The intracellular death pathways involved in zoledronic acid-induced apoptosis of human breast cancer cell lines

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Introduction. Bisphosphonates (BP) are used in the treatment of metastatic bone disease in women with breast cancer (BC). In addition to their established role as inhibitors of osteoclast activity and bone resorption, BPs can directly induce tumor cell death and inhibition of tumour cell migration (1). Among BPs, zoledronic acid (ZA) demonstrated the highest antitumor activity (2). Some reports indicate that ZA can induce BC cell death, but its mechanism of action is still not completely understood (3), although the inhibition of the mevalonate pathway has been suggested (4). The aim of this study is to investigate the effects of ZA treatment in human BC cell lines with different hormonal receptor expression.
Methods. BC cells were treated for 72hrs with ZA in the presence of 3,7,11-trimethyl-2-6,10-dodecatrien-1-ol, farnesol mixed isomer (FOH), according to the experimental conditions. A double staining with Acridine Orange and Ethidium Bromide (AO/EtBr) was performed to visualize and quantify the number of viable, apoptotic and necrotic cells (5). The apoptotic cell death of MCF7 and MDA-MB-231 cells was confirmed using the M30 CytoDeath ELISA (Vinci-Biochem, Firenze, Italy). Expression level of miR-21 was determined by qRT-PCR (Qiagen, Milano, Italy) and the expression of PTEN was measured by Western blot (6). To investigate the molecular pathway involved in ZA-induced apoptosis, the Human Apoptosis Array was performed (R&D Systems, Milano, Italy). Data analysis and graphycs were obtained with the GraphPad Software (La Jolla, CA, USA), using one-way ANOVA and Bonferroni's Multiple Comparison test, considering $p<0.05$ as statistically significant.
Results. MCF7 (ER+,PR+,Her2-), MDA-MB-231 (triple negative) and SKBR3 (ER-,PR-,Her2+) cell lines were treated with the calculated ZA IC_{50} (Fragni et al. unpublished results). The maximal induction of apoptosis was found in MCF7 and MDA-MB-231 cells, that was 76.18±0.89% and 58.55±2.93%, respectively, while ZA induced in SKBR3 cells a necrotic death. The MCF7 and MDA-MB-231 apoptotic cell death was confirmed by a significant increase in the M30 antigen level. We thus drawn our attention to the apoptotic pathway activated by ZA. The oncomir miR-21 was significantly decreased by ZA exposure (-28±0.01% in ZA treated-MCF7, -34±0.07%, in ZA treated-MDA-MB-231), while the expression of its pro-apoptotic target protein PTEN was increased of about 30±2.9% in MCF7 and 57±6% in MDA-MB-231 cells. The Human Apoptosis Array conducted in untreated- and ZA treated-BC cells revealed that in MCF7 cells the expression level of 27 proteins (pro-apoptotic, anti-apoptotic, death receptors, involved in adaptative response to stress and cell cycle regulation) was modified by ZA, while in MDA-MB-231 cells, 6 proteins (pro-apoptotic, anti-apoptotic and death receptors) were modified. The mevalonate pathway inhibition induced by ZA can be bypassed by FOH; indeed, pretreatment of BC cells with 40uM FOH reverted the ZA induced apoptotic cell death.
Conclusions. In human BC cell lines, ZA treatment induced an expression level modification of many proteins involved not only in the apoptotic pathway, but also in the cell cycle and in the adaptative response to stress. These effects were reverted by the addiction of FOH, thus indicating that the mechanism of action of ZA was linked to the inhibition of the farnesyl-PP synthase enzyme, involved in the mevalonate pathway. Present results suggested a mechanism of action of ZA-induced BC cell death and give support to the current use of ZA as a coadjuvant drug in BC chemotherapy (7).

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