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## NGS with Molecular Barcodes Uncovers Somatic Mosaicism

### Dr. Jiannis Ragoussis

Dr. Ragoussis, associate professor of human genetics at McGill University, co-authored a study of childhood cancers associated with a rare DICER1 syndrome.

#### Introduction

Dr. Ragoussis collaborated with Professor Will Foulkes, director of the program in cancer genetics at McGill University, to study four children with multiple primary tumors associated with DICER1 syndrome.



They investigated and characterized DICER1 RNase IIIb mutations by deep sequencing both tumor and normal tissues from the four cases. A HaloPlex<sup>HS</sup> panel incorporating molecular barcodes was used by the team to confirm its hypothesis that DICER1 RNase IIIb mosaicism was the cause of the rare DICER1-associated tumors.

### Zooming In: The Research

**Q1: What is your lab's focus?** I oversee the genomics platform strategy of the Innovation Center. My lab focuses on technical developments in sequencing technology and new assay development, particularly for cancer applications and difficult situations such as somatic mutation detection, for example, related to the problem of tumor heterogeneity. I also work on technology for single cell genomics.

We collaborate with the William Foulkes' group at McGill on DICER1 syndrome, which is typically caused by heterozygous germline mutations in DICER1. This gene encodes a small RNA endoribonuclease responsible for processing precursor miRNAs into mature miRNAs. Mutations in DICER1 are associated with a range of rare cancers and developmental phenotypes.

**Q2: How did you get into this line of research?** I got interested in the DICER1 work, because there were some instances in which no germline mutation that predisposes to the syndrome were identified, and it was proving to be very difficult to identify somatic mutations. It was technically challenging and got me really interested in solving the problem of finding causal mutations that may be present at very low frequencies. We studied four cases of DICER1 syndrome that were particularly interesting (1).

**Q3: What challenges did you encounter?** We initially tried deep sequencing to find causal somatic mutations. We sequenced deeper and deeper, until we did identify possible mutations, but it was very difficult to prove that these mutations were real and not sequencing artifacts. This is what led us to looking at next-generation sequencing with molecular barcodes—using HaloPlex<sup>HS</sup> as a means to identify very low frequency mutations with a high degree of certainty. We also compared HaloPlex<sup>HS</sup> to other platforms.



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#### Q4: How did molecular barcodes solve this problem?

HaloPlex<sup>HS</sup> technology involves the attachment of a 10-nucleotide long molecular barcode to the captured DNA sample molecules. Up to eight different restriction fragments per targeted base in the region of interest are captured. This redundancy increases the accuracy and sensitivity of detecting rare mutations. During the analysis, barcode sequence data are used to build a consensus at which time artifacts and errors are removed. In the platform comparison, we found that HaloPlex<sup>HS</sup> offered us the greatest precision with its ability to remove duplicate reads, thereby increasing base calling accuracy. It also fits very nicely into our workflow.

HaloPlex<sup>HS</sup> managed to actually help us solve all of the problems that we faced. Using the molecular barcode technology and high efficiency of capture that the assay can deliver, we confirmed all of the low frequency mutations in all of the tumor samples that we analyzed. The negative results obtained in the non-tumor samples were also confirmed. I would recommend HaloPlex<sup>HS</sup> to anyone who would like to analyze any sample of DNA at a very high accuracy and sensitivity and is interested in detecting low frequency mutations.

#### Q5: What did sequencing with barcodes reveal?

We found that unlike many previously reported cases of DICER1 syndrome involving germline truncating mutations, what we had in three of the cases was somatic mosaicism. We also hypothesized that two-hit tumorigenesis like that seen in most dicer-related tumours could also be at work here. In fact we did find second somatic mutations.

#### Q6: How does finding low frequency alleles help with the understanding of cancer?

HaloPlex<sup>HS</sup> helps us understand tumor heterogeneity with a high level of detail and accuracy. It enables us to not only identify the first level mutations or "hits" in tumors, but also subsequent hits that may be associated with tumorigenesis.

### Zooming Out: What is next for your laboratory?

With Will Foulkes, we would like to use HaloPlex<sup>HS</sup> to study tissue DNA at a much higher depth in order to identify not only the first, but the second hit that may be associated with tumorigenesis, not only in DICER1, but also in many other syndromes.

In addition, I am really interested in understanding tumor heterogeneity. To do that, I am applying high-resolution techniques, such as HaloPlex<sup>HS</sup>, because it can help us understand heterogeneity at a very high level of detail and accuracy, but also single cell genomics approaches. Research focused on understanding heterogeneity is very important, because we need to study see how mutations behave in terms of response to treatments and therefore guide future treatment regimes.

Kock, LD *et al.* High sensitivity sequencing reveals multi-organ somatic mosaicism causing DICER1 Syndrome. *J Med Genet* 2016; 53:43-52.



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