FUNCTIONAL SIGNIFICANCE OF MOLECULAR INTERACTIONS BETWEEN THE ANTI-APOPTOTIC PROTEINS BCL-XL, BCL-2 AND MORTALIN

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Introduction:

The two anti-apoptotic proteins, Bcl-xl and Bcl-2, have been looked upon as attractive anti-cancer targets since their high levels in cancer were found to increase resistance to a wide spectrum of therapeutic agents and radiation therapy. The two proteins predominantly reside in mitochondria and function to prevent loss of mitochondrial membrane potential, cell death and mitochondrial integrity. Mortalin/mthsp70/GRP75 (mot-2)/PBP74 is a member of heat shock protein 70 (hsp 70) family also predominantly found in mitochondria and is involved in mitochondrial import, control of membrane permeability and ROS production.

Background:

Bcl-2 and Bcl-xl overexpression in cancer cells have shown to increase resistance to a wide range of chemotherapeutic agents and radiation therapy. It was reported that mortalin interacts with and inhibits p53, causing its cytoplasmic sequestration and inactivation of wild-type p53 function. Cells overexpressing mortalin were shown to acquire malignant properties including tumor formation in nude mice and metastasis. It was previously reported that p53 translocates to the mitochondria and promotes mitochondrial membrane potential disruption leading to apoptosis. Further, mitochondrial p53 was also shown to interact with members of the Bcl-2 family and mortalin.

Purpose:

In view of the anti-apoptotic function of these mitochondrial proteins, we suspected that these proteins might form a complex that may have functional consequences on the proliferative or apoptotic phenotype of cells. Therefore, we undertook this study to resolve their molecular interactions and functional relevance to p53 activity and cell proliferation control.

Methods:

We transfected cancer cells with Bcl-xl and Bcl-2 expression vectors followed by selection with G418-supplemeted medium to produce Bcl-xl and Bcl-2 overexpressing cells. To elucidate the biochemical and cellular interactions between p53, mortalin, and Bcl-xl or Bcl-2, immunoprecipitation, Western blotting, and immunocytochemical staining were performed.

Results:

The interactions between mortalin and Bcl-2 or Bcl-xl were confirmed by immunoprecipitation assays. When Bcl-2 and Bcl-xl were overexpressed in cancer cells, p53 was downregulated while mortalin was upregulated, both in a dose-dependent fashion. Immunoprecipitation assays using both overexpressing cell lines showed that mortalin was not detected in p53 immunocomplexes which suggested that increase in p53 levels in Bcl-2 and Bcl-

xl overexpressing cells were due, at least in part, to abrogation of its interaction with mortalin. An increase in p53 nuclear localization and thus activation was observed in Bcl-2 and Bcl-xl overexpressing cells. The overexpressing cells also showed expanded morphology and a shift in mortalin staining pattern from the perinuclear to pancytoplasmic type, the latter of which is associated with a non-proliferative phenotype. SA β-galactosidase assay of Bcl-xl and Bcl-2 overexpressing cells showed an increase in β-galactosidase activity, which suggests induction of senescence.

Conclusion

Interactions between Bcl-xl or Bcl-2 and mortalin abrogate mortalin-p53 complex, allowing p53 nuclear localization and subsequent activation, thus leading to senescence.