TARGETING TUMOR CELLS WITH GD(III) CHELATES BY MEANS OF THE GLUTAMINE TRANSPORTING SYSTEM

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Introduction.

The development of new Gd-based contrast agents (CAs) with high contrast ability and targeting capability is the key step for the set-up of innovative magnetic resonance-molecular imaging (MRMI) protocols. In fact, in MRMI procedures, one has to visualize epitopes that are present at very low concentration (typically in the 50-100 nM range) and therefore it is necessary to design proper methods to amplify the response upon recognition of the target of interest.

We have recently exploited the glutamine transporting system as a route to deliver a large number of Gd(III) contrast agents to the tumor cells. It is well known that proliferating cells consume more glucose and amino acids (and their derivatives) than their benign counterparts. Transport of glucose and amino acids into cells is mediated by specific membrane proteins called transporters, which are responsible for the translocation of the substrate from one side of the membrane to the other. The increased expression or up-regulation of these transporters correlates with the greater transport of glucose and amino acids and it is strictly related to the cells growth. We have chosen glutamine as it is the most abundant amino acid in the body (0.5-0.8 mM in serum) and is the physiological non-toxic ammonium vehicle between different mammalian tissues; therefore glutamine is the main source of nitrogen for tumor cells which transport glutamine at a faster rate than normal cells[1].

Methods.

Extensive synthetic chemistry has been necessary to synthetize the compounds reported in fig.1. In particular novel systems for efficient conjugation have been synthetized, namely DOTAMAC_6OH and DOTAMAC_6NH_2 . The latter compound has been used in the synthesis of DOTAMA/Gln multimers both in solid phase and in solution. Gln phospholipid was synthesized by conjugation of activated PEG 2000 spaced phospholipid with Gln, GdHPDO3A loaded liposome was prepared using this Gln phospholipid as targeting unit. All Gd-complexes were tested in vitro on HTC, C6 and Hepatocytes cell lines and the best compounds also in vivo on A/J mice grafted with the murine neuroblastoma cell line Neuro-2a and in Her-2/neu transgenic mice developing multiple mammary carcinoma

Results.

Several glutamine and Gd chelate containing systems have been prepared (Fig.1). Namely: a) Gd-DOTA monoamide (Gd-DOTAMA)derivatives in which the glutamine residue is conjugated through different functionalities; b) Gd-DOTAMA derivatives endowed with a different spacer between the chelate and the glutamine moieties; c) Multi-valent systems containing more glutamine residues per Gd complex; d) A Gd-loaded liposome functionalized with glutamine vectors on its outer surface.

A thorough investigation on different cell lines has allowed to assess the main determinants of cellular uptake through the glutamine transporter system. Important parameters appear to be the length of the spacer between the glutamine and the chelate moiety and the way through which the glutamine residue is conjugated to the imaging reporter. No advantage has been found with the use of the glutamine multimeric system. Finally the liposome based system works very well “in vitro” but it has still to be optimized for “in vivo” applications.

Conclusions.

This work has allowed the identification of the determinants of cellular binding and uptake through the glutamine transporters. This target appears very promising for the MRI visualization of tumor cells.

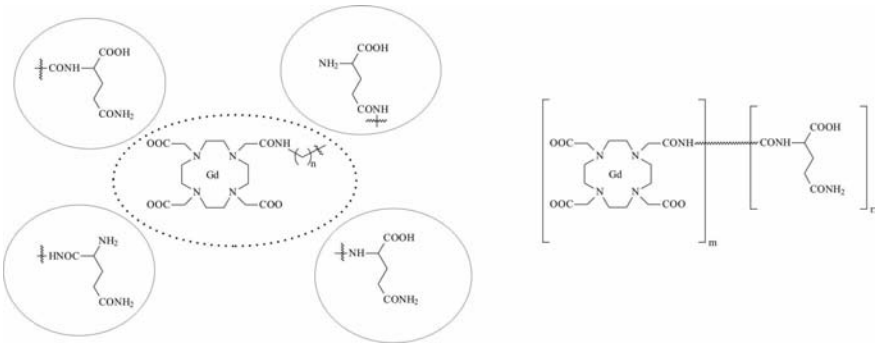

Figure 1: New DOTAMA derivatives bringing a glutamine residue as targeting vector

References

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