Nuclear localization and new isoforms detection give new insights on Hsp10 functions in normal and cigarette smoke-stressed lung cells

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Heat-shock protein (Hsp)10 is the co-chaperone for Hsp60 inside mitochondria, but it also resides outside the organelle. Variations in its levels and intracellular distribution have been documented in pathological conditions, e.g. cancer and chronic obstructive pulmonary disease (COPD). Cigarette smoke (CS) is a potent stressor for the respiratory system, but its effects on the expression, function, and cellular localization of mitochondrial chaperonins are still largely unknown.

We studied in vivo (airways biopsies) the localization of Hsp10 and Hsp60 in patients (smokers and non-smokers) affected by mild-moderate COPD, and characterized the effects of non-lethal doses of CS extract (CSE) on the expression of these molecules in two human cell lines: lung fibroblasts (HFL-1) and bronchial epithelial cells (16HBE). We applied various in vitro methods: IHC, subcellular fractionation analyses (SFA), western blotting (WB), ICC, transmission electron microscopy (TEM) immunogold, chromati protein extracts (CPE), as well as 2D-gel based proteomics analyses. Bioinformatics was used to gather structural in silico data.

IHC showed that Hsp10 occurred in nuclei of epithelial and lamina propria cells of bronchial mucosa from non-smokers and smokers. ICC, SFA, and WB showed that 16HBE and HFL-1 cells featured nuclear Hsp10, before and after CSE exposure; TEM immunogold further confirmed this observation. Proteomics data showed that CSE stimulation did not increase the levels of Hsp10 but did elicit qualitative changes as indicated by molecular weight and isoelectric point shifts. Bioinformatics analyses indicated that Hsp10 can localize in extramitochondrial sites, such as the nucleus, even if Hsp10 lacks known DNA-binding motifs or nuclear import signals. Hsp10 nuclear levels increased after CSE stimulation in HFL-1, indicating cytosol to nucleus migration, and although Hsp10 did not bind DNA, it bound a DNA-associated protein as suggested by CPE/gel retardation experiments.

Data reported here indicate that in human cells of the respiratory mucosa there are at least three different intracellular locales for Hsp10: mitochondrial, nuclear, and cytosolic. Further experiments are en route for the definition of the mechanisms underlying the transfer of Hsp10 to the nucleus and other cellular/extracellular compartments.

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Keywords

Hsp10; lung cells; COPD; cigarette smoke; nuclear localization.