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Malignant Bone Tumours with *H3F3A* Mutations: A Genomic and Epigenetic Study

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Introduction and aim of study

Giant cell tumour of bone (GCT) is an uncommon neoplasm of osteoblastic origin, characterised by highly recurrent missense mutations in the histone H3.3 gene (*H3F3A*), usually p.G34W (Behjati et al., 2013; Fletcher et al., 2013; Amary et al., 2017). The majority behave in a benign fashion, but a small subset show malignant behaviour and have a poor prognosis. Clinical features and histology do not reliably distinguish between benign and malignant GCT. Our aim was to characterise the genomic and epigenomic landscape of malignant GCT, with the purpose of identifying molecular prognostic features.

Methods

The following investigations were performed on groups of histologically 'conventional' (n = 38) and 'malignant' (n = 13) GCT: whole genome sequencing (WGS), and/or DNA methylation profiling (Illumina 450K/EPIC arrays), and/or SNP array profiling (Illumina Omni2.5). Data were also compared with whole genomes generated from osteosarcomas without H3.3 mutations (n = 35) (Behjati et al., 2017). The data were analysed without knowledge of the histological subtypes or the clinical outcome.

Results and discussion

Malignant GCT genomes differ from those of conventional GCT and osteosarcoma.

- Malignant GCT has a heterogeneous burden of DNA structural variants (DNA insertions, deletions, tandem duplications and inversions). SV burden varied widely between individual cases of malignant GCT (median 10.5; range 0-102), but SVs were virtually absent from conventional GCT.
- Chromothripsis, a catastrophic shattering of chromosomes and a hallmark of malignant tumours including osteosarcoma, was founded in 2/10 malignant cases. Kataegis, a localised hypermethylation phenotype and another hallmark of malignancy was seen in 4/10 genomes.
- Besides the canonical *H3F3A* mutation, virtually no recurrent mutations in known cancer drivers were detected in malignant or conventional GCT. In contrast to conventional osteosarcoma, *TP53* and *RB1* mutations were absent.
- Conventional and malignant GCT both showed global DNA hypermethylation relative to a cohort of 87 benign bone tumours without G34 mutations. However, conventional and malignant GCT could not be reliably separated on the basis of principal component analysis, or by looking at differentially methylated regions.

Ploidy and copy number score are prognostic markers for H3.3-mutated bone tumours.

Amongst GCTs classified histologically as malignant, we noticed a biphasic distribution of structural variation. To investigate this further, we assigned each sample a copy number (CN) score (Davoli et al., 2017), to quantify the genome-wide prevalence and magnitude of copy number alterations. This score does not account for sample ploidy, which was called separately from WGS, methylation and SNP array data using the ASCAT algorithm (van Loo et al., 2010).

Plotting these combined data revealed two molecular subgroups of malignant GCT: diploid samples with near-zero CN score (group 1), and samples with abnormal CN score and/or ploidy (group 2). All conventional GCT showed the former profile, which was confirmed by an extension study incorporating SNP array data from 16 further conventional GCT. Further analysis of clinical and genomic data supported these findings by showing that the samples in the malignant GCT molecular 'group 1' were identical to that of conventional GCT. This was also supported by analysis of survival data.

Conclusions

- Distinguishing conventional from atypical/malignant GCT (*H3F3A*-mutated bone tumours) can be challenging on histology. This work shows that copy number alterations and ploidy are prognostic indicators for these tumours. *H3F3A*-mutated bone tumours with high copy number score and/or ploidy are associated with a poor clinical outcome.
- H3.3-mutated bone tumours (GCT and chondroblastoma) can be identified by their methylation profiles, and distinguished from each other and all other histological bone tumour types. The methylation profiles of H3.3 p.G34-mutated bone tumours do not distinguish between benign and malignant lesions. The methylation profiles of H3.3-mutated bone tumours are not valuable as prognostic markers.

References

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