MOLECULAR IMPLICATIONS OF PROHIBITIN-1 DEFICIENCY IN LIVER HOMEOSTASIS

E. Bigaud [(1)]***, V. Sánchez-Quiles*** [(1)]***, E. Santamaría*** [(1)]***, F. Corrales*** [(1)]***.***

[(1)] Centro de Investigación Médica Aplicada (CIMA)

Prohibitin (Phb) is a multifunctional protein participating in a plethora of essential cellular functions, such as cell signalling, apoptosis, survival and proliferation,and might play a prominent role in hepatocellular carcinoma (HCC) progression, although the molecular mechanisms are still obscure. Phb1 silencing was carried out on human hepatoma cell line PLC/PRF5 using siRNA oligonucleotides. Phb1 deficiency leads to an increase of apoptosis and severely compromised the capacity of PLC cells to proliferate in a semisolid substrate. Both cell counting and formazan production revealed a significant reduction of the proliferation rate of prohibitin deficient (siPHB) cells. 2D-DIGE and MS/MS analysis of cytosolic and microsomal fractions allowed the identification of 24 and 6 unique proteins.Down-regulation of Calreticulin, ERp29 and in siPHB cells suggest ER stress that, in turn, might participate in the apoptotic response of PLC cells to Phb1 silencing. In agreement to this hypothesis, we found increased CHOP levels, PARP cleavage and activation of caspase 12 and downstream caspase 7. ER stress might result from proteasome malfunction leading to the accumulation of miss-folded polypeptide chains in the cell. Phb1 silencing induced down-regulation of proteasome activator complex subunit 2 and stathmin suggesting impairment of proteasome activity. Deficient proteasome activity was evidenced by the accumulation of ubiquitinated proteins upon Phb silencing. The pivotal role of Phb1 in liver cell homeostasis is further supported by findings indicating that partial deficiency of this protein leads to an increased sensitivity of murine liver to a choline and methionine deficient diet. Over production of proinflammatory cytokines might mediate this effect. Taken together, these results provide evidences supporting the participation of prohibitin in central cellular pathways regulating the fate of hepatocytes.