

**P012** Investigation of a novel serine protease involved in cell survival

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Proteases play a critical role in the regulation of cell survival and in the execution of cell death, particularly apoptosis. In this study we have attempted to identify novel serine proteases through the application of a library of 'in-house' and commercially available inhibitors. Inhibitor screening detected a chymotrypsin-like protease targeted by the cell-permeable inhibitor, tetramethylrhodamine-phenylalanine-diphenyl phosphonate (TMR-F-dFP). Incubation with this inhibitor reduced cell viability to 9% of control values in both HeLa (3 hr post-treatment) and U251 (12-18 hours post-treatment) cell-lines. This reduced cell viability correlated with a time-dependent increase in caspase-3 activity and PARP cleavage. Additionally, phosphatidylserine translocation was detected, commencing 3 hours post-incubation in HeLa cells. Uptake and localisation of TMR-F-dFP in cells was observed through confocal microscopy, with early detection showing potential endosomal trafficking/location. Following SDS-PAGE, a fluorescent band was detected between 52 and 60 kDa, however sequencing this labelled protease has proved relatively problematic. To date, immunoprecipitation methods have been unsuccessful, potentially due to the close coupling of the tetramethylrhodamine label to the phenylalanine residue. Alternative identification methods are currently being followed utilizing a biotin-labelled phenylalanine diphenylphosphonate which we have also demonstrated binds to the active site of our target protease.