhD, and CU Cancer Center colleagues show that whole-exome sequencing predicts bladder cancer sensitivity to cisplatin. Credit: University of Colorado Cancer Center

Much of basic cancer research is based on studies with cultured cancer cells. However, the usefulness of these studies greatly depends on how

accurately these cancer cells grown in a dish represent human tumors. A University of Colorado Cancer Center study published in the journal *Oncogene* used next-generation sequencing technologies to perform the most detailed DNA-based analysis to date of 25 commonly used bladder cancer cell lines, allowing researchers to match patient tumors with their closest genetic cell line match, and demonstrated genetic alterations that may make cells more or less sensitive to common therapies.

"The idea is very simple but very important," says Dan Theodorescu, MD, PhD, director of the CU Cancer Center. "With our sequencing and transcriptional data, we can figure out which of these cell lines most closely match the human tumors. Once we know which ones match, we should only use these in our experiments in order to have the best chance that our experimental findings then apply to patients and their tumors."

The study used whole-exome sequencing to characterize genetic alterations that occur at the single nucleotide level for all genes in 25 cell lines commonly used as models of bladder cancer. The human genome contains about 3 billion base pairs, but only about 2 percent of these base pairs represent protein-coding genes, meaning that whole-exome sequencing measures the genetic alterations focused on a small but very important fraction of the genome (as opposed to techniques of whole genome sequencing, which measures every nucleotide across the entire genome, regardless of whether these genes are expressed or silent).

In combination with a separate technique that measures the degree to which a gene is expressed, the researchers then identified genes that were either mutated or functionally altered through expression levels in these bladder cancer cell lines. In all, the study found and validated 76 alterations in cancer-associated genes in the cell lines, many of which are involved in activating known oncogenes including TERT, TP53 and PIK3CA. Importantly, this information can then be used to compare genetic aberrations in cell lines to human tumor samples.

However, not all 76 genes were altered in all 25 cell lines, resulting in "signatures" of genetic changes that differed between lines. Like these cell lines, not all human bladder cancers share the same genetic changes. When the researchers compared these cell lines to the data of human tumors stored in The Cancer Genome Atlas (TCGA), they found that some cells lines better modeled some human tumors.

"Instead of saying 'these cells look like oranges and these patients look like oranges, so they must be similar' this is an experiment to functionally show how similar or dissimilar these human tumors are to these cell lines at a molecular level," Theodorescu says.

Along with differences in gene alterations, bladder cancer patients also show differences in how well they respond to certain therapies. In this case, researchers wondered whether the signatures that describe the genetic alterations in these 25 cell lines could predict the outcomes of patients treated with the common bladder cancer chemotherapy, cisplatin.

"In other words, we wanted to make sure these signatures were meaningful in real, human tumors and not just an artifact of being grown in a dish," says James Costello, PhD, investigator at the CU Cancer Center and assistant professor in the CU School of Medicine Department of Pharmacology.

It turned out that, based on comparing the alterations found in these 25 cell lines with alterations found in a patient's tumor, the researchers could predict who responded favorably and who did not respond to cisplatin treatment.

"We don't propose this as a current diagnostic or prognostic tool," Costello says, "but showing that these alterations have real effect in human tumors allows us to explore the mechanisms that tumors use to

resist therapies like cisplatin."

For example, genes in the CDK family regulate the cell cycle and are commonly mutated or deleted in many cancers. Alongside the CDK genes, CDKN2A and CDKN2B, sits the gene MTAP. Because cancers commonly delete the CDKN2A/2B genes, nearby MTAP is often deleted as well. The question has been whether MTAP deletion is functional in bladder cancer or whether the deletion is just a passenger along with CDKN2A/2B deletion. In this study, Theodorescu, Costello and colleagues were able to explore bladder cancers without CDKN2A/2B deletion, with only CDKN2A/2B deletion and with paired CDKN2A/2B and MTAP deletion. It turned out that independent of CDKN2A/2B deletion, MTAP-deficient cells act differently than cells with CDNK2A/2B loss only. This observation has been recently supported by independent research that indicates tumors with MTAP/CDKN2A/2B loss can be therapeutically targeted.

"MTAP was only recently described in the context of cancer and our work further supports the involvement of this gene in the development and progression of the disease," Costello says.

The study describes the mutational landscape of bladder cancer cell lines . It demonstrates that alterations in these cells lines do indeed match changes in samples of human bladder cancer. And it demonstrates genes and gene pathways that may be functionally involved in the ability of bladder cancer to resist therapy.

"Philosophically, we wanted to provide the bladder cancer field an atlas of cells that mirror human tumors so our collective experiments could be most relevant to patients. The study also adds to our understanding of disease development and therapy resistance," Theodorescu says.

More information: M L Nickerson et al, Molecular analysis of

urothelial cancer cell lines for modeling tumor biology and drug response, *Oncogene* (2016). DOI: 10.1038/onc.2016.172