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abstract book

Abstract Book

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ONCOBIOLOGY 08

Role of Autophagy in breast cancer cells following exemestane treatment

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Introduction: Breast cancer is the most common cause of cancer cell death in Women Worldwide. Nowadays, there are several therapeutic approaches for hormone-dependent (estrogen receptor positive [ER+]) breast cancers, being one of them based on the inhibition of the enzyme aromatase. Aromatase inhibitors (AIs), which block the conversion of androgens to estrogens, correspond to an effective alternative to the classical tamoxifen in the treatment of ER^+ breast cancers. Exemestane is a third-generation steroidal AI that is a mechanism-based inhibitor that binds covalently and irreversibly, inactivating the enzyme aromatase. Though, the biological effects of exemestane in breast cancer cells are not totally understood. In that way, the effects of exemestane on the mechanisms of cell death were studied using an ER+ aromatase-overexpressing breast cancer cell line (MCF-7aro).
Methods: The biological effects on MCF-7aro cells treated with testosterone and different concentrations of exemestane (10 – 15 μM) with or without 3-methyladenine (3-MA), an inhibitor of autophagy, were studied during different times of incubation (3 - 6 days). Phase contrast microscopy, Giemsa and acridine orange staining were used to study the morphological alterations induced by exemestane. Cell viability was evaluated by MTT assay, determination of caspase-9 and caspase-8 activities were performed by luminescent assays and production of intracellular reactive oxygen species (ROS) by a fluorescent assay. The formation of acid vesicular organelles (AVOs) and the presence of LC3 protein were studied by flow cytometry and Western-Blot, respectively.
Results: In MCF-7aro cells, exemestane induced chromatin condensation and fragmentation, a decrease in cell viability, activation of caspase-9 and production of ROS, which suggest the occurrence of cell death by apoptosis via mitochondrial pathway. This was accompanied by cytoplasm vacuolization, and the appearance of AVOs and by the formation of LC3-II, typical features of autophagic cell death. Cells treated with exemestane plus 3-MA, presented a reduction on cell viability, an activation of caspase-8 and an increase in intracellular ROS, when comparing to exemestane treated cells. It also induced a reduction in AVOs and LC3 II formation.
Conclusions: Exemestane induced mitochondrial-mediated apoptosis and autophagy in MCF-7aro cells. Studies with 3-MA, suggested that autophagy act as a pro-survival process to protect breast cancer cells from cell death by apoptosis.

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ONCOBIOLOGY 09

Phenolic extract from the fruiting body and spores of a medicinal mushroom - *Ganoderma lucidum*: investigating their antioxidant potential and antitumor cell growth inhibitory effects

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Background: Mushrooms are a source of bioactive compounds, some of them with antioxidant properties or with antitumour potential (1). *Ganoderma lucidum* is one of the most extensively studied mushrooms due to its medicinal properties, as functional food and as chemopreventive (2). Some of the pharmacological properties of its compounds (e.g. polysaccharides, glucoproteins, triterpenes and steroids) have been related to antitumor activity, including cell cycle arrest and induction of apoptosis (3). Nevertheless, the bioactive properties of its phenolic compounds remain unevaluated.
Aim: It was intended to investigate the bioactive properties (antioxidant and antitumor potential) of the phenolic extract of the fruiting body and spores from this mushroom.
Methods: *Ganoderma lucidum* was collected in Northeast Portugal, and methanolic extraction was performed from the fruiting body and the spores of the mushroom. The extracts were submitted to evaluation of their antioxidant activity (radical scavenging activity, reducing power and lipid peroxidation inhibition in animal cell homogenates) and cell growth inhibitory activity in four human tumour cell lines (MCF-7, NCI-H460, HCT15 and AGS), by the sulforhodamine B assay.

Results: The fruiting body phenolic extract revealed higher antioxidant properties (EC_{50} values ranging between 0.10 and 0.62 mg/mL) than the corresponding spores extract (EC_{50} values ranging between 0.58 and 1.61 mg/mL). Furthermore, the fruiting body phenolic extract presented a moderate growth inhibitory activity in all the tested cell lines (GI_{50} values ranging between 93.3±18.1 and 112.6±11.7μg/mL), while the spores extract presented poor cell growth inhibitory activity in the H460 and HCT15 cell lines and GI50>400 μg/mL in the other cell lines in study.

Conclusion: In spite of the well-known medicinal properties of *Ganoderma lucidum*, so far we found no evidence, in the phenolic extract of the fruiting body and spores, for a strong cell growth inhibitory potential. Further studies will be performed to optimize extraction procedures in order to obtain compounds with higher bioactivity.

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ONCOBIOLOGY 10

Looking for ALDH expression and activity in canine mammary tumours and cell lines

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Introduction: Mammary cancer represents approximately 25 to 50% of all neoplasias in female dog, constituting an important disease in the veterinary setting. Currently, one of the most motivating concepts that is being explored in the cancer research field is the cancer stem cell hypothesis, which states that a minority of transformed cells, with acquired stem or progenitor properties, are the source of tumour cell renewal and thereby determine tumour behaviour. In humans, breast cancer stem cells have been identified using sphere-forming assays, expression of surface markers (1, 2) and by aldehyde dehydrogenase (ALDH) activity by Ginestier (2007), who showed that normal and cancer human mammary epithelial cells with high ALDH activity have stem/progenitor properties (3). Cancer stem cells in canine mammary tumours have been identified using the sphere forming assay (4-6) and, most recently, a unique study identified and characterized cells expressing $ALDH^+$ in established canine mammary carcinoma cell lines (7). **Material and Methods:** A series of 103 mammary carcinomas obtained from female dogs were immunohistochemically stained for ALDH1 (Abcam). Both epithelial and stromal ALDH1 expression was evaluated. Epithelial expression was classified as positive when more than 1% of neoplastic cells showed clear cytoplasmic staining. Stromal expression of ALDH1 was classified in two categories: none/weak, or moderate/strong. In three canine mammary carcinoma cell lines (CMT-1m, CMT-2p and CMT-3p), previously established by our group, ALDEFLUOR assay was performed (Stem Cell Technologies, Grenoble, France), in order to analyse the cell population with ALDH enzymatic activity, using a FACS Calibur (BD Biosciences), according to the manufacturer's instructions. We also investigate ALDH1 expression through western blot analysis with the same antibody that was used in immunohistochemistry analyses. **Results and Discussion:** Immunohistochemical staining of ALDH1 showed 36 (34,3%) cases with epithelial expression, 4 of them exhibiting more than 25% of positivity. Moderate to strong stromal expression was observed in 23 cases (21,9%). These results demonstrated that in canine mammary tumours, cells have higher expression of ALDH1 than in human breast cancer where, in a total of 464 cases, only 33 (7,1%) were positive (1). The ALDH1 activity was evaluated in cancer cell lines: CMT-1m was negative for its activity (0%), whereas CMT-2p and CMT-3p demonstrated activity (about 5% and 1,2%, respectively). The presence of the protein of interest was found in CMT-2p by western blot. **Conclusions:** Although preliminary, this data showed that the frequency of ALDH1 expression is higher in canine mammary tumours compared with human breast carcinomas. Based on these observations, we consider that ALDH1 can have an important role in canine carcinogenesis, and more studies are needed to further explore this issue in the near future.

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