potential antineoplastic activity against leukemias. **Aim:** To evaluate the in vitro cytotoxicity of *Piper nigrum* (black pepper) oil and its isolated alkaloid, piperine, in the Nalm6 cell line derived from acute lymphoblastic leukemia. **Material and methods:** Cells were cultured in flasks containing RPMI medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin and maintained at 37°C in a 5% CO_2 atmosphere. The MTT assay was performed to evaluate the effect of the substances on Nalm6 cell viability. Cells were seeded in 96-well plates at a concentration of 30,000 cells/well and treated with black pepper oil and isolated piperine, previously dissolved in dimethyl sulfoxide (DMSO) to obtain initial concentrations of 15.4 and 12.2 mg/mL, respectively. A dose-response assay was then carried out with concentrations ranging from 100 to 1.56 μg/mL. After 72 hours of treatment, the supernatant was removed, and an MTT solution at 5 mg/mL was added and incubated for 3 hours in a CO_2 atmosphere. Subsequently, the solution was removed, and DMSO was added to fully dissolve the formazan crystals. Data analysis was performed using GraphPad Prism 9.0 software, with the experiment conducted in triplicate. **Results:** The main results were obtained from the evaluation of the mean inhibitory concentrations (IC50) of *Piper nigrum* compounds on cell growth. Both substances significantly reduced the viability of the Nalm6 cell line, with IC50 values of 21.6 μg/mL for black pepper oil and 14.1 μg/mL for the isolated alkaloid piperine, the latter showing greater cytotoxic effect. **Discussion and conclusion:** Cell viability assays demonstrate that both *Piper nigrum* essential oil and its major alkaloid, piperine, have relevant cytotoxic effects against the Nalm6 cell line, representative of ALL. The lower IC50 value for piperine compared to the essential oil suggests that the alkaloid is one of the main constituents responsible for the antineoplastic activity of *Piper nigrum*. These findings are consistent with recent investigations that address piperine as a potential antineoplastic agent in different types of cancer, particularly gastric cancer (RAMOS et al., 2023). Therefore, it is of great interest to conduct further biological tests comparing the use of the oil and the isolated compound, as well as to evaluate these substances in non-neoplastic hematological cells.

Referências:

Ramos INF, da Silva MF, Lopes JMS, et al. Extraction, characterization, and evaluation of the cytotoxic activity of piperine in its isolated form and in combination with chemotherapeutics against gastric cancer. Molecules. 2023;28:5587.

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ID - 2139

DECODING STROMAL ARCHITECTURE IN PANCREATIC TUMOR THROUGH SPHEROMAP CYTOMETRY: A PLATFORM FOR THERAPEUTIC DISCOVERY

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Introduction: Pancreatic ductal adenocarcinoma is characterized by a dense stroma predominantly formed by cancer-associated fibroblasts (CAFs). These cells are key drivers of tumor progression, immune exclusion and therapy resistance. While CAFs exist as two functionally distinct and reversible phenotypes—myofibroblastic (myCAFs) and inflammatory (iCAFs)—their spatial distribution and the mechanisms underpinning this heterogeneity remain poorly understood. **Aim:** Here, we present SpheroMap Cytometry, an innovative spatial flow cytometry method that enables high-resolution analysis of spatially organized cellular phenotypes within spheroids. This approach leverages Image-iT Green Hypoxia, a fluorescent probe that accumulates in hypoxic cells, enabling spatial mapping of cells dissociated from spheroids. **Material and methods:** Therefore, heterotypic spheroids composed of CAPAN-1 pancreatic tumor cells and HS-5 bone marrow stromal or umbilical-cord-derived mesenchymal stromal cells (UC-MSCs); or homotypic spheroids composed solely of HS-5 or UC-MSCs cells were cultured in ultra-low-adhesion 96-well plates. After 48h aggregation, spheroids were incubated with the hypoxia probe and after 72h they were dissociated and stained with CD73 and CD140B antibodies. **Results:** We identified a robust enrichment of $CD73^+$/$CD140B^+$ myCAF-like cells within the hypoxic core. In tumor-HS-5 spheroids, co-expression of CD73 and CD140B reached 59.27% in hypoxia *vs* 49.37% in normoxia ($p < 0.01$). Similarly, UC-MSC spheroids showed even stronger co-expression in hypoxia 80.57% *vs* 38.5% in normoxia ($p < 0.0001$). We observed the presence of a distinct subpopulation of small stromal cells (FSClow/SSClow) lacking expression of CD73 and CD140B. Upon exposure to hypoxia, these cells acquired CD73 and CD140B expression transitioning toward a myCAF phenotype as they also increased in size and complexity (FSChigh/SSChigh). Our findings point to the hypoxic tumor microenvironment (TME) not only as a trigger for phenotypic reprogramming, but also as a niche where immature or quiescent fibroblast cells are converted into immunosuppressive, tumor-supportive myCAFs. Moreover, while hypoxia alone was sufficient to drive myCAF differentiation in heterotypic (~70%) and monotypic spheroids (~40%), we found that normoxic induction of myCAF occurred only in tumor cells presence, reaching 47% (tumor–HS-5) and 30% (tumor–UC-MSCs), but dropping to ~10% and ~1% in monotypic spheroids. **Discussion and conclusion:** Our results raise the possibility that the spatial proximity of myCAF cells to tumor cells may not result solely from paracrine signaling. Instead, it may reflect their shared occupancy of hypoxic tumor regions that actively promote a myCAF phenotype. This has profound implications for understanding how CAF heterogeneity is shaped and how spatial organization influences immunosuppressive dynamics within the TME. By preserving hypoxia-derived spatial information post-dissociation, SpheroMap Cytometry bridges the gap between functional imaging and high-throughput

phenotyping. Overcoming limitations of both static microscopy and conventional flow cytometry, opening new avenues for preclinical assessment of stroma-targeting therapies and the development of immunotherapeutics that reprogram the TME. This study was funded by the São Paulo Research Foundation (FAPESP), Brasil, process #2022/12856-6; the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES); and the National Council for Scientific and Technological Development (CNPQ).

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DENDRITIC CELL MATURATION INDUCED BY MAGHEMITE-BASED NANOPARTICLES ASSOCIATED WITH METHYLENE BLUE FOR THE IN VITRO TREATMENT OF BREAST AND OVARIAN CANCER

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Introduction: Breast cancer, the second most common type, and ovarian cancer, the most lethal among tumors of the female reproductive system, stand out due to their high incidence and mortality rates. The development of new therapeutic strategies supported by nanotechnology has gained prominence owing to the controlled and targeted delivery of drugs. In parallel, the role of the immune system in cancer treatment has driven the advancement of numerous studies in the field of immunotherapy, particularly those focused on eliciting an effective immune response against tumors, such as approaches based on dendritic cell activation and maturation. Previous studies have demonstrated the in vitro efficacy of maghemite nanoparticles associated with methylene blue (MAGCIT-AM) for the treatment of breast and ovarian cancers. **Aim:** Based on these findings, the present study investigated the application of MAGCIT-AM with the aim of promoting an antitumor immune response in vitro for the treatment of breast and ovarian cancers. **Material and methods:** For this work was used two human breast carcinoma cell lines (MDA-MB-231) and one ovarian cancer cell line (A2710), MAGCIT-MB was synthesized through hydrothermal coprecipitation in an alkaline medium at 100°C, surface functionalization was performed using a citric acid solution, by applying the solution aggregation method (0.05 M) to maghemite nanoparticles at a concentration of 90 mg/L. The analysis was performed using flow cytometry (FACSCanto II and FACSCalibur). **Results:** Two human breast carcinoma cell lines (MDA-MB-231 and T-47D) and one ovarian cancer cell line (A2780) were used. MAGCIT-AM exhibited a hydrodynamic diameter of 60.93 nm, a polydispersity index of 0.199, and a zeta potential of −20.9 mV. Analysis of flow cytometry revealed modulation of lipid droplet biogenesis by BODIPY, reduction in mitochondrial membrane potential by JC-1 dye, and dendritic cell maturation, evidenced by increased expression of specific markers, like CD11c, CD86, HLA-DR, CD25 and CD40. **Discussion and conclusion:** Suggesting that cell death induced by MAGCIT-AM is capable of promoting dendritic cell maturation, thereby reinforcing its potential as a therapeutic strategy for breast and ovarian cancer.

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ID - 892

DIFERENCIAÇÃO ERITROCITÁRIA *IN VITRO* A PARTIR DE CÉLULAS-TRONCO DE PLURIPOTÊNCIA INDUZIDA UTILIZANDO CORPOS EMBRIOIDES: ESTABELECIMENTO E CARACTERIZAÇÃO DE PROTOCOLO

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Introdução: A diferenciação eritrocitária a partir de células-tronco de pluripotência induzida (iPSCs) tem se consolidado como abordagem promissora frente à crescente demanda por sangue e às limitações associadas à dependência de doadores. Diante da escassez global de bolsas e das restrições de armazenamento, a produção de eritrócitos *ex vivo* a partir de iPSCs surge como alternativa viável para aplicações em pesquisa e suprimento experimental. Esse modelo permite gerar eritrócitos sob condições biológicas controladas, favorecendo estudos em hematologia, infecções e modelagem de doenças. Além disso, supera limitações de métodos baseados em células mononucleares de sangue periférico, como baixa enucleação, variabilidade entre amostras e necessidade contínua de doadores. **Objetivo:** Este trabalho visa desenvolver e caracterizar um protocolo eficiente de diferenciação eritroide *in vitro* a partir de iPSCs, utilizando corpos embrioides (EBs) como etapa inicial, com foco na geração de reticulócitos maduros. Os EBs são estruturas tridimensionais formadas em suspensão, derivadas de iPSCs, que mimetizam os estágios iniciais do desenvolvimento embrionário e permitem a diferenciação celular nos três folhetos germinativos: ectoderma, mesoderma e endoderma, o que favorece a indução da linhagem hematopoética. **Material e método:** Foram utilizadas linhagens de iPSCs geradas em nosso laboratório. As células foram cultivadas até 80% de confluência, dissociadas com *enzyme-free* para formar aglomerados de 30–50 células e plaqueadas em meio StemFlex, em placas *ultra-low attachment*, para formação dos EBs, induzidos à diferenciação após 24 horas. O protocolo foi estruturado em três etapas. Na fase 1, os EBs permaneceram em suspensão em meio RPMI com KOSR, BMP4, IWP2, VEGF, Y-27632 e b-FGF, promovendo o comprometimento mesodermal. Na fase 2, foram transferidos para placas aderentes e cultivados com BMP4, VEGF, b-FGF, SCF, IGF-2, heparina, IBMX e SR1, favorecendo a expansão hematopoiética. Na fase 3, utilizou-se transferrina, insulina,