In vivo labelling of xenografted B16 melanoma cells with a thiol-responsive Gd(III) based MRI contrast agent

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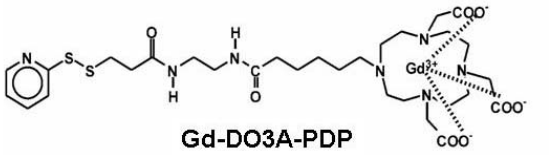
Mammalian cells display a large number of reactive protein thiols on the extracellular side of the plasma membrane (Exofacial Protein Thiols, EPTs). These EPTs are quite reactive and can be chemically labeled by suitably designed MRI contrast agents. We have recently shown [1,2] that compound Gd-DO3A-PDP, containing the 2-pyridinedithio chemical function for the recognition of EPTs, can label cells *ex vivo* through the formation of disulfide bridges with the cell's EPTs. The Gd(III) chelate thus anchored on the cell surface is transported into the cytoplasm, and by this route up to $1.2x10^{10}$ Gd atoms per single cell can be internalized. The amount of internalized Gd is dependent upon the availability of EPTs, whose levels are influenced by the extracellular redox microenvironment.



In solid tumors, cells proliferate under a hypoxic (hence reducing) microenvironment, therefore they are expected to display high levels of EPTs. The extent of labeling of these cells *in vivo* by Gd-DO3A-PDP would therefore report about the tumor extracellular redox, readily seen as DCE in MRI images. To assess whether Gd-DO3A-PDP could be used to label tumors *in vivo*, murine melanoma B16 cancer cells have first been assayed *ex vitro* for their ability to take up Gd-DO3A-PDP as a function of the EPTs levels. Then, Gd-DO3A-PDP has been delivered to mice grafted with a B16 melanoma derived tumor (direct intra tumor injection) and the signal enhancement monitored over time. The signal enhancement in tumors treated with Gd-DO3A-PDP has been shown to decay very slowly with time (it is still clearly detectable at 48-72 hours post injection), especially if compared with that of tumors treated with control Gd-DO3A or ProHance. The long lasting signal enhancement of Gd-DO3A-PDP in tumors is in line with the compound being internalized into cells through the EPTs route. We think that the results herein presented constitute a good proof-of-concept about the possibility to obtain molecular images of EPTs *in vivo*, that in turn can give information about the tumor extracellular redox.

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References

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