mitochondrial complex I. However, F1,6BP also activates Ras and its downstream targets ERK and MEK [5]. In turn, small GTPases of the Ras family directly bind to the catalytic subunit p110α of PI3K, activating PI3K-AKT signaling [6]. As a result, a vicious cycle is created between F1,6P and major oncogenic drivers (Ras and PI3K-AKT) acting with HIF-1α to promote the Warburg effect [7]. Moreover, PFKFB and dimeric PKM2 translocate into nucleus, where F2,6BP represses p27Kip1, a strong inhibitor of cell cycle progression [2], while dimeric PKM2 promotes c-Myc expression, favoring the Warburg effect and cell cycle progression [8].

Unlike regulation occurring in normal cells, where citrate closely adjusts anabolic and catabolic flows according to nutrient availability and ATP production, the Warburg effect reduces the mitochondrial synthesis of citrate, while the continuous consumption of the molecule in fatty acid synthesis (FAS) reduces its cytosolic level. Therefore, the negative and positive feedbacks of citrate on PFK1-2 and FBPase, respectively are avoided [9]. To sustain their continuous need for acetyl-CoA supplying lipid synthesis and/or protein acetylation, proliferative cancer cells can form citrate by alternative pathways, such as direct carboxylation of α-ketoglutarate (α-KG) (a molecule derived from glutamate), or directly use acetate as a substrate for acetyl-CoA synthesis [9]. Consequently, the inhibition of ATP citrate lyase (ACLY), the cytosolic enzyme transforming citrate into acetyl-CoA and oxaloacetate (OAA), leads to an increased level of citrate and results in the inhibition of mitogen-activated protein kinase (MAPK) and IGF-1R/PI3K/AKT proliferative pathways [10]. *In vitro* studies [11,12] showed that high-dose citrate: (i) inhibits PFK and decreases ATP production; (ii) inhibits the growth of numerous cultured cancer cell lines; (iii) promotes apoptosis with the activation of various caspases and extinction of expression of the antiapoptotic factor Mcl-1; (iv) reverses dedifferentiation (in particular through Snail inhibition and E-cadherin expression); and (v) increases sensitivity to cisplatin. Furthermore, experiments involving tumor-bearing mice (Ras-driven lung and Her2/Neu mammary cancers) showed that citrate inhibited tumor growth and additional benefit was achieved when combined with cisplatin [12]. For all these arguments, citrate should be considered as a tool to better understand and disrupt metabolism sustaining cancer cell proliferation, in particular truncated gluconeogenesis.

Author Contributions

P.I. wrote the letter, Z.W. prepared the figure, and L.F. and M.A. contributed to the literature review and comments and editing of the letter.

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Spotlight

Solid Stress in Brain Tumors

Daniele M. Gilkes[1,2,*] and Denis Wirtz[1,2]

A solid brain tumor mass places compressive forces on adjacent normal brain tissue, and clinically presents as impaired motor performance in cancer patients, ultimately limiting their quality of life. In a recent article by Jain and colleagues (*Nat. Biomed. Eng*. 2019;3:230–245), the biological consequences of mechanical forces imparted by a growing tumor mass are explored in both mouse models and human brain tumors, revealing a novel opportunity for therapeutic intervention.

The role of biomechanics in cancer has drawn widespread attention [1]. Studies with a focus on tissue stiffness have considered breast, prostate, liver, and pancreatic cancer whose stiffness is altered by aberrant extracellular matrix deposition

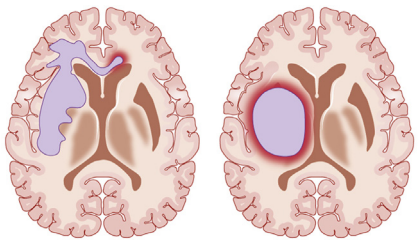

Figure 1. Nodular (Right) Brain Tumors Exert Approximately Twofold Greater Circumferential and Radial Solid Stress on Surrounding Normal Tissue than Do Infiltrative (Left) Brain Tumors. This leads to increased neuronal damage which correlates with motor impairment in patients with primary and metastatic brain tumors.

and remodeling [2,3]. Less is known about the biomechanics of brain tissue compression that occurs as the result of a growing tumor mass. The so-called 'mass effect' has prognostic significance for glioblastoma (GBM) [4], the most common form of adult brain cancer. Brain tumors compress, or even destroy, surrounding normal tissue and increase intracranial pressure, resulting in headaches, seizures, speech, and/or vision impairments in patients.

In the most recent edition of Nature Biomedical Engineering, Rakesh Jain and colleagues report measurements and neurological consequences of tumor-induced brain deformation or the 'pushing' of healthy brain matter by a burgeoning tumor [5]. Using two mouse models, one that reflects nodular tumor growth (growth as a single, well-defined mass) and one that mimics infiltrative tumor growth, the researchers demonstrate that nodular tumors have higher gradients of compression and tension that are capable of locally deforming tumor-adjacent healthy brain tissue, whereas infiltrative brain tumors impart lower forces. Consistent with these findings, magnetic resonance imaging (MRI) of tumors from 64 GBM patients reveals that patients with nodular tumors display more functional impairment, as measured by Karnofsky performance scores (KPS), compared with those patients with infiltrative tumors. It would also be interesting to measure the lateral ventricle displacement (LVd) volume in the same cohort of patients using methods reported by Chen and colleagues [6] to determine if LVd also differs in nodular versus infiltrative type brain tumors. It is interesting that the study by Chen and coworkers showed that GBMs with high LVds are associated with increased expression of genes related to increased cellular proliferation, whereas tumors with low levels of LVd expressed genes involved in cell migration. It is tempting to speculate from these results that the gene expression patterns also correlate with nodular versus infiltrative tumor types (Figure 1).

To determine how mechanical stresses generated in the local tumor microenvironment by nodular tumors may cause performance reduction, mice bearing GBM tumors and mice bearing brain tumors derived from metastatic breast cancer cells were assessed for vascular perfusion ability. Using optical coherence tomography in mice outfitted with transparent cranial windows, Jain and colleagues found that nodular tumors impair vascular perfusion, which occurs concomitantly with compressed neuronal nuclei leading to neuronal loss. To test whether these findings were recapitulated in patients with brain tumors, the perfusion levels of normal tissues adjacent to tumors were also measured. Fifty-three percent of patients displayed reduced perfusion in their surrounding brain tissue. Notably, patients with reduced perfusion had significantly worse KPS scores. A cohort of 34 breast cancer patients with brain metastasis that were nodular in shape had reduced vascular perfusion that correlated with impaired performance levels, suggesting that not only primary but also metastatic brain tumors suffer from mechanical compression forces.

To decouple potential biological interactions of the tumor microenvironment with healthy tissue from the direct impact of mechanical stimulus, the group devised a strategy to compress the brains of non-tumor-bearing mice. The rate of compression used in the experimental set-up modeled the rate of nodular tumor growth measured in the mouse model. The compression force gradually deformed the cortex, mimicking the effect of a growing nodular tumor in causing reduced vascular perfusion and density. Ultrastructural analysis of the altered cortex revealed signs of cell distress, with reduced neuronal nuclei size. To assess performance following compression, the mice were subjected to Rotarod and gait tests which showed reduced motor coordination and movement.

To model the changes that occur following tumor resection, the brains from mice bearing nodular tumors with cranial windows were decompressed by removal of the cranial window. Removal of the window led to an increase in perfusion of tumor-adjacent tissue. Likewise, non-tumor-bearing mice whose brains were decompressed by removing the solid stress had increased vascular perfusion and, over time, displayed an increase in neuronal cell numbers together with improved, albeit temporary, motor coordination. This is in keeping with post-surgery performance results from GBM patients.

Taken together, the data suggest that treatment regimens that can reverse or protect from mechanically induced neuronal cell loss would be beneficial for patients with brain tumors. To this end, Jain and colleagues used their mouse compression device to screen compounds for neuroprotective effects against solid stress. They assessed drugs that (i) were capable of crossing the blood–brain barrier, and (ii) had previously been shown to provide neuroprotection in ischemia [7,8], a condition

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in which blood perfusion is also compromised. Treatment with lithium, but not necrostatin-1, valproic acid, or dexamethasone, reduced neuronal death and protected from cortical tissue damage, leading to improved motor coordination during compression. To identify a potential mechanism for the protective properties of lithium, RNA sequencing and gene set enrichment analysis were used to compare gene expression profiles from the cortex of lithium-treated and control mice. Gene expression in lithium-treated cortex was enriched for pathways associated with ion channels, mitochondrial function, and neuronal differentiation, as well as protection from apoptosis, autophagy, or ischemia – pathways that are indicative of protection from neuronal damage. Future studies are warranted to determine if lithium treatment will be efficacious for patients with brain tumors. Studies focused on enhancing vascular perfusion in the brain may also prove useful.

Brain tumors not only have a poor prognosis but also lead to a reduced quality of life before morbidity. Neuro-oncologists are increasingly aware that preserving neurological function is important for improving the quality of life of their patients [9]. The current study shows that patients with nodular tumors may benefit from neuroprotective therapies, such as lithium, to spare normal brain tissue. This would be particularly beneficial for patients with advanced or inoperable tumors who will be unable to gain relief from a surgical intervention. Given that the brain has been shown to have heterogeneous stiffness profiles that are dependent on spatial localization [10], it is also likely that compression of different areas of the brain may result in differing degrees of mechanical and neurological response, and this would need to be considered before deciding on an individualized treatment regimen. Moreover, the resulting neurological effect will likely also be dependent on the function of the specific area of the brain that is under compression. Overall, the study offers promising hope to improve the quality of life of patients with nodular-type brain tumors.

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Forum

DUX4 Pathological Expression: Causes and Consequences in Cancer

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DUX4, a double homeobox transcription factor, has been mostly studied in facioscapulohumeral dystrophy (FSHD), a pathology linked to a deletion of subtelomeric repeats on chromosome 4q. More recently, however, the gene has been associated with various sarcomas and haematological malignancies. Drugs developed for FSHD could be tested on cancer cells to develop efficient treatment strategies for both pathologies.

DUX4 is a double homeobox transcription factor encoded within the D4Z4 subtelomeric repeat element on chromosome 4q. Recently, *DUX4* rearrangements were reported in a frequent paediatric subtype of B cell precursor acute lymphoblastic leukaemia (BCP-ALL) (reviewed in [1]), in Ewing-like sarcoma [2] and rhabdomyosarcoma (RMS) [3]. Previously, aberrant expression of *DUX4* was identified as a major factor in the aetiology of facioscapulohumeral dystrophy (FSHD), an autosomal dominant disorder. Below, we discuss features and consequences of *DUX4* gene rearrangements in malignancies and new therapeutic approaches in the context of FSHD that might prove useful for cancer treatment.

DUX4 Expression and Gene Rearrangements

In humans, an aberrantly expressed *DUX4* has been observed in numerous malignancies, including renal, breast, and testicular cancers (source: Human