THE NON-APOPTOTIC ROLE OF CASPASE-2 IN CELL CYCLE REGULATION AND DNA REPAIR

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Background: Caspase-2 is a tumor suppressor but the mechanisms of this are unclear. It is traditionally known as a pro-apoptotic protease but has been implicated in non-apoptotic processes. Interestingly, caspase-2-deficient tumors have comparable basal apoptosis levels to wild-type tumors, suggesting that non-apoptotic mechanisms may underlie the role of caspase-2 as a tumor suppressor.

Materials/Methods: I used Casp2-/- mouse embryonic fibroblasts (MEF) and CRISPR/Cas9 generated caspase-2 deficient HeLa with wild type to examine caspase-2 functions. I used a viability stain to compare cell growth and BrdU-FITC/7-AAD staining to quantify the percentage of cells in each phase of the cell cycle to determine proliferative rate and cell cycle recovery after arrest. I employed bimolecular fluorescence complementation (BiFC) to visualize caspase-2 activation in relation to cell cycle with AmCyan-hGeminin as a marker of S/G2/M. We examined DNA protection by caspase-2 before and after S-phase arrest with DNA fiber analysis to visualize replication forks and by pH2AX staining to quantify double strand DNA breaks (DSB). To determine dependence on the apoptotic pathway of caspase-2, we used the stable expression of Bcl-XL, a potent inhibitor of this pathway.

Results: In caspase-2 deficient cells, we observed increased proliferation, indicating that caspase-2 limits cellular proliferation. Time-lapse microscopy revealed the majority of BiFC-positive cells divided and this activation occurred prior to expression of hGeminin, indicating that caspase-2 is activated in G1 in dividing cells. Caspase-2-deficient cells demonstrated a delayed exit from S-phase following S-phase arrest, indicating an S-phase defect. Consistent with this, DNA fiber experiments demonstrated that after S-phase arrest caspase-2-deficient cells had an increased percentage of: stalled DNA replication forks, new replication origins, and DSB. Overexpression of Bcl-XL to block caspase-2-induced apoptosis failed to phenocopy the cell cycle profile or increased DNA damage seen in Casp2-/- MEF, suggesting that this role in cell cycle and DNA repair is independent of the pro-apoptotic function of caspase-2.

Conclusions: Altogether, these results support an important non-apoptotic role for caspase-2 in cell cycle regulation that results in stabilized DNA replication forks and DNA repair. These data suggest that this less studied role of caspase-2 in cell division may be the primary driver of caspase-2-associated tumor suppression.

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