ABSTRACT

TP53 is a tumor suppressor gene whose product is involved in cellular growth inhibition, apoptosis and senescence. Two new classes of proteins recently discovered, p63 and p73 proteins, share with p53 a high structural homology and overlapping yet specific functions.

p63 and p73 can be expressed as TA forms that have the Transcriptional Activation Domain, behave as p53-like proteins and show variability at the C-terminus due to alternative splicing. Indeed p63 and p73 can be also expressed from an alternative promoter or by N-terminal alternative splicing and the products are ΔNp73 or ΔTAp73 forms that are truncated at the N-teminus and act as dominant negative proteins of the other p53 family members.

Although p53 family proteins share the main functional domains and activate the transcription of a subset of common genes, unlike p53, p73 and p63 do not have clear features of tumor suppressors. Actually their activity is more complex and still not well defined. The entire p73 network of proteins, in fact, is involved in neuronal differentiation, in the apoptotic response to damaging agents (cisplatin, IR, doxorubicin) and in tumorigenesis.

TP73 gene is transcriptionally regulated by E2F1, in the G1/S transition and in the DNA damage or oncogenes activated apoptotic response. The p73 protein functions are modulated by post-translational modifications and protein-protein interactions in different physiopathological cellular contexts.

The aim of this PhD thesis has been the characterization of post-translational modifications that regulate p73 transcriptional functions upon DNA damage and in physiological contexts.

A new post-translational modification induced by Doxorubicin treatment has been identified during the first part of my PhD experience. In fact, upon Doxo treatment p73 protein is induced and acetylated by p300 acetyltransferase. The region involved in acetylation has been found using deletion and point mutation mutants: it maps between the DNA Binding Domain and the Oligomerization Domain and the Lysines involved are K321, K327 and K331. The not acetylable p73RRR mutant binds DNA and activates the transcription of p21 target gene but is unable to activate DNA damage apoptotic response. Thus DNA damage-induced p300-mediated acetylation of p73 seems to be important to recruit p73 transcription factor directly on apoptotic genes (p53AIP1) rather than on growth arrest genes (p21). Moreover p73 acetylation is dependent on tyrosine-kinase c-Abl activity, as well involved in pro-apoptotic p73 functions induced by damaging agents (IR and cisplatin).

The study of p73 post-translational modifications continued with the attempt to assess how p73 activity is regulated during the cell cycle.

p73α and p73β are phosphorylated in normal mitotic cells or in spindle drug arrested mitotic cells and the $p34^{cdc2}$/cyclin B1 complex has been identified, by *in vitro* kinase assays, as responsible for this phosphorylation. Moreover p73 interacts with and is phosphorylated by the $p34^{cdc2}$/cyclin B1 complex in the amino-terminal region on the Threonine 86 residue. Thr86 phopshorylation reduces p73 DNA binding and transcriptional activities during mitosis and delocalizes the protein from condensed mitotic chromatin. In late telophase p73 lacks phopshorylation and is able to localize again with deconsensed chromosome likely resuming its transcriptional activity. Interfering with p73 expression and function, by specific siRNAs or by the use of the dominant negative p73DD mutant, results in an altered mitotic progression, formation of mitotic abnormilities and increase in binucleated cells. Thus p73 seems to be involved in M/G1 transition and in the correct cell division.

Taken together these results suggest that p73 activating transcriptional functions can be specifically modulated by post-translational modifications in different physiopathological contexts.

ABSTRACT

INTRODUCTION

CHAPTER 1.
p73 and the p53 family: a complex network of proteins

CHAPTER 2.
p73 functions are modulated by post-translational modifications

RESULTS

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INTRODUCTION