Identification of a novel biomarker of HIF-1alpha-mediated doxorubicin resistance in 3D cancer spheroid models

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Background: Chemotherapy resistance is a major limiting factor in cancer field, requiring the use of extensive chemotherapeutic agents to achieve an effective anti-tumour properties, and promoting severe cardiotoxicity. Because hypoxia is associated with chemoresistance, we investigated whether a hypoxia-regulated transcription factor (HIF-1) regulates the expression of CD73, a purinergic-ecto enzyme implicated in cancer pathogenesis and chemoresistance. We also hypothesized that a cytokine, prokineticin-2 (PK2) which has a HIF-binding site at its promoter, may be an adverse prognostic indicator of chemoresistance in cancer. To investigate this, 3D breast and lung cancer spheroids were exposed to doxorubicin (Dox), an anthracycline applied in clinic as a first-line chemotherapeutic agent for the treatment of a variety of solid cancers.
Methods: We generated two different types of 3D human breast cancer spheroids by co-culturing MCF7 or MDAMB231 cells with endothelial cells (EC) and fibroblasts (FB) on ultra-low attachment plates. To form the lung cancer spheroids, lung adenocarcinoma cells (A549), EC and FB were used. After 7 days, the spheroids were treated with different Dox concentration at the indicated time, then spheroid viability (CellTiter-3D assay), apoptotic cell rate (tunel assay/active caspase-3 staining) and HIF-1alpha protein expression (immunofluorescence labelling) were assessed. The enzymatic activity of CD73 for the conversion of extracellular ATP to adenosine was also examined on the spheroid cryosections. The release of PK2 in the conditioned medium of spheroids was evaluated by ELISA assay.
Results: We observed a significant dose-dependent decrease in viability of cancer spheroids only after 72 hours of Dox treatment. However, no changes in spheroid viability were observed after 24- or 48-hours of treatment even with the highest concentration of Dox, underscoring the chemoresistance of our 3D model. The presence of a mechanism able to counteract Dox cytotoxicity was confirmed by dose-indipendent increase in the rate of active caspase-3 positive cells. In these cancer spheroids, HIF-1alpha was significantly and dose-dependently upregulated and contributed to Dox resistance by stimulating the CD73 enzymatic activity. Moreover, the release of PK2 in spheroid-conditioned medium directly correlated with HIF1-alpha level and inversely correlated with Dox-mediated reduction in spheroid viability.
Conclusions: Our study demonstrated that 3D models can mimic Dox resistance of *in vivo* human breast and lung cancer. Specifically, we have shown that HIF-1alpha is involved in Dox resistance via stimulation of the CD73 pathway. Although further studies are needed, we have, for the first time, identified PK2 as a potential biomarker of HIF-1alpha-mediated Dox resistance, at least in breast cancer and lung adenocarcinoma.