Cellular neurothekeoma is driven by copy number deletions, providing potential diagnostic and therapeutic avenues

Dear Editor, To-date our knowledge of the genomic landscape of cellular neurothekeoma (cNTK) remains incomplete, with only microarray analysis of differentially expressed genes between neurothekeomas and nerve sheath myxomas[1], or case reports of targeted sequencing or whole-exome sequencing (WES) performed on individual patients. These cases reported somatic mutations in *PI3K, ALK, SMO*, and *ERBB3* in a patient with cNTK[2], a somatic mutation in *NF1* in a patient with atypical cNTK[3] and a somatic mutation in *FLCN* in a patient with a cutaneous plexiform hybrid tumour of perineurioma and cNTK[4]. Thus, in order to comprehensively elucidate the mutational profile of this rare tumour type and identify driver events, we sequenced DNA (WES) and RNA (pulldown transcriptome) from an international, multi-institutional cohort of cNTK matched tumour-normal pairs (n = 20), that were independently assessed by two dermatopathologists. Consistent with the clinical presentation of this tumour type, our cohort was predominantly composed of females (7 males and 13 females), with the lesions located primarily on the head and neck area (Fig. 1). The median patient age was 26.5 years (range: 4 – 58 years). Sequencing data has been deposited in the European Genome and Phenome Archive (EGA; Accession IDs: EGAS00001006749-50).

As gene fusions are driver events in many tumour types, we looked for their presence in the RNA-sequencing reads of the cNTKs. Six novel fusion genes were identified (Fig.1). However, given these fusions were found in one tumour sample each, it would suggest they are not major driver events. As a potential role for viruses has been established in some skin cancers, we also used the RNA-sequencing reads to look for the presence of viruses. However, we did not find statistically significant levels of sequencing reads associated with any viruses, suggesting they are unlikely to be drivers of cNTK.

In keeping with the benign nature of this tumour type, the tumour mutational burden was low (median: 0.124 mutations per megabase, range: 0.020 – 0.352), and only two genes were recurrently mutated, specifically the serine/threonine kinase, *MAPK1* (also known as *ERK2*) and the E3 ubiquitin-protein ligase, *MKRN1* (n = 2 samples each; Fig. 1). No genes were identified as significantly enriched for non-synonymous mutations (i.e., under positive selection in the tumour), making these somatic mutations unlikely to be driver genes.

Next, we sought to identify likely gene targets of somatic copy number (CN) alterations. By analysing regions commonly amplified or deleted within the cohort, we

identified focal regions (<10 Mb) with statistically significant recurrent CN alterations in cNTK (Fig. 1a). Notably, one of these was a 595 kb region with CN deletion on chromosome 9 at position 21,379,301 – 21,974,390 bp (9p21.3; q-value = 0.00018) that encompassed the *MTAP* gene and the two C-terminus exons of the tumour suppressor gene *CDKN2A*. Ten of 19 cases (52%) had CN deletion in the region; 3 with homozygous, focal deletions (~0.4-6.2 Mb) and 7 with heterozygous deletion of a larger region (~33-42 Mb) encompassing the distal end of chromosome 9p. The homozygous deletions are derived from focal deletion of one allele and deletion of an overlapping, broader region (20.2-42.6 Mb) on chromosome 9p, as seen in other tumour types. Analysis of The Cancer Genome Atlas (TCGA) pan-cancer database has shown that loss of 9p21.3 is frequently observed in cancer and is associated with shorter survival[5]. Of the genes located within that chromosomal region of CN loss, *CDKN2A* (~13%) and *MTAP* (~9%) are the most commonly deleted[5]. These findings raise the possibility that p16 protein loss could be a diagnostic immunohistochemical finding to assist distinguishing cNTK from histiocytic neoplasms that typically express this marker[6]. In addition, preclinical studies have shown that *MTAP*- homozygous deleted (deficient) cells are vulnerable to PRMT5 inhibition, and PRMT5 inhibitors (PRMT5i) in clinical trials are showing promising results as a synthetic lethal approach for *MTAP*-deleted tumours (by selectively targeting MTAP-deficient tumour cells to prevent the methylation activity of PRMT5 when complexed with MTAP[7-8]).

As we sequenced matched normal tissue for the tumours we were able to search for putative pathogenic germline variants predisposing to cNTK. To focus on clinically relevant genes, we considered known cancer-predisposition genes, with variants of moderate/severe functional impact, that are classified in the ClinVar database as ‘pathogenic’. Using these criteria, we identified a patient carrying a heterozygous premature stop codon in *CHEK2* (p. W454* in patient PD66656).

In summary, our study has provided the first comprehensive characterisation of the genomic landscape, and identified CN alterations as a driver event of this tumour type. The most recurrent CN alteration was a deletion on 9p21.3 that encompassed *MTAP* and the c-terminal exons of *CDKN2A*. Further studies may be warranted to assess whether p16 could be a useful diagnostic biomarker for cNTK, and PRMT5i represent a future therapeutic option for those with recurrent cNTK lesions that are MTAP-deficient.

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Figure legend

Fig. 1. Overall mutational landscape of cellular neurothekeomas (cNTKs). Tumour mutational burden is shown as mutations per megabase (Mb). The second panel shows genes for which a somatic mutation was identified in multiple samples or present in OncoKB. For tumour site, the locations are either the extremities (arms, hands, legs and feet) or trunk (shoulders, torso and buttocks/genital areas). The significant copy number altered GRCh38 regions shown include focal (<10 Mb) regions of amplification/deletion. Indicated are the cases that have an amplification or deletion overlapping the significant region. For tumour site, the locations are either the head and neck, extremities (arms, hands, legs and feet) or trunk (shoulders, torso and buttocks/genital areas). Abbreviations: chr, chromosome.