Mobile lipids and apoptosis

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Tumor responses to treatment are still assessed largely from imaging measurements of reductions in tumor size. However, this may take several weeks to become manifest and in some cases may not occur at all, despite a positive response to treatment. There has been considerable interest, therefore, in non-invasive techniques for imaging tissue function that can give an early indication of treatment response. These could be used in clinical trials of new drugs to provide evidence of drug efficacy, and subsequently in the clinic to select the most effective therapy at an early stage during treatment [1].

There has been considerable interest in the development of non-invasive and clinically applicable magnetic resonance-based methods for detecting the early responses of tumors to therapy. A primary focus has been on the development of methods for detecting tumor cell apoptosis, or programmed cell death, since the level of tumor apoptosis following drug treatment has been shown, in preclinical and clinical studies, to be a good predictive indicator for treatment outcome. Thus by monitoring tumor cell death an oncologist may get an indication of whether a particular drug is working very early during treatment, possibly within 24-48 hours, and long before there is any evidence of tumor shrinkage. The MR methods available for detecting apoptosis, including ^{1}H MRS measurements of mobile lipid accumulation, have been reviewed recently [2].

Tumor tissue usually contains high concentrations of NMR-visible mobile lipids. Furthermore, lipid metabolism is known to alter during apoptosis, making MRS detection of changes in lipid concentrations a potential means of detecting cell death *in vivo* [3]. Several lipid-associated signals are visible in proton spectroscopy, the most prominent ones being the methylene ($-CH_2-$) signal at 1.3 ppm and the methyl ($-CH_3$) signal at 0.9 ppm. Blankenberg *et al*. [4, 5] first demonstrated that the methylene signal intensity increased following induction of apoptosis *in vitro*, although an association between an increase in this signal and cell death had been noted previously [6]. In later studies, an increase in the CH_2/CH_3-ratio, as well as increases in the signals from polyunsaturated fatty acids (PUFA, at 2.8 ppm and 5.4 ppm) [7], were suggested as potential markers of apoptosis. Numerous studies *in vitro* have now shown that increased lipid signals are observed in a range of cell lines and following a variety of different apoptosis-inducing treatments, suggesting that altered lipid metabolism is a relatively common consequence of apoptosis. However, cellular lipid levels are not only affected by cell death, as significant changes have also been reported as a consequence of cell growth conditions, cell cycle stage and acidotic stress [2]. In general, however, these *in vitro* studies indicated that lipid accumulation is a potentially useful marker of apoptosis.

The current consensus is that the majority of this lipid signal is produced by lipids located in cytoplasmic lipid bodies (reviewed in [2]). This supported by the close correlation between the accumulation of cytoplasmic lipid droplets and increases in lipid intensities observed in several cell lines during apoptosis and under other conditions. The processes leading to lipid body formation in apoptosis are only partially understood. Increased catabolism of membrane phospholipids, through phospholipase activation, can produce free fatty acids that are then converted to triacylglycerols, which are stored in lipid bodies. Especially phospholipase A_2 activity, which is known to increase during apoptosis, has been linked to increased lipid signals. Delikatny *et al*. suggested that breakdown of mitochondrial membranes may be a potential source of the mobile lipids [8]. An alternative route to lipid droplet accumulation could result from inhibition of phospholipid biosynthesis, which would lead to the accumulation of diacylglycerols and triacylglycerols. Inhibition of phosphatidylcholine biosynthesis was observed in cells undergoing apoptosis, resulting in the accumulation of CDP-choline, which is an intermediate in this pathway [9, 10]. The role of lipid accumulation in cell death is still poorly understood. Lipid accumulation in apoptotic HuT 78 cells can be prevented by using an acyl-CoA synthetase inhibitor, which inhibits triacylglycerol formation from free fatty acids. However, this had only minor effects on the progress of the apoptotic programme, indicating that lipid accumulation may be simply an epi-phenomenon rather than an integral part of the process [11].

The use of ^{1}H MRS measurements to detect apoptosis, via the increase in mobile lipid resonances, is more complex *in vivo* as tumor cells are surrounded by fatty tissues and other cells with active lipid metabolism, such as immune cells, which can enter tumors following chemotherapy. The presence of a background lipid signal in viable tumor cells, which can be high when compared to the increases induced by cell death, adds to the difficulty of detecting cell death from increases in the NMR signals of cell lipids. Despite these challenges, there have been several *in vivo* studies that have demonstrated lipid accumulation in cell death. Ross *et al*. noted increased signal intensities at 0.9-1.3 ppm in a Herpes simplex virus thymidine kinase gene therapy model of 9L gliosarcomas, and tentatively assigned the signals to mobile lipids and/or lactate [12]. Working with a similar model, Hakumäki *et al*. reported increased intensities in the same region during gene therapy-induced tumor cell eradication in BTC4 glioma, and assigned the signals to mobile lipids, including those from PUFA [7]. Further work in the same tumor model has confirmed these observations and indicated accumulation of intracellular lipid droplets as a source of these lipid signals. We compared the effects of chemotherapy on lipid metabolism in cultured EL4 lymphoma cells, and in the solid tumors formed from these cells following their subcutaneous implantation [13]. In the cells, increased lipid signals were associated with apoptosis and increased lipid droplet content. Similarly, we were able to confirm increased lipid signals in the tumors during chemotherapy. Increased NMR-detectable lipid signals, however, are not a specific marker of apoptosis. For example, increased lipid signals, coming from intra- and extracellular lipid bodies, have also been associated with necrosis [14].

The utility of *in vivo* spectroscopy for detecting apoptosis via mobile lipid signals is hampered by the low spatial resolution achievable using single-voxel spectroscopy and spectroscopic imaging. This makes detection of relatively low levels of cell death very difficult, as the majority of cells inside the voxel may not be undergoing apoptosis at the same time. Another disadvantage of low resolution is the vulnerability to contamination from lipid signals from surrounding tissue, which may be much larger than those in the tumors. Finally spectroscopic

methods suffer from relatively long data acquisition times. These limitations, combined with the difficulties in assigning increases in lipid signals unambiguously to the presence of apoptotic cells makes it difficult currently to use MRS methods to detect apoptosis *in vivo*. Nevertheless, the method has the virtue that it is "label-free" and utilizes a relatively intense ^{1}H signal, which could aid translation to the clinic.

References

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