TCOF1 as a nucleolar caspase substrate: a tale of two (or three) caspases

Alexandra Brown-Suedel
Karen Zuniga
Lisa Bouchier-Hayes, Ph.D.

Texas Children's Hospital, Department of Pediatrics and Hematology, Baylor College of Medicine

BACKGROUND

- Caspase-2 is a tumor suppressor in multiple cancer models[1]
- Caspase-2 has independent roles in apoptosis and cell cycle progression[2]
- Caspase-2 protects from DNA damage[2]
- Caspase-2 is activated in the nucleolus by DNA damage[3]
- Substrates of caspase-2 are understudied and are poorly linked to the known functions of caspase-2[4]
- TCOF1 is a nucleolar protein that is involved in ribosome production and responds to nucleolar DNA damage
- TCOF1 is a proposed substrate of caspases-2, -3, and -6, but how caspase cleavage impacts its function is unknown.

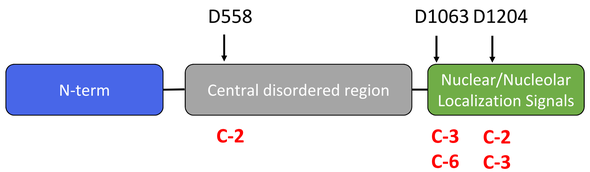

Fig 1: Schematic of proposed caspase cleavage sites in TCOF1

PURPOSE

The goal of this project is to verify the nucleolar protein TCOF1 as a caspase substrate and determine how cleavage by caspases; particularly cleavage of caspase-2 in the nucleolus impacts TCOF1 functions that could relate to caspase-2's role as a tumor suppressor.

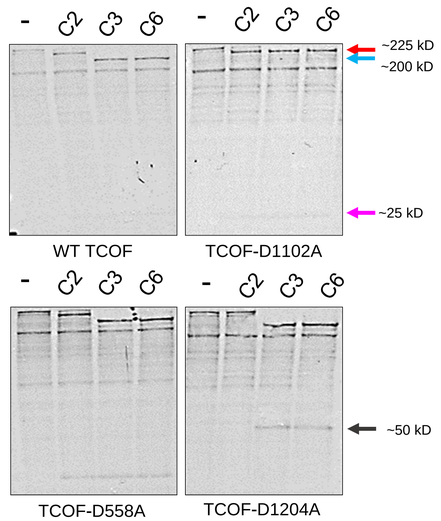

Fig 2: TCOF1 is a caspase-2 substrate in vitro, but is also readily cleaved by caspases-3 and -6. Wildtype and cleavage defective mutants (D558A, D1063A, and D1204A) were radiolabeled with S-35 and incubated with the indicated recombinant caspases (3h). Cleavage products are indicated by colored arrows.

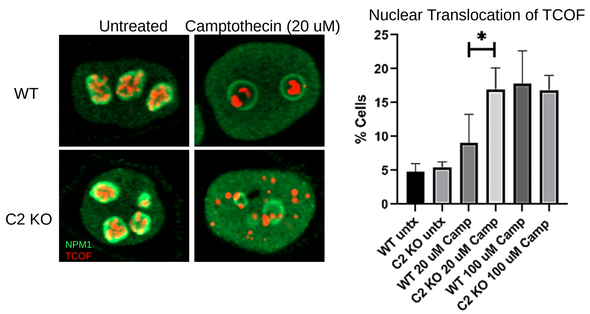

Fig 4: Endogenous TCOF1 translocates to the nucleus after DNA damage in the absence of caspase-2. Wildtype and caspase-2 knockout Hela cells were treated with Camptothecin to induce DNA damage, then fixed and stained for endogenous TCOF1 (red). NPM1 is used as a nucleolar marker, but also weakly stains the nucleus (green).

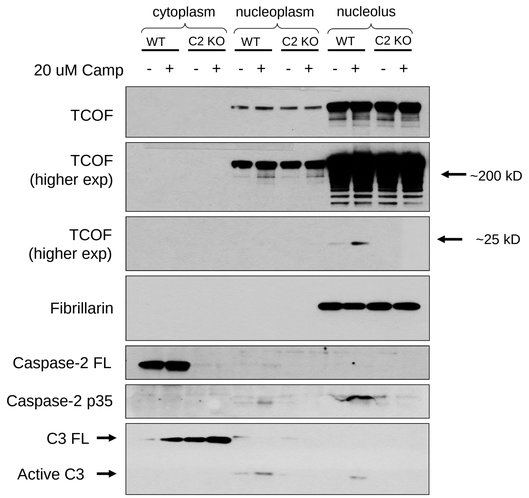

Fig 3: TCOF1 is cleaved after DNA damage induced by Camptothecin in Hela cells in both the nucleus and nucleolus. Wildtype and caspase-2 knockout Hela cells were fractionated into cytoplasmic, nucleoplasmic, and nucleolar components. Western blotting was performed against the proteins indicated.

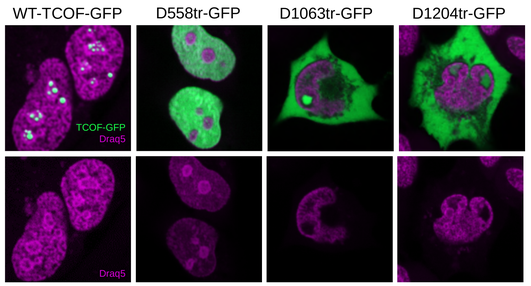

Fig 5: N-terminal cleavage fragments of TCOF1 localize to the nucleus and cytoplasm. The potential fragments (N-terminal) resulting from TCOF1 cleavage at either D558, D1063, or D1204 were tagged with GFP and transiently expressed in U2OS cells and compared to full length TCOF1 tagged with GFP. TCOF1 variants are visible in green, with Draq5 (purple) staining the nucleus.

RESULTS

- TCOF1 is cleaved by caspases-2, -3, and -6 at D1204
- TCOF1 is cleaved by caspases-3 and -6 at D1063
- In our hands, D558 (a reported caspase-2 cleavage site), is not cleaved by caspase-2
- TCOF1 does appear to be cleaved in cells after DNA damage, and observed cleavage patterns are consistent with detected activated caspases via western blot
- TCOF1 translocates to the nucleus after DNA damage in the absence of caspase-2
- Nuclear localization of various cleavage fragments could explain the observed translocation of endogenous TCOF1

FUTURE DIRECTIONS

- Determine the functional consequences of TCOF1 cleavage by caspases
  - Create stable cell lines in which endogenous TCOF1 is knocked down and cleavage defective mutants (or truncations) are expressed
- Determine which caspases are responsible for TCOF1 cleavage in cells
  - Perform fractionation in cells lacking caspase-3 and -6
- Visualize TCOF1 cleavage microscopically
  - Create a double fluorescently tagged TCOF1 construct

REFERENCES

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