MITOPHAGIC/AUTOPHAGIC INHIBITORS AS NEW STRATEGY TO OVERCOME CISPLATIN RESISTANCE IN CANCER

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Introduction: Cisplatin (CDDP) is employed to treat a multitude of cancer including sarcomas, ovarian and cervical cancers. The main problems in the clinical use are the severity of the side effects and the onset of resistance. It is well established that cisplatin resistance is a multifactorial phenomenon, but the mechanism is not completely understood. Typically, CDDP is known for its DNA-damaging properties, although recent studies highlight its ability to interact with additional nucleophilic targets such as phospholipids, proteins, RNA and mitochondrial DNA. The mtDNA, unlike the nDNA, does not possess effective repair systems, consequently it is more susceptible of mutations and oncogenic transformations. Mitochondria dysfunctions have been implicated in tumorigenesis but little is known about the their role in chemoresistance. Our study is aimed to explore the mitochondrial dynamics in CDDP resistant cells, in order to identify new potential targets to overcome resistance.

Material and methods: For this study it has been used an ovarian cancer cell line 2008e the corresponding CDDP-resistant clone C13, and a model of osteosarcoma sensitive (U2OS) and resistant to CDDP (U2OS pt). The mitochondrial network organization was analyzed by confocal microscopy and the results were confirmed by TEM analysis. Based on the different morphology observed in the resistant clones, we verified the expression of key players of the mitochondrial dynamic both in terms of mRNA levels and protein expression. Having identify Bcl-2/adenovirus E1B 19kDa interacting protein 3(BNIP3) as one of the possible actors in the resistant phenomena onset, BNIP3has been silenced and the knocked down cells were treated with CDDP to verify the effect on drug sensitivity. Moreover, we tested two selective inhibitors of vacuolar sorting protein 34(Vps34), involved in the initial stages of autophagosomes formation.

Results: The analysis of the mitochondrial network morphology show a network less interconnected and more fragmented in C13and U2OS-PTcells (CDDP resistant) than in 2008and U2OS cells. The evaluation of the key regulators of fusion and fission processes in cells, suggests that in cisplatin resistant cells there is an imbalance toward fission process. In fact, resistant clones express higher levels of fission proteins (H-Fis1mRNA levels and Mffs protein in C13, H-Fis1and DRP1mRNA and Mffs proteins inU2OS-Pt). Mitochondrial fragmentation could help to segregate dysfunctional mitochondria and their removal through mitochondria-selective autophagy (a process named mitophagy). Results regarding BNIP3in our cell lines pointed out a higher expression of BNIP3mRNA and protein in the resistant clones. Thus, to verify if the upregulation of mitophagy is implicated in the resistance to CDDP, we silenced BNIP3in our cells. Treatment with CDDP showed similar cell viability curves in resistant and sensitive cell line indicating a potential action of mitophagy in promoting cancer survival. Since in commerce there aren't specific mitophagic inhibitors, we use two highly specific small molecule inhibitors of Vps34and co-treated the cancer cells with CDDP in association with them. Results showed that they are able to increase the sensitivity to the CDDP cytotoxicity

Discussion and conclusions: From this work we deduce that the mitophagic process could avoid accumulation of mtDNA damage caused by cisplatin, thus promoting cell survival. BNIP3ablation and the inhibition of autophagy could restore CDDP sensitivity to CDDP sensitivity to resistance cells. Thus, the combination of CDDP with mitophagic/autophagic inhibitors could represent a novel therapeutic approach to counteract CDDP resistance.