Abstract

Plexiform neurofibromas (PNs) are present at birth in 25-50% of children with NF1. They are typically highly vascular, involve multiple, large nerve segments and can only be incompletely resected. Our goal is to provide the first in vitro organotypic models of PNs to allow for identification of potential therapeutic targets.

PNs are initiated by aberrant activity of the NF1-containing Schwann cell (SC) followed by an influx of nearby cells associated with nerve tissue including fibroblasts. Observations in NF1 mouse neurofibroma models and observed generally in tumor formation in multiple tissue types have demonstrated increased fibroblast infiltration/proliferation, fibroblast production of Type I collagen and soluble protein secretion affecting the phenotype of neighboring cells. To define the effects of changes in microenvironment on tumor progression imposed by SCs, fibroblasts and their potential interaction we have used a live-cell proteolysis assay that reports matrix degradation by 3D cultures. The results show a redistribution of cells from a random spread across the matrix (monoculture) to a centralized aggregate of NF1-SC fibroblasts surrounded by NF1-SCs (co-culture). This complex multicellular structure generated a 9 to 11-fold increase in proteolysis compared to the proteolysis of the same cells as monocultures grown in parallel. These combined results suggest a strong interaction between the NF1 fibroblasts and the NF1 Schwann cells that may recapitulate the in vivo processes of tumor development. Interactions such as this are missing in traditional 2D monotypic culture on plastic dishes. Robust 3D co-culture models will allow preclinical drug screening that is much more representative of likely translational effectiveness than can be achieved by testing in 2D monolayers.

Many drugs that successfully block proliferation of tumor cells preclinically are a failure under clinical conditions. This can be attributed in part to the limitations of 2D culture. To determine the differential effects of chemotherapeutic and potential targeted agents on 2D and 3D cultures we tested a series of drugs using a monoculture of wildtype or NF1-SCs. This format allows the attribution of differences between the 2 culture types to be due solely to the presence of a matrix. For the four drugs shown, the presence of RbM lessens the sensitivity of the cells to the action of the drug. This effect is drug and cell line dependent. Subsequent testing with comparisons between 3D mono and co-culture systems with a focus on either proliferation, matrix degradation or survival will give additional essential information. It is the parsing out of the drug response with regard to several tumor phenotypes and the presence of an RbM that mimics in vivo conditions that promises to make the 3D model an indispensable pre-clinical tool.

Cell interaction in 3D culture

Drug response: 2D culture vs. 3D culture

Fig. 1 Schematic for organotypic models of plexiform neurofibroma

Fig. 2 Increased proteolysis and cell redistribution. NF1 SCs (95.11b C-RFP) and NF1 primary fibroblasts were grown for 2 days in RbM supplemented with a proteolytic substrate conjugated with fluorescein. SCs were visualized with RFP and fibroblasts with far-red cell trace, total cell number with Hoescht nuclear dye. MetaXpress 3D analysis software package was used to identify proteolysis degradation product on a cell-by-cell basis, and classified based on cell type (SCs or fibroblasts). (A) SCs or (B) fibroblasts in monoculture where blue-nuclear stain, pink-cytoplasmic stain, green-proteolysis; (C) SC, blue and fibroblasts, pink in co-culture; (D) Total proteolysis/green; (E) Total fluorescence intensity was measured in 3D for each cell type under monoculture and co-culture conditions, with the average intensity quantified along the y axis.

Fig. 3 The presence of tumor RbM in culture decreases drug sensitivity. Cells were plated at 1500 (2D) or 4000 (3D) cells per well of a 96-well plate. Twenty four hrs later the drug was added at concentrations indicated. Two days after drug addition the cell number was quantified using a luciferase-based assay (Promega). DMSO, the drug vehicle, is the negative control. Doxorubicin was used as a positive control. The cell lines tested were pNp02.3 RFP (NF1 wild-type SC line); pNpNF5.11b C-RFP and pNpNF5.6.21 RFP (NF1 null SC lines). These cell lines have been stably transduced (lentiviral) with tdTomato, a red fluorescent protein. Sapitinib is a reversible, ATP competitive inhibitor of EGFR, ErbB1 and ErbB2. BI-2536, Ispinesib, and colchicine are chemotherapeutic drugs that target specific proteins (polo-like kinase 1, kinesin spindle protein and mitotusubules, respectively) essential to mitosis thereby halting cell cycle progression.