Monitoring tumor response to the direct or indirect targeting of choline

Lionel Mignion[1], Pierre Danhier[1], Paolo E. Porporato[2], Julie Magat[1], Pierre Sonveaux[2], Vincent Gregoire[3], Bernard Gallez[1], and Benedicte F. Jordan[1]

[1]Biomedical Magnetic Resonance Unit, Louvain Drug Research Institute, Université Catholique de Louvain, Brussels, Belgium, [2]Pole of pharmacology, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels, Belgium, [3]Pole of Molecular Imaging, Radiotherapy and Oncology, Université Catholique de Louvain, Brussels, Belgium

Target audience :

MR scientist with interest in the monitoring of the response to anti-tumoral treatment.

Purpose :

The aim of the current study is to assess response to the modulation of the choline pathway, that is known to be involved in oncogenesis, by using specific choline kinase and transporters inhibitors and shRNA of choline kinase. Conventional anatomically and histologically based endpoints may be inadequate to monitor the tumor response to targeted agents. Therefore, the identification and use of more sensitively, non-invasive biomarkers that can detect treatment response before there is any change in tumor size are needed to optimize the schedule and dosage of novel therapeutic approaches.

Activated choline metabolism, which is characterized by increased total choline-containing compounds (tCho), is a metabolic hallmark of cancer. This change in the metabolism can be monitor using magnetic resonance spectroscopy (MRS) of tumors (1). The major molecular causes of the increased tCho levels in cancer cells and tumors include an increased expression and activity of choline kinase alpha, a higher rate of choline transport, and increased activities of phosphatidylcholine specific phospholipase C (2). All these molecular changes have consequences on tumor growth. Both Diffusion Weighted MRI and choline spectroscopy can be used as as an evaluation of cell density and proliferation marker of response to treatment, respectively.

Methods :

In this study, direct and indirect modulators of the choline pathway are tested in vivo on xenografts (mammary MDA-MB-231 tumor model) using multi-modal imaging endpoints. The modulators include three direct choline modulators : an inhibitor of choline transporters (Tetraethylammonium : 20mg/kg/day; intraperitoneal injection); a pharmacologic inhibitor of choline kinase (H89 : 20mg/kg/day; intraperitoneal injection) and a SH-RNA modulating choline kinase, as well as an indirect inhibitor of choline kinase via the MAP kinase pathway (Sorafenib : 40mg/kg/day; intraperitoneal injection) (3).

Results :

After 2 days of direct or indirect inhibition of choline kinase, we observed a decrease of the total choline from 44% to 53% for all the modulators. This was not the case for the inhibitor of the choline transporter OCT (Table 1). Results were similar after 5 days of treatment. Regarding the tumor growth delay endpoint, we measured a significant growth delay ($p<0.01$) in the Sorafenib group (after 3 days of treatment) and in the SH-RNA ChK group in comparaison with control groups ($p<0.001$; 52 days after tumor implantation). The choline kinase inhibitor H89 was not able to induce any growth delay. Using Diffusion MRI we observed a significant increase ($p<0.01$) of the apparent diffusion coefficient (ADC) after 5 days of Sorafenib treatment (Table1), but not after treatment with H-89, wich is in line with tumor growth delay data.

Method	TEA	H-89	SH-RNA ChK vs Ctrl	Sorafenib
1H MRS	ns (-8.3%)	tCho ↓ (-44%)	tCho ↓ (-53.29%)	tCho ↓ (-49.32%)
DW-MRI	ns	ns	nd	ADCw ↑
Growth delay	ns	ns	Growth ↓	Growth ↓

Table1 : Treatment response using multi-modal imaging endpoints

Discussion :

These results illustrate that the assessment of total choline with 1H-MRS is able to confirm the inhibition of the target but is not sufficient to predict tumor response to the targeted treatment. Indeed, using H89 we observed a significant decrease of the total choline but this was not associated with tumor shrinkage or stabilization. Adding DW-MRI as a marker of tumor response to choline inhibition improves specificity of the monitoring, since ADC was only modified when tumor growth delay was increased. Ex vivo histological studies confirm the increase in cell death observed using DW-MRI.

Conclusion :

In this study we proposed a combination of choline spectroscopy and diffusion markers to predict the tumor evolution after treatment targeted at the choline cycle. Using these combined markers we can assess the actual inhibition of the target in vivo and assess early tumor response non invasively, in order to avoid drug resistance with the ultimate goal of improving therapy individualization.

References :

1. Glunde K, Bhujwalla ZM, Ronen SM. Choline metabolism in malignant transformation. Nat Rev Cancer. 2011; 11(12):835-48.
2. Iorio E, Ricci A, Bagnoli M, et al. Activation of phosphatidylcholine cycle enzymes in human epithelial ovarian cancer cells. Cancer Res. 2010;70(5):2126-35.
3. Clem BF, Clem AL, Yalcin A et al. A novel small molecule antagonist of choline kinase-α that simultaneously suppresses MAPK and PI3K/AKT signaling. Oncogene. 2011;30(30):3370-80.