The British Journal of Radiology, 84 (2011), S159–S167

Imaging biomarkers of brain tumour margin and tumour invasion

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ABSTRACT. Invasion of tumour cells into the normal brain is one of the major reasons of treatment failure for gliomas. Although there is a good understanding of the molecular and cellular processes that occur during this invasion, it is not possible to detect the extent of the tumour with conventional imaging. However, there is an understanding that the degree of invasion differs with individual tumours, and yet they are all treated the same. Newer imaging techniques that probe the pathological changes within tumours may be suitable biomarkers for invasion. Imaging methods are now available that can detect subtle changes in white matter organisation (diffusion tensor imaging), tumour metabolism and cellular proliferation (using MR spectroscopy and positron emission tomography) occurring in regions of tumour that cannot be detected by conventional imaging. The role of such biomarkers of invasion should allow better delineation of tumour margins, which should improve treatment planning (especially surgery and radiotherapy) and provide information on the invasiveness of an individual tumour to help select the most appropriate therapy and help stratify patients for clinical trials.

DOI: 10.1259/bjr/26838774

Recent studies combining maximal resection, chemotherapy and radiotherapy have at last provided significant improvements in survival for patients with high-grade gliomas [1]. Despite these advances, most patients will still die from progressive disease. One of the major factors for treatment failure is the invasion of glioma cells into normal brain, a key feature of gliomas. These infiltrating tumour cells mean that surgical resection is rarely curative; even attempts at removing entire hemispheres have failed to halt tumour progression [2]. Radiation oncologists add a 2 cm margin to the apparent tumour to produce a clinical target volume (CTV) that encompasses these infiltrating cells. As this volume includes normal brain tissue that is sensitive to radiation injury, the total dose has to be reduced to within the tolerance limits of the normal brain [3]. This dose is insufficient to sterilise tumour cells, resulting in most tumours recurring within the high-dose treatment volume [4, 5]. In addition, these infiltrating cells are predominantly migrating and not proliferating [6], so treatments that disrupt dividing cells (especially radiotherapy and chemotherapy) will have less effect on these cells.

Although tumour invasion is a key feature of gliomas, the degree of invasion is variable. Post-mortem studies show that between 20% and 27% of glioblastomas have limited invasion (*i.e.* infiltrating cells less than 1 cm from the edge of the gross tumour) [7, 8]; 20% have more extensive invasion (*i.e.* invasion of more than 3 cm from the gross tumour) [8] with 8% showing disseminated spread [9]. It is clear that these groups should be treated differently and raises the question of whether these tumours should be considered as local disease (requiring aggressive local therapy) or diffuse disease (requiring systemic therapy) [10]. At present we cannot separate glioblastomas based on their extent of invasion. As a result we must treat all of them the same, despite the fact gliomas with limited invasion are likely to respond better to local therapies than those with diffuse invasion.

Attempts to better understand the molecular differences in more infiltrative tumours have suggested a number of genes that are upregulated in these tumours [11, 12, 13]. The problem with using this approach to determine the invasiveness of an individual tumour is that it requires tissue. As a result it cannot guide surgical treatment or local therapies at the time of resection. It is also unable to demonstrate the tumour margin. Development of imaging biomarkers for the non-invasive study of tumour invasion could potentially provide this information.

Problems with conventional imaging

With the advent of improvements in brain imaging the hope was that the margin of gliomas could be accurately determined. Biopsy and post-mortem studies, have shown that gliomas extend further than could be determined using conventional imaging techniques. For CT imaging, tumour cells extend beyond the area of CT enhancement and are frequently seen in regions of peritumoural oedema [14, 15]. Tumour cells may be found in areas that appeared normal on CT in 20% of serial stereotactic biopsy specimens [16]. Tumour cells can be detected up to 6 cm from abnormal areas on CT [17].

The improved soft-tissue resolution of MR has failed to improve the identification of the tumour margin. Biopsy studies have shown that the tumour extends

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beyond the margin of T_2 signal change in most glioblastomas [17, 18], in some cases tumours extended up to 2.5 cm beyond the area of T_2 signal change. Tumours can be identified in regions with a normal T_1 signal in 16% of biopsies and have a normal T_2 signal in 4% of biopsies [16]. It is clear from these studies that tumour cell invasion extends at least as far as the abnormal T_2 signal in both high- and low-grade gliomas.

Studies have also focused on the border of the T_2 weighted abnormality. In oligodendrogliomas the sharpness of the T_2 weighted abnormality did not predict invasive behaviour, but did predict the presence of loss of heterozygosity of chromosomes 1p and 19q, which is a marker of good prognosis and response to chemotherapy [19]. In glioblastomas, tumours with a low T_2 weighted border sharpness and a high ratio of the volume of the T_2 weighted abnormality to the T_1 weighted area correlated with increased expression of epidermal growth factor receptor (EGFR) [20], a molecular marker that is known to be associated with invasive behaviour [21].

If conventional imaging fails to identify invasion, novel methods based on our understanding of the biology of glioma invasion are needed to address this problem.

Biology of glioma invasion

Much of our understanding of the process of glioma invasion has come from the careful examination of post-mortem brains by Hans Scherer in the late 1930s. He showed that individual cells disperse predominantly along white matter tracts, with some spreading along blood vessels and along the ependymal and pial lining [7, 22]. Spread along white matter tracts involves individual cells spreading within (intrafasicular), around (parafasicular) and between (interfibrillary) the axonal processes within the white matter. Little damage is caused at this stage, and the white matter tract remains intact. As the tumour develops and the number of tumour cells increases, the white matter tracts are destroyed by tumour. Scherer referred to this as neurophagic growth [22].

Glioma cell invasion of normal brain is a multistep process. One of the first stages is binding of tumours to the extracellular matrix (ECM) or other cells. Much of our understanding of this process comes from other cancer models and shows that the tumour binds via a number of receptor systems (especially integrin receptors) to matrix glycoproteins, such as fibronectin, laminin, vibronectin and collagen. In gliomas, however, these glycoproteins are only found on the basal membrane of blood vessels and the glial limitans, but are not found in white matter [23]. Although culture experiments suggest that glial tumours can secrete matrix proteins [24], the main matrix component surrounding neurons and glia are glycoaminoglycans, especially hyaluron. A number of adhesion molecules will bind to this [25].

Once tumours bind to the ECM it needs to create space to allow the cells to move in. The various proteases include the matrix metalloproteinases (MMPs) (especially MMP-2, MMP-9 and the membrane-bound MT1-MMP [26], the serine proteases (especially plasminogen activators [27]) and cysteine proteases (especially cathepsins [28]). Once there is space, the cells will migrate in to complete the process. Tumour invasion is accompanied by growth of vessels (angiogenesis) and all these processes are controlled by autocrine and paracrine communication between tumour cells, glial cells, endothelial cells and various immune cells [29].

Potential biomarkers of tumour invasion

Recent advances in imaging can now provide information that is not possible on anatomical ''conventional'' imaging. These techniques allow us to probe pathological changes within tumours. These methods provide information on cellularity (diffusion MR and MR spectroscopy), angiogenesis (perfusion MR), metabolism [various methods using positron emission tomography (PET) and MR spectroscopy] and cellular proliferation (both PET and MR spectroscopy) [30]. As all of these processes are involved in tumour invasion they can potentially be used as both direct and indirect markers of invasion.

Imaging white matter disruption

As has been previously discussed, gliomas preferentially spread along white matter tracts. This is in contrast with metastases, which tend to spread along vascular planes and form a tumour that is separate from the surrounding brain. Diffusion tensor imaging (DTI) is very sensitive in detecting disruption of white matter in regions that appear normal in a number of diseases [31–35]. Initial studies in brain tumours have shown a 30° deviation of corona radiata fibres in a patient with a low-grade glioma, suggesting that the fibres had been displaced but not infiltrated by the tumour [36]. Using directionally encoded colour maps, Mori et al [37] could differentiate tumour displacement of adjacent tracts from tumour infiltration in two patients with anaplastic astrocytomas. This method was further refined to identify four patterns of white matter involvement [38].

- *White matter disruption by the tumour*: isotropic or near isotropic diffusion so that tract is not identifiable on fractional anisotropy (FA) or directionally encoded colour maps.
- *Tumour infiltrated white matter tracts*: reduction of FA (>25%) with increased apparent diffusion coefficient (ADC) with abnormal colour hues not as a result of bulk movement.
- *Oedematous white matter tracts*: reduction of FA (>25%) with increased ADC with normal direction and location (*i.e.* colour hues) on directionally encoded maps. There is some doubt if this differs much from the infiltrated tracts described above.
- *Displacement of white matter tracts*: normal or mildly decreased (<25%) FA values compared with contralateral side, but alteration in either position or direction of fibres on directionally encoded colour maps.

Examples of these patterns are demonstrated in Figure 1. At present, these studies lack histological confirmation, but using this information for intra-operative

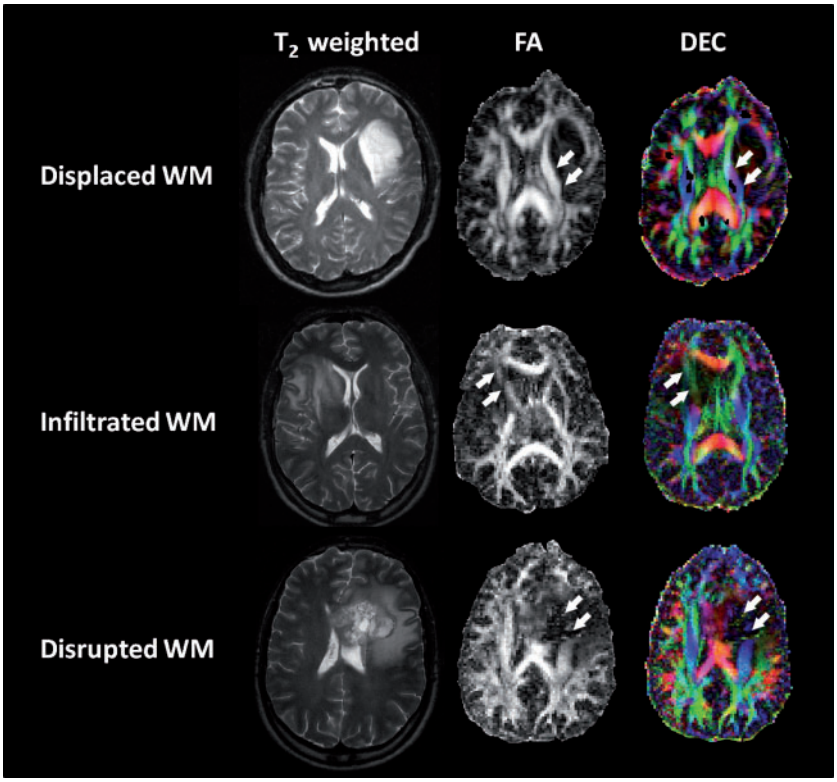

Figure 1. Example of the use of diffusion tensor imaging (DTI) to understand the effect of the tumour on white matter (WM) tracts. The upper row shows a tumour deviating a tract (arrows). In this case the fractional anisotropy (FA) values are similar to the contralateral side but the directionally encoded colour (DEC) maps shows the tract colour is different to the contralateral side. The middle row shows tumour invasion of a tract (arrows). The tract can still be seen but with reduced FA and hue on DEC. The lowest row shows tumour disruption (arrows), where no tract can be identified on either method.

planning has allowed safe resection without worsening the neurological deficit [38].

Attempts have been made to differentiate the effects of invasive gliomas on white matter tracts from non-invasive tumours (*e.g.* meningiomas and metastases). The results appear mixed, with some studies showing a larger reduction in FA in the peritumoural region [39–42] while other studies only show significant increases in mean diffusivity (*D*) [43,44]. One study found no change in FA values but did find a decrease in the magnitude of the principal eigenvalue in the peritumoural tissue of gliomas [45]. Another study described visual differences in FA surrounding gliomas compared with metastases but failed to demonstrate changes in FA values [46] (an example of this visual difference is shown in Figure 2). Their study, however, failed to measure the FA in the peritumoural tissues.

Reduction of FA is not the only finding surrounding brain tumours. Longitudinal studies in a rat C6 tumour model (a model that does not infiltrate normal brain and acts more like a metastasis [47]) show an increase in anisotropy at the tumour margin, suggesting compression of surrounding white matter tracts [48]. This has been confirmed histologically in a rat model with C6

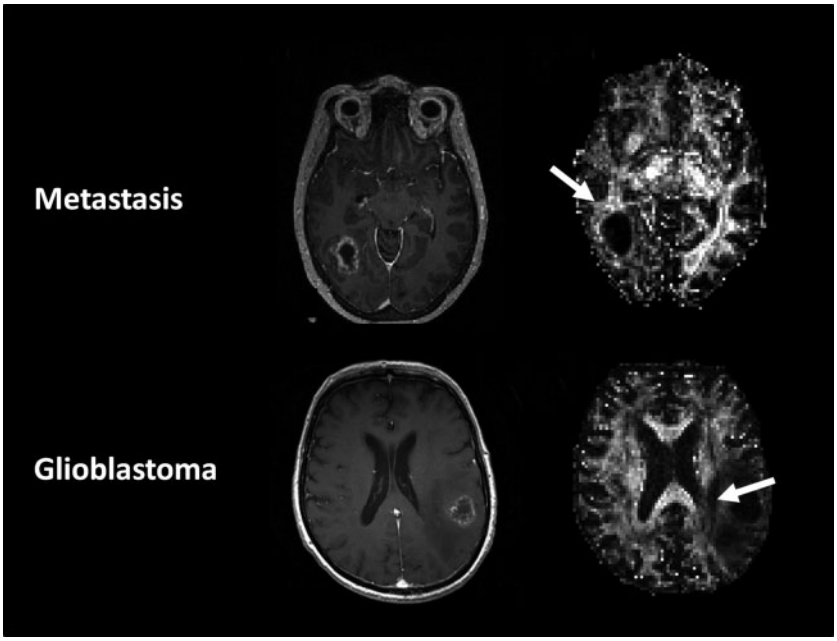

Figure 2. Visual differences in fractional anisotropy between a metastasis (upper row) and a glioblastoma. With the metastasis there is still intact white matter surrounding the tumour (arrows) whereas in the glioblastoma there is disruption of the white matter over a larger area. This difference, despite the marked oedema from both tumours, has been suggested as a result of tumour invasion.

glioma cells engrafted into the spinal cord [49]. A similar finding has been reported at the edge of some glioblastomas and meningiomas in patients [41].

As it appears FA alone is too insensitive to identify occult white matter infiltration completely, various groups have developed new ways of analysing the tensor information. Zhou and Leeds [50] described a regional fibre coherence index that is based on the fibre orientation in a cluster of voxels around the voxel being investigated. The sum of these vectors are weighted by the proximity of the voxel to the one investigated. They found that, in some areas with low FA values, there was a low coherence index (*i.e.* surrounding voxels had random orientation of white matter fibres). Follow-up imaging revealed DTI abnormalities persisting up to 3 months later. In other areas they found a high index (*i.e.* fibres had similar orientations); in these regions anisotropy returned on follow-up. They suggested that a low coherence index represents tumour infiltration whereas a high index represents oedema. This finding was not confirmed with histology.

Lu et al [51] found that mean diffusivity (D) and FA in peritumoural regions were linearly related in a cohort of patients with non-invasive tumours (*e.g.* metastases and meningiomas). For gliomas this relationship is not linear. This difference could be quantified to produce a novel parameter called a tumour infiltration index derived from the difference of the expected FA (from the linear regression model) for a given D and the observed FA. They suggested that the differences between these two groups were due to tumour cell infiltration. This technique, however, could not differentiate between high- and low-grade gliomas. Morita et al [52] studied the peritumoural region in a number of tumours using lambda chart analysis method that plots the largest eigenvalues (*i.e.* λ_1, called the longitudinal lambda, λ_L) *vs* the transverse lambda (the average of the two remaining eigenvalues, λ_2 and λ_3 is the transverse lambda, λ_T). The values for high-grade gliomas and other tumours (low-grade gliomas, metastases and meningiomas) were different. This would suggest reduced anisotropy in high-grade gliomas owing to tumour infiltration of the white matter.

The obvious question from these studies is whether the difference in diffusion around high-grade gliomas is actually as a result of tumour cell invasion. There is some evidence that this is indeed due to tumour. Follow-up studies have shown that DTI abnormalities can predict the presence of tumour [53, 54] and can even predict the pattern of recurrence [55]. Multimodal imaging studies have shown that the reduction in anisotropy correlates with reduction in *N*-acetylaspartate (NAA) concentrations as measured with MR proton spectroscopy, suggesting it is due to the reduction in neuronal integrity [56]. A number of studies have tried to correlate image-guided biopsy material with DTI measurements. Pauleit et al [57] tried to correlate these peritumoural ADC measurements with histology determined by image-guided biopsies. Although the ADC from the peritumoural tissue was higher than the tumour tissue ($1.23 \pm 0.21 \times 10^{-3}$ $mm^2 s^{-1}$ *vs* $1.11 \pm 0.3 \times 10^{-3} mm^2 s^{-1}$) it was not significant. This study pooled the ADC values from tumour and peritumoural brain from a variety of tumour grades (and only included two glioblastomas) and is likely to be confounded by the variation of ADC values with tumour grade. Another study comparing diffusion tissue signatures [58, 59] with tissue from image-guided biopsies avoided this problem by comparing the values with histology in individual patients [60]. Using this method it was possible to differentiate normal tissue from tumour or tumour-invaded brain with a sensitivity of 98% and specificity of 81%. One other study found FA values correlated better than ADC with tumour cellularity and infiltration [61].

The use of DTI to look at white matter disruption appears to be a sensitive marker of tumour margin. What is not certain is whether it tells us about invasiveness in an individual patient; however, some work suggests it might. Analysis of diffusion tissue signatures found that in 20% of the studied patients there was little DTI disruption of the surrounding white matter tracts [55]. The progression-free survival in this group of patients was markedly increased in this group, and follow-up imaging suggested they had limited invasiveness. This study was retrospective and contained a very heterogeneous group of tumours that had received a variety of treatments. Confirmation by a large prospective study is needed.

Tumour metabolism

Malignant tumours are hypermetabolic compared with the surrounding normal brain. There is an increase in glycolytic metabolism, increased protein synthesis and an increase in membrane synthesis to maintain the rapidly dividing malignant cells. There are various imaging methods that study these processes.

MR spectroscopy can differentiate between tumour and normal brain, and studies have attempted to determine tumour margins. The region of oedema surrounding a glioma has a similar spectroscopic pattern as the centre of the tumour with increased choline (Cho) peaks and reduced NAA compared with normal brain [62, 63]. An example is shown in Figure 3. This was not seen in non-invasive tumours (such as meningiomas) where the spectra in regions of oedema resembled normal brain tissue [63]. Some of these areas of oedema identified on T_2 weighted imaging have Cho/NAA ratios greater than 2, which is within the range seen with tumours [64]. In an image-guided biopsy study, Croteau et al [65] showed that the Cho/NAA and normalised Cho ratios correlated with the degree of tumour infiltration. Although spectroscopy was better than conventional MRI at defining the tumour margin, they were unable to differentiate between normal brain and mild tumour infiltration. Follow-up imaging has shown that these areas of spectroscopic abnormality could predict the later development of contrast enhancement [66]. More recent studies have shown that the spectroscopic abnormality is 20% larger than the volume of increased T_2 signal and biopsies of this region show evidence of tumour invasion [67]. Other studies have detected increases in myoinositol (a spectroscopic marker of glia) and glutamate (implicated with anabolic pathways upregulated in tumours) in the contralateral hemisphere of glioblastomas [68]. This change was not seen in either low-grade gliomas or normal controls.

Measuring the uptake of amino acids with PET imaging using either ^{11}C-methionine (MET) or

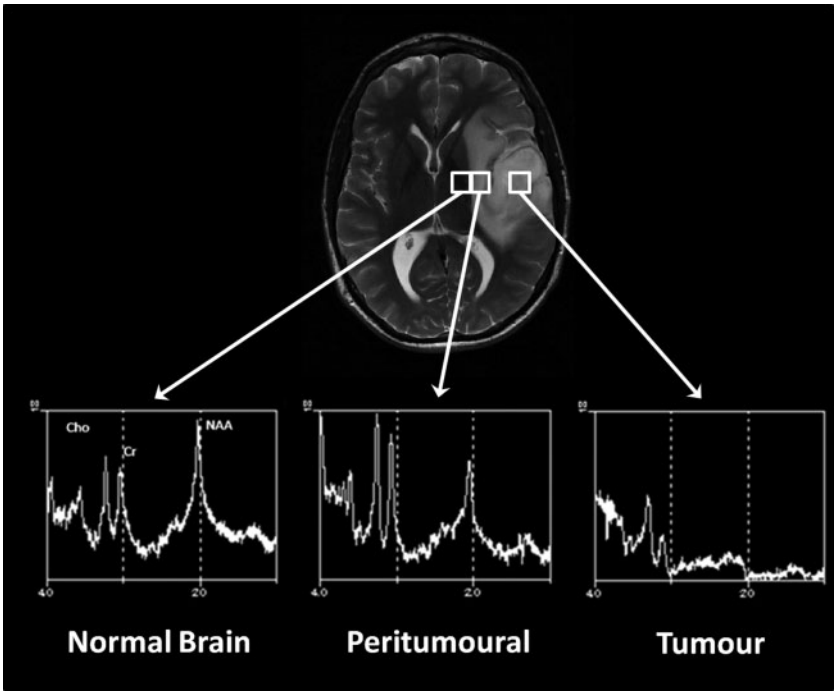

Figure 3. An example of the proton spectra (echo time=30 ms) within and surrounding a World Health Organization grade III anaplastic astrocytoma. The first region is within normal brain and the normal spectra can be seen with high *N*-acetylaspartate (NAA) peaks and a lower choline peak. The second spectrum is taken from the peritumoural tissue where there is a much higher choline peak and a lower NAA peak. NAA is still detectable suggesting viable neurons are still present. Within the tumour there is a very high choline peak but NAA is undetectable.

18F-fluoroethyl-L-tyrosine (FET) has been shown to be a more sensitive method of tumour detection than 18F-fluorodeoxyglucose (FDG) PET [69]. Studies that fuse MR and methionine PET images have shown that the volume of increased methionine uptake is greater than the volume of gadolinium enhancement on T_1 weighted MR, and, although smaller than the volume of increased T_2 weighted signal, it extends beyond it in most cases [70]. Other studies have shown the region of increased amino acid uptake correlates well with areas of increased Cho/NAA [71] and DTI abnormalities in white matter tracts [72]. To see if methionine could better determine the margin of gliomas, Mosskin et al [73] compared methionine uptake with image-guided brain biopsies. They found that in a cohort of 38 patients that mainly had low-grade gliomas, the tumour extended beyond the area of methionine uptake in 5 out of 38 cases (13%), and that the methionine uptake overestimated the tumour size in a further 5 of 38 cases. In a later study comparing methionine uptake with unenhanced MRI, they found that the tumour extended beyond the area of methionine uptake in 2 out of 9 (22%) patients [74]. In other words, the methionine abnormality cannot accurately predict the tumour margins, suggesting that either the peripheral infiltrating cells do not take up methionine, or that other, reactive/inflammatory cells have increased uptake. Other biopsy studies suggest that a ratio of MET uptake (normalised to normal brain) of greater than 1.3 could detect tumour tissue with a sensitivity of 87% and specificity of 89% [75]. Interestingly, in low-grade gliomas, the infiltrating cells had a higher uptake than regions of solid tumour.

Cellular proliferation

Cellular proliferation is a cardinal feature of malignant tumours. In the brain, as the surrounding normal structures has a very low proliferation rate, attempts have been made to use these markers to better identify the tumour margin. 18F-fluorothymidine (FLT) is actively taken up into dividing cells and has an excellent contrast-to-background ratio [76–78]. The area of abnormality is larger than the abnormality seen on MR [77]. An example is shown in Figure 4. Studies measuring FLT uptake have shown it correlates well with tissue markers of proliferation [77, 78, 79]. A recent study comparing uptake of FLT to the appearance of tumour in image-guided biopsies has shown that FLT underestimates the extent of tumours in half of the cases [78]. This can be explained by the finding that dividing and infiltrating cells appear to be two distinct tumour phenotypes, and that the most invasive cells will not be dividing [6].

Perfusion changes owing to angiogenesis

Tumour growth and invasion is dependent on developing a suitable blood supply. Studies have shown that there is an increase in rCBV in regions adjacent to the tumour that appeared normal on conventional imaging [80]. Biopsies of these abnormalities have confirmed infiltrating tumour [81] and are not seen in non-infiltrating tumours (*e.g.* meningiomas) [82].

Molecular markers of gliomas

All of the methods so far discussed are indirect methods of detecting tumour cells. The advance of molecular imaging may allow the direct detection of these cells. It has already been mentioned that the expression of EGFR is an important molecular marker of *de novo* glioblastomas, which have the tendency to be more invasive [83]. A number of PET tracers are being developed to study these processes using both ^{18}F- and ^{11}C-labelled irreversible EGFR inhibitors [84, 85], as well as ^{64}Cu-labelled EGFR blocking drugs (*e.g.* Cetuximab)

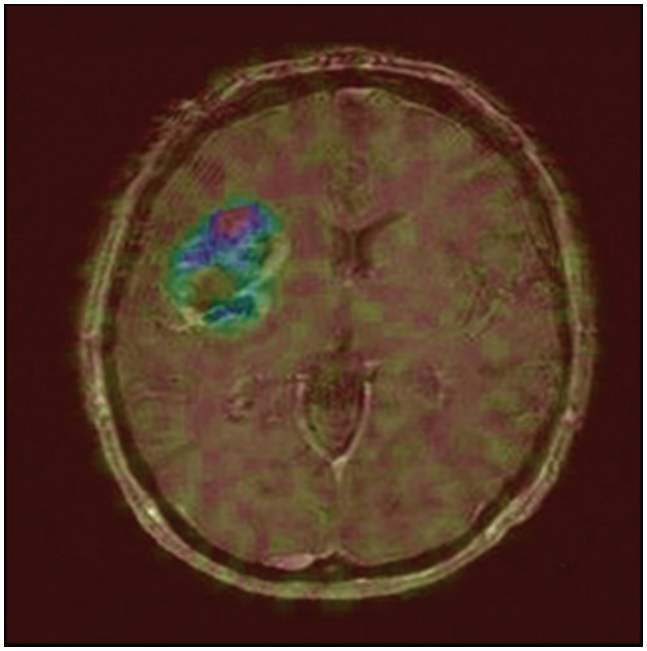

Figure 4. An example of a 18F-fluorothymidine (FLT) positron emission tomography (PET) image overlying a contrast-enhanced T_1 weighted image of a glioblastoma. Although the FLT uptake is largely in areas of contrast enhancement, the uptake extends into the surrounding brain. Histological analysis of image-guided biopsies, however, showed that the FLT uptake underestimated the region of tumour invasion in this case.

[86]. Animal studies suggest good identification of EGFR-positive tumours [86].

One of the key molecular components of the invasive behaviour is the production of MMPs. PET tracers of MMP activity are currently being developed using ^{125}I- and ^{18}F-labelled analogues of the non-peptidyl broad-spectrum MMP inhibitor CGS 27023A [87, 88]. Animal studies suggest this preferentially detects activity of MMP-1, -2 and -9 [87], the MMPs with the most important activity in gliomas [89]. Such PET markers may help determine the degree of invasiveness in an individual glioma.

Conclusion

Tumour invasion is a key stage of gliomas that is poorly detected using conventional imaging methods. Using methods that look at changes in white matter structure (DTI), metabolism and proliferation we can begin to detect the edge of the tumour and get an idea of the degree of invasiveness for an individual patient. The utility of these methods in radiotherapy planning has already been shown; using DTI to plan radiotherapy may lead to reduction of planning target volumes of 35% and allow dose escalation by a mean of 7 Gy (range 4–14 Gy) for the same risk of normal tissue injury [90]. Similarly, Pirzkall et al [66] have shown that radiotherapy volumes derived from spectroscopy extended beyond the T_2 signal abnormality in 60% of cases and should thus be used for planning radiotherapy. Further studies to show if these techniques could be used to improve radiotherapy planning are underway.

Acknowledgments

Stephen Price is funded by a Clinician Scientist Award from the National Institute for Health Research.

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