CARF PLAYS A VITAL ROLE IN SENESCENCE AND APOPTOSIS, THE TWO MAIN TUMOR SUPPRESSION MECHANISMS IN HUMAN CELLS

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Introduction: CARF (Collaborator of ARF) was cloned as an ARF-interacting protein, and was shown to enhance both ARF-dependent and -independent p53 functions, which are central to the control of tumor suppression via cellular senescence and apoptosis of human cells. However, the mechanisms governing whether p53 pathway leads to senescence or apoptosis are still unclear. We have previously found that CARF regulates the p53 pathway, undergoes MDM2-mediated degradation, and is self-regulated by feedback loops.

Background: Tumor suppression is largely controlled via cell death and growth arrest mechanisms. The p53 pathway is known to be a central regulator of tumor suppression, and activation of this pathway can either induce cell death via apoptosis or irreversible growth arrest (senescence). The p53 pathways involves a vast multitude of molecules and interacting networks. Of particular interest is the involvement of ARF (alterative reading frame) protein in the activation of p53 and subsequently p21 to promote either apoptosis or senescence. HDM2 is an E3 ubiquitin ligase that has been shown to be a negative regulator of this pathway through proteasomal degradation. Recently, CARF was found to regulate the p53 pathway through feedback loops, whereby HDM2 inhibits both CARF and p53 through proteasomal degradation, but CARF stabilizes and activates p53 via direct interaction as well as transcriptional repression of HDM2.

Purpose: Considering the role of CARF in regulation of the p53 pathway, we set out to determine the involvement of CARF in the tumor suppression mechanisms that govern the decision between apoptosis and senescence.

Methods: To determine if CARF is involved in senescence, human cells were (1) passaged until they reached growth arrest to produce premature senescence, (2) treated with H_2O_2 or MNNG for stress-induced senescence, or (3) transfected with mutant Ras to induce oncogenic senescence. Further, CARF was overexpressed or silenced by siRNA in human cells to determine its effect on cell growth. Western blotting and immunocytochemical staining techniques as well as senescence-associated β-galactosidase assay were performed to determine the effect of CARF and associated molecules in the activation of senescence or apoptosis.

Results: Here, we report that CARF is upregulated during replicative, oncogenic, and stress-induced senescence, and its overexpression induces premature senescence that is mediated by upregulation of p53 and p21, as well as p16 and pRb proteins. Knockdown of CARF resulted in mitotic arrest and caspase-dependent cell death, suggesting that CARF regulates the delicate balance between growth arrest and apoptosis. Whereas its overexpression caused growth arrest, its presence is essential for cell survival and its absence caused cells to either undergo apoptosis, excessive chromosomal condensation or aneuploidy, a prominent characteristic of cancer cells.

Conclusion: Thus, we demonstrate a vital role of CARF during senescence and apoptosis, and propose it as a crucial player in p53-mediated tumor suppression and cancer therapeutics.