Executive summary
Tumours do not grow in isolation, but are supported by normal cells in the body, sometimes called the ‘tumour microenvironment’. The findings highlighted in this article describe a mouse model in which the brain microenvironment (neurons and astrocytes) can artificially stimulate the growth of implanted human glioblastoma cells. A specific protein, neuroligin-3 (NLGN3) was shown to be responsible for this growth, and the investigators identified a way in which its production can be stopped using a small molecule drug. Although there are technical reasons why this specific type of drug may not be clinically useful, the target NLGN3 itself is of great interest and may offer an effective way to treat glioblastoma and other brain cancers in the future.

Background
Most attention in cancer research has understandably been focussed on the transformed cells that form the tumour mass and subsequent metastases. However, it is also appreciated that the tumour microenvironment is of critical importance in supporting tumour growth through crosstalk via signalling molecules. Aggressive brain cancers, such as glioblastoma (GBM), are no exception to this, as highlighted by work showing how neuronal activity stimulates the growth of two cell types thought to give rise to glioma, namely oligodendroglial precursor cells and earlier neural precursor cells. A subsequent paper by Venkatesh et al in 2015 took this one step further and identified the synaptic adhesion molecule neuroligin-3 (NLGN3) as a direct stimulator of glioma proliferation through the PI3K–mTOR signalling pathway. This Window on Glioblastoma article focusses on a subsequent (2017) paper by the same group in which they inhibit the secretion of NLGN3 from neuronal cells thereby preventing glioma cell growth in vivo. This inhibition was achieved using inhibitors of the ADAM10 sheddase enzyme, both of which are in clinical development for other cancers and therefore may also be of use in GBM treatment.

Investigating neuron-glioma interactions using optogenetics
The stimulatory effects of neuronal activity of glioma growth and the involvement of NLGN3 were discovered using mice engineered to express the excitatory opsin channelrhodopsin-2 (ChR2) in deep cortical projection neurons. Light at 473nm can be used to stimulate action potentials in vivo in only those cells expressing the ChR2 ion channel. Venkatesh et al crossed these animals with immunodeficient mice to produce a system for testing human xenografts. They found that light-stimulated neurons enhanced the growth of implanted human glioblastoma cells. This effect was due to the action of a soluble factor, since cortical slices stimulated by light in vitro released a growth promoting molecule into the conditioned medium.

Neuroligin-3
The secreted factor derived from light-activated neurons was identified as the secreted ectodomain of the protein neuroligin-3 (NLGN3), using two-dimensional gel electrophoresis and mass spectrometry of candidate proteins. The neuroligins are a family of synaptic proteins that are ligands for the neurexin family, the latter being first identified as targets for the black widow spider venom latrotoxin. The role of secreted neuroligin in GBM growth was further validated using recombinant NLGN3 protein in vitro. The genes induced in GBM cells by NLGN3 were identified by transcriptomics and were consistent with activation of the PI3K–mTOR signaling pathway to trigger cell proliferation. Interestingly, NLGN3 also stimulates its own production in a ‘feed forward’ loop also involving the PI3K–mTOR pathway. Thus, soluble NLGN3 appears to be acting in both a paracrine and autocrine way to promote GBM growth.

In the 2017 paper, Nlgn3 knockout mice were used to explore the action of NLGN3 on tumour growth in vivo, demonstrating the complete dependency on the growth of the initial GBM implants on this molecule. This strong dependency led to the authors to speculate that the PI3K–mTOR signaling pathway was only one of several pathways activated in GBM cells.

Analyzing the phosphoproteome with phospho-antibody arrays after NLGN3 exposure revealed focal adhesion kinase (FAK) phosphorylation and numerous downstream phosphorylation events dependent on FAK activation. These include activation of the SRC kinase, PI3K–mTOR and SHC-RAS-RAF-MEK-ERK pathways.

In summary, the findings described in this article demonstrate that NLGN3 secreted from neurons in the brain microenvironment may provide a promising target to block glioma growth. Further work to explore this therapeutic option is currently ongoing.
Therapeutic potential of NLGN3 inhibition

The data presented in 3 and 5 and summarised above, provide evidence that inhibitors of neuroligin-3 production or action could be used to treat malignant brain diseases such as glioblastoma. This evidence includes an analysis of paediatric glioblastoma samples from the Cancer Genome Atlas where expression levels of NLGN3 is negatively correlated with patient overall survival.3 Neurons and oligodendrocyte precursor cells (OPCs) both express NLGN3, but the latter were shown to be the predominant in vivo source by selectively deleting the gene in each cell type using the Cre-Lox system in transgenic mice.5

Inhibition of NLGN3 secretion

The NLGN3 protein consists of a large N-terminal ectodomain, single pass transmembrane domain, and smaller C-terminal cytoplasmic domain. The presence of potential protease cleavage sites within the C-terminal transmembrane region was determined using the PROSPER database.69 Two candidate metalloproteinases were identified, MMP9 and ADAM10, with ADAM10 being responsible for cleaving membrane-associated NLGN3 in neurons and oligodendrocyte precursor cells (demonstrated using transgenic mice deficient in either enzyme). Fortunately, ADAM10 inhibitors such as GI254023X have been under investigation for oncology (and other) targets10, so Venkatesh et al could use them to show penetration and GBM growth arrest in xenografts.5 Other ADAM10 inhibitors, XL-784 (Exelixis) and INCB7839 (Incyte), have been shown to be safe and well tolerated in early stage clinical trials11 so these compounds were used in the glioma study.5 Brain penetration of INCB7839 was superior to XL-784, inhibited ADAM10 enzymatic function, and robustly inhibited the growth of glioma cells derived from adult and paediatric patients.

Concluding remarks

Through a series of elegant in vivo optogenetic experiments in mouse brains (summarised in the Figure), Venkatesh et al have shown that neuronal activity can stimulate the growth of human glioma cells in xenografts, and that the molecule responsible is neuroligin-3.34 The enzyme ADAM10 was shown to be a druggable target for the inhibition of NLGN3 production and therefore of potential use as a therapeutic agent for glioblastoma.

The findings are clearly interesting, but there are caveats, not least over the consequences of inhibiting NLGN3 production in patients. For example, the neuroligin family (NLGN1-3) is strongly implicated in astrocyte function and synaptogenesis12 and NLGN3 mutations have been implicated in autism.13 Even assuming that NLGN3 inhibition is not problematical in a clinical setting, how it is to be inhibited warrants further investigation. The druggable target ADAM10 is superficially attractive, but the field of metalloprotease inhibition has been anything but straightforward in drug development. Furthermore, the inhibitors XL-784 and INCB7839 (Aderbasib) have both failed to meet their endpoints in Phase II clinical trials and have been dropped by their sponsors14,15 so it is unlikely that their performance will be much better in treating glioblastoma. Despite these considerations, NLGN3 does appear to be a potential glioblastoma target, perhaps better approached using an alternative strategy to inhibiting ectodomain shedding.

Finally, Venkatesh et al describe the growth inhibition of GBM xenografts in NLGN3 knockout mice and include a single sentence without further comment: “By 4.5 months, a subset of tumours circumvented this apparent NLGN3 dependency and began to exhibit growth”.6 Whether this outgrowth could occur in patients where NLGN3 has been inhibited pharmacologically remains to be seen; however, it is yet another sign that effective cancer treatment with drugs will most likely be achieved through combination therapies rather than single targeted agents.

References

15) http://www.wikiwand.com/en/Aderbasib