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Ultrastructure of immunogenic cell death in vivo

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Identification of effective combinations of immunotherapies with chemotherapies and targeted anti-cancer agents requires an integrated understanding of the effects of the combination on the tumor and associated stromal cells. We have developed an integrated technological platform for this purpose that uses an implantable microdevice for delivery of dozens of drugs or drug combinations into spatially separate regions of a single living tumor and multiplexed histology analyses with probes for 30 proteins to assess the effects of each spatially defined treatment on the tumor and on the tumor microenvironment. We demonstrated the utility of this platform by assessing the effects of seven FDA approved drugs (paclitaxel, doxorubicin, lenvatinib, olaparib, palboclib, panobinostat and venetoclax) and combinations thereof on two late stage breast cancer mouse models with intact immunity (MMTV-PyMT and ErbB2DEx16). These studies indicated that the most effective, immunogenic, cell kill was produced by the epigenetic modulator, panobinostat, and was linked with upregulation of Calreticulin and MHC-I expression on tumor cells, and recruitment of professional antigen presenting macrophages and cytotoxic neutrophils. Increased expression of Galectin-3 and Neuropilin-1 on cancer stem cells and neutrophils, respectively, correlated with the therapeutic response three days after drug exposure suggesting a functional role of these two proteins in early events of anti-tumor immunity. Our spatial analyses also suggested the density of cancer stem cell and the formation of fibroblasts/ECM barriers as potential resistance mechanisms. Our findings in this pilot study demonstrate the ability to use effect signatures of locally administered drug microdoses, to predict the most effective, systemically administered combination regimens.

To delineate the ultrastructure of immunogenic cell death we combined the implantable screening microdevice with focused ion beam-scanning electron microscopy (FIB-SEM) to collect large format 2D images over a large area. A cross-section of the tumor/microdevice cavity was imaged at 4nm/pixel using the Thermo Fisher Maps software and FEI Helios NanoLab G3 DualBeam. Scanning conditions used were 3keV, 200pA and 4 mm working distance with the Concentric Backscatter (CBS) detector. This method allowed a very large block face to be imaged to access phenotypic relationships with respect to the microdevice. The overall map has the ability to be panned and zoomed into, providing a truly multiscale view of the tumor response similar to "Google Street View." The tissue was fixed, post-stained, and Epon-embedded using a typical heavy metal-based protocol described elsewhere.[1] We show local cellular morphologies to phenocopy the histological readout and to provide deeper understanding of the panobinostat drug mechanism of action. We measured the majority of immune cells present in the drug affected assay area to be neutrophils and macrophages (54% and 39%, respectively). Neutrophils function to release cytotoxic granules and mediate collateral damage by committing beneficial suicide. Both mechanisms mediate direct killing of surrounding tumor cells. All three main elements of innate immunity (neutrophils, macrophages and dendritic cells) then phagocytose components of dying tumor to activate adaptive immunity. At this early day 3 time point, a single T cell was observed in the assay forming immunological synapse with a necroptotic cell. Cancer stem cells and/or fibroblasts attempt and fail to die by apoptosis in the resistant tumor regions. Importantly, we used morphological and cytochemical features to determine the mode of tumor cell death. We measured 51%, 34%, 5.8% and 1.5% of cells dying by

autophagy, necroptosis, necrosis and apoptosis, respectively. To our knowledge, this is the first study providing direct evidence of immunogenic cell death in vivo with quantitative assessment of critical immune and cell death components. Further understanding of early events of anti-tumor immunity by implementing e.g. 3D electron microscopy or correlative light-electron microscopy will help to potentiate and optimize the use of immune checkpoint blockade in mammary carcinoma.

References

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