Targeting of Tumor Cells with Glutamine Containing Carriers

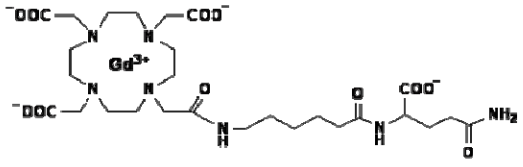
S. Geninatti-Crich[1], R. Stefania[1], L. Tei[2], A. Barge[1], I. Szabo[1], S. Lanzardo[1], C. Bianco[1], and S. Aime[1]
[1]University of Torino, Torino, Italy, [2]University of piemonte orientale, Alessandria, Italy

Introduction: In respect to other molecular imaging modalities the low sensitivity is the main limitation of the Magnetic-Resonance-Molecular-Imaging approach. Therefore, the success of a MR-Molecular Imaging protocol strongly relies on the amplification effects associated to the accumulation of the agents at the cells of interest. Efficient routes to accumulate imaging probes in tumor cells may be found by exploiting the up-regulation of trans-membrane transporting systems. In fact, rapidly growing tumors require an increased and continuous supply of aminoacids and other nutrients. Glutamine appears an interesting candidate as it is considered the main source of nitrogen for tumor cells. Thus tumor cells have been targeted with MR imaging probes bearing glutamine residues as targeting vectors.

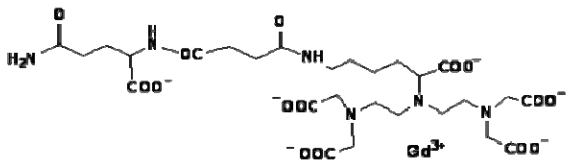
Methods: ESI mass spectra were recorded on a Waters Micromass ZQ spectrometer in murine and human serum, in PBS and in the presence of pyroglutamate aminopeptidase. Magnetic Resonance Imaging (MRI) was performed at 7T. The internalized Gd was determined by ICP-MS analysis.

Results: Magnetic resonance imaging probes that target glutamine transporters have been synthesized. They consist of a Gd-DOTA monoamide moiety linked through a six carbon atom chain to a vector represented by a glutamine residue bound through α-carboxylic or an α-amino functionalities (scheme 1). Their uptake by different tumor cell lines (HTC, Neuro-2a, C6, B16 and healthy rat hepatocytes) has shown that the system containing the glutamine vector bound through the α–carboxylic group (compound 2) displays a markedly higher affinity for tumor cells. On the contrary, different results have been obtained "in vivo". The uptake of compound 1 by tumor cells in mice grafted with the murine neuroblastoma cell line Neuro-2a and in Her-2/neu transgenic mice developing multiple mammary carcinoma,, is significantly higher than that obtained with compound 2. This different behavior could be the consequence of an improved enzymatic cleavage of glutamine or/and of a different biodistribution caused by the different residual charge of the two complexes. Mass spectrometry studies performed in the presence of the enzyme pyroglutamate aminopeptidase (a proteolitic enzyme present in serum) and in serum indicate that the cleavage of the glutamine moiety occurs only in the case of compound 2. Compound 1 remains stable in all the conditions used in this study. Similar results have been obtained in serum without the addition of any enzyme. Work is in progress on complexes with different residual charge (DTPA derivatives). Preliminary results show us that compound 4 is taken up by tumor cells more efficiently. Probably, the negative charge and the more flexible structure of the coordinating cage of this complex is responsible of the increased affinity for the glutamine transporter. The stability of the glutamine residue present on compound 3 and 4 will be checked in different media and in the presence of specific proteolytic enzymes.

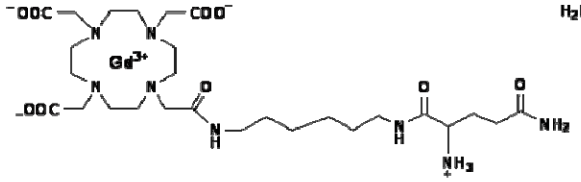
Conclusions. Compound 2, containing glutamine functionalized through the α–carboxylic group, showed a high internalization in tumor cells "in vitro", but also a high degradability in serum that avoid tumor accumulation. To improve tumor uptake also under "in vivo" conditions, new structures have to be tested in order to reduce or delay the enzymatic cleavage.



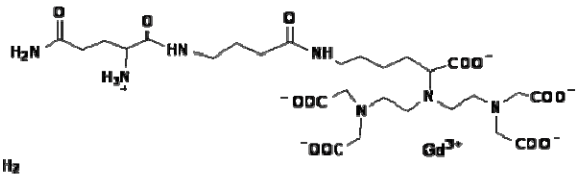
Compound 1



Compound 3



Compound 2



Compound 4