Entrega de l'Abstract

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Títol de la recerca: Aberrant activation of DFF40/CAD endonuclease causes distinct apoptotic nuclear phenotypes in human neuroblastoma-derived cells.

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Abstract:

Background: Apoptosis is a highly regulated cellular program of cell demolition. Its deregulation conducts to several diseases, including cancer. Caspases (cysteine proteases) are activated during the execution of the apoptotic program. They selectively cleave vital cellular substrates, conducting to the two apoptotic hallmarks: nuclear chromatin condensation and internucleosomal DNA fragmentation, achieved by the specific activation of an endonuclease (DFF40/CAD). Unveiling the role of DFF40/CAD should be crucial for understanding the relevance of apoptosis ending in cancer development.

Methodology: Cellular viability and survival were assessed by MTT and Trypan Blue exclusion assays, respectively. Caspase activation was determined by Western-blot, and by *in vitro* cleavage of a fluorescent substrate. Subcellular protein distribution was performed by a novel subfractionation protocol. *Cell-free* assays were also performed. Hoechst-stained nuclei were visualized under an UV-fluorescence microscope.

Results: In this work we have analysed both oligonucleosomal DNA fragmentation and apoptotic nuclear morphology in different human neuroblastoma-derived cells cultured in the

presence of staurosporine, a well-known apoptotic trigger. Among them, we found that SH-SY5Y cells presented both characteristics, SK-N-AS cells showed apoptotic nuclear fragmentation without displaying internucleosomal DNA fragmentation and LAI-5S cells lacked both phenotypes. Firstly, we found that DFF40/CAD was present in both the cytosolic and the nuclear fractions of SH-SY5Y cells. However, DFF40/CAD was poorly localized in the cytosolic fraction of healthy SK-N-AS cells. In these cells, the overexpression of DFF40/CAD restored their cytosolic levels and recovered their ability to degrade chromatin. Secondly, LAI-5S cells demonstrated a certain resistance against staurosporine-induced cell death, which correlated with a partial activation of both caspase-3 and 7 and a partial translocation of DFF40/CAD to the nucleus. However, pro-apoptogenic proteins were properly released from the mitochondria and caspase-9 and 6 were efficiently activated. Overexpression of the endonuclease restored the classical apoptotic nuclear morphology, but the most striking fact was that it could be restored by the sole transfection of an empty pcDNA3 plasmid.

Conclusions: The levels of DFF40/CAD in each subcellular compartment are cell-specific: in cells displaying oligonucleosomal DNA degradation (SH-SY5Y cells), the endonuclease is located in both cytosol and nucleus, whereas in cells defective in DNA laddering generation (SK-N-AS and LAI-5S cells) DFF40/CAD is not enriched in the cytosolic fraction. Then, the cellular resistance to complete apoptosis can be due to an alteration in the subcellular localization of DFF40/CAD. Therefore, a new mechanism that could in part explain the resistance to apoptosis after chemotherapy displayed by aggressive neuroblastomas is unveiled.

Authorship: Firstly, I have actively contributed in the performance of some experiments in the part of SH-SY5Y and SK-N-AS cells, which has been recently published in *The Journal of Biological Chemistry* and where I signed as third author. Secondly, I have contributed in the design of the project of LAI-5S cells and in carrying out all the experiments. Data obtained from LAI-5S results will be compiled as a manuscript and sent to a peer review international journal shortly. This work has been performed in the context of *Beca de col·laboració, curs 2011-12* (MEC-AGAUR).