Chapter 3

Identification of Oxidative Stress-Induced Tyrosine Phosphorylated Proteins by Immunoprecipitation and Mass Spectrometry

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Summary

Oxidative stress is the result of an increased presence of reactive oxygen species (ROS) in cells and is able to promote, among others, protein and lipid oxidation, DNA damage, mutagenesis, oncogenic activation, or inhibition of tumour suppression, resulting in pathological processes such as myocardial dysfunction or carcinogenesis. External treatment of cells with oxidants such as H_2O_2 or high intracellular levels of ROS has been shown to trigger protein tyrosine phosphorylation. This occurs, at least in part, through the oxidation of reactive cysteine groups in protein tyrosine phosphatases resulting in an inhibition of their activities. Herein, we focus on the characterization of stress-induced protein tyrosine phosphorylation events in a cellular model of human mammary luminal epithelial cells (HB4a cells) stimulated with H_2O_2, in an attempt to better understand the mechanisms by which oxidative stress could promote such phenomena. Thus, immunoprecipitation with anti-phosphotyrosine antibodies and mass spectrometry have allowed us to identify a number of phosphorylated proteins that respond to oxidative stress and thereby further probe the effects of these changes on cellular function.

Key words: Oxidative stress, H_2O_2, Tyrosine phosphorylation, Immunoprecipitation, Mass spectrometry, LC-MS/MS.

1. Introduction

In the last decades, ROS have been shown to play a pivotal role in aging, degenerative diseases, immuno-defence, and cancer *(1–3)*. Apart from the well-known defensive role assigned for these oxygen species in phagocytic cells *(4)*, they also seem to participate in many diverse biological systems. For example,

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