Long Glucocorticoid-Induced Leucine Zipper (L-GILZ) acts as a tumor suppressor by activating p53

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Glucocorticoid-Induce Leucine Zipper (GILZ) protein plays an important role in immune response as regulator of the anti-inflammatory and immunomodulatory effects of Glucocorticoids (GC). GC effects are in part due to the GILZ-mediated modulation of cell proliferation and differentiation. In particular GILZ directly interacts with many signaling proteins such as NF-kB, Ras and Raf. Notably GILZ inhibits MAPK/ERK/AKT pathways, thus functioning as a physiological brake of cell proliferation.

The GILZ locus gene is characterized by two main isoforms, GILZ and a longer isoform, long GILZ (L-GILZ), that are expressed differentially in various tissues and that share about 70% homology. The high homology in most of the functional domains suggests that L-GILZ can acts as a suppressor of cell growth and differentiation.

The tumor protein 53 (p53) functions as a transcription factor and through its target genes it regulates a variety of cellular functions, including DNA repair, senescence, cell differentiation, cell-cycle progression and apoptosis. Activation of p53 can inhibits neoplastic transformation repressing the propagation of tumor cells. Of note, p53 regulation and expression is well modulated and Mdm2 plays the major role in regulating p53 levels.

We here show that a direct interaction of L-GILZ with both p53 and Mdm2 can modulate p53 activation. Briefly we demonstrate that the affinity binding between L-GILZ and Mdm2 is higher than that for p53 thus preventing p53/Mdm2 interaction and allowing to p53 activation.

Overexpression of L-GILZ in human colorectal carcinoma (HCT116) $p53^{+/+}$ results in inhibition of cell proliferation and induction of apoptosis. Moreover, in a mouse xenograft tumor model, the suppression of tumor growth was observed in L-GILZ-transfected $p53^{+/+}$ but not in $p53^{-/-}$ HCT116 cell line demonstrating that the L-GILZ antiproliferative effect depends on p53.

To demonstrate that L-GILZ induces p53 activation we investigated the expression of major targets downstream p53. We found that PUMA and p21 were upregulated specifically in L-GILZ-transfected $p53^{+/+}$ HCT116 cell and that L-GILZ increases the activity of a firely-luciferase p53 dependent promoter.

To further investigate the molecular mechanisms underlying L-GILZ effects on p53 activation, we perform WB and Real-Time PCR of p53 expression in cells expressing L-GILZ. We found that L-GILZ modulates p53 protein but not RNA expression. Furthermore, in L-GILZ-transfected $p53^{+/+}$ cells treated with CHX, the level of p53 were higher than that found in controls, suggesting that L-GILZ stabilizes p53 half-life. Finally we also found that L-GILZ induces ubiquitination of Mdm2 and de-ubiquination of p53.

These data suggest that L-GILZ acts as p53 regulator and may represents a promising novel anti-tumor therapeutic strategy.