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Resumo	Three-dimensional (3D) cell culture technologies, which more closely mimic the complex microenvironment of tissue, are being increasingly evaluated as a tool for the preclinical screening of clinically promising new molecules, and studying of tissue metabolism. Studies of metabolites released into the extracellular space (secretome) allow understanding the metabolic dynamics of tissues and changes caused by therapeutic interventions. Although quite advanced in the field of proteomics, studies on the secretome of low molecular weight metabolites (< 1500 Da) are still very scarce. We present an untargeted metabolomic protocol based on the hybrid technique of liquid chromatography coupled with high-resolution mass spectrometry for the analysis of low-molecular-weight metabolites released into the culture medium by 3D cultures and co-culture (secretome model). For that we analyzed HT-29 human colon carcinoma cells and 3T3-L1 preadipocytes in 3D-monoculture and 3D-co-culture. The putative identification of the metabolites indicated a sort of metabolites, among them arachidonic acid, glyceric acid, docosapentaenoic acid and beta-Alanine which are related to cancer and obesity. This protocol represents a possibility to list metabolites released in the extracellular environment in a comprehensive and untargeted manner,

	opening the way for the generation of metabolic hypotheses that will certainly contribute to the understanding of tissue metabolism, tissue-tissue interactions, and metabolic responses to the most varied interventions. Moreover, it brings the potential to determine novel pathways and accurately identify biomarkers in cancer and other diseases. The metabolites indicated in our study have a close relationship with the tumor microenvironment in accordance with the literature review.
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