Electron microscopic (EM) characterization of mice in situ and tissue organoids models of YAP1 and ZFTA1 fusion-associated ependymomas generated via mosaic analysis by dual recombinase-mediated cassette exchange (MADR) technology.

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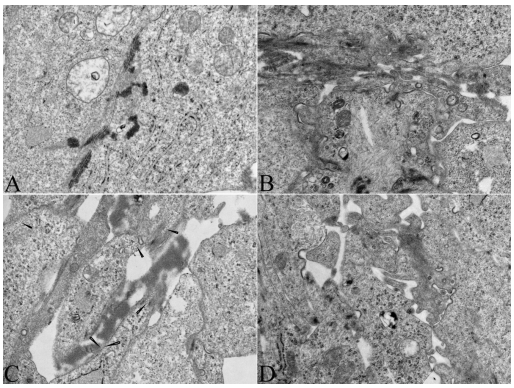
Abstract

In mice and tissue organoid modeling of glioma helps to decipher key drivers in tumor formation and allows a screen for potential targeted therapy. With the use of MADR technology we introduced cassettes containing TetOff transactivator (tTA2) and a response plasmids, of either tet-ZFTA::RELA, or tet-YAP1::MAMLD1 into the brains of newborn mice. Two months after transfection the tumors were analyzed by histology, sc-RNAseq and TEM. The TEM findings were compared to one seen in human ZFTA::RELA fusion positive ependymomas. No molecularly characterized YAP1-fused human tumors were available for EM. Both brain models showed formation of well circumscribed GFAP+ masses. EM of ZFTA::RELA mutant showed rudimentary ependymal features with presence of perivascular basal membrane, adhesive junctions, lumen studded with microvilli and cilia. Additionally, YAP1::MAMLD1 only showed striking proliferation of clathrin coated plaques and pits and also caveolae, structures, whose formation depends on activation of YAP1 and which is integral in Hippo Pathway. Similar findings were also identified by a use of in vitro organoids. sc-RNAseq data confirmed strong over-expression of components of clathrin coat, as well caveolin 1 & 2, suggesting that either of these proteins can be used as a surrogate marker for supratentorial ependymoma with YAP1-fusion.



Representative EM images of YAP1::MAMLD1 mice model. Focally developed junctional complexes (A), conspicuous caveolae (arrowheads) (C). Luminal surfaces containing no apparent cilia, but many microvilli and very prominent clathrin patches, many pits and occasional vesicles (B, D).