Aggravating the aggressor

DigitalStock

The most common type of brain tumour is glioblastoma multiforme (GBM), which often develops resistance to its standard chemotherapy (temozolomide). It is thought that tumour cells with stem cell-like properties confer resistance to chemotherapy and radiotherapy and that this can be accompanied by the overexpression of ATP-binding cassette (ABC) transporters and an increased capacity to repair damaged DNA. Eric Holland and colleagues now provide evidence linking stem cell-like tumour cells (SCLCs) to GBM and its resistance to chemotherapy.

Cells with stem cell-like properties are able to efflux the dye Hoechst 33342, an effect mediated in part by ABCG2, and appear as a side population when analysed by fluorescence-activated cell sorting. Using transgenic mice that develop platelet-derived growth factor (PDGF)-inducible gliomas, Bleau and colleagues found that the side

population was increased to 6–8% compared with 0.5–0.8% in normal adult brain tissue. In addition, they found that the expression of the endothelial marker CD31 (also known as *PECAM1*) and putative SCLC marker CD133 (also known as *PROM1*) was not limited to this fraction. To assess the stem cell-like characteristics of the side population, the authors cultured side population cells in neurosphere medium and found that more neurospheres were formed compared with tumour cells from the main population. Analysis of the glioma neurospheres showed that cells expressed stem cell markers, such as *nestin*, OCT4 (also known as *POU5F1*) and *SOX2*, and the side population increased to 10–20% of the total. Moreover, cells from side population neurospheres exhibited self-renewal properties and were also more resistant to mitoxantrone, a drug that is exported by ABCG2. Inhibition of ABCG2 reduced the number of cells in the side population. Together, these data indicate that the side populations of gliomas contain SCLCs that are more resistant to therapy.

PTEN expression is reduced and Akt is activated in advanced GBM so the authors investigated the role of this pathway in PDGF-induced gliomas in *PTEN*-deficient mice. They observed an ~2-fold increase in the side population, which was accompanied by increased Akt phosphorylation and resistance to mitoxantrone. This was reversed when neurospheres were cultured in the presence of a PI3K inhibitor (LY294002). Moreover, when they intercranially injected PDGF-induced *PTEN*-deficient side population glioma cells into newborn pups, the authors found that loss of

PTEN reduced the latency of tumour development. Next, the authors treated mice with increasing doses of temozolomide for 2 weeks and found that the side population increased 1.5-fold and increased 2.2-fold when *PTEN* was lost. In addition, neurospheres of gliomas treated with temozolomide were more tumorigenic, as shown by intercranial injection into newborn pups. Therefore, temozolomide appears to enrich the side population. Interestingly, they showed that ABCG2 activity was increased by the PI3K–Akt pathway but this did not confer temozolomide resistance; instead, expression of the DNA repair enzyme O⁶-methylguanine DNA methyltransferase (*MGMT*) was responsible. To assess the relevance of the side population to human disease, the authors produced neurospheres from four human GBM samples. Confirming the observations from mice, they found that the side population was eliminated by treatment with an ABCG2 inhibitor or LY294002. Stereotactic injection of the human GBM side population cells after neurosphere culture into the brains of nude mice decreased the latency of tumour development.

Therefore, these data indicate that the side population contains GBM SCLCs and — consistent with medulloblastoma and breast cancer — increased activity of Akt appears to be associated with the acquisition of stem cell-like properties and therapeutic resistance.

Gemma K. Alderton

ORIGINAL RESEARCH PAPER Bleau, A.-M. et al. *PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. Cell Stem Cell* 4, 226–235 (2009)