Defining protein function and activity associated to different pathologies by combining molecular, cellular, biochemical and analytical methods

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Although recently, new strategies such as balanced diets, reduction of alcohol consumption and tobacco, exercise, limited UV exposure are proposed as prevention measures for different maladies such as cancer, neurological, cardiovascular diseases etc., their incidence is still growing worldwide. Thus, the molecular and biochemical investigation of different proteins dysregulated in such pathologies is of high interest for the prevention, stadialization and for the treatment of different disorders.

Taking advantage of different available tools in gene regulation such as knock-down, stable-overexpression or knock-out, we modulate expression of different proteins in order to study their function or control their activity in cell lines. We have achieved these goals by coupling cellular tools with biochemical methods such as: SDS-PAGE, Western Blot, Immunofluorescence, gradient ultracentrifugation and analytical ones such as Mass Spectrometry (MS) and Liquid Chromatography (LC).

More precisely, we showed that tyrosinase N-glycans, a tumor antigen overexpressed in melanoma cells, are important for the immune response activation by using single or triple tyrosinase glycosylation mutants overexpressed in A375 amelanotic melanoma cells.

Additionally, since perturbation of Endoplasmic Reticulum (ER) homeostasis it is associated with various pathologies, we focused our attention on modulating the expression of Endoplasmic Reticulum Associated Degradation (ERAD) components able to restore ER homeostasis. Our results demonstrated that downregulating or stable overexpression of ER degradation–enhancing α-mannosidase-like proteins (EDEM1 and EDEM2), highlighted new proteins from the degradation complex involved in cellular homeostasis restauration, such as SEL1L and DERL1, components of retrotranslocation complex. Moreover, by identifying new ERAD substrates our studies contributed to the enrichment of the immune repertoire associated to melanoma, able to elicit an immunological response.

All these data suggest that combining genetical tools with biochemical and analytical methods can result in the identification of new components potentially involved in prognostic and treatment of different pathologies.

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