Primary breast tumor spheroids express an organized extracellular matrix limiting doxorubicin efficacy

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Tumor microenvironment and specifically the extracellular matrix (ECM) modulates cancer cell behavior and response to chemotherapy. Collagens are the main components of ECM, and many collagen proteins are overexpressed in tumors, giving the characteristic stiffness of the tumor microenvironment. We developed an *in vitro* model to mimic the *in vivo* microenvironment and specifically the interaction of tumor mass with the surrounding ECM. Breast carcinoma in rats was induced with a single dose of 7,12-Dimethylbenzatracene (DMBA), intraperitoneally administered in 5 weeks old female Wistar rats. The explanted tumor mass was treated with recombinant collagenases (Class I and Class II) and thermolysin, and a heterogenous cell population, such as epithelial-tumor cells and fibroblast, was isolated. Tumor spheroids were generated in low attachment plate and after 6 days of culture, confocal microscopy revealed the presence of a collagen matrix. Proteomic analysis identified a complex matrix organization on 3D spheroids compared to 2D cell culture. Therefore, to investigate in vitro the ECM involvement in the modulation of Doxorubicin effects, spheroids were treated with recombinant collagenases for the digestion of the ECM to reduce the thick and dense collagen matrix around them. Doxorubicin cellular uptake studies highlighted the role of ECM as a barrier, limiting drug penetration, and consequentially, collagen degradation increases doxorubicin cellular internalization. Moreover, treating spheroids with collagenases prior to Doxorubicin incubation has been shown to significantly enhance drug cytotoxicity effects. Overall, isolated primary cancer cells were used as reliable sources to generate *in vitro* 3D spheroid that mimic the tumoral ECM. These spheroids have a distinct ECM organization, which enhances chemoresistance, impairing the efficacy of anticancer drugs.