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Heterocyclic chalcone derivatives: Synthesis and biological activity evaluation

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Aim: Synthesis of new heterocyclic chalcone derivatives with promising antitumor activity.

Introduction: Natural chalcones have been intensively studied for their wide range of biological activities, namely antitumor.[1] Possessing two electrophilic reactive centers at α,β-unsaturated ketone group, chalcone derivatives can participate in addition reactions leading to the synthesis of promising bioactive compounds with a more rigid structure, like isoxazoles and pyrazoles.[2]

Methods: Chalcones were synthesized by base catalysed Claisen Schmidt condensation via microwave assisted organic synthesis (MAOS). The antiproliferative activity was assessed using sulforhodamine B assay.[3]

Results: Seventeen chalcone derivatives were synthesized and identified as having in vitro growth inhibitory activity on three human tumor cell lines from breast, lung and melanoma (MCF-7, NCI-H460, and A375-C5).

Conclusion: Most of the synthetized chalcones revealed to be promising growth inhibitors of human tumor cell lines. The molecular mechanisms involved in their antiproliferative effect are being evaluated.

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PS209

A posttranslational modification in histones as prognostic/predictive marker in Estrogen-Positive Breast Cancer

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Aim: This work aims to evaluate H3K27me3 expression in luminal-like breast tumors, using immunohistochemistry assay, to assess the prognostic value of this epigenetic alterations in estrogen positive breast cancer (BrC).

Introduction: BrC is the second most incident cancer worldwide. In Portugal, in 2012, BrC was simultaneously the leading cancer in incidence and mortality in women.[1] Around 70% of all BrC are hormone-receptor positive, that is the major part of breast tumors is luminal-like.[2] H3K27m3 is a gene repression marker[3,4] and is associated with gene silencing, playing a crucial role in cell proliferation and differentiation.[3] H3K27me3 may have some clinical value in several types of cancer since it can be used as a biomarker. This histone modification has been associated with poor prognosis of some BrC subtypes.[5]

Methods: It was used a cohort of BrC patients of the Portuguese Oncology Institution of Porto (IPO-Porto), diagnosed between 1994 and 2002. A total of 102 luminal-like tumor cases were assessed by immunohistochemistry, to H3K27me3 expression. To verify the prognostic value of H3K27me3 levels, Cox regression with a log rank test was performed for both disease-specific and disease-free survival.

Results: Through the result analysis, it was established that only tumor grade ($p = 0.021$) was significant associated with disease-specific survival. Nevertheless, both luminal subtype ($p = 0.016$) and H3K27me3 expression ($p = 0.012$) were significantly associated with disease-free survival. Indeed, H3K27me3 high expression is associated with higher recurrence risk, especially in Luminal A.

Conclusion: We could confirm the prognostic value of H3K27me3 expression in luminal A subtype BrC patients. Therefore, higher H3K27me3 expression in luminal A tumors is associated with a greater probability of recurrence.

However, studies in larger cohorts are mandatory to validate its clinical utility.

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PS212

Is P-glycoprotein relevant for the release of microvesicles by tumor cells?

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Aim: In this study, we aimed to verify if MDR cells without expression of P-gp also produced more microvesicles and less exosomes than their DS counterpart cells.

Introduction: Cancer multidrug resistance (MDR) is a major cause of chemotherapy failure and is highly associated with overexpression of drug-efflux pumps such as P-glycoprotein (P-gp). The identification of mechanisms specific of P-gp overexpressing cells may contribute to the identification of biomarkers of MDR.

It was recently discovered that a drug-resistant phenotype may be horizontally transferred from drug-resistant (DR) to drug-sensitive (DS) cells, mediated by the cargo of extracellular vesicles (EVs) released by DR cells and captured by DS cells. These EVs include smaller exosomes and larger microvesicles. Our previous work showed that MDR cells with overexpression of P-gp released more microvesicles than exosomes, unlike their DS counterpart cells. However, it is not known if this phenomenon is restricted to MDR cells with overexpression of P-gp or if it is extensive to all DR cells (with other mechanism of drug resistance).

Methods: Drug-response curves of MDR and DS counterpart cells were obtained, using resazurin and trypan blue assays, to confirm the resistant or sensitive phenotype of the cell lines. Confirmation of their P-gp status was possible by Western-Blot. EVs released by both DS and MDR cells were isolated by ultracentrifugation and characterized by transmission electron microscopy, dynamic light scattering, nanoparticle tracking analysis and Western blot analysis.

Results: We confirmed that MDR cells without expression of P-gp release EVs with similar sizes to the ones released by their DS counterparts.

Conclusion: So, P-gp may be associated with the release of larger EVs by MDR cells. These results will be further confirmed by characterizing the EVs released by P-gp overexpressing MDR cell lines following downregulation of P-gp expression and the EVs released by DS cell lines following transfection of P-gp.[1–4]

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PS215

Uterine protein oxidative modifications may condition trophoblast function

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Aim: Evaluate whether protein carbonylation resulting from uterine altered redox imbalance interferes with extravillous trophoblast viability.

Introduction: Local redox homeostasis is believed to have a pivot role in uterine transformation necessary for blastocyst implantation and placenta development. By contrast, redox status imbalance plays a role in deficient placentation and the development of pregnancy-related complications (e.g. preeclampsia or gestational diabetes) with increased incidence in older women. Thus, it was hypothesized that at an older reproductive age, loss of redox homeostasis is a contributor to disruption of foetal/placental interactions and the development of such complications.

Methods: Uterine human samples were collected at delivery by elective caesarean section. The protocol was approved by the ethical committee of "Centro Materno-Infantil do Porto", volunteers gave written consent to be included in the study. Total protein carbonylation was detected by oxyblot and protein expression was quantified by western blotting. Specific protein carbonylation was verified by immunoprecipitation. Albumin was carbonylated using hydrogen peroxide (H2O2), followed by dialysis, and western blotting to confirm carbonylated albumin. HST-8SV neo extravillous trophoblasts were treated with carbonylated/non-carbonylated albumin, followed by cell viability assay. A P value less than 0.05 was assumed to denote significant difference.

Results: At the placental site, carbonylated albumin normalized to total albumin expression showed a positive and significant association with maternal age. ($r = 0.6909$, $P = 0.0021$) In vitro, carbonylated albumin displayed a cytotoxic effect, at concentrations ranging from 10 to 100 μg/ml. Lower concentrations did not affect trophoblast viability.

Conclusion: Uterine aging is accompanied by selective albumin oxidative modifications, which appears to interfere with trophoblast ability to invade and transform the maternal placental site.

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