Introduction
IDH1 and IDH2 mutations have recently been shown to be present ab initio in ~60% of chondrosarcoma (CS) cases (Figure 1) and retained throughout disease progression including trans formation into dedifferentiated CS.1 The ability to detect IDH1/2 mutations in biopsy material from patients with CS may aid diagnosis.

There is currently significant interest in the discovery that solid tumours release DNA, cell-free (ctDNA), into the circulation. By screening for IDH1/2 mutations in blood, it may be possible to detect and quantify the tumour fraction for a number of uses, including non-invasive diagnosis, prognostication, monitoring of disease, and residual disease detection.2,3 Until now, the ability to detect ctDNA in patients with CS has not been tested.

We have developed digital PCR (dPCR) assays for highly sensitive detection of IDH1/2 mutations in FFPE tissue and blood plasma.

Digital PCR

dPCR is currently the most sensitive and accurate method for detecting and quantifying mutant DNA molecules. dPCR is achieved by partitioning a standard PCR into many replicate reactions prior to thermal cycling. This results in the physical separation of DNA molecules, thus allowing binary classification of compartments that either contain the amplified target sequence, or do not. Copy numbers can then be calculated by applying the Poisson distribution. We have used the Bio-Rad QX200 dPCR platform (Figure 2).

Assay characteristics

We have developed and optimised singleplex assays for all common IDH1 and IDH2 mutations (Figure 1), and a multiplex for the IDH1 mutations (Figure 3).

The IDH1-R132C assay has been chosen to initially generate data for tissue DNA analysis, as it is the most common mutation in central CS. The observed mutation is a C>T transition, which can occur as an artefact in wild-type formalin-fixed tissue due to cytosine deamination. Quantification is accurate over 4 orders of magnitude (Figure 4a). The limitation of detection is constrained by the background false positive rate which has been shown to be ~0.04% in good quality high molecular weight DNA and up to 0.38% in FFPE DNA (Figure 4b).

Results – tumour analysis

76 chondrosarcomas and 79 wild-type sarcomas have been analysed using the IDH1-R132C dPCR assay. Of the chondrosarcomas, 37 were classified as mutant and 39 as wild-type for the mutation in a previous study using a combination of sequencing and genotyping techniques. All samples were analysed in duplicate, and the assay is highly repeatable between PCR replicates and experiments (Figure 5).

Cut-off values of 0.4% mutant copies and > 2 mutant-only droplets were applied to each set of duplicate reactions. From these data, the sensitivity of the test is 97.2% and the specificity is 99.1% (Table 1).

The only discrepant ‘false positive’ result was called as mutant at 1.5% by dPCR. DNA from this patient had previously been determined to be wild-type using Sanger sequencing, a technology with a sensitivity of approximately 15-20%. This sample highlights the potential of dPCR as a more sensitive analytical test for detection of cancer-specific mutations in biopsy tissue.

The discrepant ‘false negative’ mutant result is being investigated. It was previously analysed by Sanger sequencing, whereas dPCR detected the mutation at 0.12% (within background noise).

Results – circulating tumour DNA in plasma

In a pilot study we have analysed pre-treatment ctDNA levels in 14 patients with CS. ctDNA was detectable in all G11 samples, half of GII samples and not in G1 samples (Figure 6a). ctDNA levels did not correlate with tumour size.

In samples from 4 patients we also assessed ctDNA levels serially throughout treatment. In all ctDNA levels dropped post treatment (Figure 6b).

Conclusions

• Digital PCR is both a sensitive and specific method for IDH1/2 mutation detection
• The IDH1 multiplex assay is able to detect all common IDH1 mutations
• Chondrosarcomas release DNA cell free into patients’ circulation
• Ongoing studies are assessing the different clinical uses of chondrosarcoma ctDNA analysis

References

Contact details - tфоршев@ucl.ac.uk