ISSN 0025-7680

SIMPOSIO 7.
APOPTOSIS

MEDICINA (Buenos Aires) 2004; 64 (Supl. II): 41-42

DEGRADATION AND FUNCTION OF P73 AND P63IS INHIBITED BY PML

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p73 and p63, similarly to their homologue p53, regulate apoptosis during DNA damage. The activity of p73/p63 depends on its steady state protein levels, and a number of evidence suggest that post-transcriptional regulation rather than transcriptional control plays a major role in p73/p63 response to DNA damage; both TAp73 (pro-apoptotic) and DNp73 (anti-apoptotic). p73 has recently been identified as a structural and functional homologue of p53. However, the molecular mechanisms underlying the regulation of p73/p63 protein stability remain largely unknown. p73/p63 steady state protein levels increase in the presence of proteasome inhibitors, suggesting a role for this pathway in p73/p63 degradation. Here we report that p73/p63 stability is directly regulated by the ubiquitin/proteasome pathway and p73/p63 is degraded through specific mechanisms, different from that of p53.

(1) we found that upon DNA damage (UV, doxorubicin, etoposide) «Np73 is rapidly degraded releasing the block exerted on p53 and TAp73 allowing cell cycle arrest and apoptosis to proceed.

(2) we found that p73 is degraded through a NEDD8-mediated mechanism and that the tumor suppressor protein PML modulates p73 half-life by inhibiting its degradation. PML-mediated stabilization of p73 is nuclear body (NB)-dependent, as PML mutants that do not localize to the NBs are drastically less effective inpreventing inducing p73 accumulation. We found that p300-mediated acetylation and consequent stabilization of p73 is impaired in PML-/- cells.

(3) we show that the protective effect of PML requires p73 phosphorylation by the p38 MAP kinase pathway. As a result, PML significantly increases the ability of p73 to transactivate the p53-responsive promoters of the bax and p21 genes and potentiates p73-dependent apoptosis and tumor suppressive activity. In turn, p73 pro-apoptotic function is markedly impaired in PML-/-cells. Thus, our findings demonstrate that PML plays a crucial role in modulating p73 function and provide further insights for the involvement of PML in tumor suppression.

(4) the PY motif-containing C-terminal region of p73a (not the isoform b, g, d) binds to Aip4/Itch, an E3 Hect domain-containing (NEDD-4like) ubiquitin-protein ligase, resulting in ubiquitination and degradation of p73. The PY motif of p73 interacts with the WW domain of Itch. Interestingly Aip4/Itch is down-regulated upon DNA damage in a p73 dependent fashion allowing TAp73 levels to raise in response to this type of stress, and apoptosis to occur.

(5) p63 is also degrades by ubiquitin-dependent proteosome degradation, via PML.

(6) consistend with the defect described in KO mice, TAp73 / «Np73 differently regulate spontaneous and induce differentiation of primary oligodendrocyte precursors cells purified from the rat optical nerve, while TAp63 / «Np63 differently regulate epithelial differentiation.

In conclusion, at least two distinct mechanisms of degradation exists for p73, NEDD8- and NEDD4-dependant. The former is finely regulated by PML in the nuclear body, resulting in a strict control of its function. The relative ratio of TAp73/p63 (pro-apoptotic) and DNp73/p63 (anti-apoptotic) finely regulates the sensitivity of cancer cell to chemotherapy. Therefore their relative degradation is crucial for the outcome of cancer cells. We showed that they are different regulated upon DNA damage, finely regulating apoptosis and chemosensitivity. Finally, the stabnility and balance of the p73 protein isoforms regulates the differentiation and development of neural tissue.

NF-KB AS A REGULATOR OF THE PROAPOPTOTIC ACTIVITY OF CYCLOPENTENONE PROSTANOIDS

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Cyclopentenone prostaglandins (cyPG) are potent bioactive molecules that possess anti-inflammatory and antiviral activity. In addition to these effects, cyPG have been shown to induce cell growth arrest and apoptosis in a number of cancer cell types. In particular, the terminal derivative of prostaglandin J_2 (PGJ_2) metabolism, 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2), is emerging as the most potent anti-neoplastic agent of this class of prostaglandins. Anticancer activity of 15d-PGJ_2 has been reported both *in vitro* and *in vivo* in a multiplicity of tissues including breast, prostate, colon, lung and lymphoid. In most types of cancer, 15d-PGJ_2 inhibits tumor cell proliferation and induces apoptosis; however, the mechanism of 15d-PGJ_2 anti-neoplastic activity has not been fully elucidated as yet. We have previously shown that cyclopentenone prostaglandins are potent inhibitors of NF-?B, a transcription factor with a critical role in promoting inflammation and connected with multiple aspects of oncogenesis and cancer cell survival. In particular, NF-?B activation has been shown to suppress cell death pathways by switching on genes that dampen pro-apoptotic signals. Moreover, NF-?B has been recently shown to be constitutively activated in different types of haematological malignancies, including multiple myeloma (MM) and Burkitt's lymphoma (BL), and has been proposed as a potential therapeutic target for these types of neoplasia. We now report that 15d-PGJ_2 potently induces apoptosis in MM and BL cell lines. 15d-PGJ_2-Induced apoptosis occurs through multiple caspase activation pathways involving caspase-8 and caspase-9 and is prevented by treatment with the pan-caspase inhibitor ZVAD. In both types of B-cell malignancies,15d-PGJ_2-induced apoptosis is associated with inhibition of constitutive NF-?B activity, followed by rapid down-regulation of NF-?B-dependent anti-apoptotic gene products, including cellular inhibitor-of-apoptosis cIAP-1, cIAP-2, XIAP and cFLIP. These effects were mimicked by the proteasome inhibitor MG-132, while the conventional chemotherapeutic drug dexamethasone had no effect. The results indicate that inhibition of NF-?B activity and of NF-?B-dependent expression of cell survival proteins plays a major role in the pro-apoptotic activity of 15d-PGJ_2 in aggressive B-cell malignancies characterized by aberrant regulation of NF-?B.