

**P023** Quantification of repair of localised oxidative damage in live cells induced by focused near infra-red multiphoton absorption: direct evidence that the polymorphic variant [Cys326] of OGG1 is repair deficient

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Previous studies suggest that 8-oxo-2'-deoxyguanosine glycosylase 1 (OGG1) plays a major role in the repair of 8-oxo dG. A common polymorphism of OGG1, [Ser326Cys], may be a risk factor for human cancer. We have developed a novel technique using multi-photon focused induction of oxidative DNA damage and OGG1 knock out mouse embryonic fibroblasts transfected with GFP tagged OGG1 to measure the kinetics of repair of oxidative DNA damage by OGG1 in live cells. The repair of damage by Cys326 OGG1 was significantly slower compared to the wild type protein. The accumulation of OGG1-GFP protein at the targeted sites of irradiation revealed the location of oxidative damage. After irradiation, wild type cells appeared to repair damage completely within 1200 s while Cys326 transfected cells still had substantial damage remaining at this time. Interestingly, when cells were pre-treated with L-buthionine sulphoxamine (BSO; 100  $\mu$ M, 24 h) which causes a significant reduction in levels of GSH (approximately 80 %) and an elevation of ROS (almost 300 %) there was an almost complete inhibition in the ability of both wild type and Cys326 OGG1 to accumulate at the sites of damage. This suggests that under these conditions OGG1 is not able to recognise damage and that oxidative modification to the protein structure may be responsible for an inhibition in damage recognition. Although the mechanism remains to be determined, this is a potentially important observation and is the subject of current investigations in our laboratory.