DECIPHERING TUMOUR TISSUE ORGANIZATION BY 3D ELECTRON MICROSCOPY AND MACHINE LEARNING

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INTRODUCTION AND AIM

Despite recent progress in the characterization of tumour components, the tri-dimensional (3D) organization of this pathological tissue and the parameters determining its internal architecture remain elusive. Here, we analysed the spatial organization of patient-derived xenograft tissues generated from **hepatoblastoma**, the most frequent childhood liver tumour, by serial block-face scanning electron microscopy using an integrated workflow combining 3D imaging, manual and machine learning-based semi-automatic segmentations, mathematics and infographics.

RESULTS

By digitally reconstituting an entire hepatoblastoma sample with a blood capillary, a bile canaliculus-like structure, hundreds of tumour cells and their main organelles (e.g. cytoplasm, nucleus, mitochondria), we report unique 3D ultrastructural data about the organization of tumoral tissue. We found that the size of hepatoblastoma cells correlates with the size of their nucleus, cytoplasm and mitochondrial mass. We also discovered that the blood capillary controls the planar alignment and size of tumour cells in their 3D milieu. Finally, a set of tumour cells polarized in the direction of a hot spot corresponding to a bile canaliculus-like structure.

FIGURES

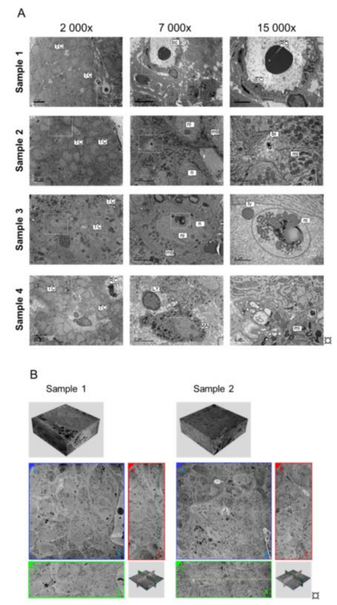


Figure 1: Analysis of HB-PDX by EM. *(A) Analysis of four HB-PDX by TEM at different magnifications. White dotted-line frame: ROI corresponding to immediately higher magnification. bc, blood capillary; bi, bile canaliculus-like structure; EC, endothelial cells; MA, macrophage; lv, lipid vesicles; mb; plasma membrane; mi, mitochondrion; n, nucleus; ni, nuclear invagination; nl, nucleolus; LY, lymphocyte; RC, red cells; TC, tumour cell. (B) Top panels: SBF-SEM stacks from two samples. Bottom panels: Extracted images of orthogonal plane from different faces of reconstructed volume (XY, YZ, XZ), whose positions are displayed on orthoslice view positions inside stack.*

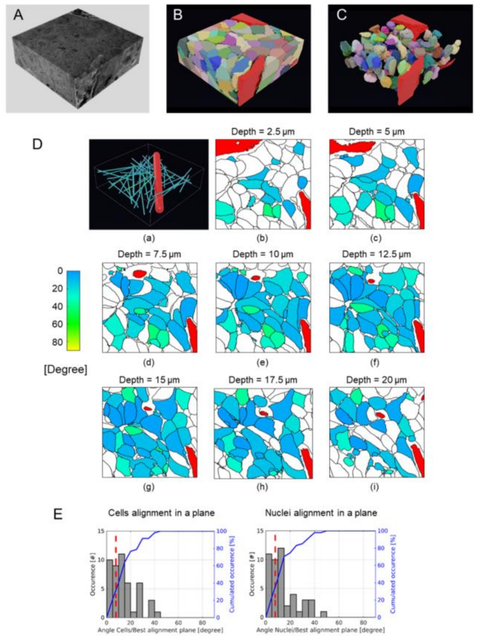


Figure 2. Alignment of HB cells and nuclei. (A) Sample 2 stack. *(B-C) Digital representation of A with 182 cells (B) and 113 nuclei (C). (D) Alignment of cells and blood capillary in a plane: (a) coloured sticks = main axis of tumour cells; red stick = main axis of blood capillary. (b-i) Angles between each main cell axis and the best alignment plane. 2D cross-sectional maps for increasing depth along Z-axis. (B-D) Blood capillary portions are in red. (E) Histograms of cells (left panel) and nuclei (right panel) alignment angles. Red dashed line: alignment angle of blood capillary.*

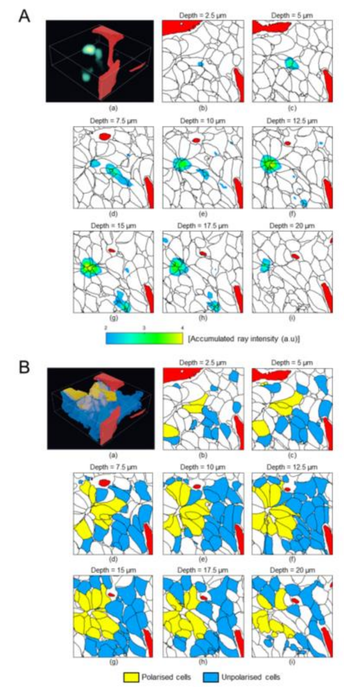


Figure 3. Tumour cell cluster with polarized shape orientation. *(A-B) Only tumour cells with a complete nucleus are considered here. Blood capillary portions are in red. (A) Accumulation map of virtual rays [Accumulated ray intensity [a.u]; voxel-by-voxel basis; see yellowish voxels] emitted by each cell along its main axis. (b-i) 2D cross-sectional maps for increasing depth along the Z-axis. (B) Binary classification of polarised/unpolarised cells: 17 cells (in yellow) emitted virtual rays reaching the main accumulation region observable in panel A-(g) (accumulated ray intensity >3.5). a.u., arbitrary unit.*

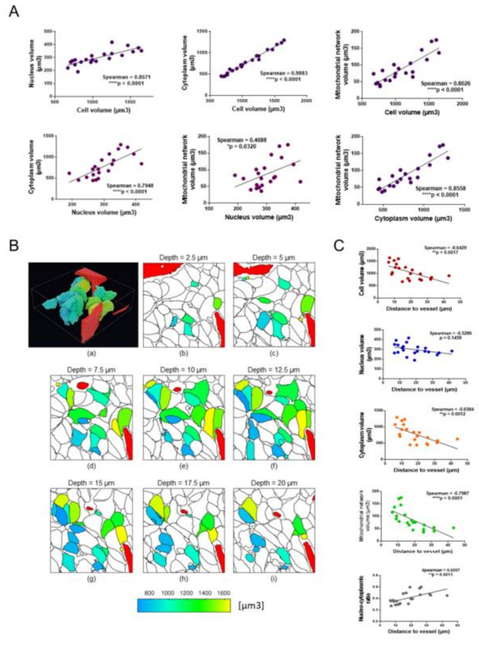
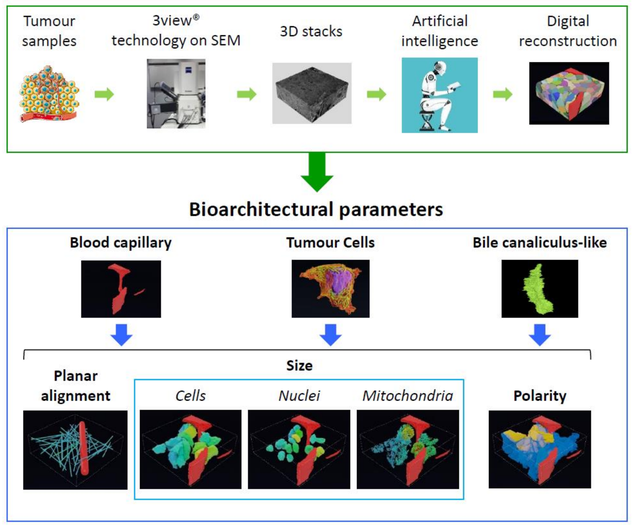


Figure 4. 3D organization of typical cellular and subcellular structures. *Only 21 entirely contained cells are considered here. (A) Volumetric correlations between cytoplasm, nucleus and mitochondrial network. (B) Cell sizes related to distance from blood capillary: (a) Reconstructed 3D image of cells and blood capillary. Cell colour is related to its volumetric size. (b-i) 2D cross-sectional maps for increasing depth along the Z-axis. (a-i) Blood capillary portions are in red. (C) Correlation between distance to blood capillary and cellular/subcellular structures or nucleocytoplasmic ratio. (A and C) Spearman correlations. r and p values are as indicated in corresponding graph.*

TECHNICAL WORKFLOW



CONCLUSIONS

This pilot study allowed the identification of bioarchitectural parameters that shape the internal and spatial organization of tumours, thus paving the way for new investigations in an emerging field called **onconanotomy**. The paper is available as a pre-print on *BioRXiv* website: https://www.biorxiv.org/content/10.1101/2021.06.15.446847v1

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