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KIDNEY CANCER

Intratumoral differences analysed

Detailed genetic analysis of clear cell renal cell carcinoma tumours provides new insight into intratumoral heterogeneity with implications for tumour development, biopsy, drug resistance and future therapeutic targets. Marco Gerlinger and a team of researchers have built upon their groundbreaking study published in *The New England Journal of Medicine* in 2012, expanding their analysis from two tumours to 10, and revealing a high number of spatially separated subclonal driver events.

In their original study, published nearly 2 years ago, Gerlinger and colleagues performed multiregion sequencing of the entire exome on multiple biopsy samples taken from two patients with metastatic clear cell renal cell carcinoma. They found that approximatly two-thirds of the mutations detected were expressed heterogeneously within tumours, and some mutations were unique to specific regions, indicating continuous regional clonal evolution. "Our next aim was to study a larger number of patients to understand exactly how frequently heterogeneity occurs in renal cell carcinoma, and whether we can start to understand the general principles governing cancer evolution," explains Gerlinger.

For their updated study, published in *Nature Genetics*, the researchers used a standard exome sequencing approach, with a depth that is sufficient for the analysis of cancer specimens, to analyse tumours from eight additional patients with clear cell renal cell carcinoma, and combined the data with their previously reported patients. In total, seven patients had stage T3 disease, two patients had stage T2 and one patient had stage T4 renal cell carcinoma; eight patients presented with metastatic disease. To define the regional distribution of mutations, they re-sequenced each mutation that was found in at least one tumour region in all regions of the same tumour, using a different next-generation sequencing platform, at a much greater depth. "All 10 tumours were heterogeneous and showed a branched evolution pattern with multiple co-existing subclones, confirming the importance of our previous report," explains Gerlinger. The authors conclude that intratumoral heterogeneity is a common feature of stage T2–T4 clear cell renal cell carcinoma.

Gerlinger and his colleagues constructed a phylogenetic tree for each tumour and mapped nonsynonymous mutations to the trunk or branches, to investigate their evolutionary patterns. They were surprised to find that all common clear cell renal cell carcinoma driver aberrations—with the exception of *VHL* mutations and loss of chromosome 3p—were predominantly subclonal (in other words, they mapped to branches rather than the trunk). "This finding indicates that most driver mutations do not define intrinsic clear cell renal cell carcinoma subtypes but that distinct subclones with driver mutations—for example in *PBRM1*, *SETD2*, *BAP1* and *TP53*—can all co-exist within the same tumour," Gerlinger explains. "These subclones were predominantly spatially separated, and single biopsies would have failed to detect the complex subclonal architectures that we identified by multiregion exome sequencing."

In an attempt to understand how much information is missed if clinicians rely on a single biopsy for treatment decisions, the investigators calculated the number of mutations that would have been detected in each tumour by sampling one to *n* biopsies. They found that the number of aberrations identified increased with the number of biopsies; in seven cases the number of mutations did not plateau, indicating that this research probably underestimates the total number of mutations present.

"We are increasingly utilizing circulating tumour DNA and circulating tumour cell sequencing technologies for biomarker discovery, which might provide more uniform sampling than a single biopsy," says Gerlinger. "We think it will be important to develop therapies that target genetic changes present in all cancer cells within a tumour, so-called truncal alterations, which might be difficult in renal cancer because there are only a few drivers located on the trunks of the phylogenetic trees."

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Original article Gerlinger, M. *et al.* Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. *Nat. Genet.* doi:10.1038/ng.2891