Application Note

Mass Density: A Predictive Biomarker for Cell Permeation and Cytotoxicity in Cancer Immunotherapy

This study ventures into the intricate interplay between natural killer (NK) cells and colorectal cancer (CRC) spheroids within a three-dimensional culture environment, underscoring the pivotal role of spheroid biophysical properties in mediating immune cell permeation and cytotoxic activity. Employing the W8 system for the precise quantification of spheroid biophysical characteristics, such as mass density, alongside confocal microscopy for visualizing NK cell infiltration, our research delineates how the structural attributes of cancer spheroids can significantly influence the effectiveness of NK cell-induced cytotoxicity.
The scope of our investigation encompasses generating CRC spheroids from various cell lines, assessing their biophysical properties, and examining the dynamics of NK cell engagement and cytotoxic response. Through detailed analysis, we discovered that spheroids with lower mass density, exemplified by DLD-1 cell-derived spheroids, facilitated greater NK cell penetration and exhibited higher susceptibility to NK cell cytotoxic effects compared to denser spheroids, such as those derived from SW620 cells.
Our findings highlight the potential of utilizing spheroid mass density as a predictive biomarker for the permeation capability of NK cells and their cytotoxic effectiveness. This approach not only enhances our understanding of the biophysical determinants of immunotherapy efficacy but also opens avenues for optimizing cancer treatment strategies by considering the structural properties of tumor spheroids. Consequently, this work contributes to the broader vision of personalized medicine, where the structural characteristics of tumors could guide the selection and optimization of immunotherapeutic interventions.

frontiers in Immunology

Physical Characterization of Colorectal Cancer Spheroids and Evaluation of NK Cell Infiltration Through a Flow-Based Analysis. Sargeti et All 2020

MATERIAL AND METHODS

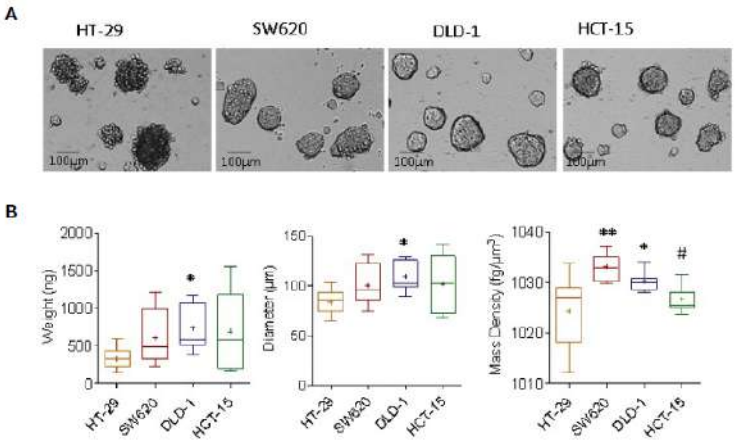
CRC Spheroid Generation
CRC spheroids were formed from four human colorectal cancer cell lines: HT-29, SW620, DLD-1, and HCT-15. These spheroids were cultivated in ultra-low attachment plates to promote three-dimensional growth.

Spheroid Biophysical Characterization
The biophysical properties of the CRC spheroids, including mass density, weight, and diameter, were quantitatively assessed using W8 system.

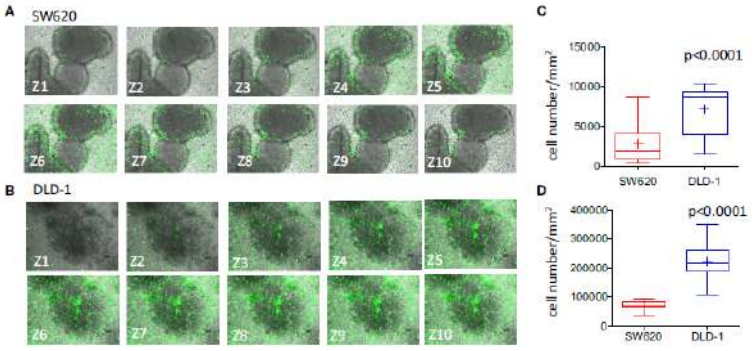
NK Cell Co-culture
NK cells were added to the CRC spheroids at an effector:target (E:T) ratio of 1:1. This co-culture setup was designed to investigate the cytotoxic response of NK cells against the cancer cells in a three-dimensional context.

Evaluation of NK Cell Permeation
The infiltration of NK cells into the CRC spheroids was analyzed using confocal microscopy. This technique allowed for the visualization of NK cell distribution and penetration within the spheroids, providing direct evidence of immune cell interaction with tumor cells.

RESULTS



Measurement of mass density, weight and diameter of CRC spheroids. (A) CRC spheroids were generated with HT-29, SW620, DLD-1, and HCT-15 CRC cell lines cultured in ultra-low adherent flat-bottomed microplates and analyzed on day 6 by inverted IX70 microscope (Olympus); images were taken with 20x objective NA 0.40 (200x magnification). Bar in each panel: 100mm.
(B) CRC spheroid samples were fixed with 4% PFA and analyzed with the W8 system. Data are graphically depicted in box-and-whisker plots and the lines, extending from the boxes, indicate variability outside the upper and lower quartiles. Results are expressed as the weight (ng, left graph), diameter (mm, central graph) and mass density (fg/mm3, right graph). *p< 0.05 and **p< 0.001 vs HT-29. #p<0.05 vs DLD-1.



Infiltration of CRC spheroids by NK cells.I. (A, B): SW620 (A) or DLD-1 (B) spheroids were seeded into a Matrigel dome in Cell Imaging plates (Eppendorf) and incubated with CFSE-labeled NK cells (E:T ratio of 1:1) for 24h. Samples were run under the FV500 confocal microscope and analyzed with FluoView 4.3b software (Olympus). Images were taken at different Z planes (Z1-Z10) every 10mm with a 20x objective NA 0.40 and shown as green CFSE+ NK cells merged with bright field spheroids. (C, D): NK cells present in each Z plane were counted with the Multipoint Analyze Particle tool of the Image J software and plotted as the number of NK cells/mm2 infiltrating SW620 (red boxes and whiskers) or DLD-1 (blue boxes and whiskers) and the mean±SD of 10 Z plans of a single spheroid (C) or mean±SD of NK cells/mm2 infiltrating 20 spheroids evaluated each at 10 different Z positions (D).

This Application Note highlights only a small portion of the results obtained. For a deeper understanding, we encourage you to read the associated paper at: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2020.564887/full

DISCUSSION

In our investigation into the interactions between natural killer (NK) cells and colorectal cancer (CRC) spheroids, we generated and characterized CRC spheroids: HT-29, SW620, DLD-1, and HCT-15 , with a particular focus on their biophysical properties. Notably, our analyses revealed that SW620 spheroids exhibited the highest mass density, thus indicating a more compact structure, whereas DLD-1 spheroids were found to be less dense and, consequently, less compact. This distinction in compactness between the spheroids of different cell lines led to a deeper investigation into how such biophysical characteristics could influence immune cell penetration and cytotoxicity, the details of which are extensively elaborated in the official paper.

Following the characterization phase, we conducted experiments where SW620 and DLD-1 spheroids were exposed to NK cells at a 1:1 effector:target ratio. After a 24-hour exposure period, confocal microscopy analysis provided insightful observations; notably, in the less compact DLD-1 spheroids, NK cells were able to infiltrate deep into the core of the spheroids. In contrast, within the more dense SW620 spheroids, NK cell penetration was largely confined to the surface. This differential infiltration underscores how the biophysical properties of spheroids can significantly influence the penetrating capabilities of immune system cells.

Moreover, the viability assays conducted post-treatment revealed a heightened cytotoxic efficacy on DLD-1 spheroids compared to SW620, further supporting the notion that the structural properties of spheroids can affect the outcome of immune cell engagement. These findings underscore the importance of considering the biophysical context in the development of immunotherapy strategies. For an in-depth exploration of these results and their implications, we encourage readers to refer to the official paper.

CONCLUSION

This study underscores the pivotal role played by the W8 system in biophysically quantifying CRC spheroids, shedding light on the interaction dynamics between NK cells and cancerous structures. The varied structural composition of spheroids significantly influences NK cell permeation and cytotoxicity. Understanding these structural properties is crucial, offering invaluable insights into complex biological processes and aiding in predicting treatment efficacy. Key biophysical characteristics such as spheroid mass density and compactness emerge as pivotal factors in determining NK cell infiltration and subsequent cytotoxic impact. For instance, less compact DLD-1 spheroids facilitate deeper NK cell penetration, resulting in more pronounced cytotoxic effects compared to densely structured SW620 spheroids. This underscores the importance of recognizing and analyzing the structural nuances of cancer spheroids, providing valuable guidance in tailoring and predicting the effectiveness of immunotherapeutic strategies.