Supplementary

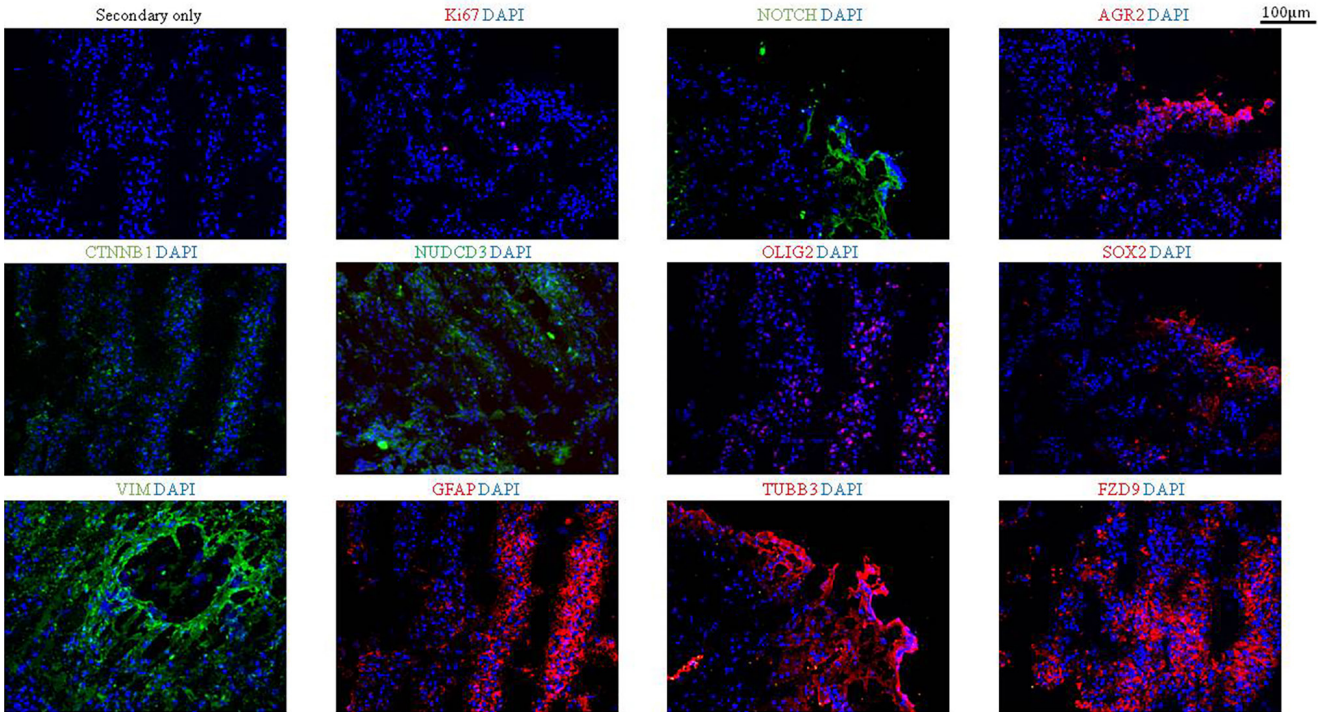

Figure S1 Immunofluorescence images showing the highest expression seen for each tested marker in the tissue. Shown in red are rabbit antibodies against Ki67, AGR2, OLIG2, FZD9, SOX2, GFAP and TUBB3. Shown in green are mouse antibodies against NOTCH, CTNNB1, VIM and NUDCD3. DAPI is shown in blue. All images were taken at ×20. AGR2, anterior gradient 2; CTNNB1, catenin beta 1; FZD9, frizzled class receptor 9; GFAP, glial fibrillary acidic protein; NUDCD3, NUDC domain containing 3; OLIG2, oligodendrocyte transcription factor 2; SOX2, SRY-Box transcription factor 2; TUBB3, tubulin beta 3 class III; VIM, vimentin.

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Appendix 1 Material and methods

Tumour sampling and cell culture methods

Specimen collection and cell line induction were performed as previously reported (12,19). Cell line drug treatments was performed as previously described (19). The drugs' ranges for the clonogenic assay were 3.1, 6.3, 12.5, 25 and 50 µM for cisplatin, and 1, 2, 4, 8 and 16 nM for taxol.

WES analysis

Libraries were generated in the Roya Laboratories, as per their standard protocols (ThermoFisher Scientific, Massachusetts, USA). The samples had an average total read depth of 119.9× for blood, 136.3× for tissue and 113.7× for the cell line. The pipeline used for the variant call format (VCF) analysis is shown in *Figure 4*. The files were annotated in the BaseSpace Variant Interpreter (accessed on 03/02/2021). Only catalogue of somatic mutations in cancer (COSMIC) variants that had damaging coding consequences, as called by BaseSpace Variant Interpreter platform provided by Illumina, or checked manually using PolyPhen-2 Wiki, and had a population frequency of less than 0.01 for all population sources, were selected. COSMIC variants detected in tissue, cell line and blood (TCB) were selected, a process previously shown to potentially enrich for predictive, tumorigenic and progressive genes (14). A final filter was applied based on genes previously published to be relevant in the posterior temporoparietooccipital region (PTR) of embryonic brain development as outlined in the SCDevDB (20).

Pathway analysis

Biological functions and implicated pathways were interpreted by employing both Panther and Metascape platforms.

Immunofluorescence staining

Frozen tissue was sectioned to generate 10 consecutive sections at 4-µm thickness. Each section was processed as previously mentioned (12). Sections were stained with only secondary rabbit anti-Ki67 (1:200, ab16667, Abcam), rabbit anti-anterior gradient 2 (AGR2) (1:100, ab227584, Abcam), rabbit anti-oligodendrocyte transcription factor 2 (OLIG2) (1:500, ab42453, Abcam), rabbit anti-frizzled class receptor 9 (FZD9) (1:100, ab150515, Abcam), rabbit anti-SRY-box transcription factor 2 (SOX2) (1:200, 130-095-636, Miltenyi), mouse anti-NOTCH (1:100, ab44986, Abcam), mouse anti-catenin beta 1 (CTNNB1) (1:500, ab18207, Abcam), rabbit anti-glial fibrillary acidic protein (GFAP) (1:500, ab7260, Abcam), rabbit anti-tubulin beta 3 class III (TUBB3) (1:500, ab18207, Abcam), mouse anti-vimentin (1:100, ab8978, Abcam), mouse anti-NUDC domain containing 3 (NUDCD3) (1:100, ab89080, Abcam), mouse anti-nestin (1:50, ab6142, Abcam), mouse anti-CD133 (1:100, 130-092-395, Miltenyi), mouse anti-P53 (1:500, ab26, Abcam) and mouse anti-SRY-box transcription factor 11 (SOX11) (1:200, ab154138, Abcam). For each section, five coordinate-fixed dispersed regions were selected for imaging. For cell line staining, cells were co-stained with the aforementioned antibodies in the following pattern: rabbit anti-Ki67 with mouse anti-Vimentin, rabbit anti-FZD9 with mouse anti-CTNNB1, rabbit anti-SOX2 with mouse anti-NOTCH, rabbit anti-GFAP with mouse anti-NUDCD3, rabbit anti-OLIG2 with mouse anti-CD133, rabbit anti-TUBB3 and rabbit anti-AGR2 with mouse anti-BMI1 proto-oncogene (BMI) (1:100, ab14389, Abcam). All images were taken and processed as previously described (12). Manual counting was performed twice by two independent scientists for three randomly selected regions, and indications for positivity for each marker and final counts were confirmed with a neuropathologist.

Statistical analysis of the data

The results were analysed using SPSS version 21.0. The percentage similarity of single nucleotide variants (SNVs) in exomes for DNA collected from cell line and tissue was calculated as: the number of unfiltered SNVs in exomes present in both DNA collected from the cell line and the tissue, as a percentage fraction of the total number of unfiltered SNVs present in the tissue. One sample *t*-test was used to test for significant differences between the percentage similarity of SNVs in exomes for cell line's and corresponding tissue's DNA, and the average percentage similarity of SNVs in exomes for cell line's and non-corresponding tissue's DNA.

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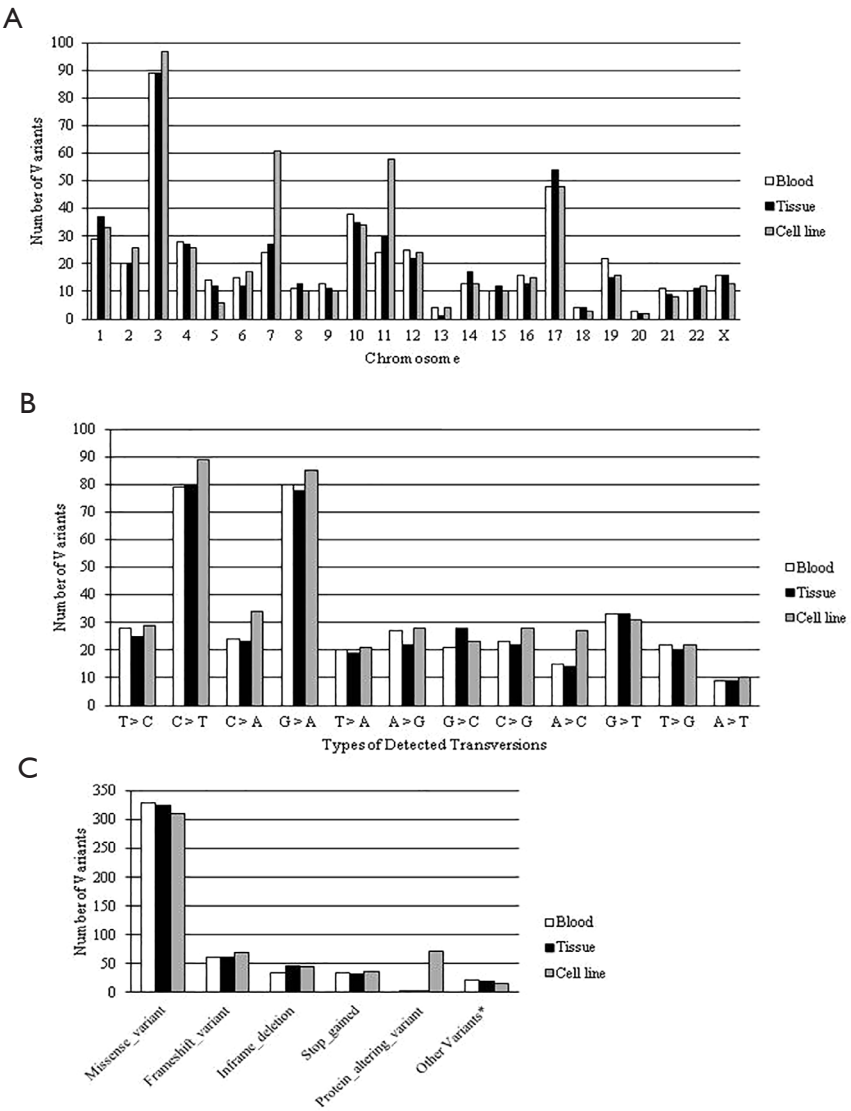

Figure S2 Basic characteristics of the COSMIC variants detected in each of the blood, tissue and cell line. (A) Number of variants detected in each chromosome. (B) Types of detected mutations in the exomic regions. (C) Number of all detected variant types based on their consequence. All selected variants had PolyPhen-damaging consequences, according to BaseSpace or PolyPhen-2 Wiki. *, XXXXX. COSMIC, catalogue of somatic mutations in cancer.

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