NPM1-MEDIATED CASPASE-2 REGULATION IN ACUTE MYELOID LEUKEMIA

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Background: Acute Myeloid Leukemia (AML) is the second most common pediatric blood cancer. Nucleophosmin (NPM1) mutations, a frequent genetic lesion in adult AML, occur in about 10% of pediatric cases. Typically, a nucleolar protein, mutations in NPM1 cause an aberrant cytoplasmic delocalization of NPM1 (NPM1c+). Strikingly, NPM1c+ mutants show higher sensitivity to chemotherapy. NPM1 forms a scaffold with PIDD in the nucleolus for caspase-2 activation under genotoxic stimuli. Caspase-2 is a pro-apoptotic protein and a known tumor suppressor. Our preliminary results indicate that nucleolar caspase-2 activation facilitates cell survival yet, apoptosis in NPM1c+ AML cells is caspase-2-dependent, revealing a dual role for caspase-2 governed by NPM1 localization. Hence, I hypothesize that NPM1-mediated caspase-2 activation is the pivotal regulator of cell death and survival cascades in AML cells.

Materials/Methods: A panel of caspase 2-deficient OCI-AML-2 (NPM1wt) and OCI-AML-3 (NPM1 C+) cell lines were used to determine the apoptotic index, cell cycle phase, and immunophenotypes via flow cytometry. The type of substrate cleavage by caspase-2 upon DNA damage was determined by immunoblotting. BiFC and time-lapse confocal microscopy were used to ascertain the subcellular location of caspase-2 activation. The student's t-test was used for statistical analysis.

Results: Caspase-2 activation was observed in the nucleolus of NPM1wt, but caspase-2 BiFC was exclusively cytosolic in the NPM1C+ cells. Caspase-2 deletion in NPM1C+ displays higher apoptotic cells and growth cycle arrest in the G1 phase than parental cells, which predominantly reside in S-phase (Parental vs. Knockout, G1: 38.6%vs.83.4%; S: 48.7%vs.13.8%). Immunophenotyping results suggest that caspase-2 deletion causes AML blasts to become macrophages in NPM1C+ cells (Parental vs. Knockout, %CD14+, n=2, 1.35vs.23.81, $P<0.05$), resulting in severe growth defect. No such effects were seen in NPM1wt cells. Cytosolic apoptosis substrates such as BID, caspase-9, caspase-3 were cleaved significantly less in caspase-2 deleted NPM1C+ cells than NPM1wt. Introduction of exogenous NPM1C+ mutation in cells containing NPM1wt triggers cell lysis resembling late apoptosis/necrosis.

Conclusions: Caspase-2 activation is dependent on NPM1 localization. Abrogation of caspase-2 results in terminal differentiation of NPM1C+ AML. Modulation of NPM1/Caspase-2 cascade alters AML cell fate forming a novel basis for sensitizing otherwise resistant pediatric AML.

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