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TRANSLATION OF METABOLOMICS MARKERS INTO NON-INVASIVE METABOLIC IMAGING IN GLIOBLASTOMA.

Natalie J. Serkova.
Colorado Imaging and Metabolomics Core, Anschutz Medical Center, University of Colorado. 12800 East 19th Ave. Aurora CO 80045, Denver Colorado.
Natalie.Serkova@ucdenver.edu
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Background: A radiological phenomenon of abnormal enhancement on magnetic resonance imaging (MRI) in gliomablsatoma (GBM) patients in the first weeks after completion of radio- and chemotherapy is called "pseudoprogression" (PsP). PsP is defined as treatment-induced inflammation, which leads to edema/ radiation necrosis resulting in abnormal "GBM-like" MRI appearances. Conventional ^{18}F-FDG (^{18}F-fluorodeoxyglucose) positron emission tomography (PET) is unreliable at predicting the tumoral nature of a lesion, because of the unspecific glucose uptake in inflammation and normal brain. Presently, PsP and true GBM progression are radiologically indistinguishable.
Materials and Methods: To address this highly relevant clinical question, a quantitative ^{1}H-NMR (nuclear magnetic resonance) untargeted metabolomics was first applied on mouse, rat, and human glioma cells, mouse GBM xenografts and human recurrent GBM surgical specimens. The metabolic phenotypes of these GBM models were then compared with metabolism of normal rat astrocytes, rat brain slices and, most importantly, of activated pro-inflammatory macrophages. The distinguished glioma-associated metabolites (related to amino acid metabolism) were then validated using targeted ^{13}C-amino acid NMR flux analysis and *in vivo* ^{18}F/ ^{11}C-amino acid PET.
Results: From over 75 endogenous metabolites quantified by ^{1}H-NMR in surgical recurrent GBM specimens, 12 metabolites were highly different from the normal brain using partial least square discriminant analysis (PLS-DA). In addition to well know GBM markers (decreased NAA and increased phospholipids), dramatic changes in amino acid metabolism were observed, related to a decreased amino acid synthesis, decreased TCA cycle, and a concurrent high protein synthesis. Using human GBM cells in-silico, ^{13}C-amino acid uptake and incorporation into protein synthesis was then evaluated. The ^{13}C-NMR studies revealed a highly elevated uptake for glutamine, glycine, tyrosine, and methionine in GBM cells as compared to normal astrocytes. These findings were also confirmed using ex-vivo dynamic ^{13}C-MRS in perfused rat brain slices and human recurrent GBM slices (obtained during surgeries). Highly up-regulated L-type amino acid transporters (LAT1) were also reported in GBM. Even though glutamine revealed the highest abundance/ uptake rate in GBM cells and slices, ^{13}C-glutamine up-take was even higher in isolated human macrophages ($p<0.05$). Presently, ex-vivo discovered metabolic biomarkers (glycine, tyrosine and methionine) are validated in GBM mouse xenogratfts using ^{13}C-amino acids (for NMR metabolomics on biopsies) and ^{11}C-/ ^{18}F-amino acids (for non-invasive PET).
Conclusions: GBM cells and recurrent GBM tissue specimens revealed highly decreased rates of amino acid synthesis accompanied by an elevated up-take of several amino acids using quantitative ^{1}H-NMR metabolomics. Glutamine is taken up in high amounts by both GBM cells as well as by activated macrophages and is, therefore, disqualified as a potential imaging end-point for distinguishing GBM progression from pseudo-progression (inflammation). Animal PET can reliably distinguished between GBM progression (high PET signal) and radiation-induced PsP (no PET uptake) using radiolabelled amino acid tracers. The first US and German clinical trials are underway, utilizing ^{11}C-methionine and ^{18}F-tyrosine as "GBM" PET tracers and yielding promising results in recurrent GBM cases as early as 5 days after radiation/chemotherapy treatment.