Right at your fingertips!

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Records of the detection and management of cancer date as far back as 3000 BC [1]. It wasn't for another ~5000 years that a link of cancer to a genetic etiology was established with the discovery of the Philadelphia chromosome in 1960 [2, 3]. The pace of discovery has been astronomical since 1960, and advances in sequencing technologies have played a critical role in significantly improving the diagnosis and management of cancer patients (Figure 1). Although sequencing of the tumour and germline DNA has traditionally been performed separately, paired tumour:normal analysis by next generation sequencing (NGS) is emerging as a strategy that is faster and more efficient in distinguishing germline variants from acquired somatic changes in the tumour, thus enabling more accurate analysis of the variants [4-7]. The source of germline DNA is most often peripheral blood, as it is easily obtainable; however, it is not suitable in the analysis of haematologic malignancies, as there can be contamination of the blood with tumour cells that could result in inaccurate distinction between germline and tumour variants. Other sources of germline DNA, such as buccal swabs, saliva and direct skin biopsies, can also be contaminated with tumour cells. Furthermore, the time required to culture skin fibroblasts to eliminate possible blood tumour contamination leads to significant delay of the results, possibly impacting patient management. Hence, an alternative source of germline DNA is required in the analysis of haematologic cancers. In this issue of *Haematologica*, Krystel-Whittemore et al. [8] report on one institution's extensive experience in using cell free DNA (cfDNA) obtained from nail clippings as a source of normal control for paired tumour:normal genomic analysis of haematologic malignancies. In addition, they describe a rapid protocol for extraction of cfDNA from nail clippings, and discuss the benefits and disadvantages, as well as highlight some interesting findings, using cfDNA obtained from nail.

In this study, 2,610 diagnostic tumour samples (blood, bone marrow, tumour biopsies), with nail as the normal control, underwent molecular profiling using MSK-IMPACT, a custom 400-gene NGS panel used for the detection of somatic mutations and copy number changes [9]. Analysis of these tumour:normal pairs yielded 10,942 somatic variants, of which 4,640 were in myeloid and 6,302 were in lymphoid disease categories. Mutations in the nail were detected in 13.9% of the patients tested. Interestingly, the majority of these mutations were found in patients with myeloid neoplasms (20.5%) as compared to lymphoid neoplasms (5.4%).

While the average variant allele frequency (VAF) of the tumour variants was 26.7%, the average VAF of the nail variants was significantly less at 4.4%. However, in 19 patients (0.7%), the nail variant VAFs were surprisingly close to and even slightly higher than the corresponding tumour variant VAFs, suggesting a high tumour contamination in the nail. Why would this be? The authors attribute three of these cases
to gaps in collection. In other words, these cases correlated with nails collected at the time of greatest
disease burden, but the tumour collected after interim therapy, a time of very low disease burden.
Despite this high level of contamination, the authors were readily able to distinguish germline vs.
somatic variants in all, except in one unique case.

Mutations in nails were significantly biased towards genes frequently altered in myeloproliferative
neoplasm (MPN) and myelodysplastic syndrome (MDS). While the VAFs observed in nail were generally
<5%, those observed at VAFs >5% (92 patients) were associated with the presence of marked bone
marrow fibrosis and osteosclerosis (33%) and myeloid neoplasms with monocytic features (13%). Loss of
heterozygosity (LOH) was observed for those mutations with the highest VAFs. In stark contrast, among
patients with lymphoid and plasma cell neoplasms, mutations in genes recurrently associated with these
neoplasms were absent in nail. Furthermore, in a select group, generally with nail mutation VAFs >3%,
only mutations of myeloid origin were identified in the nail. Interestingly, these patients were
determined to have an emerging or coexisting clonal myeloid process.

Krystel-Whittemore et al. [8] looked at a subset of 50 patients with nails collected post-allogeneic stem
cell transplantation. Donor DNA could be identified in 22% of nails. The authors determined that a
history of graft-vs-host-disease (GVHD) was significantly higher in patients with donor DNA in their nails
(63.6%) as compared to no donor (15.4%). They suggest GVHD as a potential cause of donor cells in the
nail, among others that have been documented in the literature. To use nail as a normal control, it will
be important to determine the presence and level of chimerism in the nail. In the presence of minimal
or no donor contamination, the authors found that sequencing both the nail and donor DNA was
extremely useful in the analysis of difficult post-transplant samples.

To routinely use nail as a source of normal DNA in the clinical setting, there is a need to efficiently extract
high quality DNA from nail. Using a commercial kit, Krystel-Whittemore et al. [8] compared two different
nail fragmentation methods on 20 validation samples. The first used cut-up nail fragments and
overnight (sometimes several) proteinase K digestion. With the second method, nail clippings were
pulverized using a tissue homogenizer. The authors found that method 2 had a significantly shorter
procedure time with improved results. Specifically, method 2 produced larger fragment sizes, a higher
yield of DNA and greater targeted coverage as compared to method 1. Furthermore, nail cfDNA was
found to be high-performing for hybrid-capture-based NGS assays, as well as for short amplicon PCR-
based assays, thus confirming the clinical utility of cfDNA extracted from nail.

In addition to using nail cfDNA in paired tumour:normal genomic analysis, it can also be used in
identifying hereditary predisposition in individuals with haematologic malignancies, as illustrated by
Ceyhan-Birsoy et al. [10] in an accompanying paper in this issue of Haematologica. Using nail cfDNA for
germline testing of patients with haematologic malignancies, they found that only 0.08% of patients
tested had somatic contamination in nail at levels that could potentially make unequivocal distinction of
germline and somatic variants difficult in the absence of a matched tumour. One of these patients had
MPN and fibrotic bone marrow while the second had MDS, reminiscent of the results obtained by
Krystel-Whittemore et al. [8]. Hence, Ceyhan-Birsoy et al. [10] show that nail cfDNA is a reliable source
of germline DNA for the testing of patients with haematologic malignancies.

The impressive work by Krystel-Whittemore et al. [8], supported by the study of Ceyhan-Birsoy et al.
[10], demonstrates that nail cfDNA is a robust source of germline DNA in the genomic analysis of
patients with haematologic disorders. These results would have a particularly significant impact on the
paediatric population, for whom haematologic neoplasms are the most commonly occurring cancer [11, 12]. Parents would be less hesitant to provide nail clippings as the source of germline control, whereas an invasive skin biopsy procedure would add considerable anxiety to an already devastating and stressful situation. Furthermore, since acute lymphoblastic leukemia is the most common cancer in children [12], one would not expect lymphoid tumour contamination in the nail cfDNA of these patients if the findings of this current study can be extrapolated to the paediatric population, making nail cfDNA preferable. In summary, Krystel-Whittemore et al. [8] show that there can be tumour contamination in nail cfDNA, notably in patients with myeloid neoplasms with marked fibrosis and in patients post-allogeneic stem-cell transplantation, but that these are readily resolved. Tumour contamination in nail could also provide important diagnostic information for the patient. Despite some potential confounding factors, nail cfDNA should be considered a reliable and robust source of germline DNA for patients with haematologic malignancies.
REFERENCES


Figure 1. Evolution of cancer diagnostics. The earliest written record of human cancer, a breast cancer, was documented in ~3000 BC [1]. Chronic myelogenous leukemia was the first cancer linked to a genetic etiology upon discovery of the Philadelphia (Ph) chromosome in 1960 [2, 3]. The exact molecular mechanism of the Ph chromosome rearrangement, the fusion of the BCR gene with the ABL1 gene, was not elucidated until 1980 [13], after Sanger had developed a reliable method of sequencing DNA [14]. Advances in molecular technologies led to the discovery of the first hereditary cancer genes in 1994 [15, 16], followed by the initial completion of the Human Genome Project in 2001 [17]. While traditionally the tumour and germline DNA have been sequenced separately, the development of next generation sequencing [18], which allowed for the massively parallel sequencing of large amounts of DNA, revolutionized cancer diagnostics such that the tumour and germline DNA could be paired and sequenced/analyzed together [4-9]. This current study [8] confirms that cfDNA extracted from nail clippings is a robust and reliable source of germline DNA in the genomic analysis of haematologic malignancies.