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5-Fluorouracil mediated cell death signaling in colon carcinoma cells

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av
Birce Akpinar
M.Sc.

Huvudhandledare:
Magnus Olsson, PhD
Karolinska Institutet
Institute of Environmental Medicine
Division of Toxicology

Bihandledare:
Professor Boris Zhivotovsky
Karolinska Institutet
Institute of Environmental Medicine
Division of Toxicology

Fakultetsopponent:
Professor Karin Öllinger
Linköping University
Department of Clinical and Experimental Medicine
Experimental Pathology

Betygsnämnd:
Professor Sonia Lain
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology

Professor Stig Linder
Karolinska Institutet
Department of Ongology-Pathology

Docent Ingvar Ferby
Uppsala University
Ludwig Institute for Cancer Research

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Abstract

Colorectal cancer (CRC) is the third most common form of cancer in the world. 5-Fluorouracil (5-FU) belongs to the fluoropyrimidine-type of drugs and is commonly used in the treatment of several solid tumors. It has become the mainstay of CRC treatment at stage III and high risk stage II, either alone or in combination with other drugs. Primary drug effects include both DNA damage and RNA stress but the relative importance of each triggering points remains elusive.

In Paper I we investigated the signaling cascade leading to 5-FU-induced cell death in the colon carcinoma cell line HCT116 in more detail; especially, with respect to the early response and ensuing apoptosis. Upon 5-FU treatment, the death-inducing signaling complex (DISC) was formed on the plasma membrane and this process was facilitated by p53. Increase in intracellular levels of Ca^{2+}, at least partially via L-type channels, was an early response which led to phosphorylation of at least three p53 serine sites (S15, S33, S37) upstream of caspase-8. Mutational analysis concluded that S15 phosphorylation was necessary for the processing of caspase-8 and PARP. Analyses using small molecule inhibitors indicated that Ca^{2+}-dependent calmodulin served as an intermediate factor preceding p53 phospho-activation.

Altogether, obtained results present the evidence for a novel apoptotic signaling mechanism induced by 5-FU, dependent on extracellular Ca^{2+}, involving DR-DISC and regulated by p53, p53 phosphorylations and calmodulin.

In Paper II we focused on cell death signaling pathways in 5-FU-stressed *p53*$^{-/-}$ cells. Using the human colon carcinoma parental cell line HCT116 and its variant lacking p53, we found that the cell death *per se* induced by 5-FU is independent of p53. However, in the absence of the tumor suppressor, the apoptotic response is delayed. In addition, the lack of p53 was associated with the formation of reactive oxygen species (ROS) in mitochondria which resulted in necrotic characteristics. Co-treatment with zVAD-fmk and 5-FU revealed that DNA damage, reflected in phosphorylation of the histone H2AX (γH2AX), is a consequence rather than a cause of apoptosis. Finally, our data suggested that silencing of PARP-1 function may be used as an approach to selectively sensitize p53-deficient tumor cells to 5-FU.

In Paper III we examined the possible crosstalk between apoptosis and autophagy upon 5-FU treatment. In contrast to cells in which apoptosis was blocked, either at the DISC or the mitochondrial level, p53 deficiency was associated with deregulation of autophagy in response to 5-FU. Disruption of lysosomal function with chloroquine (CQ) caused a profound reduction in the appearance of apoptotic markers, as a consequence of death receptor (DR) accumulation in lysosomes and autophagosomes.

Since RNAi targeting of critical regulators of autophagy or inhibition of lysosomal cathepsins reversed apoptosis in different manners, it is unlikely that autophagy *per se*, but rather correct receptor transport is an important factor for 5-FU-induced cell death. Interestingly, apoptosis activated via TRAIL, the cognate ligand for DR5, remained unaffected in the presence of CQ, indicating that 5-FU activates the receptor by a discrete mechanism. Through depletion of membrane cholesterol or inhibition of cholesterol transport, the cytotoxicity of 5-FU was drastically reduced, thereby supporting the idea that correct trafficking of the receptor is important for 5-FU-mediated elimination of cells.

In conclusion, this study indicates a novel chemotherapy-induced mechanism for activation of DR5, which may have important ramifications on research conducted in the apoptosis and tumor treatment field.
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